JOURNAL OF NUTRITION
FASTING AND HEALTH

Mashhad University of
Medical Sciences

Summer 2022 / Volume 10 / Issue 2

JOURNAL OF NUTRITION FASTING AND HEALTH

http://jnfh.mums.ac.ir

Online ISSN: 2345-2587

jnfh@mums.ac.ir

JOURNAL OF NUTRITION FASTING AND HEALTH

Evaluation of the Intention of Nutritional Behavior in Women with Prediabetes Based On the Theory of Planned Behavior

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 02 Jan 2022
Accepted: 13 Mar 2022
Published: 30 May 2022

Keywords:
Planned behavior theory
Nutritional behavior
Prediabetes
Women

ABSTRACT

Introduction: Diabetes is one of the most common non-communicable diseases that can be prevented and controlled by following a healthy lifestyle. This study aimed to apply the theory of planned behavior in determining the predictors of nutritional behavior in women with prediabetes referred to the comprehensive health service centers of Mashhad University of Medical Sciences.

Methods: This descriptive-analytical study was conducted in 2020. A total of 196 pre-diabetic women referred to Mashhad Comprehensive Health Service Center (Iran) were selected by multi-stage random sampling. To collect the data, a researcher-made questionnaire including demographic information based on the theoretical structures of planned behavior [attitude, subjective norms, behavioral control, and intention to follow a healthy diet] was used. Data were analyzed in SPSS-25 software at a significance level of 0.05.

Results: The mean of total scores obtained in Attitude constructs 4.1 ± 0.50, subjective norms 3.67 ± 1.06, perceived behavioral control 4.13 ± 0.55 and behavioral intention 4.00 ± 0.39 and healthy eating behavior 3.00 ± 0.65 was reported. The construct of the subjective norm (β = 0.254) was a strong predictor in the structure of intention and intention (β = 0.419) and perceived behavioral control (β = 0.240) was a strong predictor of nutritional behavior ($p < 0.001$). Planned behavior theory was able to predict 40.5% of eating behavior in women with prediabetes.

Conclusion: It seems that by applying the planned theory in the design of educational programs, it is possible to improve the observance of a healthy diet in women with prediabetes.

► Please cite this paper as:
Mardanpour F, Abdollahi M, Jamali J, Vahedian Shahroodi M, Mahdizadeh M. Evaluation of the Intention of Nutritional Behavior in Women with Prediabetes Based On the Theory of Planned Behavior. J Nutr Fast Health. 2022; 10(2): 86-93. DOI: 10.22038/JNFH.2022.62586.1371.

Introduction

The incidence and prevalence of diabetes have been increasing rapidly in the last century. More than 7 million people worldwide are diagnosed with diabetes each year, with 3.8 million deaths from diabetes occurring, meaning one death from diabetes every 10 seconds (1). The prevalence of diabetes in the Middle East will increase significantly by 2030, and it is estimated that the annual growth rate of diabetes in Iran by 2030 will be the highest-second in the region after Pakistan (2). According to the latest reports of the World Health Organization in 2016, the prevalence of this disease in Iran is 9.6% for men and 11.1% for women (3).

The hereditary background and environmental factors are necessary for the development of type 2 diabetes, which is the most common type of

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diabetes. In many cases, poor nutrition and a sedentary lifestyle lead to pre-diabetes and then overt diabetes. Prediabetes is defined as a disease in which fasting glucose in patients with between 100 and 125 mg/dL and a two-hour glucose tolerance test between 140 and 199 mg/dL [4].

In epidemiological studies conducted in Mashhad in 2018, Isfahan in 2013, and Ahvaz in 2014, the prevalence of pre-diabetes was 15, 19.5, and 18.3%, respectively. These statistics show the high prevalence of pre-diabetes, which in all cases Prediabetes was more common in women [5-7].

90% of people with pre-diabetes are unaware that they have the disease, and about 30% of these people will develop type 2 diabetes [8]. This stage can be important and sensitive in the development of diabetes because it is detectable and its treatment may be effective in preventing or delaying diabetes [9]. Studies have shown that by modifying the pre-diabetes stage through lifestyle modification, the chance of developing type 2 diabetes in people with pre-diabetes can be reduced by up to 60% [10]. The Progressive Importance of Type 2 Diabetes and Its Chronic Complications Provide complex conditions that require lifestyle modification interventions to prevent and control type 2 diabetes, and lifestyle modification is the cornerstone of type 2 diabetes control. Therefore, the need for intervention in the pre-infection stage, and creating a low-cost strategy to prevent it is felt more than ever [5, 11].

The theory of planned behavior consists of structures of attitude (a person's cognitive and affective evaluation of a healthy behavior such as nutritional behavior as being good for them to manage their Diabetes prevention);), subjective norms (the important people in a person's life who encourage the person to perform healthy behaviors, such as my spouse would like me to Have a healthy diet to prevent diabetes and perceived behavioral control (The confidence or capability to perform a healthy behavior, such as 'I am to Have a healthy diet to prevent diabetes.), which are effective on behavioral intention [12]. The more favorable a person's attitude towards a behavior is, the more others approve of doing that behavior, and the more control a person feels over performing a behavior, the more it affects the intention and the more likely the person is to perform that behavior [13].

This theory has been used in many studies, including: in explaining the adoption of healthy eating behavior in type 2 diabetic patients [14], performing self-care behaviors in type 2 diabetic patients [15], as well as physical activity and drug adherence in patients with type 2 diabetes. [16]. Systematic review studies show the need for formative research to investigate the effect of the structures of the theory of planned behavior on the intention and adoption of nutritional behavior [17, 18]. Diabetes has not been reported in Iran and considering that the statistics show a high prevalence of pre-diabetes in women, to evaluate the status of nutritional behavior and improve nutritional behavior in the prevention of pre-diabetes to diabetes, effective social factors should be identified. Therefore, the present study aimed to apply the theory of planned behavior in determining the predictors of nutritional behavior in women with prediabetes in comprehensive health centers in Mashhad.

Materials and Methods

This cross-sectional descriptive-analytical study was conducted in 2020. The statistical population was women with prediabetes referred to Mashhad Comprehensive Health Service Centers who had health records.

Due to the lack of a completely similar study in Iran, the sample size was determined using the effect size formula and taking into account 5% error, test power 80%, and effect size 0.2, using the formula below 196 people.

$$n = \left(\frac{z_{1-\frac{\alpha}{2}} + z_{1-\beta}}{\frac{1}{2} ln \frac{1+r}{1-r}}\right)^2 + 3 = \left(\frac{1.96 + 0.84}{\frac{1}{2} ln \frac{1+r}{1-r}}\right)^2 + 3 = 196$$

In this study, a multi-stage sampling method was used to examine women with prediabetes, so that out of three health centers in Mashhad, three centers were selected by simple random as the main cluster. Following that, four comprehensive health service centers from each of the above health centers were selected by simple randomness (12 comprehensive health service centers in total were reviewed).

Finally, from each comprehensive health service center about the population of people with prediabetes, the number of people with prediabetes was selected from the list. Patients covered by comprehensive health service centers were selected by simple random sampling using a random number table.

Inclusion criteria included informed consent, willingness to participate in the study, women with pre-diabetes (with a diagnosis of blood sugar level 125-100), the age range of 30-59 years, no other chronic diseases and debilitating problems (Such as cancer, psychological problems, and high blood pressure, etc.), was at least the fifth elementary education and Iranian nationality. Individuals who had completed the questionnaires incompletely and were unwilling to cooperate during the study were excluded from the study.

Data were collected after obtaining the necessary permits from the Vice-Chancellor for Health of Mashhad University of Medical Sciences to complete the questionnaires.

Due to the prevalence of coronavirus and the limited number of patients referred to comprehensive health care centers in person, the questionnaires were completed by telephone. Thus, after justifying the study and stating the goals, and ensuring the confidentiality of the information and with their consent, it was completed within 15-20 minutes for each person. Individuals who were unable to respond by telephone were completed in person at the Comprehensive Health Services Center.

The tool for measuring the study variables was a researcher-made questionnaire that was developed based on the study of scientific sources and the opinions of professors in the Department of Nutrition and Health. After preparing the questionnaire, the face and content validity method was used to determine validity.

The questionnaire consisted of two parts, the first part contains personal characteristics such as age, body mass index, marital status, education, job, family income, disease, family history of diabetes and the second part is related to questions the constructs of the theory of planned behavior consisted of 16 questions. To construct the attitude, 6 questions were used. For example, "I think pre-diabetes is not a serious problem and will improve without any specific action" and "I think diabetes is completely inherited and nutrition has no role in preventing it." To measure the structure of the subjective norm, ask 2 questions: "The doctor encourages me to follow a healthy diet (including daily consumption of fruits and vegetables, milk and dairy products, not eating fatty and sweet foods, fast foods, etc.)" and " The staff of the health center encouraged me to follow a healthy diet (including daily consumption of fruits and vegetables, milk and dairy products, not consuming fatty and sweet foods, fast foods, etc.). Two questions were used to measure the perceived behavioral control structure, for example, "It is entirely up to me to decide whether to eat fatty foods." 3 questions were used to measure intentional behavior. For example, "I plan to eat less unhealthy foods (fast foods, soft drinks, sugary drinks, cakes, and chocolates, etc.) in the next month", all of which are on a Likert scale with a range of 1 (strongly disagree) It was up to 5 (strongly agree). Three questions related to the structure of behavior were assessed based on a five-point Likert scale from 1 (never) to 5 (always). For example, "I eat at least 2 servings of fruit a day."

Questionnaire scores ranged from 16 to 80, with higher scores indicating higher behavior in preventive nutrition than diabetes. To determine the face validity index in this study, the views of a panel of ten experts including two nutritionists and eight health education experts were used and their suggestions for correcting the questionnaire items were taken into account. The content validity of the questionnaire was calculated by calculating the content validity index (CVI) and content validity ratio (CVR), which were 0.89 and 0.90 for the whole questionnaire, respectively. The reliability of the instrument was assessed by calculating internal consistency and stability by calculating Cronbach's alpha and the interclass correlation coefficient between test and retest (ICC), respectively. Cronbach's alpha results were calculated to assess internal consistency for attitude structure of 0.730, subjective norms of 0.958, perceived behavioral control of 0.745, the intention of the behavior of 0.736, behavior of 0.722, and the whole questionnaire of 0.748. Intra-class correlation coefficient (ICC) was calculated for attitude 0.868, subjective norm 0.916, perceived behavioral control 0.942, intention behavior 0.960, behavior 0.948, and the whole questionnaire 0.925.

Height was measured using a tape measure fixed on the wall, standing and without shoes, and weight with minimal coverage and without shoes was measured using a digital scale.

Data were entered into SPSS statistical software version 25 and using descriptive statistics, indices of a tendency to center and dispersion such as mean and standard deviation of values

related to quantitative variables and determination and frequency distribution and percentage of qualitative variables were determined. Linear regression was used to determine the structures that had a more predictive effect on the nutritional behavior of women with prediabetes. The significance level in this study was considered 0.05. This article is taken from a research project with the ethics code IR.MUMS.REC.1399.324.

Table 1. Demographic information of the participants in the study marital status

variable		N	%
Marital status	Single	1	0.5
	Married	169	86.2
	divorced	10	5.1
	Deceased wife	16	8.2
Level of Educational	High school	113	57.7
	Diploma and above	83	42.3
Marital status	housewife	188	95.9
	Employed	6	3.1
	Retired	2	1.0
Household income	Less than enough	134	68.4
	Enough	61	31.1
	More than enough	1	0.5
Family history of diabetes	yes	70	35.7
	no	126	64.3

Table 2. Descriptive statistics for and inter correlations among variables attitudes, subjective norms, perceived behavioral control (PBC), Intention and Behavior.

Variable	Correlations					Means± SD	Range Max,Min
	1	2	3	4	5		
1.Attitude	-	-	-	-	-	4.01±0.50	5.00,2.83
2.Subjective Norms	0.109	-	-	-	-	3.67±1.06	5.00,0.00
3.Perceived behavioral control	0.226**	0.273**	-	-	-	4.13±0.55	5.00,2.00
4.Intention	0.145*	0.372**	0.506**	-	-	4.00±0.39	5.00,3.00
5.Behavior	0.075	0.051	0.302**	0.341**	-	3.00±0.65	5.00,1.67

* Correlation is significant at p <0 .05, ** Correlation is significant at p <0 .01

Results

The mean age of study participants was 47.41 ± 7.01. 36.7% (n = 72) were overweight and 49.5% (n = 97) were obese and 13.8% (n = 27) had normal body mass index. Table 1 shows the other demographic information of the study participants.

The mean standard deviation of the scores of the structural model of the theory of planned behavior and nutritional behaviors preventing type 2 diabetes in women with prediabetes is shown in Table 2. Among the model constructs, perceived behavioral control had the highest and behavior had the lowest mean percentage of the highest score.

The correlations between the structures of the theory of planned behavior and nutritional behavior in women with pre-diabetes are shown in Table 2.

The results show that there was a direct and significant correlation between the scores of perceived behavioral control structures and intention and behavior. Also, the results in Table 2 show that there was no direct correlation between the scores of attitude structures and subjective norm with behavior and it was not statistically significant.

Table 3. Simple linear regression results in predicting the intention of nutritional behavior based on the structures of the theory of planned behavior

Variable	Non-Standard Coefficient (Standard Deviation)	Standardized Coefficient	P-Value	R2
Attitude	0.133(0.046)	0.195	0.005	
Subjective Norms	0.184(0.048)	0.254	<0.001	0.212
Perceived behavioral control	0.191(0.066)	0.206	0.004	

Table 4. Linear regression results in predicting nutritional behavior based on the structures of the theory of planned behavior

Variable	Non-Standard Coefficient (Standard Deviation)	Standardized Coefficient	P-Value	R2
Attitude	0.147(0.055)	0.159	0.009	0.405
Subjective Norms	0.031(0.059)	0.031	0.605	
Perceived behavioral control	0.300(0.079)	0.240	<0.001	
Intention	0.564(0.085)	0.419	<0.001	

The findings of Table 3 show that the structure of subjective norms has the strongest power in predicting the intention of nutritional behavior in women with prediabetes. According to the results of the constructs of subjective norms, perceived behavioral control and attitude were able to predict 21.2% of changes in behavioral intention.

The findings of Table 4 show that the strongest construct in predicting nutritional behavior in women with prediabetes was intentional structure and was able to predict 41.9% of behavior changes; after that, the perceived behavioral control construct could predict 24% of behavior changes and then the attitude construct could predict 15% of behavioral changes. The lowest power of structures in predicting nutritional behavior was related to the structure of subjective norms. Also, the findings of the study showed that the predictive power of nutritional behavior of all structures was 40.5%.

Discussion

In this study, social cognitive predictors of nutritional behavior in women with prediabetes were determined based on the theory of planned behavior. As it is clear from the results, the highest correlation between the constructs of the theory of planned behavior and the nutritional behavior was related to the structure of perceived behavioral control and intention of behavior, and the weakest correlation was related to subjective norms and attitudes. Among the constructs of the theory of planned behavior, perceived behavioral control showed the highest correlation with behavioral intention, and intention had the highest correlation with nutritional behavior. Consistent with these findings, the results reported from the Satisfaction Study of Rezabeigi et al. In 2018 show that perceived behavioral control had the highest correlation with behavioral intention, and construct intention had the highest correlation with nutritional behavior [19].

In Close et al. study in 2018, the effect of perceived behavioral control on intention was the strongest [20]. The results of the study by White et al. In 2012 also showed the highest correlation between perceived behavioral control and intention, and perceived intent and behavioral control were a strong predictor for the consumption of saturated fat foods in the subjects [21]. Also, in Mazloomy et al. study in 2018, the perceived behavior control construct showed the highest correlation with weight loss intention and behavior, and the highest correlation of planned behavior theory constructs with weight loss behavior was related to perceived behavioral control structure and behavioral intention. And the weakest predictive power was reported for subjective norms and attitudes [22]. In the results of the study of Bagheri et al. In 2019, which was conducted to determine the factors affecting healthy eating behaviors in diabetic patients, the structures of the theory of planned behavior had a suitable fit. Findings of this researcher's study showed that perceived behavioral control structures, subjective norms and attitudes had a significant relationship with the intention to follow a healthy diet and the theory of planned behavior was a good predictor of the intention to follow a healthy diet in diabetic patients. [23].

The findings of the present study show that the constructs of attitude, subjective norm, and perceived behavioral control predict a total of 21.2% of the variance of intention. In this study, in general, the structures of the theory of planned behavior were able to predict 40.5% of nutritional behavior. According to studies, various factors can affect a particular behavior, including socio-economic factors, socio-cultural norms, and family characteristics that can affect nutritional behavior [24]. Consistent with these findings, the ability to predict nutritional behavior in the study of satisfaction of judges and colleagues was 44% [19]. Also in Babazadeh et al. study in 2015 on the predictors of fruit and vegetable consumption in students using the theory of planned behavior, model structures

were able to predict 39% of changes in fruit and vegetable consumption [25]. In Sadat Navabi et al. stud, regarding the prediction of weight loss behavior, the variables of attitude, subjective norms, perceived behavioral control and intention predicted a total of 44% of the behavior [26]. In Peyman et al. study in 2015 on the consumption of prepared foods in students, the results showed that the constructs of attitude, subjective norm, and perceived behavioral control together predicted 56.5% of intentional behavior and 20.32% of behavior [27]. The reason for the difference in the results of these studies may be due to differences in the study group, the type of behavior studied, and the research environment and data collection tools.

Based on the findings of the present study on the effect of attitudes of women with prediabetes on having nutritional behaviors that prevent type 2 diabetes, the results showed that attitudes were significantly associated with the intention of eating behaviors, although this relationship was not very strong. Contrary to these findings, in the study of Hossein Rouhani et al., Attitude had no significant relationship with the behavioral intention [14].

The findings of our study showed that the determining role of subjective norms of women with prediabetes is important in the intention of nutritional behavior but does not play an important role in following their eating behavior and women's attitude has a more important effect on following behavioral norms than subjective norms. . Subjective norms reflect a person's perception of whether or not others approve of a behavior. In this study, the influential people on the intention to perform the behavior of women with prediabetes, physicians, and staff of comprehensive health services. Therefore, the greater the supportive and facilitative role of abstract norms, the more likely it is that these individuals will intend to behave correctly. Similarly, the results of a systematic review and meta-analysis show that subjective norms are the most important determinants of nutritional intent and behavior [18]. Contrary to these findings, another study reported that subjective norms have a more significant effect on individuals' attitudes toward adhering to eating behavior [28].

In this study, perceived behavioral control after the intention was the most important determinant of diabetic preventive eating behavior. Perceived behavioral control reflects a person's beliefs about the availability or non-availability of resources and opportunities to engage in the behavior. People are motivated to engage in healthy behaviors when they feel they have control over that behavior [29]. In studies related to nutrition education, the role of perceived behavioral control as a predictor of nutrition-related intention and behavior has been highly emphasized [30]. Contrary to the present findings, the results of Malik et al.'s study in 2017 showed that perceived behavioral control structures and subjective norms were the most important factors determining the intention and adherence to healthy eating behaviors in pregnant women [26]. This discrepancy in study findings may be due to differences in the characteristics of study target groups and study tools.

According to the results, the intention was a strong predictor of behavior. Behavioral intention is the thought of performing a behavior that is the immediate determining factor of a specific behavior. High Predictability of Intention in this Study, was consistent with the Results of the Rezabeigi et al. Study(2018) Aiming at Factors Affecting Nutritional Behavior Related to Heart Disease [19] and Bagheri et al. Study(2019) Aiming to Investigate Healthy Eating Behavior in Diabetic Patients that intended was a strong predictor of behavior [23]. It seems that by promoting perceived behavioral control and intention, we can help increase the nutritional performance of preventing type 2 diabetes in women with prediabetes.

To our knowledge, the lack of a similar study in the field of application of the theory of planned behavior to predict nutritional behavior in pre-diabetic individuals is the strength of the study. One of the limitations of this study is the small sample size and considering that the present study was conducted only on women, so the results cannot be generalized to other age and sex groups and it is recommended that future studies be conducted in this field.

The present study is a cross-sectional study and it was not possible to analyze the causal relationships due to the nature of the study. The self-report of the questionnaire used and the researcher's lack of control over the accuracy of the report can cause bias. Also, the effect of individual differences and personality traits of individuals, the level of interest of individuals

when answering questions was beyond the control of the researcher. Another limitation of the present study was the selection of research samples from patients covered by Mashhad Comprehensive Health Service Centers, which certainly limits the generalization of the study results due to the cultural and economic context and demographic characteristics. Therefore, similar studies are recommended in other cities.

Conclusion

Considering that in the present study, the construct of perceived intention and perceived behavioral control was a good predictor of food behavior, by strengthening these structures, an effective step can be taken to improve nutritional behavior. Subjective norms were also the strongest structure affecting intention, so it seems that providing educational programs through the staff of comprehensive health service centers has an impact on behavioral intention and it is suggested when designing interventions to improve nutritional behaviors to prevent type 2 diabetes. Influential social cognitive factors should be considered.

Acknowledgments

This research is part of the master's thesis on health education and health promotion entitled "Study of the effect of education based on the theory of planned behavior on the promotion of nutritional behaviors of women with prediabetes referred to Mashhad health centers" in 2020. And it has been implemented with the support of the Research of Mashhad University of Medical Sciences. The authors of this article thank the university officials and the cooperation of the staff of Mashhad Comprehensive Health Service Centers and all study participants.

For its support and cooperation.

Conflicts of Interest

None declared

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JOURNAL OF NUTRITION FASTING AND HEALTH

Modelling of *Staphylococcus Aureus* under the Effect of *Carum Copticum* Essential Oil, pH, Temperature, and Inoculum Level

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 12 Feb 2022
Accepted: 13 Mar 2022
Published: 20 May 2022

Keywords:
Carum copticum essential oil
Predictive modeling
Staphylococcus aureus

ABSTRACT

Staphylococcus aureus is among the major causes of foodborne outbreaks globally. To limit its potential risks and predict its growth behaviors, it is crucial to define the growth boundaries of *Staphylococcus aureus*. So, this experiment was designed to estimate the growth behavior of *Staphylococcus aureus* in brain heart infusion (BHI) broth while affected by various concentrations of *Carum copticum* EO (0, 0.015, 0.030, 0.045%), pH (5, 6, 7), temperature (25, 35 ˚C), and inoculum levels (10^3, 10^5 CFU ml^{-1}). The assay was performed with 48 treatment conditions in triplicate. Visible turbidity represents growth onset was checked daily during 30 days of trial. According to the accelerated failure time (AFT) approach, a parametric survival model was chosen to predict the impact of selected variables on *Staphylococcus aureus* growth. GC-MS assay had quantified sixteen (16) compounds constituting 98.88% of pure oil. Based on our findings, the major components of essential oil were identified as thymol (57.18%), ρ-cymene (22.55%), γ-terpinene (13.07%), and trans-anethole (1.7%). The MIC value of the EO was 0.625 µl ml^{-1}. The median time to detection of bacterial growth was six days. All the predictor variables showed a significant effect on time to initiate the bacterial growth ($p < 0.05$). The ultimate model could precisely estimate the growth responses of *Staphylococcus aureus.*

▶ Please cite this paper as:
Mohamadi S, Khanzadi S, Jamshidi A, Azizzadeh M. Modelling of Staphylococcus Aureus under the Effect of Carum Copticum Essential Oil, pH, Temperature, and Inoculum Level. J Nutr Fast Health. 2022; 10(2): 94-102. DOI: 10.22038/JNFH.2022.63675.1377.

Introduction

Staphylococcus aureus (*S. aureus*) is a facultative, gram-positive foodborne pathogen, which gives rise to numerous complications like gastroenteritis, cutaneous infections, furunculosis, impetigo, scalded skin syndrome, and toxic shock syndrome [1-3]. It has been distinguished as the second cause of food poisoning outbreaks worldwide and a marker of insufficient hygiene of food handlers and inadequate storage temperature [4, 5]. As long as *S. aureus* is relatively poor in competition with other microorganisms, food poisoning from raw foods is rare [6]. Staphylococcal food poisoning is caused by the ingestion of foods that contain heat-resistant staphylococcal enterotoxins A, B, C, D, E, and F (SEA-SEF). Subsequently, heat treatment of food before consumption would eliminate the bacterial cells, but not their toxins [6]. Staphylococcal intoxication is induced by ingestion of staphylococcal enterotoxins (≤ 1.0 µg), which are secreted by > 10^5 CFU g^{-1} of cell bacteria [7]. *S. aureus* has the capability of increasing and surviving in a large variety of environmental conditions for a long time because of its ability to cultivate in a broad spectrum of thermal conditions (7- 48.5 °C) and pH (4.0-10.0) [7]. Furthermore, *S. aureus* is among the antibiotic resistance bacteria, which is even served as indicator for this phenomenon [8]. So, this growing problem of the emergence of antibiotic-resistant bacteria, along with the adverse effects of many antibiotics in humans (i.e., hypersensitivity and allergic reactions) has turned this issue into a significant public health concern [9]. Therefore, surging for new treatment options such as exploring the medicinal plants for their bioactive molecules with antimicrobial properties has gained lots of interests. Plant extracts such as essential oils (EOs) can serve as safe alternatives to synthetic antibiotics [10]. The antibacterial property of EOs against various bacteria has been defined to be at concentrations between 0.2 and 10 µl ml^{-1}. But the critical point is to identify the lowest level of EO, which could prevent the growth of pathogens, as well as being organoleptically accepted when used in food [11]. Among traditional medicinal herbs, *Carum copticum*

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(syn: *Trachyspermum Ammi*), belonging to the *Apiaceae* family, is an annual herbaceous plant. It is commonly known as a flavoring agent, also known to possess anti-allergic, anti-inflammatory, antimicrobial, hypocholesteremia, and antioxidant activities [9, 12, 13]. In Iran, their fruits, commonly known as '*Zenyan*' or '*Ajwain*' have been used widely in traditional Iranian medication to cure several diseases such as gastrointestinal, inflammatory, and rheumatic complications [14]. Its EO (2.5-5%) consists of phenolic components like thymol, p-cymene, γ-terpinene, carvacrol, and β-pinene, which represent antimicrobial properties [14, 15].

Taken together, the growth of foodborne pathogens can be affected by many intrinsic and extrinsic factors. To minimize their development and limit their potential risks, it is crucial to scrutinize the environmental effects on them and realize their growth boundaries. In this regard, predictive microbiology is typically applied to develop models to predict the growth responses of particular microorganisms. The basic premise of predictive microbiology is the reproducibility of the microbial responses to environmental factors [16]. These microbial performance models were initially illustrated by [17]. After that, it gains more and more attention worldwide and has been widely studied in recent years [1, 7, 16, 18, 19]. This study was also designed to estimate the growth behavior of *S. aureus* in brain heart infusion (BHI) broth while affected by various concentrations of *C. copticum* EO, pH, temperature, and inoculum levels.

Material and Methods

Experimental Approach

To evaluate the growth responses of *S. aureus*, while affected by *C. copticum* EO, pH, temperature, and inoculum levels, the present trial was conducted in a multidimensional matrix in BHI broth (Merck). This matrix (4×3×2×2×3 equal to 144 conditions) consisted of 4 levels of the EO (0, 0.015, 0.03, and 0.045%), 3 values of pH (5, 6, and 7), 2 incubated temperature (35 and 25°C), and 2 concentrations of inoculums of the bacteria (10^3 and 10^5 CFU ml^{-1}), which were observed daily during 30 days for the possible growth.

Bacterial Strain

The bacteria which were undergone in this experiment was *S. aureus* subsp. *Aureus* ATCC 25923 (Mast International Inc-England).

Inoculum Preparation

The inoculums were ready by plating the reference bacteria on Baird Parker agar plates followed by 24 h storage at 37°C. Then, second subcultures were plated for 24 h at 37°C. After that, to acquire the optical density (i.e., absorbance) of 0.669 at 600 nm, a full loop of bacterial cells was transferred to sterile cuvettes, which had been filled with isotonic saline solution, and then placed in a spectrophotometer apparatus (Jenway 6105, Essex, England). This acquirement is equal to a cell level of 1.2×10^9 CFU ml^{-1}. Tenfold serial dilution was performed to estimate the concentration of the bacteria cells in the suspension.

Plant Material

The pure steam distillation extraction of *C. copticum* EO was acquired from the Agro-Industry corporation, Mashhad, Iran (Nader-Co®).

GC-MS Identification of EO

The EO constituents were identified by employing an Agilent Technologies HP-6890 Gas chromatography-mass spectrometry (Palo Alto, CA, USA), with a capillary column (Model HP-5MS; 30 m × 0.25 mm internal diameter, 0.25 µm membranous thickness), coupled with a mass spectrometer (Model HP 5973; Agilent Technologies, Palo Alto, CA, USA), which have an electron ionization potential (70 eV). The oven temperature was held at 50°C for about 5 min at the beginning and gradually was elevated at the rate of 3°C per min until it reached 240°C. Eventually, it was raised at the rate of 15°C per min until reached 290°C, then maintained at this degree for 3 min. The inert gas was Helium which flowed past by a speed of 0.8 ml per min. The injector mode was run in the splitless mode, so 1.0 µl of each sample was injected manually. Quantitative data were obtained by using the percentage of the area of the peaks. Retention indexes were measured for each constituent using a similar sequence of hydrocarbons (i.e., n-alkanes series) injected in the same situations as the samples. The EO ingredients were quantified by assimilating their retention indexes in proportion to the n-alkanes series (C8 to C22), with those presented in the literature [20].

In Vitro Antibacterial Assay: MIC Test

The standard tube dilution technique was applied to define the minimum inhibitory concentration of the used EO [21]. The method

was carried out by binary serial dilutions of EO in BHI broth, using 5% (v/v) dimethyl sulfoxide (DMSO, Merk, Hohenbrunn, Germany) as an emulsifying agent. To attain EO concentrations from 1 % to 0.001 %, EO was serially diluted 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.4, 0.2 and 0.1 µl 10 ml^{-1}, respectively. Subsequently, the test organism (10^6 CFU ml^{-1}) was inoculated into the test tubes with various concentrations of EO. The tubes, which included various amounts of EO but not the bacteria were assumed as the negative control tubes. Then, all tubes (both controls and tests) were stored at 37°C for 24 h. Finally, the tubes were checked daily to observe the presence or lack of turbidity. Accordingly, the tube which had the lowest amount of EO that showed no evident bacterial growth (i.e., absence of turbidity) was considered as MIC. In the current research, four distinct values of EO, which were below MIC, were chosen as analyte amounts.

Performing the Trial

At first, 3.7 g of BHI powder (Merck, Darmstadt, Germany) was diluted in 90 ml distilled water using gentle heating in a 250 ml containing flask to prepare BHI broth medium. To produce and maintaine the stability of oil emulsion in BHI broth medium throughout the study period (30 days), the revised technique elucidated by Mann C and Markham JJ (1998) [22] were applied. In this regard, an emulsifying agent named dimethyl-sulfoxide (5% v/v, from Merck, Darmstadt, Germany), along with a stabilizing agent named agar agar (0.05% w/v, from Merck, Darmstadt, Germany) was added to the broth medium of all combinations, even to combinations without EO (0.0%), to recognize any probable influence of them on the growth responses of the tested bacteria. By adding distilled water, the ultimate content of BHI broth was 100 ml. The pH values were adjusted using the normal solution of HCL as an acidifying agent and a pH meter (Jenway, Staffordshire, UK) [5-7]. Then, all flasks were sterilized at 121°C for 15 min. After getting cold, the pH degree of every tube was remeasured and readjusted by using 1 N purified sterilized NaOH (or HCl). Then, the sterile BHI broth in the flask was portioned out into 3 ml amounts in sterilized capped tubes (16 × 100 mm; Becton Dickinson, Durham, USA). After that, different amounts of filtered sterilized EO (0, 0.015, 0.030, 0.045%), were added. The tubes were infected with *S. aureus* culture (10^3 and 10^5 CFU ml^{-1}) and stored at 35 and 25°C for 30 days. Throughout this period, the tubes were verified daily for possible observable growth (turbidity). At every observation, the number of tubes exhibiting growth was recorded. A negative control (uninfected tube) was considered for each combination. All experiments were performed with three repetitions. The whole numbers of conditions were 144 (4 × 3 × 2 × 2 × 3).

Analysis of the Statistics

The time to the nearest visual turbidity (TTD) was determined as a dependent variable for further consideration in this work. Considering the combinations that did not grow during the entire period of the experiment (30 days), standard regression approaches were recognized to be incompatible. As an alternative, an event-time (survival) assay was applied, which was capable of using all the observational data regardless of whether or not growth occurred. According to the accelerated failure time (AFT) method [23], a parametric survival model was chosen to evaluate the impact of each of the predictor parameters on time to detection. The inclusive type of AFT model is as follows (equation No. 1):

$$\log(t)=(\alpha+\beta_1 x_{1i}+\ldots+\beta_m x_{mi})+\log(\tau) \quad (1$$

Where log (t) is stands for the Napierian logarithm of the duration of time to growth inhibition, α is an intercept term, $\beta_1 x_{1i} + \ldots + \beta_m x_{mi}$ is a linear regression of the m predictor variables and their constant coefficients, and log(τ) is an error term. The AFT coefficients indicate the predicted alteration in log (t) for variations in the explanatory levels. In this survey, the Weibull, exponential, log-logistic, and log-normal distributions that could be elucidated in the AFT metric were estimated. To assess the degree of fitness of nominated distribution to the present findings, the mean square error (MSE) was calculated and matched up based on equation No. 2. The lower MSE value represents a more suitable match.

$$\mathrm{MSE}=\frac{\sum(\mathrm{Predicted}-\mathrm{Observed})^2}{(\mathrm{n}-\mathrm{p})} \quad (2)$$

Where n is the number of parameters to be observed, and p is the number of variables to be predicted.

To choose those predictor variables that best interpreted time to detection, a backward stepwise technique was utilized. Predictor variables that were not statistically significant were eliminated from the model one at a time, starting with the lowest significant, until the predicted regression coefficients for all remaining variables were significant at an alpha level of < 0.05.

Result

Determination of EO Constituents

The ingredients of *C. copticum* EO, along with their retention time and percentages, were summarized in Table 1. The GC-MS assay had quantified sixteen (16) compounds constituting 98.88% of pure oil. The major components were identified as thymol (57.18%), ρ-cymene (22.55%), γ-terpinene (13.07%), and trans-anethole (1.7%) (Table 1).

Table 1. Essential oil composition of *C. copticum* identified by GC-MS.

No.	Phytochemical	Percent	Retention index (RI)
1	*α-Pinene*	0.29	11.35
2	*ß-Pinene*	0.43	13.45
3	*ß-Myrcene*	0.34	14.28
4	*α-Phellandrene*	0.065	14.89
5	*α-Terpinen*	0.311	15.54
6	ρ-Cymene	22.55	16.21
7	β-Phellandrene	0.541	16.29
8	*γ-Terpinene*	13.07	17.93
9	*α-Terpinolene*	0.095	19.18
10	*α-Terpineol*	0.155	24.92
11	L-Carvone	0.908	27.97
12	*trans* -anethole	1.7	28.68
13	Thymol	57.18	29.73
14	Carvacrol	0.524	29.84
15	3-Dodecen-1-al	0.161	36.51
16	Apiol	0.566	42.73
	Total identified	98.886	

MIC Analysis

In vitro antibacterial properties of *C. copticum* EO was evaluated by standard tube dilution approach versus *S. aureus*. The antibacterial property was described as MIC value. In the present survey, the MIC value of the EO was 0.625 µl ml^{-1}. Thus, four distinct values of EO below MIC were chosen as analyte amounts.

Table 2. Accelerated failure time model of factors influencing time to detection of bacterial growth.

Variables	β (SE)	P-value	TTD ratio (95% CI)
Intercept	-0.536 (0.056)	< 0.01	
Essential oil (%):			
0	0		1
0.015	1.096 (0.052)	< 0.01	2.99 (2.70-3.31)
0.030	1.984 (0.061)	< 0.01	7.27 (6.45-8.19)
0.045	2.994 (0.071)	< 0.01	19.98 (17.38-22.97)
pH:			
7	0		1
6	0.367 (0.050)	< 0.01	1.44 (1.30-1.59)
5	0.885 (0.042)	< 0.01	2.42 (2.17-2.70)
Inoculum level (CFU/ml):			
10^5	0		1
10^3	0.305(0.048)	< 0.01	1.35 (1.24-1.47)
Temperature (˚C):			
35	0		1
25	0.896 (0.044)	< 0.01	2.45 (2.24-2.67)
P	4.541 (0.327)		
1/p	0.220 (.015)		

β: Regression coefficient; SE: Standard error; CI: Confidence interval; TTD: Time to detection.

Definition of Growth/No Growth

Close to 81.25% of conditions (117 out of 144) displayed turbidity throughout the entire survey, and 18.75% of conditions (27 out of 144) did not show turbidity, so regarded as censored observations. According to the MSE value, the Weibull model represented the most suitable match to the obtained findings. The lower the value of the MSE, the better the fit. The MSE value of the Weibull model was 116.44. The MSE values were 139.98, 125.98, and 647.56 for log-normal, log-logistic, and exponential models, respectively.

Time to Detection (TTD) Assessment

The median time to detection of bacterial growth was 6 days. Kaplan-Meier survival curve for predictor variables is indicated in figures 1-4. The ultimate model displayed that all predictor variables had significant correlation ($P < 0.01$) with time to detection (Table 2).

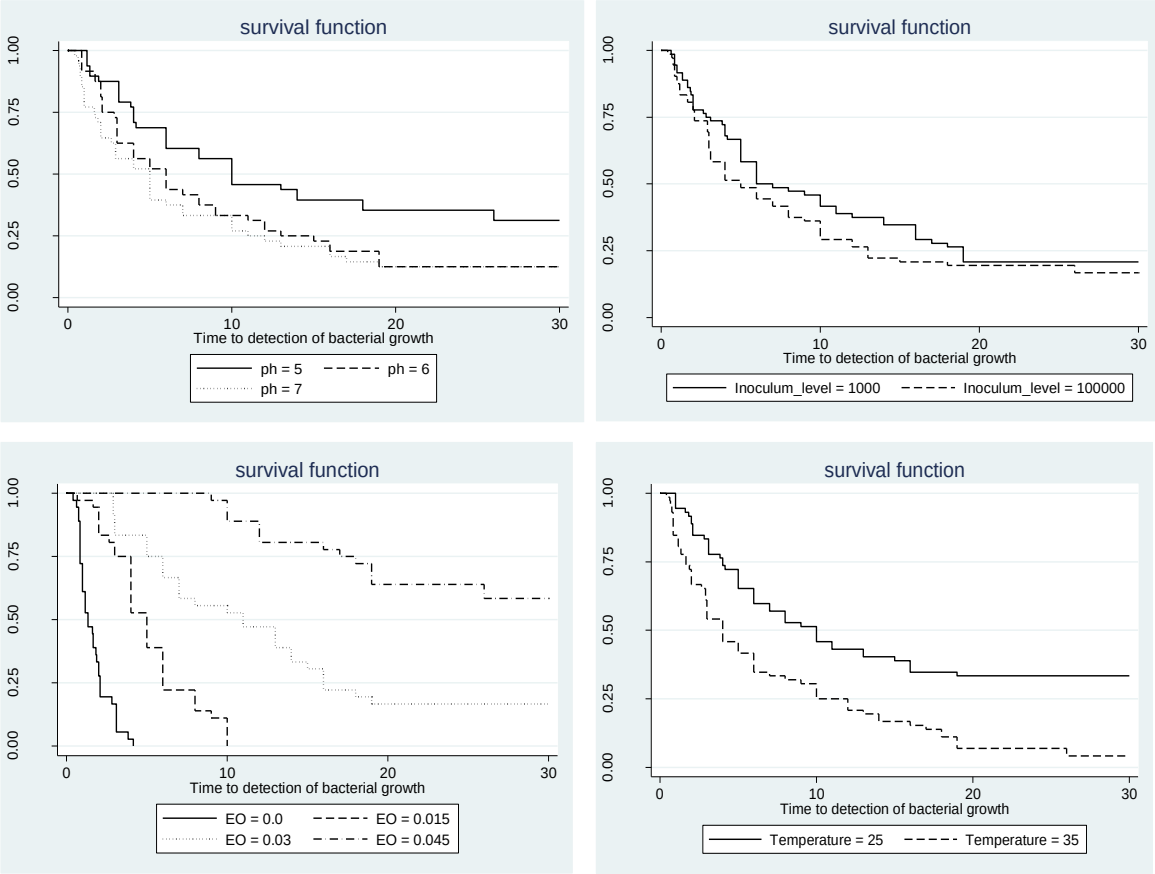

Figure 1. Kaplan-meier survival curves displaying the proportion of no growth combinations for various levels of pH, inoculum size, essential oil, and temperature.

Taken together, TTD for treatment conditions that contain 0.015%, 0.03%, and 0.045% of *C. copticum* EO was 2.99, 7.27, and 19.98 times higher than those without it, respectively. Moreover, TTD for those treatment conditions with pH values of 6 and 5 was 1.44 and 2.42 times higher than those with 7, respectively. In addition, this period for treatment conditions with an inoculum level of 10^3 was 1.35 times higher than combinations with an inoculum size of 10^5. Moreover, this period for conditions with incubator temperature of 25°C was 2.45 times higher than combinations with incubator temperature of 35°C. The final model equation No. 3 is as below: (3)

$$TTD = \left[-\ln 0.5\right]^{0.22} \times e^{-0.54+0.89T25+0.31IL1000+1.09EO0.015+1.98EO0.03+2.99EO0.045+0.88PH5+0.36PH6}$$

Where "TTD" is time to detection, "ln" is the natural logarithm (logarithm to the base e), "e" is a constant coefficient roughly identical to 2.718281828, "T" is temperature," IL" is inoculum size, and" EO" is essential oil. The model well estimates the value of TTD, which described the growth behaviors of *S. aureus* as environmental conditions were modified. Figure 2 shows the relationship between the observed and predicted values by the Weibull model for time to detection (TTD) of bacterial growth in designed combinations.

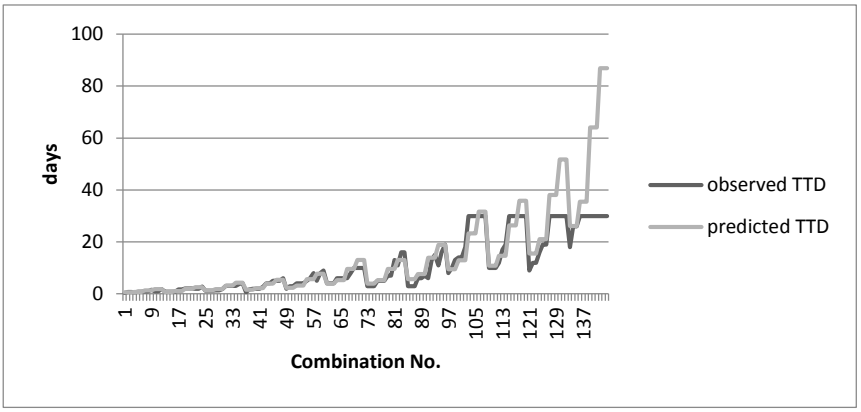

Figure 2. Observed and predicted days needed for growth onset of *S. aureus* (TTD) according to the weibull model.

Discussion

A key point for controlling *S. aureus* and other foodborne pathogens is identifying the parameters which affect their growth and manipulating those parameters to limit their potential risks [24]. With respect, multifactorial combinations have been designed to stop microbial growth, particularly *S. aureus* [6, 7, 16, 24], which was the aim of the multiple hurdle effect proposed by Leistner *et al*. [25]. After that, the concept of predictive microbiology came into existence to combine the mathematical modeling with the experimental data within a matrix of conditions that influence the growth responses of foodborne pathogens to predict the growth fate of micro-organisms [1, 6, 26, 27]. Recently, predictive modelling has been significantly expanded. However, the number of works arranged for modelling the effects of EOs, is scarce [16, 18, 26, 28]. So, the purpose of this work was to estimate the growth behaviors of *S. aureus* in BHI broth while affected by *C. copticum* EO, pH, temperature, and inoculum levels. Based on the concept of TTD in predictive microbiology, the Weibull model was nominated to estimate the growth onset of *S. aureus* in other situations. Our findings disclosed that in the ultimate model, all predicted variables had a significant correlation with TTD. Respecting the impacts of the predictor variables on *S. aureus* growth, the lower the values of temperature, pH, and inoculums, but higher levels of the EO would lead to the higher values of TTD. Similar data have been obtained by other scientists [7, 11, 16, 18]. Moreover, the evidence provided by another scientist [19] showed that elevated temperature would enhance TTD while increasing the pH and inoculum size would decrease TTD.

C. copticum EO is a potentially valuable source of phenolic compounds, which possess high levels of antimicrobial activity [18]. These phenolic components are naturally hydrophobic, hence quickly diffusing into the cell and induce destabilization and destruction of the cell wall, then lead to leakage of vital intracellular components, and eventually end it up with inactivation of enzyme mechanisms [29, 30]. There is remarkable attention to the potential application of these compounds as food additives to postpone and, or inhibit the growth of food spoilage. Among which *S. aureus* is of great significance [16]. In this research, the principal component was thymol (57.18%) and the second dominant constituent was p-cymene (22.55%). In accordance with our findings, a recent study [31] also indicated thymol (48.4%), p-cymene (21.8%) as the major components. Whereas another survey [30] reported γ-terpinene (39.8%), thymol (34.1%), and p-cymene (24.8 %) as the major compounds. Moreover, in another similar research [14], the major components were detected to be carvacrol (1%)

and p-cymene (23%). These differences may be attributed to the various geographic regions, climatic conditions, cultivation time, and genetic variations [30, 32]. The current research illustrated that the antimicrobial activities of plant EO are related to concentration, which is in good agreement with another study [31]. Well coped with our data by elevating the content of EO, the growth onset of *S. aureus*, and the number of no growth combinations raised [16]. Scientists [33] have demonstrated that the application of EOs along with other inhibitory methods like low temperature or low pH could arise synergistic effects to the current method.

Another inhibitory parameter was pH, which could significantly influence the growth onset and metabolism of microorganisms [16]. It was observed that *S. aureus* could grow at pH = 4.5, which defined the capability of *S. aureus* to cultivate at low pH [6]. Although, its growth onset at low pH can be affected by other parameters like the type of acidulants [1, 7]. It was disclosed that in acidic situations, *S. aureus* modulates its gene expression to improve defense mechanisms against acidity, which would result in growth retardation [29]. Apart from the direct impact of pH on the activity and stability of macromolecules, the hydrophobicity of EO is also higher at lower pH, which promotes their dissolution in the bilayer lipid membrane [18, 29]. Similar to our findings, the growth decline of *S. aureus* at lower pH was also demonstrated by other researchers [11, 16, 24].

Temperature is another most relevant hurdle applied to control microbial growth. *S. aureus* is a mesophilic bacterium that grows within a temperature range (7 - 48.5°C) depending on other environmental conditions, with an optimum of 30 - 37°C. Based on our findings, increasing the storage temperature positively affects the rate of proliferation. In the similar conditions, the time to detection of the bacterial growth for conditions with a storage temperature of 25 ˚C was 2.45 times higher than those of 35 ˚C. In keeping with the above observation, various researchers have shown that the growth rate of *S. aureus* at refrigeration temperatures (< 8 °C) was inhibited. And the slowest growth was noticed to be at 7°C. While, the most accelerated growth was discovered to be at 43°C [5, 7, 28]. Furthermore, it has been proven that low temperature, in combination with low pH has been more effective against *S. aureus* [7]. To sum up, temperature mainly affects the shape of the intracellular enzymes required for metabolism hence lower temperature would lead to lower metabolic activity [1, 16].

Another significant hurdle on the growth onset of the microbial population was inoculum size, which was well documented by different scientists [11, 16]. Our findings also revealed the extension of TTD for combinations with lower inoculum levels. Moreover, it has been indicated that greater inoculum size led to growth onset at the lower pH. By elevating the inoculum size, the possibility of detecting cells in the appropriate physiological condition to initiate growth increases hence the intensity of other inhibitory parameters should be increased [11].

Conclusion

The results disclosed that the obtained TTD of bacterial growth and the value estimated by the Weibull model equation was appropriately estimated the growth onset and prevention situations of *S. aureus* as affected by EO, pH, temperature, and inoculum levels. This sort of studies can supply food manufacturers with reliable methods to predict the growth boundaries of *S. aureus*, prevent enterotoxin production along with the extension of their shelf-life, and hence contribute to the safety of relevant foods.

Acknowledgments

The authors sincerely appreciated the financial support acquired from the Faculty of Veterinary Medicine, FUM, for performing this study (Grant no: 2059610). We also thank Mrs. Samira Khajenasiri for her technical support.

Conflict of Interests

The authors declare no conflict of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Impact of High-Intensity Interval Training with Physical Fitness Course Using Royal Jelly Supplementation on Lipid Profile in Overweight and Obese Middle-Aged Men

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 12 Feb 2022
Accepted: 13 Mar 2022
Published: 20 May 2022

Keywords:
Royal Jelly
High intensity interval training
Lipid profile
Obesity

ABSTRACT

Introduction: Royal jelly contains large amounts of phenolic compounds from the flavonoid family, which can improve the lipid profile with exercise. This study aimed to study the effect of high intensity interval training (HIIT) using royal jelly consumption on triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in overweight and obese middle-aged men.

Methods: This study was conducted on 60 middle-aged men, who were randomly divided into four groups: 1) control + Placebo, 2) training, 3) royal jelly supplementation, and 4) training + royal jelly supplementation. The subjects of training and training + royal jelly supplementation groups performed the training protocol. The HIIT protocol was implemented for eight weeks with high intensity of 85-95% of the maximum heart rate, and active rest periods included 60-70% of the maximum heart rate. The participants in the royal jelly supplementation groups received a 1000 mg capsule once a day. The SPSS software version 22, one-way ANOVA, and Tukey's *post hoc* tests were utilized to perform intergroup data analysis, and a dependent sample t-test was used to carry out intra-group data analysis at a significance level as much as $P \leq 0.05$.

Results: LDL, TC, and TG serum levels were reduced and HDL was increased in HIIT training, consumption of royal jelly, and training + royal jelly supplementation in overweight and obese middle-aged men ($p \leq 0.05$).

Conclusion: According to the results, HIIT combined with royal jelly supplementation could improve lipid profile in obese or overweight people prone to cardiovascular disease and various types of diabetes.

▶ Please cite this paper as:
Hazrati Nejad H, Khajehlandi A, Mohammadi A. The Impact of High-Intensity Interval Training with Physical Fitness Course Using Royal Jelly Supplementation on Lipid Profile in Overweight and Obese Middle-Aged Men. J Nutr Fast Health. 2022; 10(2): 103-109. DOI: 10.22038/JNFH.2022.62978.1373.

Introduction

Globally, being overweight and obese is a significant public health concern, which puts a heavy financial burden on different communities. There were nearly 2 billion adults over 18 in 2016, and among them, 39 and 40% were obese. In other words, 11% of men and 15% of women, i.e., more than half a billion people, were obese worldwide. The rate of overweight and obesity has increased over the past 40 years [1]. Obesity is a complex, multifaceted disease, whose rate has been estimated to be doubled since 1980 worldwide, so approximately one-third of the world's population is now overweight or obese. The obesity rate has increased among all ages and genders, regardless of geographical location, ethnicity, or socioeconomic status. However, the prevalence of obesity is much higher in older people [2].

According to epidemiological data, heart and blood-related disease (CVD) is the primary cause of mortality and morbidity. People who develop CVD experience severe suffering and a decline in their quality of life, placing a heavy economic burden on their families and communities [3, 4]. Triglycerides, cholesterol, and related lipoproteins are the main components of human body fat, playing essential physiological roles including cell membrane stability, energy storage, hormone and bile acid synthesis, uptake and assembly of dietary fats, stress response, total cellular signaling, and calcium metabolism [5, 6]. Lipid metabolism disorders may result in several metabolic disorders, including instances of cardiovascular disease [7]. Plasma levels of

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total cholesterol (TC), low-density lipoprotein (LDL), and triglycerides (TG) increase the risk of cardiovascular disease, while high concentrations of high-density lipoprotein (HDL) can have a protective effect [8]. The reverse cholesterol transport process in which excess cholesterol from peripheral cells is transported and removed in the liver is one of HDL's most essential functions. Numerous epidemiological studies have clearly indicated that high concentrations of plasma HDL are associated with a lower risk of CVD. Therefore, increasing HDL levels is considered a promising treatment strategy [9].

High intensity interval training (HIIT) has recently become popular among cardiovascular patients, defined by performing high-intensity workouts, as well as active or inactive rest intervals with spending less time than traditional endurance exercises [10,11]. Keating et al. (2014) found that three months of HIIT had no significant effect on body fat in overweight adults [12]. In contrast, Gillen et al. (2013) concluded that the HIIT course for six weeks improved body composition in obese adults [13].

Although HIIT is performed with different intensities, durations, and frequencies, its effect has not yet been well established on obesity, weight, and serum levels of lipid profile factors (TG, TC, LDL, and HDL). Royal jelly supplementation can improve lipid profile factors, a good treatment for obese people prone to cardiovascular disease. The following study is conducted to evaluate the effect of training exercises and specifically high intensity interval training using royal jelly supplementation on some major cardiovascular causes (triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in overweight and obese middle-aged men.

Materials and Method

This quasi-experimental study was conducted with four groups (one control group and three experimental groups). First, the researcher made an announcement to identify and invite the overweight and obese middle-aged men in Gachsaran who wanted to work out to improve their weight and physiological conditions.

The inclusion criteria were being male in an age range of 40-55 years, and the exclusion criteria were suffering chronic diseases, smoking in the past six months, and exercising regularly in the past six months.

In the next step, individuals were invited for initial examinations, among whom 60 people were selected who were physically and mentally healthy based on the general health questionnaire results and clinical symptoms by a physician. Then, the subjects were randomly assigned to four groups, including 1) control (C), 2) training (C), 3) royal jelly supplementation (RJ), and 4) training + royal jelly supplementation (T+RJ).

Subjects in the experimental groups of T and T + RJ supplementation groups performed the training protocol, and the C group continued their daily activities without intervention. A briefing session was held initially in which the research conditions, including benefits and potential risks, were explained. The necessary recommendations were made for each subject, and an informed consent was obtained to participate in all research stages. During this study, the subjects were asked not to participate in out-of-the-protocol activities and inform the researcher in case of lifestyle change. The subjects were also asked to follow their usual diet under the pre-program research. In addition, subjects were prohibited from taking any supplements, medications, or diet. Initial assessments of height, weight, and body environment in the experimental conditions were conducted before starting the program.

The high-intensity interval training protocol included four 4-minute intervals with 85-95% of the maximum heart rate (HR_{max}) and 3-minute bouts of active rest with 60-70% of the maximum heart rate (HR_{max}). The subjects started the training with 85% of the maximum heart rate (HR_{max}) and improving the subjects' preparation by adding 5% to the intensity of training every week. Following the subjects' attaining 95% of the maximum heart rate, the training condition was kept constant until the end of the protocol.

In each session, warming up and cooling down were performed similarly for both T groups and included 15 minutes of mild aerobic activity with static and dynamic stretching. The subjects' maximum heart rate was calculated by the formula (220 – age) [14, 15], who took a 1000 mg capsule (Royall jelly Sofgel, Defenvit OPD Pharma) once daily with a fixed diet for eight weeks in the royal jelly supplementation groups.

Moreover, the control group used a capsule containing starch powder [16].

Blood sampling was performed in two stages. The first stage was performed at the training site by taking 8 cc of the blood sample 48 hours before starting the training program at 8-9:00 a.m., following approximately 10 hours of overnight fasting. The second stage of blood sampling was performed 48 hours after the last training session in the intervention groups with the same conditions as the initial test. The subjects were prevented from participating in case of symptoms such as dizziness, fever, nausea, and absence of more than two sessions.

Kolmogorov-Smirnov test (KS) was used to determine the normality of research data distribution.

Given the normality of data distribution in the variables, one-way ANOVA and Tukey's *post hoc* tests were run to examine the intergroup analysis of data, and dependent samples t-test was carried out to examine the intragroup analysis of data. In addition, SPSS software Version 22 was used to perform data analysis. The significance level of the statistical analysis of the present study was considered as much as P ≤0.05.

Results

Table 1 presents the mean and standard deviation for weight in the study groups. The results of dependent sample t-test showed no significant difference in the pre-test and post-test levels of LDL (P =0.83), HDL (P =0.96), TC (P =0.53), and TG (P =0.80) in the C group. However, posttest levels of LDL (P =0.001), HDL (P =0.001) ,TC (P =0.001), and TG (P =0.005) in the JR group, as well as LDL (P =0.001), HDL (P =0.001) , TC (P =0.001), TG (P =0.001) in the T group, and posttest levels of LDL (P =0.0001), HDL (P =0.0001), TC (P =0.0001), TG (P =0.0001) in the T+JR group were significantly different (Figures 1-4).

Table 1. The mean and standard deviation of weight in the pre-test and post-test of the research groups

Group	Weight (kg) Pretest	Weight (kg) Posttest
C	90.16 ± 2.75	90.42 ± 3.09
RJ	90.83 ± 2.48	88.91 ± 2.28¶ €
T	91.41 ± 2.55	88.83 ± 2.55 ¥*
T+RJ	91.33 ± 2.60	88.25 ± 3.26 ¥*

* Significant decrease compared to the pre-test (p < 0.001).
€ Significant decrease compared to the pre-test (p < 0.01).
¥ Significant decrease compared to the C group (p < 0.001).
¶ Significant decrease compared to the C group (p < 0.01).

The one-way ANOVA results marked some significant differences in LDL (P =0.001, F=6.72), HDL (P =0.001, F=8.33), TC (P =0.001, F=11.76), and TG (P =0.001, F=11.35) in the study groups.

The post hoc test findings revealed that LDL blood levels in the JR (P = 0.025), T (P = 0.012), and T + JR (P = 0.001) groups were statistically lower than group C. LDL blood levels in the T + JR group (P = 0.040) were significantly lower than the JR group (0.044) (Figure 1).

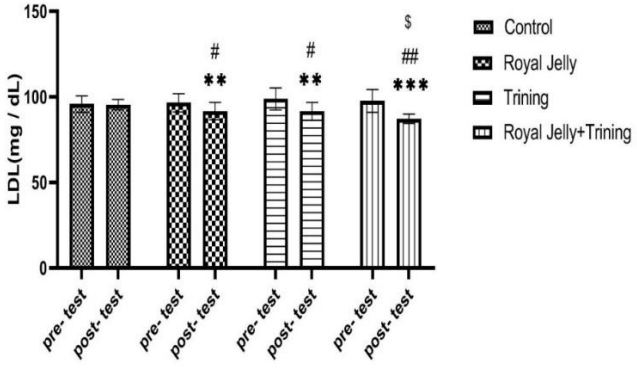

Figure 1. Mean and standard deviation of low-density-lipoprotein (mg / dL)
(P = 0<001) ; *(P = 0<0001)Significant decrease compared to the pre-test
#(P < 0.01) ; ##(P < 0.001) Significant difference compared to the C group in the post-test.
$ (P < 0.01) Significant difference compared to the RJ supplementation group in the post-test.

Furthermore, post hoc test results showed that HDL blood levels in the JR (P = 0.003), T (P = 0.002), and T + JR (P = 0.001) groups increased significantly compared to group C. There was no significant difference in the blood levels of HDL in the T + JR (P =0.905), JR, T (P = 0.925), and group T (Figure 2).

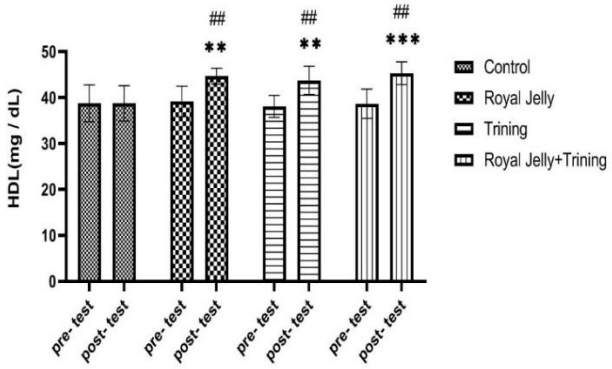

Figure 2. Mean and standard deviation of high-density lipoprotein (mg / dL)
(P = 0<001) ; *(P = 0<0001)Significant decrease compared to the pre-test
##(P < 0.001) Significant difference compared to the C group in the post-test.

The results of the Tukey's post hoc test revealed that the blood levels of TC in the JR (p=0.030), T (p=0.001), and T + JR (p = 0.001) groups were significantly much lower than group C. The levels of TC blood in the T + JR group (p = 0.028) were significantly different from the JR groups (Figure 3).

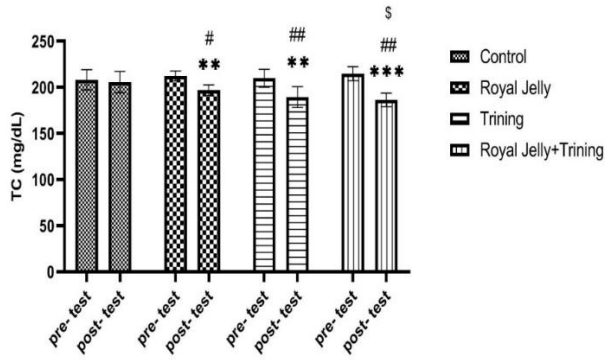

Figure 3. Mean and standard deviation of total cholesterol (mg / dL)
(P = 0<001) ; *(P = 0<0001)Significant decrease compared to the pre-test
#(P < 0.01) ; ##(P < 0.001) Significant difference compared to the C group in the post-test.
$ (P < 0.01) Significant difference compared to the RJ supplementation group in the post-test.

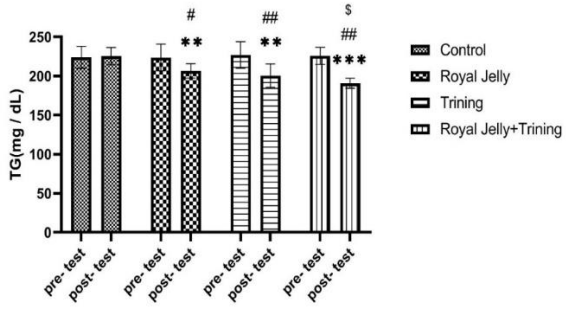

Figure 4. Mean and standard deviation of triglyceride (mg / dL)
(P = 0<001) ; *(P = 0<0001)Significant decrease compared to the pre-test
#(P < 0.01) ; ##(P < 0.001) Significant difference compared to the C group in the post-test.
$ (P < 0.01) Significant difference compared to the RJ supplementation group in the post-test.

The results of the Tukey post hoc test showed that the blood levels of TG in the JR (p = 0.034), A (p = 0.001), and A + S (p = 0.001) group were significantly lower than group C. The levels of blood TG on the T + JR (p=0.044) group differ significantly from that of JR group (Figure 4).

Discussion

Our findings revealed a noticeable reduction in the subjects' weight regarding the pre-test and post-test in the RJ supplementation, training, and T + RJ supplementation groups.

In contrast, LDL, TG, and TC levels significantly decreased between post-test groups and pre-test/post-test groups. In contrast, HDL levels significantly increased between post-test and pre-test/post-test groups alike.

Proper physical activity is one of the least expensive ways to maintain health and prevent non-contagious diseases like high blood pressure and cardiovascular disease. Sports experts and physiologists have proven that appropriate physical activity can promote human health, vitality, and vigor [17]. In this regard, Khammassi et al. (2018) studied the effect of four months of high-intensity training, excluding the calorie restriction on body composition and lipid profile in overweight/obese youth. The HIIT protocol included three sessions of training per week (30 seconds of work at maximum speed) consisting of 30 seconds of recovery. The results revealed a significant reduction in TC and TG levels, while LDL/HDL levels remained unchanged [18].

The results of Khammassi et al. (2018) were consistent to ours concerning TC and TG levels because both studies showed a decrease in levels of some markers. However, the results of these two studies are opposite in terms of LDL and HDL levels because in the present study, LDL levels decreased and HDL levels increased, while in Khammassi et al. (2018), both remained unchanged. The training protocol of both studies was HIIT, but the training time of Khammassi et al.'s study was four weeks longer than the present study.

Another difference between the two studies was the age and type of subjects. Although the subjects were overweight and obese in both studies, only middle-aged people were selected in the present study, while young ones were only studied by Khammassi et al. (2018).

One of the main characteristics of HIIT is the intensity of training, which can change many physiological factors in the human body. In this regard, Kannan et al. (2014) investigated the role of intensity training on lipid profile (LDL, HDL, and TG levels) in inactive older adults. The subjects performed moderate-intensity training on the treadmill for 40 minutes 5 days a week with high-intensity training for 20 minutes a day for three days a week for 15 weeks. The results indicated that moderate and high-intensity training significantly effected lipid profile, reduced LDL/TG levels, and increased HDL levels [19].

The intensity of HIIT can be a significant factor affecting lipid profile levels. Kannan et al. (2014) and the present study revealed that HIIT could significantly reduce LDL/TG and increase HDL

Factors such as duration of the training period, characteristics and principles of training, type of subjects, as well as type, intensity, and duration of the training in each session can change lipid profile levels as a reason for conflicting research results.

Other research studies have found that aerobic, resistance or combined training can modulate LDL, TC, and TG levels and improve HDL levels. In this regard, Dianatinasab et al. (2020) investigated the role of aerobics, resistance, and combined training on LDL, HDL, TC, and TG levels in women. Aerobic training included stretching, walking, and running on a treadmill and stationary bike. Resistance training included bodybuilding exercises. Aerobic, resistance training, and combined training led to significant reductions in weight, LDL, TC, and TG levels. HDL levels significantly increased only in the combined T group [20].

The results of Dianatinasab et al. (2020) were not consistent to those of the present study. Both results showed a significant decrease in LDL, TC, and TG levels and increase in HDL levels. Hence, the results can improve the lipid profile when the intensity, duration, and other factors regarding training principles are considered appropriate in training programs.

In this study, the effect of RJ supplementation and HIIT on lipid profile were also examined.

According to findings, taking RJ supplementation like exercise can improve lipid profile levels, and the best results are obtained when taking RJ supplementation is used concurrently with exercise. In line with the effectiveness of RJ supplementation, a study showed that RJ consumption significantly reduces the TC, TG,

and LDL levels in the blood serum of diabetic rats. In addition, it was shown that HDL levels increased significantly following RJ supplementation. The researchers suggested that RJ can be used to C and reduce the complications of diabetes and cardiovascular disease. The hypoglycemic and hypolipidemic effects of RJ are probably due to various antioxidants in it [21].

Saritas et al. (2011) investigated the effect of different levels of RJ supplementation on biochemical parameters in swimmers. Participants practiced swimming 20 km in 2 hours, five days a week for four weeks. No significant change was detected in LDL, HDL, TC, and TG levels [22].

The results of Saritas et al. (2011) were not aligned to those ours. In the current study, in TC, TG, and LDL levels decreased in the blood serum of overweight or obese subjects following HIIT/RJ supplementation. On the contrary, in Saritas et al. (2011), exercise training and RJ supplementation could not make a change in the lipid profile levels. The first important factor was the type of exercise activity, which was HIIT on a treadmill in the present study and swimming training in Saritas et al. c. Another important factor was the type of subjects and the training duration.

The current study was conducted on overweight and obese people for eight weeks, while Saritas et al. (2011) was performed on swimmers for four weeks. RJ contained large amounts of phenolic compounds from the flavonoid family, the most important of which were quercetin, camphor, apigenin, and luteolin [23]. Flavonoids regulate carbohydrate and lipid metabolism and reduce hyperglycemia, dyslipidemia, and insulin resistance, and reduce levels of oxidative stress and inflammatory responses.

Flavonoids, especially quercetin played a role in weight regulation and prevent weight loss in people with conditions such as diabetes [24, 25]. Research has shown that taking RJ supplementation can be an effective solution to overweight, high blood sugar, and hepatic steatosis by enhancing metabolic thermogenesis in rats' brown adipose tissues. The researchers suggested that RJ supplementation may be a promising new nutrient in the fight against obesity and metabolic disorders [26]. Therefore, taking RJ supplementation can increase the metabolism of brown adipose tissue by converting white adipose tissue to this tissue to improve the lipid profile in obese people. Eventually, eight weeks of HIIT regulated the lipid profile in overweight and obese men, which meant that HIIT could be prescribed for these people as a great way to regulate weight and improve their lipid profile. In the current study, the small size of the sample could be regarded as a limitation. Therefore, working on a bigger sample size can increase the reliability of the findings in the related studies. The lack of complete diet monitoring throughout the study is another setback. It should be noted that some other non-athletic physical tasks were not fully observed. Therefore, an analogous study is recommended to fully observe the participants' diet control and physical activity. A meticulous study should examine and explain the mechanisms in the variables studied as well as the results obtained.

Conclusion

The present study results showed that RJ supplementation could improve lipid profiles, like HIIT. In addition, taking this jelly and HIIT could be more effective for obese people.

Conflicts of Interest

The authors declared no conflict of interest.

Acknowledgments

The researchers appreciate the spiritual assistance of the Department of research of this university.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Intermittent Fasting on Body Weight, BMI, Waist Circumference, and Fat Mass Percentage in Adults with Overweight or Obesity

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 08 Mar 2022
Accepted: 09 May 2022
Published: 20 May 2022

Keywords:
Intermittent fasting
Overweight
Anthropometric parameters

ABSTRACT

Introduction: Fasting is a state of negative energy balance. Different fasting regimens have been used to achieve weight loss and other health benefits. To evaluate the effects of intermittent fasting on body weight, body mass index (BMI), waist circumference and fat mass percentage in people with overweight or obesity.

Methods: This experimental study was conducted on 22 adults of both genders aged 18-60 years, who were overweight or obese. A gradual intermittent fasting intervention was performed for 4 weeks with 10 hours of fasting in the first week and 16 hours of fasting in the last week. Variables such as body weight, BMI, waist circumference, and fat mass percentage were evaluated before and after the intervention in two groups, with and without dietary recommendations (ad libitum).

Results: At the end of the intervention, body weight, BMI, and waist circumference were significantly reduced in both groups, except for fat mass percentage. On the contrary, no statistically significant differences were found for all the parameters between groups ($p>0.05$).

Conclusion: According to results, body weight, BMI, and waist circumference decreased significantly in the two groups after four weeks of intermittent fasting.

► Please cite this paper as:
Meza Miranda ER, Castillo Ocampos F, Domínguez Colman D, Ramírez Chamorro M. The Effect of Intermittent Fasting on Body Weight, BMI, Waist Circumference, and Fat Mass Percentage in Adults with Overweight or Obesity. J Nutr Fast Health. 2022; 10(2): 110-115. DOI: 10.22038/JNFH.2022.64271.1385.

Introduction

Obesity and a poor diet are essential and modifiable factors contributing the development of chronic non-communicable diseases, such as cardiovascular diseases, with an estimated risk attributable to cardiovascular mortality as much as 13%. Several dietary interventions have improved obesity, including calorie restriction (CR) and limiting calories (1).

Given that adherence to CR in humans is low (2), other alternative feeding methods were developed with the same benefits as CR. Intermittent fasting (IF) can be mentioned as one of these methods, which is also known as periodic energy restriction (3) and comprises eating patterns in which fasting (negligible energy intake without deprivation of essential nutrients) is followed for hours.

The best-known forms of IF include periodic fasting (PF) (4) and 5:2 intermittent fasting (fasting two days per week), time-restricted feeding (TRF) (5), in which the daily feeding is reduced, and alternate-day fasting (ADF) (6), in which the fasting day can be altered (7).

The health benefits of IF have been demonstrated through several clinical trials, especially weight loss in obese people, diabetes, and cardiovascular diseases, as well as improving cardiometabolic parameters (3). Several systematic reviews and meta-analyses of randomized clinical trials (RCTs) (8-11) have supported the IF benefits. However, the overall strength and quality of the evidence are low because the existing RCTs focused on FI subtypes or particular health outcomes.

Patirkon et al. (12) concluded that the IF can have positive results in terms of anthropometric and cardiometabolic parameters, even in overweight adults. This review included meta-analyses of RCTs investigating the effects of FI in adults.

This umbrella review included 11 meta-analyses with 130 RCTs. Participants followed three

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months of IF (13-15) and evaluated 104 relationships of different FI types and its effects on health, specifically in people with excess weight (3,14,15).

Beneficial results were observed on anthropometric parameters, such as lipids, glucose, and pressure of blood, and 28 statistically significant relationships were obtained. six relationships of moderate quality evidence were found from these studies. Study evidence was low in the other studies analyzed with significant results (12).

This study selected time-restricted feeding (TRF), restricting eating times to less than 10 hours per day without reducing daily calories during permitted eating times (13).

The novelty of this study is the use of TRF interventions gradually in two groups, with and without dietary recommendations (ad libitum). The results showed that the gradual TRF-type IF in both intervention groups benefits anthropometric parameters and body composition (fat mass).

No clinical trials have been conducted so far to evaluate this type of fasting and the effect of this type of feeding during the hours on the mentioned parameters.

Materials and Methods

Study design

This experimental study was conducted on a randomized clinical trial in both genders with overweight or obesity from Asunción – Paraguay, aged 18-60 years without underlying diseases in June 2021.

The study was carried out for a month (starting in June 2021) by researchers from the Multidisciplinary Center for Technological Research - National University of Asunción and Universidad del Norte - Paraguay in three base, follow-up, and final visits after the intervention. The researchers explained the nature, objective, and methodology in choosing the participants, and obtained informed consent from each volunteer, who wished to participate in the study. The assessments were not invasive and performed according to the corresponding clinical practice with no risk for the participants.

Clinical Endpoints

The primary endpoint of this study was evaluating nutritional status by IMC and assessing the abdominal circumference and body fat before and after the intervention. The secondary endpoint after the intervention was to show the efficacy of the intermittent fast in anthropometric parameters in people with excess weight.

Study Participants

The inclusion criteria were being adults aged 18-60 years who are overweight or obese and reside in Asunción or the Metropolitan Area and give their informed consent. The exclusion criteria were pregnant or lactating women, people with comorbidities, such as hypertension and diabetes, and who carry out programmed physical activities.

Each researcher included the first five consecutive participants who met the inclusion criteria to avoid selection bias. No sample size calculation was performed due to the exploratory nature of this study.

Intervention and Evaluation

The non-probabilistic convenient sampling was performed after taking their informed consent. A total of 30 people were enrolled to participate in the study, who were divided into two groups of 15; the first with ad libitum intermittent fasting (IFA) and the second with intermittent fasting with dietary recommendations (IFD).

Sociodemographic variables such as gender, age, origin, occupation, marital status, and academic level were evaluated. On the other hand, anthropometric variables, including (body mass index) BMI, waist circumference and percentage of fat mass (electrical bioimpedance) were evaluated before and after the intervention.

The IFA group was subjected to intermittent fasting, but they were allowed to consume the foods without restrictions. The IFD group was subjected to intermittent fasting with recommendations for a healthy diet in the hours allowed for food consumption. This group had to avoid fried foods, junk food, excess salt, carbonated and sugary drinks, alcohol, processed foods, increase the intake of fruits, raw and cooked vegetables, at least two liters of water per day and choose healthy menus.

The intermittent fasting was prescribed in the form of time-restricted feeding and gradually for four weeks. The first week fast was 10 hours with 14 hours permission to eat, and the second week fast was 12 hours with 12 hours permission to eat. The third week fast and eat was 14 and 12 hours, and the last week was 16 and 8 hours, respectively. The fast began in the

afternoon/night in the hours of sleep to prevent their excessive hunger and match circadian changes in the body during the day. The volunteers were evaluated every week and given continuous advice during the intervention. Compliance with the dietary recommendations was carried out through an adherence survey. The student's t-test was used to determine significant differences before and after intermittent fasting of the study variables with p-value <0.05 using SPSS© software version 21.0. The research was carried out according to the ethical principles of the Declaration of Helsinki (16) and approved by the Ethics Committee of the Universidad del Norte.

Statistical Analysis

Statistical analyzes were performed based on the available data, and no substitution was performed for missing values. The student's t-test was used to analyze differences between the study variables before and after supplementation with p-value <0.05 using SPSS© software version 21.0.

Table 1. Sociodemographic characteristics

VARIABLE		n (%)
Gender	Female	14 (63.6)
	Male	8 (36.4)
Total		**22 (100)**
Education Level	Elementary	0 (0)
	High school	7 (31.9)
	College education	15 (68.1)
Total		**22 (100)**
Marital Status	Single	11 (50)
	Married	9 (41)
	Separated	2 (9)
Total		**22 (100)**
Job Occupation	Student	4 (18.1)
	Employee	7 (31.8)
	Businessman	2 (9)
	Professional	8 (36.3)
	Housekeeper	1 (4.8)
TOTAL	**22 (100)**	
AGE (AVERAGE ±SD)		
38.8±10,0		

Results and Discussion

In the study, 30 volunteers signed up, of whom eight people were dropped out due to force majeure. The rest received the intermittent fasting group with (13 people) and without (9 people) recommendations.

The majority of the sample (63.6%) was female, 68.1% had a university education, 50% were single, 36.3% had a profession as a work occupation, and the mean age was 38.8±10.0 (Table 1). This study was similar to the Wegman et al. (17) in terms of gender percentage, which examined the effect of intermittent fasting on oxidative stress (17), but this percentage was less than Trepanowski et al. (18) (86%) for intermittent fasting in weight loss and maintenance (18). The average age was lower than Trepanowski et al. (18), who found an average of 44 years in their study population (18).

The educational level exceeds the 50.2% observed by Machado et al. (19) on sociodemographic characteristics concerning cardiovascular health (19). In this study, 50% were single, a percentage exceeding the Savas (20) study (14.7%) on the effect of sociodemographic factors on fasting in diabetic patients (20).

Significant differences were found between the initial body weight, BMI, and waist circumference at the end of the intervention regarding the anthropometric variables of the group subjected to intermittent fasting with recommendations and the ad libitum group. No significant differences were found between the fat mass percentage before and after the intervention (Table 2).

The decrease in body weight was due to the restriction in food consumption. Few studies have so far shown the effects of IF on weight and the reduction of developing cardiovascular diseases. Almost all of these studies were conducted on obese people with a BMI of 30 to 39.9 kg/m^2) (21-23). Furthermore, Sundfor (24) reported comparable results for weight loss in

the intermittent and continuous energy restriction groups.

Klempel et al. (25) conducted a study on intermittent fasting and weight loss and found that BMI loss was statistically significant at 1.3 points out of 35 kg/m2 in obese volunteers, showing that intermittent fasting effectively reduces this parameter (25). On the other hand, practicing this type of lifestyle beyond a year can have a rebound effect when people go hungry or are not adequately accompanied. Fawzi et al. (26) studied people who fasted for a month as Ramadan style with and without Metabolic Syndrome. Those with Metabolic Sx had a BMI as much as 27.6 kg/m^2 one week before starting and 28.7 kg/m^2 at the end, and those without Metabolic Sx had a BMI as much as 26.2 kg/m^2 one week before starting and 26.9 kg/m^2 at the end. These results showed that the follow-up of each person who performs a specific type of immediate needs professional support to improve their health.

Table 2. Anthropometric parameters before and after fasting

IFD Group	Initial Weight (Kg)	Final Weight (Kg)	Initial Imc (Kg/M^2)	Final Imc (Kg/M^2)	Initial Waist Circum (Cm)	Final Waist Circum (Cm)	Initial% Of Fat Mass	Final % Of Fat Mass
AVERAGE	90.52	89.12	32.63	31.50	104.54	99.69	41.56	39.32
SD	15.38	15.65	5.08	5.12	9.84	9.33	10.42	10.63
IFA Group	**Initial Weight (Kg)**	**Final Weight (Kg)**	**Initial Imc (Kg/M^2)**	**Final Imc (Kg/M^2)**	**Initial Waist Circum (Cm)**	**Final Waist Circum (Cm)**	**Initial% Of Fat Mass**	**Final % Of Fat Mass**
AVERAGE	91.78	90.39	31.24	30.66	100.72	96.83	40.10	38.06
SD	20.01	19.95	5.79	5.45	19.13	17.66	4.45	5.52

IFD GROUP:
*Initial weight – Final weight **p=0.001**
*Initial IMC – Final IMC **p=0.012**
*Initial waist circum – Final waist circum **p=0.001**
* Initial % off at mass – Final % of fat mass p= 0.101
+ Student's t-analysis for related samples

IFA GROUP:
*Initial weight – Final weight **p=0.002**
*Initial IMC – Final IMC **p=0.019**
*Initial waist circum – Final waist circum **p=0.001**
* Initial % off at mass – Final % of fat mass p= 0.90
+ Student's t-analysis for related samples

Waist circumference was one of the anthropometric measurements with most reduction rate in this study. The meta-analysis by Arguin et al. (27) showed significant differences between the control and experimental group in terms of intermittent fasting on the reduction of waist circumference (27). This latter study showed a more significant reduction in waist circumference in the experimental group after one year of follow-up but not during the intervention period. Therefore, prolonged intermittent fasting effectively reduces this anthropometric measure.

Table 3. Anthropometric parameters after fasting between groups

	Final Weight (Kg) IFD	Final Wieght (Kg) IFA	Final IMC (Kg/M^2) IFD	Final IMC (Kg/M^2) IFA	Final Waist Circum (Cm) IFD	Final Waist Circum (Cm) IFA	Final % Of Fat Mass IFD	Final % Of Fat Mass IFA
Average	89.12	90.39	31.50	30.66	99.69	96.83	39.32	38.06
SD	15.65	19.95	5.12	5.45	9.33	17.66	10.63	5.52

*Final weight **p=0.72**
*Final IMC **p=0.65**
*Final waist circum **p=0.052**
*Final % of fat mass **p= 0.75**
+ Student's t-analysis for related samples

No statistically significant differences were found when comparing the results after the intervention between the two groups for all the anthropometric parameters (Table 3). Therefore, both ad libitum and dietary recommendations fasting significantly reduce weight, BMI and waist circumference in the same way.

Intermittent fasting in conjunction with healthy dietary recommendations includes several nutrients, considering the components of these foods, which has more positive effects than

including foods individually. For example, fiber is a food component that cannot be missing and is found mainly in fruits and vegetables. In addition to fiber, these foods contain optimal amounts of vitamins, minerals, and polyphenols, which are low in calories, provide satiety and have a high antioxidant capacity and a low glycemic index to control blood sugar. The Mediterranean diet is recommended in the IF since its components are healthier than other diets and includes fruits and vegetables, grains and olive oil, less meat, dairy, and solid fats. However, this diet has many variations to suit the study population from non-Mediterranean countries, and the results of randomized controlled trials provides inconsistent results (28).

On the other hand, intermittent fasting, which allows the patient to eat what they want ad libitum any time, leaves a gap in consuming foods with high fat, sugar, and calories. This diet would not lead to a severe problem in the case of consuming in short meal times. There are studies of alternate-day fasting without eating for one to two days. On eating days, they consumed high fat, sugar, and calories foods and lose weight with improved biochemical parameters (29). The prolonged fasting in the morning does not produce a compensatory intake during eating at lunch in ad libitum. Furthermore, this type of fasting does not increase appetite in the afternoon. In addition, morning IF reduces the appetite-stimulating hormone (ghrelin) levels in the afternoon. Thus, intermittent daily fasting can be safe because of the effectiveness of these mechanisms compared not eating for days (30).

One of the limitations of this study was that several participants withdrew during the study, and the sample size was reduced. In addition, biochemical parameters such as cholesterol, glycemia, and triglycerides could not be evaluated.

One of the strengths of the study is its uniqueness because it is one of the first studies on intermittent fasting intervention with two groups, one ad libitum (participant allow to eat what they want in the allowed hours) and another with healthy dietary recommendations.

Conclusion

According to results, the benefits of intermittent fasting were based on less food intake due to the restriction of eating hours without discriminating the types of food, as long as the fasting hours are respected.

Conflicts of Interest

The authors declared no conflicts of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Does Thyme Essential Oil (Zataria Multiflora) Improve Durability, the Taste and Nutritional Value of Doogh?

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ARTICLEINFO

Article type:
Short Communication

Article History:
Received: 26 Feb 2022
Accepted: 26 Jun 2022
Published: 30 Jun 2022

Keywords:
Doogh
Thyme
Escherichia coli
Minimum bactericidal concentration

ABSTRACT

Introduction: Diseases caused by the consumption of bacteria-contaminated foods have been of great importance in public health. Therefore, control of dairy product contamination with coliforms is a concern for the durability of dairy producers. Control of the contamination by natural products may have dual action; nutritive value and natural antibiotics. In this study, the antimicrobial activity of thyme essential oil against *Escherichia coli* O157: H7 in doogh (Iranian fermented dairy drink) was examined and the MIC and MBC were determined.

Methods: Antimicrobial activity of thyme essential oil was examined using different concentrations (0, 30, 60, 90, and 120 µl/ml) against bacteria with a density of 10^5CFU/ml during the 60-day at 4 °C. Moreover, the effect of different concentrations of essential oils on the taste of doogh was also studied. Taste evaluation was performed based on 5 points hedonic test to evaluate the effect of Thyme oil on the sense of taste. Duncan software was used to determine the difference between test and control groups at a confidence level of 5%. The SPSS software was also used for statistical analysis.

Results: All concentrations of thyme essential oil could stop the growth of bacteria ($P<0.05$). The inhibitory effect of oil increased with increasing concentration and time. Based on the results of sensory (taste) evaluation, treatment 2 (containing 60 µl/ml of essential oil of thyme) was selected as the best treatment.

Conclusion: Thyme essential oil can be used as a natural preservative with a positive impact on taste and nutritive value.

▶ Please cite this paper as:
Gaeini M, Pourahmad R, Haeri MR. Does Thyme Essential Oil (Zataria Multiflora) Improve Durability, the Taste and Nutritional Value of Doogh?. J Nutr Fast Health. 2022; 10(2): 116-120. DOI: 10.22038/JNFH.2022. 64038.1380.

Introduction

Diseases born from the consumption of foods contaminated with pathogenic bacteria impose significant morbidity, mortality, medical costs, and financial damage to health systems annually (1). In the meantime, many cases of human disease and death have been attributed to bacterial infections that can enter the human body through various roots such as drinking water, breathing, and food (2-4). Centers for Disease Control and Prevention reported that 9.4 million people in the United States get sick from foodborne pathogens annually. Such diseases result in near to 60000 hospitalizations and 1300 deaths each year (5).

Escherichia coli is a highly pathogenic bacterium that originates in the gut of many animals. Children, the elderly and immunocom promised people are more susceptible to infection caused by this bacterium (6 and 7). It is found in a wide variety of food products such as yogurt, milk, fruit juices, and meat (8). Pasteurization is a method of choice for eliminating the bacterium. However, heat treatment is not suitable for all kinds of foods and cross-contamination cannot always be prevented. Therefore, controlling the growth of pathogenic bacteria in food is of great importance both in terms of food quality standards and in terms of public health. One usual way to control the growth of infections in food is to use antimicrobial preservatives and compounds (9 and 10). Since chemical additives lead to allergies, undesirable side effects, and emerging antibiotic-resistant strains, there is a tendency to use natural products that not only lack side effects but also have nutritional properties. Natural preservatives like herbal essences and extracts have been shown to possess anti-bacterial effects (10 and 11). Among medicinal plants, Thyme essential oil and extract

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have been extensively studied in vitro as a source of antibacterials (12). Using plant preservatives to control the growth of pathogenic bacteria of food origin has two benefits; avoiding undesirable side effects and receiving nutritional ingredients. The present study aimed to investigate the antibacterial effect of Thyme essential oil at different concentrations as a natural flavoring and preservative on *Escherichia coli* O157H7 in pasteurized doogh. In this way, minimum growth inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Moreover, the sensory test was performed to assess the sensory properties of thyme essential oil on the taste of doogh (Iranian fermented dairy drink).

Material and Methods

Antibacterial Susceptibility Assay

The antibacterial effect of thyme essential oil was evaluated using the disk diffusion method based on the National Committee for Clinical Laboratory Standards (NCCLS). Three milliliters of sterile distilled water were added to the lyophilized *Escherichia coli* O157H7 (Iranian Biological Resource Center, Iran) and was kept under ambient temperature for 4 hours under a sterile hood. The bacteria were cultured on MacConkey agar medium and incubated at 35 °C for 24 hrs. The cultured bacteria were used for making a suspension of bacteria equal to a 0.5 McFarland standard (in normal saline) (1.5×10^8 CFU/ml). From the suspension, 0.5 ml was transferred to 500 ml pasteurized doogh (Vijeh dairy, Iran). As a result, the final concentration of bacteria in doogh was 1.5×10^5. Inoculated doogh was incubated, and a sample was taken from the suspension at the first, twentieth, fortieth, and sixtieth days of incubation for an antimicrobial susceptibility test.

Preparation of discs containing Thyme essential oil: The blank filter paper discs (6 mm) (Padtanteb, Iran) were immersed in 20 µl of different concentrations of thyme essential oil (0, 30, 60, 90, and 120 µl from essential oil per 1 ml solvent) for 24 hrs. Samples from inoculated doogh were cultured on plates and antibacterial activity was performed using discs immersed in different concentrations of Thyme essential oil.

Disc diffusion method: According to the NCCLs (2000a) standard, 150µl of bacterial suspension containing 1×10^5 CFU/ml was poured onto Mueller-Hinton agar medium. The 6-mm discs containing thyme essential oils were placed on the plate at appropriate distances. A blank disc was included in each plate as a control. The plate was incubated at 37°C and the growth inhibition zone was measured after 24 hours (13). Antibacterial activity of essential oil in doogh samples was performed at first, twentieth, fortieth, and sixtieth days of storage.

Determination of MIC and MBC

Sixty microliters of Mueller-Hinton Broth medium, 20 µl of bacterial suspension containing 1.5×10^5 bacteria, and 20 µl of different thyme essential oil concentrations (30, 60, 90, and 120 µl/ml) were transferred to the tubes. One tube containing culture media and bacteria and one tube containing culture media and essential oil were included as controls. All the tubes were incubated at 35°C for 24 hours and then examined for turbidity caused by bacterial growth and the lowest concentration at which the bacterium did not grow visibly was considered MIC (14). To measure MBC, 5 µl from each tube in which bacterial growth was not observed was transferred to a nutrient agar medium and incubated at 35°C for 24 hours. The lowest concentration of thyme essential oil at which most bacteria were killed was considered MBC.

Sensory Evaluation

A sensory evaluation was performed to evaluate the effect of different concentrations of essential oils on the taste of doogh. The test was done on the mixture of thyme essential oil and doogh on the first, twentieth, forty-sixth, and sixtieth day of mixture preparation, by seven trained panelists of the Iranian Food and Drug Administration (based on national standards 3442, 2442, 3581, 4940) based on 5 points hedonic test (from 1 to 5). For this purpose, sterile doogh was mixed with Thyme essential oil at concentrations of 30, 60, 90, and 120 µl/ml and an equal sample from each concentration was served to the assessors. Water was used to rinse the assessor's mouths before and between sample evaluations (15).

Statistical Analysis

All the experiments were done in triplicate. Data were analyzed by SPSS software and mean data were compared using a t-test. A p-value less than 0.05 was considered statistically significant.

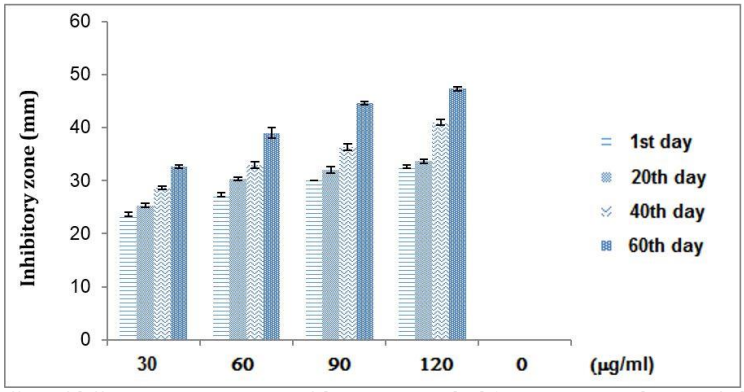

Figure 1. Antibacterial effect of different concentrations of thyme essential oil (0, 30, 60, 90, and 120 µg/ml) in different incubation times (1st, 20th, 40th and 60th day) on the growth of *Escherichia coli*. Antibacterial effects were assessed by the disk diffusion method are expressed as inhibitory zone (mm).

Results

Antibacterial susceptibility test by the disk diffusion method showed that thyme essential oil has a significant effect on bacteria. This antibacterial effect increased by increasing the concentration of the extract (data are missed apparently). MIC and MBC were measured to be 60 and 90 µl/ml respectively. Moreover, our data also show that thyme essential oil dissolved in doogh had a significant ($p < 0.05$) effect on the growth of *Escherichia coli* O157H7. As shown in figure 1, increasing the essential oil concentration and increasing incubation time (from the first day to the 60th day) had a progressive antibacterial effect on bacterial growth, therefore the highest antibacterial effect was at 120 µl/ml on the 60th day.

The effect of thyme oil extract on the taste of doogh was investigated in the first, twentieth, fortieth, and sixtieth days. As shown in table 1, the concentration of 60 µl/ml obtained the highest taste score in all four experiments (first, twentieth, fortieth, and sixtieth day). Higher concentrations were not desirable for assessors. Therefore, the concentration of 60 µl/ml of essential oil in all the studied days has the best effect on the sensory properties of doogh.

Considering both results obtained from antibacterial susceptibility and sensory test, 60 µl/ml of thyme essential oil during 60 days incubation is suggested as optimum treatment.

Table 1. Scores assigned based on the sensory test to each concentration of thyme in doogh (0, 30, 60, 90, and 120 µl/ml) over 60 days of incubation (1st, 20th, 40th and 60th day).

	1st	20th	40th	60th
30 µl/ml	2.28±0.48^{b}	2.57±0.53^{b}	2.71±0.48^{b}	2.57±0.53^{b}
60	3.57±53^{a}	3.71±0.48^{a}	3.85±37^{a}	3.42±0.53^{a}
90	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}
120	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}	1±0.00^{c}
0	2.71±1.1^{b}	2.85±1.00^{b}	2.42±0.9^{b}	2.57±1.1^{b}

Discussion

In the present study, the effect of different concentrations of thyme essential oil on *Escherichia coli* O157H7 inoculated in doogh for different incubation times was assessed. The essential oil could reduce the growth of bacteria in a concentration-dependent manner. Plant essential oils have been used to control the growth of pathogenic bacteria of food origin or spoilage bacteria and also as a food preservative (16 and 17). With excessive use of chemical preservatives, some of which are suspected to have toxic and carcinogenic effects, therefore there is a tendency to reduce or eliminate these synthesized compounds and to replace them with natural compounds (18-20). Sagdic et al. found that thyme essential oil had a dose-dependent bactericidal effect against *Escherichia coli* O157H7 and suggested it as a food preservative (21). Mahboubi et al. reported linalool, thymol, and carvacrol as three major chemotypes with the highest antibacterial activity (22). Moreover, the antimicrobial effect of thyme essential oil has been assessed on *Escherichia coli* in white-brined cheese after 60 days of cheese storage and showed that storage time and thyme concentration had a significant

effect on the logarithmic number of bacteria (23). In the present study, it was found that the storage time of inoculated doogh from first to 60th day and increasing essential oil concentration had an increasing and cumulative effect on the growth of bacteria. In another study, essential oils from four plants; bay, clove, cinnamon, and thyme showed antimicrobial effects against *Listeria monocytogenes* and *Salmonella enteritidis* in soft cheeses and reduced bacterial count to ≤1·0 log10 CFU ml−1 over 14 days (24). In another study, Shahbazi showed that Ziziphora clinopodioides essential oil could reduce 1 log CFU mL-1 of *Salmonella typhimurium* and *Staphylococcus aureus* in doogh (25). Antimicrobial effects of Thyme essential oil was also confirmed in another dairy product (Mayonnaise) against fungi and bacteria (26).

Studies show that essential oil is a rich source of thymol, which interacts with lipid bilayer resulting in changes in membrane stability, and elasticity (27) that causes disturbances in plasma membranes, loss of cellular constituents, and ultimately the death of pathogens (18, 28, and 29). Therefore, the antimicrobial activity of thyme essential oil tested in this study can be attributed to the presence of these compounds.

In the present study, MIC and MBC have measured at 60 and 90 µl/ml respectively. One of the drawbacks of aromatic compounds as food preservatives is high concentrations of them are needed to protect food against bacteria. However, it should be noted that when the amount of essential oil is high, it negatively affects the taste of the product which is not desirable to the customers. The main limiting factor to using thyme essential oil is negative organoleptic properties that lead to an unpleasant taste (30). The desirable concentration of Thyme oil seems to depend on the kind of food. Some studies have shown that a concentration of 3 µl/ml of Thyme oil is most desirable in meat products (31). However, the results of the sensory test indicated that the most desirable concentration of essential is 60 µl/ml that equal to MIC. There is no significant difference between the concentrations of 0 and 30 and also between the concentrations of 90 and 120 µl/ml. In total, considering both the antimicrobial potency and taste property, this study suggests a concentration of 60 µl/ml for doogh.

Conclusion

Our data showed that thyme essential oil can prevent the growth of bacteria and this effect is concentration-dependent, the most potent antimicrobial activity was observed to be 120 µl/ml. However, in the sensory evaluation, the most desirable concentration was 60 µl/ml. Since we cannot exceed the desirable concentration of essential oil that affects the quality of the product, we suggest the concentration of 60 µl/ml of essential oil to achieve both MIC and the best taste desirability.

Acknowledgments

This study is the result of a master's thesis approved by Islamic Azad University.

Conflict of interest

There is no conflict of interest to declare.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effects of Iron Ion Solution Consumption and Aerobic Training on Hematologic Factors among Iron Deficiency Anemia Female Patients

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 07 Mar 2022
Accepted: 23 May 2022
Published: 30 Jun 2022

Keywords:
Aerobic exercise
Iron deficiency anemia
Ferritin
CBC

ABSTRACT

Introduction: Iron metabolism is important for maintaining body homeostasis, and aerobic exercise can enhance this process. This study aimed to evaluate the effects of iron Ion solution consumption on some hematologic factors and aerobic performance among iron deficiency anemia in female patients.

Methods: This experimental research was conducted on 30 women aged 20-30 years old with iron deficiency anemia, who referred to the medical centers of Kerman, Iran using a control group in pre- and post-test with primary care. The subjects were randomly divided into three equal groups. The ISAE=10 received Iron Supplement, while the AE=10 group received Aerobic Exercise, and the Control C=10 got neither. The aerobic exercises program included eight weeks and three sessions/week. The aerobic power was measured by Astrand aerobic bike test. The complete Blood Count (CBC) measurement included HCT, MCH, MCV, MCHC, RBC, HB, chemiluminescence and Ferritin in pre-test and post-test. The statistical method included the Kolmogorov-Smirnov test for normal distribution, the Levene test for homogeneity of variances, as well as one-way analysis of variance, and Scheffe post hoc tests.

Results: ISAE and AE significantly increased serum ferritin after eight weeks of aerobic training ($F= 3.160$; $p<0.05$ and aerobic power $F= 6.23$; $p<0.01$).

Conclusion: The results showed that aerobic power and iron ion solution consumption increased significantly between the two groups compared to the control. Other variables were not significantly different between groups. Exercise and iron ion solution consumption may play a significant role in improving serum ferritin index in female patients with anemia caused by iron deficiency.

► Please cite this paper as:
Ansari Moghadam S, Aminaei M, Nikoei R. The Effects of Iron Ion Solution Consumption and Aerobic Training on Hematologic Factors among Iron Deficiency Anemia Female Patients. J Nutr Fast Health. 2022; 10(2): 121-128. DOI: 10.22038/JNFH.2022.64238.1383.

Introduction

Iron metabolism is essential for maintaining body homeostasis. Anemia caused by iron deficiency is called Iron Deficiency Anemia (IDA) (1, 2). The reasons for IDA are the amount of iron consumption in the diet, iron requirements, and abnormal menstrual cycle. Athletes' performance can be negatively affected by iron depletion because it is required for oxygen transmission, mitochondrial respiratory chain enzymes, and oxidative enzymes (3). Iron is the main element in the total metabolism of living organisms, which plays a vital role in nutrition (4). Iron is found in hemoglobin, myoglobin, and cytochromes as well as in phytonutrients (5). The Heme iron, as functional iron, is responsible for connecting oxygen to red blood cells and transferring it into the tissue, as well as for transmitting electrons in the Krebs cycle and electron transport chain (6). Non-heme iron is a catalyzer, which converts beta-carotene to vitamin A (7), and synthesis the Purine to form the nuclide acid, remove blood lipids, and collagen synthesis (8), and produce antibody in the liver (9). The results of laboratory studies on animals with iron deficiency have indicated that most animal groups have the lowest aerobic capacity (10, 11). According to the results, iron deficiency affects mitochondrial enzymes and blood hemoglobin levels (12, 13). The findings show that iron deficiency reduces mitochondrial enzymes and hemoglobin levels, while iron supplementation increases the activity of cytochrome C (35%) and Krebs cycle enzymes (15%) (14, 15). Since energy production in mitochondria is affected by iron deficiency, metabolites accumulate, function decreases, and cell death occurs (16).

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IDA reduces capacity and aerobic power among athletes (17-20). Anemia treatment has included iron supplements, artificial iron fortification, biofortification, dietary modification, nutrition education, and antiparasitic treatment in recent years (21, 22). Iron deficiency is estimated to result in a loss of US $70 billion in the global economy each year in addition to the negative effects of supplement consumption. However, access to varied diets is neither affordable nor possible for many people, and supplements are expensive, not consistently available, and often culturally unacceptable (23).

The iron supplement is found in two forms of ferrous and ferric. The first type of iron absorbs better than the ferric form in the body. Second, sulfate can cause gastrointestinal distress, nausea, heartburn, diarrhea, and constipation. Fumarate, sulfate, and gluconate are forms of iron supplements that can be absorbed in the body properly, but their digestion can be problematic (24).

The iron-containing cookware could serve to reduce IDA, which is used among children. The potential advantages of iron-containing cookware include relative cost-effectiveness and complementary combination with other interventions (25).

Charles et al. (2011) indicated that the iron ingot in boiling water is another form, which has unique properties and is used as a supplement compared to the iron chemical drugs with high absorption without gastrointestinal effects. Natural iron is the most common form of iron in nature, so its structure is similar to iron in the body. However, further research was required to assess the quantity and bioavailability of iron leached from the ingot (26).

The iron reacts readily with oxygen and water to give brown to black hydrated iron oxides, commonly known as rust. Iron is a metal that reacts with water, so dipping molten iron in water results in releasing iron ions in the water, also called iron water (27, 28).

According to a report from the Laboratory of applied research in Shahid Bahonar University of Kerman, the iron ion dissolved in this solution is 5.3 ppm. Therefore, this study evaluated the effect of iron ion solution on iron deficiency anemia treatment among female patients. The iron ion solution may change Red Blood Cell (RBC), hemoglobin levels, Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) of serum iron, and serum ferritin were also investigated.

Materials and Methods

This randomized controlled trial study was designed by pre-test and post-test with the control group and primary care. The consent form and health questionnaire were presented to control the detrimental nutritional factors. The statistical population was women aged 20 to 30 years old, who were the citizens of Kerman with iron deficiency anemia based on the results of two-factor of Iron and ferritin of serum. The leading indicators of anemia caused by iron deficiency were purposefully selected from the patients of Kerman health centers.

Table 1. Descriptive statistical analysis of variables

Characteristics		ISLE	AE	C
Age (yrs)		24.72 ± 1.01	26.72 ± 2.82	25.29 ± 1.84
Weight (kg)		59.61 ± 4.65	64.89 ± 6.31	60.88 ± 9.98
Height (cm)		161.67 ± 10.93	163.17 ± 13.75	167.65 ± 5.26
Aerobic capacity	pre	37.66 ± 1.34	34.49 ± 1.83	35.95 ± 1.48
(mL.kg.min)	post	44.28 ± 1.39	39.81 ± 1.46	36.96 ± 1.40
Hemoglobin	pre	14.66 ± 1.29	14.23 ± 1.62	13.99 ± 1.28
(g/dl)	post	15.70 ± 1.13	14.74 ± 1.17	14.61 ± 1.43
Hematocrit	pre	43.25 ± 0.74	42.01 ± 0.61	43.14 ± 1.01
	post	44.55 ± 0.49	43.82 ± 0.77	43.92 ± 0.37
MCV (fl)	pre	89.46 ± 1.82	89.33 ± 1.51	88.89 ± 1.20
	post	90.47 ± 1.09	89.66 ± 0.29	88.92 ± 0.33
MCH (Pg)	pre	28.31 ± 0.42	26.35 ± 0.75	26.54 ± 0.32
	post	29.03 ± 0.32	27.17 ± 0.12	26.92 ± 0.91
MCHC (g/dl)	pre	30.74 ± 0.25	31.28 ± 0.77	30.88 ± 0.84
	post	31.61 ± 0.14	32.99 ± 0.61	30.76 ± 0.93
Ferritin (g/dlμ)	pre	28.21±1.42	31.33±1/34	31.89±2.16
	post	39.01±2.76	39.66±1.29	33.92±4.33

Iron Supplement and Aerobic Exercise group (n=10) = ISAE, aerobic exercise group (n=10) = AE, Control group (n=10) = C

Subjects

The 75 patients in this study filled the 40-question questionnaire approved by domestic clinical experts. The exclusion criteria were laboratory, thalassemia, heart, respiratory problem, and vegetarian diet.

The patients were finally excluded from the study after the blood tests revealed that they had hematological problems. The blood tests determined the values of hemoglobin, hematocrit, MCV, MCH, and MCHC of serum ferritin in the laboratory, so the three groups were matched based on the results of ferritin levels (Table 1). The first group (n=10) received Iron Supplement and Aerobic Exercise (ISAE), the second group (n=10) got Aerobic Exercise (AE), and the third group (n=10) were control (C).

Aerobic Exercise Protocol

The aerobic exercises included three 10-15 minutes training sessions per week for eight weeks, including static stretching and dynamic warm-up. The main program was 20-35 minutes of continuous running on a fixed track with an intensity of 70-80% of the maximum heart rate for every subject. The overload program increased by 2 minutes in every session. The intensity of exercise was monitored by Polar heart rate. The cold down exercise was carried out with light jogging and stretching for 10-15 minutes. The total time was 40 minutes in the first and 60 minutes in the last session.

Measurements

The pre-test was performed by Complete Blood Count (CBC) test, which included HCT, MCH, MCV, MCHC, RBC, HB, by Symex (model: KX-21); chemiluminescence; Iron Ferozine kite; Auto Analyzer (Mindry, bs800), and Ferritin by Architect ferritin kite (7K59). The three groups were matched by ferritin levels. In addition, the aerobic power was measured by the six-minute Astrand aerobic bike tests (by Monark cycle ergometer E839). The ISAE group consumed 500 mg iron ion solution servings as 250 ml at 10 am and 250 ml at 10 pm during eight weeks of aerobic exercise protocol. AE and C groups received 500 mg of drinking water in two servings as 250 ml at 10 am and 250 ml at 10 pm as a placebo. The post-test administered the same variables after eight weeks.

Table 2. Analysis of covariance in post-test variables

	groups	Mean difference	F	P
Aerobic capacity (mL.kg.min)	ISAE	7.62	6.23	0.003*
	AE	6.32		
	C	1.1		
Hemoglobin (g/dl)	ISAE	1.4	0.301	0.892
	AE	0.51		
	C	0.62		
Hematocrit (%)	ISLE	1.30	1.24	0.287
	AE	1.82		
	C	0.150		
MCV (fl)	ISAE	1.01	1.632	0.240
	AE	0.33		
	C	0.03		
MCH (Pg)	ISAE	0.72	1.419	0.107
	AE	0.82		
	C	0.38		
MCHC (g/dl)	ISAE	0.87	1.421	0.210
	AE	0.67		
	C	0.96		
Ferritin (g/dlμ)	ISAE	10.8	3.160	0.021*
	AE	8.33		
	C	2.03		

* Significant at level p≥0.05.

Statistical Analysis

The data were analyzed by descriptive statistics, including mean, standard deviation, minimum, and maximum. The normality of variables was evaluated by the Shapiro-Wilk test. The differences in dependent variables were calculated in two pre-test and post-test stages (before and after supplementation) among three groups. The differences between groups were evaluated by the Analysis of Covariance

(ANCOVA), and the paired comparison of groups was analyzed by the Scheffe post hoc test.

Results

Table 1 present the descriptive statistical analysis variables. Consuming iron ion solution and aerobic training in the ISAE group affects aerobic capacity in female patients with IDA. The differences in aerobic power were calculated in pre and post-test (F= 6.23; p=0.003) among three groups (ISAE, AE, and C) and indicated in Tables 1 and 2.

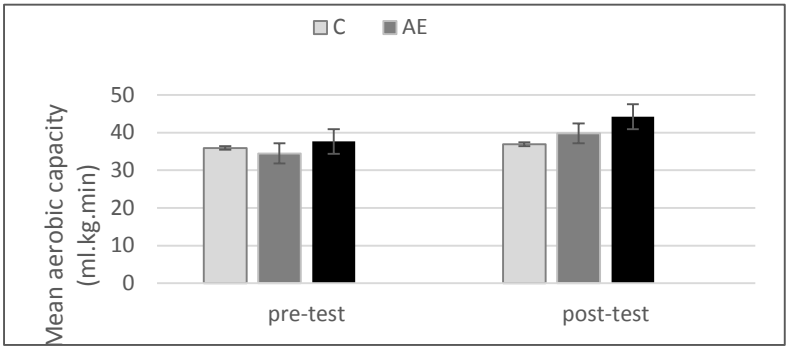

Figure 1. The alternation and comparison of aerobic capacity among groups in post-test. (C): Control group, (AE) aerobic exercise group, and (ISAE) Iron Supplement and Aerobic Exercise group. *Significant difference between groups

The Scheffe posthoc test revealed a significant difference between the ISAE group (44.28±1.39 mL.kg.min) and the C group (36.96±1.40 mL.kg.min). In addition, Figure 1 represents a significant difference between the AE (39.81±1.46 mL.kg.min) and C (36.96±1.40 mL.kg.min) groups. It means the iron ion solution and aerobic training effectively increase aerobic capacity in every group.

The supplementation of iron ion solution and aerobic training did not affect HB, HCT, MCV, MCH, and MCHC in female patients with IDA at the same time or aerobic exercise alone. No significant difference was found in HB (F= 0.301; p=0.892), HCT (F=1.24; p=0.287), MCV (F= 1.632; p=0.240), MCH (F= 1.419; p=0.107), and MCHC (F= 1.421; p=0.210) among ISAE, AE, and C groups in two stages of pre- and post-test (Table 1 and 2).

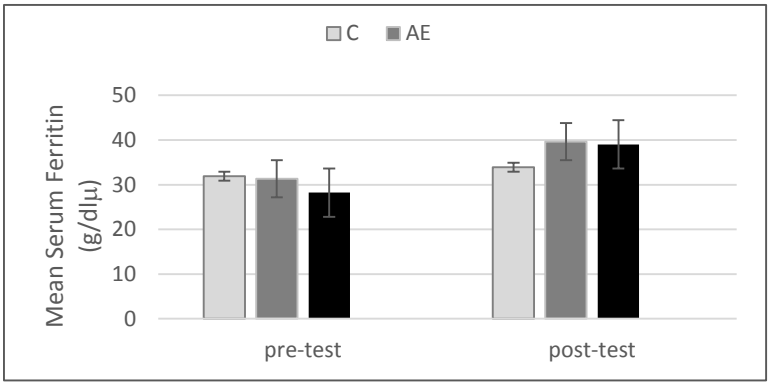

Figure 2. The alternation and comparison of serum ferritin among groups in post-test. (C): Control group, (AE) aerobic exercise group, and (ISAE) Iron Supplement and Aerobic Exercise group. *Significant difference between groups.

The iron ion solution supplement and aerobic exercise affect ferritin in female patients with IDA. There was a significant difference in serum ferritin among ISAE, AE, and C groups in pre- and post-test (F= 3.160; p=0.021). According to the Scheffe posthoc test, significant differences were observed between the ISAE and C groups, also AE and C groups. The ISAE and AE groups did not differ significantly (Figure 2). Therefore, the iron ion solution, along with aerobic training, is effective in increasing ferritin serum. In addition, aerobic training may affect ferritin serum alone.

Aerobic exercise is an essential factor in effect on Fe^2 serum (serum ferritin index).

Discussion

Iron is an essential factor in hemoglobin and myoglobin structure. Ion solution consumption and aerobic exercise affect aerobic capacity in female patients with IDA and improve iron status and endurance capacity in iron-deficient and non-anemic trained male and female subjects (29). The combination of iron in hemoglobin and aerobic exercise is fundamental to the success of athletes. Additionally, the better endurance performance of athletes in comparison with normal individuals is due to increased blood hemoglobin concentration. The function of iron in hemoglobin building increases the oxygen to the body because the prevailing system is powering through the aerobic system in endurance activities (5). Aerobic capacity may improve IDA lonely, but available cross-sectional and longitudinal studies indicate that the blood of endurance athletes is more dilute due to blood volume expansion, particularly plasma volume because of training. IDA reduces aerobic capacity, and the low hematocrit values in trained athletes represent a hydration condition rather than an iron store deficiency (30).

Biological mechanisms are sufficiently strong to justify interventions to improve iron status for the effect of IDA on aerobic capacity. Serum iron can improve exercise performance (17), and ID and IDA are common among young adolescent female athletes. Nevertheless, there was no difference between female athletes and nonathletes (19). The iron supplements may improve ventilation threshold and endurance capacity in men and women with iron deficiency (29). A few studies have reported that female endurance athletes should be screened using a serum ferritin cutoff between 20.0 and 25.0 μg/L to identify iron depletion to impair performance. Anemia or iron deficiency in athletes should be treated with supplemental and dietary guidance, as well as serial hemoglobin and serum ferritin measurements (17-20). The reduced functional observed in field studies is likely due to anemia and reduced oxygen transport. The biological mechanisms for the effect of IDA on work capacity are sufficiently strong to justify interventions to improve iron status for enhancing human capital (17).

In contrast, Garz et al. (1997) and Klingshirn et al. (1992) found that low ferritin did not affect endurance in women (31, 32). In addition, low serum iron cannot decrease endurance capacity without anemia (33). The results of these researches have not been consistent with the present study because the amount and type of supplements have differed probably.

In the present study, iron ion solution consumption and aerobic exercise did not affect blood hemoglobin in women with iron deficiency anemia. Some interfering factors, which may affect IDA, are altitude, menstrual bleeding, and renal disorder due to erythropoietin reduction and nutrition.

Hemoglobin comprises a protein section (globulin) and four granular pigments. Physical activity may cause many changes in the number, distribution, and proliferation of white blood cells. In addition, blood or hematology responses are not the same as any physical activity. Blood responses can be affected by factors such as time, intensity, duration of activities, and environmental conditions (7). Iron supplementation can raise serum ferritin levels and ferritin concentration, unaccompanied by increasing hemoglobin concentration and endurance performance (31). In addition, Zhu et al. (1997) reported that the difference in VO2max was significantly related to serum ferritin concentration, and hemoglobin value was not a significant confounder (33).

The consumption of iron ion solution and aerobic exercise did not affect blood hematocrit among female patients with iron deficiency anemia. Many factors affected blood hematocrits, such as stress, amenorrhea, climate, altitude, and activities. Drinking fluids increases blood volume and consequently decreases hematocrit. The activity in the hot environment reduces the volume of plasma serum, thereby increasing hematocrit. In general, the patients with iron deficiency anemia had normal blood factors. Aerobic training and consuming iron ion solution had no significant changes in their blood factors, especially hematocrit. The studies have indicated that the blood volume of endurance athletes is more dilute due to blood volume expansion, particularly plasma volume because of training (30).

The consumption of iron ion solution and aerobic training affected serum ferritin among female patients with iron deficiency anemia. Therefore,

using iron ion solution and aerobic training can increase serum blood ferritin. Concerning ferritin AE and ISAE groups were significantly different from the control group (placebo).

Zhu et al. (1997) indicated that the iron-sufficient group had significantly higher hemoglobin, transferrin saturation, serum ferritin values compared with the iron-depleted group with a significant greater tendency to use iron supplements (33). Garza et al. (1997) reported that screening involved in an endurance sport might be clinically useful for low iron stores at the start of a training program in female athletes given the high prevalence of iron depletion reported in this and other studies (31). In this study, iron-depleted rowers (serum Ferritin <20–25 μg/L) showed a decrease in performance time compared with normal iron stores. Bijeh et al. (2018) reported that there was a significant increase in mean platelet volume and a significant decrease in serum iron and ferritin levels in the experimental group after eight weeks of aerobic exercise (34). Eight-week moderate-intensity continuous aerobic exercises reduce Iron, hematocrit, and serum ferritin levels in the club soccer players (35). Submaximal exercise has no effect on plasma hepcidin concentration or iron metabolism immediately following exercise (36). This observation may be related to exercise duration and intensity.

The consumption of iron ion solution and aerobic exercise did not affect MCV in female patients with iron deficiency anemia. Bhatia et al. (2012) reported that MCV is unreliable as a screening parameter for the presumed diagnosis of macrocytic anemia associated with vitamin B12 deficiency (37), indicating different exercises affect MCV. A report observed that regular aerobic and strength exercises could positively influence sedentary women's body weight and BMI parameters. Additionally, a significant decrease was found in RBC, HCT, and MCV values of the strength exercise group compared to aerobic exercise (38). Although aerobic training of increasing intensity may be accompanied initially by decreases in HB, HCT, and RBC count in young women and an increase in MCV, these changes are transitory. A return to hematopoietic balance without RBC destruction or hemodilution may result in a draw upon body iron stores (39).

Consumption of iron ion solution and aerobic exercise does not affect the MCH and MCHC. The mean blood cell hemoglobin increased insignificantly since this increase was less than 1 mg/dl, so it is not worth much in all three groups. However, the consumption of ion solution and aerobic exercise had no effect on mean blood cell hemoglobin in women with iron deficiency anemia. Mousavizadeh (2009) showed that eight weeks of aerobic training decreased HCT, RBC, HB, serum iron, transferrin concentration, and serum ferritin in girls, which was consistent with the present study. However, there was no significant difference in MCH, MCV, MCHC, and TIBC among girls (40). Therefore, the changes in hematological indicators reduce result in endurance exercises due to the increase in plasma volume, which is a helpful mechanism concerning adaptation to endurance conditions. The young elite athletes with low serum ferritin and normal hemoglobin concentration and iron supplementation increased maximal aerobic performance capacity without an augmentation of RBV (41).

Conclusion

According to the results, iron supplementation increases aerobic capacity without significant changes in HCT, HB, MCH, MCV, and MCHC among young women with IDA. The iron ion supplements are easy to obtain, inexpensive, consistently available, and have adequate absorption by the body. This form of supplementation has no digestive problems and is often culturally acceptable. This study was performed without dietary restrictions, with a low number of subjects and in small groups, so it is suggested to research with at least four groups, more subjects, and a longtime protocol. The research interventions, including aerobic programs and iron supplementation, seemed to need a longer time to change.

Acknowledgments

We would like to express our appreciation to all the authors who participated, the medicine Clinic for participant recruitment, and the participants for their contribution and continuing interest in this research.

Conflict of Interest

This research received no specific grant from the public, commercial, or not-for-profit sectors funding agencies. The authors declare no conflict of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Nanoselenium Consumption during High Intensity Interval Training on IL-4 and IFN-γ Gene Expression in Thymus Organ of Dexamethasone-Induced Immunosuppressive Rats

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 09 Mar 2022
Accepted: 29 Jun 2022
Published: 03 Jul 2022

Keywords:
Nanoselnium
IL-4
IFN-γ
Dexamethasone
Interval training

ABSTRACT

Introduction: The thymus is a specialized lymphatic organ in the immune system and plays a vital role in the normal functioning of the immune system. Observed 24 and 48 hours after dexamethasone, thymocytes are reduced by 55% and 84%, respectively, which can be associated with a decrease in anti-inflammatory cytokines such as IL4 and IFN-γ. The aim of present study was to evaluate the The effect of nanoselenium consumption during high intensity interval training on IL-4 and IFN-γ gene expression in thymus organ of dexamethasone-induced immunosuppressive rats.

Methods: The study samples in the present study consisted of 40 male Wistar rats that were randomly divided into 5 groups: healthy control group (CON), immunosuppression group (DEX), immunosuppression + exercise group (DEX + TRA), immunosuppression group + nanosillenium (DEX + SEL), immunosuppression group + nanoselnium + training (DEX + TRA + SEL) were divided. Suppression of the immune system of the samples was performed by injecting of 0.4 mg / kg per day dexamethasone for three days. The training program included 4 weeks of intense intermittent training (HIIT) in the DEX + TRA and DEX + TRA + SEL groups, and supplementation with 100 mg / kg selenium nanoparticles in the DEX + SEL and DEX + TRA + SEL groups. Data were analyzed using one way ANOVA in SPSS 26 software at significance level of α <0.05.

Results: The data showed that IFN-γ gene expression decreased in all groups compared to the control group (p = 0.0001). The difference between DEX + SEL and DEX + SEL + TRA groups was less than other groups. Also, IL4 gene expression in thymus tissue was significantly reduced in DEX and DEX + TRA groups compared to healthy controls (p = 0.048 and p = 0.013, respectively).

Conclusion: In the present study, it was found that intense exercise activity in high-intensity interval training (HIIT) may inhibit immune reactions and anti-inflammatory cytokines in the thymus tissue of rats whose immune system was suppressed by DEX. Therefore, exercise for strengthening the immune system in these people should be done with more caution.

► Please cite this paper as:
Hooshangi P, Kazemzadeh Y, Shirvani H, Sedaghati S, Molanoruzi K. The Effect of Nanoselenium Consumption during High Intensity Interval Training on IL-4 and IFN-γ Gene Expression in Thymus Organ of Dexamethasone-Induced Immunosuppressive Rats. J Nutr Fast Health. 2022; 10(2): 129-135. DOI: 10.22038/JNFH.2022.65454.1387.

Introduction

Weaknesses in the immune system can be caused by two primary and secondary sources. The disease has a genetic origin of weakness of the primary immune system and a person suffers from it from birth. Secondary immune system deficiencies can also be caused by diseases such as AIDS and cancer, malnutrition, chemotherapy, or the use of corticosteroids such as dexamethasone. Today, dexamethasone is used in many inflammatory, autoimmune, and organ transplant diseases to suppress the immune system. In addition, many athletes use dexamethasone to reduce inflammation due to sports injuries, reduce pain, and improve athletic performance, which can lead to impaired immune function (Vernec et al, 2020).

The thymus is a lymphatic organ specialized in the immune system and plays a vital role in the normal functioning of the immune system. Once formed in the bone marrow, T lymphocytes travel through the bloodstream to the thymus and mature by it (Wang et al, 2020). Observed 24 and 48 hours after taking dexamethasone, thymocytes are reduced by 55% and 84%, respectively, which can be associated with impaired immune function (Ansar Ahmed et al,

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1994). In addition, dexamethasone has been shown to reduce T lymphocytes (Chen et al, 2018).

Interleukin 4 (IL-4) is a cytokine that differentiates simple helper T cells (Th0 cells) from Th2 cells. Th2 is activated by IL-4, which subsequently produces additional IL-4 in a positive feedback cycle. IL-4 is mainly produced by mast cells, Th2 cells, eosinophils and basophils. Stimulation of B and T cell proliferation is an important IL-4 task that plays a crucial role in the adaptive immune system (Yang et al, 2017). On the other hand, interferon-gamma (IFN-γ), which is secreted mainly by activated lymphocytes such as CD4 T type 1 helper cells (Th1) and cytotoxic CD8 T cells, coordinates the signaling pathway of several biological responses. These responses are primarily involved in host defense and immune monitoring, but are also effective in building adaptive immunity and in regulating inflammation, apoptosis, and the cell cycle. IFN-γ inhibits Th2 cell differentiation, and thus IL-4 production. This regulation involves inhibition of the IL-4 / STAT6 pathway required for Th2 cell differentiation, and is mediated at least by IFN-γ-induced SOCS1, which inhibits IL-4 receptor signaling (Castro et al, 2018).

Exercise as an interfering factor can play an effective role in improving immune system function. The volume and intensity of exercise are two factors that determine the rate of response and adaptation to exercise. Regarding the effect of high-intensity interval and endurance exercises, most researches shows that the negative effect of these types of exercises. But further investigation shows that intense interval training that has been going on for a long time in each position has a negative effect on immune system indices (Ito et al, 2019).

Contrary to previous reports of high-intensity interval exercise suppression, it has been reported that 6 weeks of high-intensity interval training has no adverse effect on the immune system and when combined with zinc supplementation improves immune system-related indicators in athletes (Saeedy et al, 2018). However, the results of research in the field are contradictory and more studies are needed. It seems that when intense interval training combined with the use of an antioxidant is evaluated, it has a significant impact on improving immune function (Saeedy et al, 2018; Khorasani et al, 2019). It is possible that antioxidants in foods with the help of antioxidant enzymes can improve the function of the immune system. Selenium (Se), on the other hand, is a rare mineral that is essential for human and other animal health. Many studies have shown that Se improves the immune function of cells (Radomska et al., Kurana et al., 2019). Se has been shown to increase the production of IL-2 and M and G immunoglobulins (Avery et al, 2018). Se has also been shown to play an important role in protecting T and B lymphocytes against certain toxins (Salimian et al, 2014). Taken together, these data suggest that Se could be a potential treatment for preventing immunological damage from the effects of dexamethasone or strenuous exercise. However, there is little information in this area and therefore the aim of the present study was to investigate the effect of 4 weeks of high intensity interval training along with nanoselnium supplementation on IL-4 and IFN-γ gene expression in thymus tissue of dexamethasone-induced immunosuppression rats.

Materials and Methods

Research Methods and Samples

The present study was experimental and the samples consisted of 40 male Wistar rats (180 to 220 g). Sampling was done randomly and the samples were stored in the standard laboratory environment of Baqiyatallah University of Medical Sciences after purchase from Pasteur Institute of Iran. The animals were kept in polycarbonate cages at 22 ± 4.1 ° C and the light cycle in the dark for 12:12 h and a humidity of 55.6 4 4.6%.

Methods

After transferring the samples to the laboratory environment, rats were exposed to the environment for 1 week. The rats were then introduced to the training protocol for one week and randomly divided into 5 groups: healthy control group (CON), immunosuppression control group (DEX), immunosuppression + exercise group (DEX + TRA), immunosuppression + Nanosilnium group (DEX + SEL), immunosuppression + nanosilnium + exercise group (DEX + TRA + SEL) were divided.

Suppression of the Immune System

In the present study, suppression of the immune system in samples was done by injecting 0.4 mg

/ kg / d dexamethasone (made by Osweh Iran) for three days intraperitoneally (Dehghani et al, 2021). Then Immune system attenuation samples were performed in 4 groups of immunosuppression control group (DEX), immunosuppression + exercise group (DEX + TRA), immunosuppression + Nanosilnium group (DEX + SEL), immunosuppression + nanosilnium + exercise group (DEX + TRA + SEL). In the control group, the same amount of normal saline (NS) solution was injected over three days.

Training Protocol

The training program performed in the present study included a 4-week Intense Interval Training Program (HIIT) involving running on a rodent treadmill at a speed of 24 to 34 meters per minute, equivalent to 85 to 100% of the maximum oxygen consumption of the samples. Exercises were performed six days a week (Little et al, 2011). In each session, 8-12 repetitions of 1 minute with an intensity of 24 to 34 meters per minute with active rest intervals of 75 seconds. The principle of incremental overload was implemented by increasing the speed of the treadmill and the number of repetitions per week of the training program.

Receiving Nanoselnium Supplement: To prepare selenium nanoparticles, first a 2.5 mM solution of selenium dioxide was prepared and added to a 2.5 mM solution of ascorbic acid being mixed. The resulting mixture was centrifuged and washed using filter paper (Yazdi et al, 2015). The stock solution of selenium nanoparticles was prepared and 100 mg in 250 nm size was given to mice by gavage and every other day. At the same time, normal saline solution was given by gavage in a healthy control group.

Table 1. IL-4 primer sequence, IFN-γ Genes name Primer sequences

	Genes name	Primer sequences
1	IFN-γ	Forward: CCCACAGATCCAGCACAAAG Reverse: TCTACCCCAGAATCAGCACC
2	IL-4	Forward: CAAGGAACACCACGGAGAAC Reverse: TCTTCAAGCACGGAGGTACA
3	GAPDH	Forward: CAAGTTCAAGGGCACAGTCA Reverse: CCCCATTTGATGTTAGCGGG

The maximum oxygen consumption (VO2max) of rats was assessed using a sloping treadmill (5 lanes) with a positive slope of 25 degrees (Radomska et al, 2021). After applying the independent variable, all samples with completely similar conditions and in basic conditions (48 hours after the last training session and 12 to 14 hours of fasting) by intraperitoneal injection of a combination of ketamine (60 mg / kg) and xylazine (mg / kg 5) they fainted. Then the split chest and thymus tissue were immediately divided into two parts after separation and washing and transferred to liquid nitrogen in formalin (for the tissue process of measuring gene expression). They were then stored in the refrigerator at -80 ° C until measurement.

Measurement of variables: In vitro analysis of IL-4, IFN-γ gene expression levels in rat thymus tissue was determined using special commercial kits by real-time PCR with primer sequence of Table 1.

Statistical methods: After confirming the normality of the data using Shapiro-Wilkes test, data analysis was performed using one way ANOVA and Tukey post hoc test to compare the two groups. Was used. All calculations were performed using SPSS software version 24 at a significance level of 0.05.

Table 2. Results of one-way analysis of variance in comparison of IL-4 and IFN-γ gene expression in research groups

	Sum of Squares	df	Mean Square	F	Sig.
IL-4	1.376	4	.344	4.147	.007*
INFgama	3.483	4	.871	44.599	.000*

*: Significant difference at the level of α ≤0.05

Results

The results of data analysis using one-way analysis of variance (ANOVA) are shown in Table 2. The results show that there is a significant difference between the groups in the expression of IL-4 and IFN-γ genes in the thymus tissue of the samples in different groups (P = 0.0001 and P = 0.007, respectively).

Figure 1 shows a comparison of IFN-γ gene expression in thymus tissue of different research groups. The results show that the expression of

this gene in DEX group is different from healthy control groups (p = 0.0001) and DEX + SEL (p = 0.0001). Also, DEX + SEL group showed a significant difference with control groups (p = 0.001) and DEX + TRA (p = 0.0001). IFN-γ gene expression in DEX + TRA group was significantly different from control group (p = 0.0001) and DEX + SEL + TRA group (p = 0.0001).

Figure 2 shows a comparison of IL4 gene expression in thymus tissue of different research groups. The results show that the expression of this gene in the healthy control group is significantly different from the DEX (p = 0.048) and DEX + TRA groups (p = 0.013).

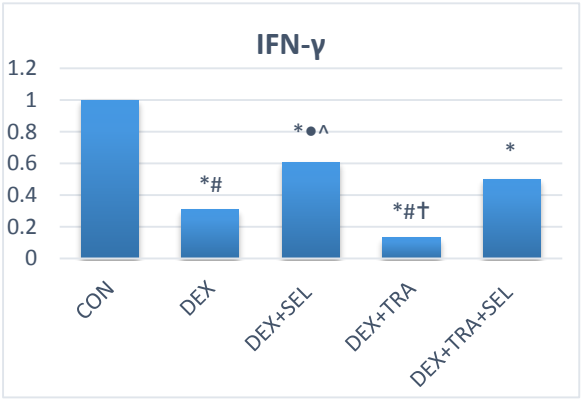

Figure 1. Tukey test results and comparison of IL-4 gene expression in different research groups

*: Significant difference with healthy control group at the level of α ≤0.05
●: Significant difference with the DEX group at the level of α ≤0.05
^: Significant difference with the DEX+TRA at the level of α ≤0.05
#: Significant difference with DEX+SEL consumption group at the level of α≥0.05
†: Significant difference with DEX +TRA+SEL at the level of α ≤0.05

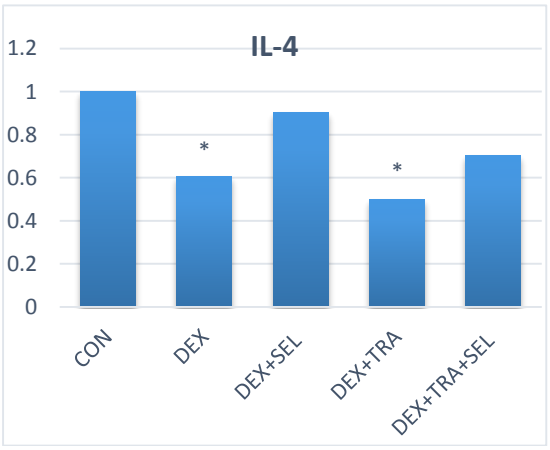

Figure 2. Tukey test results and comparison of IL-4 gene expression in different research groups
*: Significant difference with healthy control group at the level of α ≤0.05

Discussion

The results of the present study showed that weakening the immune system using dexamethasone reduces the expression of IFN-γ and IL-4 genes in rat thymus tissue. The thymus gland, as seen in mouse models, is a vital regulator for the development of various branches of lymphocytes called CD4 cells, which are necessary to ensure the formation of helper T cells. Thymocytes, positive selection, regardless of MHC specificity, lead them to become CD4 (van Vliet et al, 2017; Mitevska et al, 2015). Given the role of the thymus in the maturation of infocytes, it seems that the decrease in the expression of

these cytokines in thymus tissue leads to a decrease in the function of this tissue in the immune system's response to pathogens. On the other hand, studies have shown that dexamethasone leads to atrophy and reduction of thymus volume (Dehghani et al, 2021). Thymus atrophy in DEX-treated mice may be due to a decrease in the number of immature T cells in its cortex. The thymus has two parts, the cortex and the medulla. The cortex is made up of lymphocytes and thymocytes, which are supported by retinal epithelial cells. But there are fewer lymphocytes and more epithelial cells in the medulla (Jeklova et al, 2007). The results of studies show that most of the decrease in thymus volume is related to the volume of the thymus cortex, which has a greater role in the maturation of lymphocyte cells. The cortex and medulla contain immature and mature T cells, respectively (Pearse et al, 2006). Hussar et al. Found that DEX induced immature T cell apoptosis in the thymus cortex, while the change in lymphocyte count in the medulla was less pronounced. It is estimated that approximately 90% of immature T cells are naturally removed by intrinsic agents, but DEX plays a major role in the development of the apoptotic pathway. Sensitivity to glucocorticoids in immature T cell apoptosis has been associated with markers of oxidative stress and mitochondrial dysfunction in previous studies. Immature T cells in the thymus cortex are thought to be more sensitive to oxidative stress than lymphocytes in the medulla (Dehghani et al, 2021). They are effective in reducing IL-4 and IFN-γ (Nunes-Cabacet al, 2022).

Another result of the present study was that selenium supplementation in mice whose immune systems were weakened significantly compensated for the decrease in IL-4 gene expression. In the case of IFN-γ, although selenium intake reduced the expression of this gene slightly, its expression was still lower than in the healthy control group. These results are consistent with the findings of a study showing that selenium administration to DEX-treated mice increased total thymus volume by 37.6% and cortex by 80.5% compared with the DEX group (Dehghani et al, 2021). However, these findings emphasize that the volume of the thymus medulla remained unchanged in selenium-receiving mice. In fact, the findings show that DEX reduces the volume of all areas associated with the thymus, spleen, and lymph nodes, but selenium, as an immunomodulator, improves changes in the thymus cortex, white spleen pulp, and outer cortex of the lymph nodes. (Dehghani et al., 2021).

Selenium may exert its effect through a mechanism involving free thiols, reducing oxidative stress conditions, and increasing lymphocyte proliferation (Hoffmann et al, 2010). Hawkes et al. Also showed that selenium promotes the proliferation of B lymphocytes and possibly T cells. Kiremidjian-Schumacher et al. Also stated that selenium proliferates cytotoxic progenitor cells. Cheng et al. Reported that selenium increased the number of B and T cells. The antioxidant properties of selenium appear to cause the proliferation of immature T cells in the thymus cortex but have no effect on mature T cells in the thymus medulla. We found that selenium stimulates B cell proliferation more than the growth of mature T cells in the spleen and lymph nodes. In this study, selenium administration alone had no effect on the different structures of lymphatic organs in healthy mice. Selenium is likely to inhibit atrophic lymph nodes in immunocompromised conditions, but has no effect on the natural structure of these tissues.

Interleukin-4 (IL-4) is a peptide consisting of four short helices that is produced as a pleiotropic cytokine mainly by lymphocytes and thymocytes. IL-4 regulates various processes in cell types. In addition to its role in B cell differentiation, it enhances Th2 by inhibiting the fate of Th1 and Th17 in T cells (Nunes-Cabacet al, 2022). IL-4 is also involved in the function of T cells called CD8 because it increases their proliferation and cytotoxic activity (Oliver et al, 2012) and can also act as a negative regulator of CD8 T cell responses. (Wijesundara et al, 2013). In the thymus, IL-4 is required for the growth of CD8 cells (Jameson et al, 2015). CD8 T cells, after activation, are rapidly producing IFN-γ (Jameson et al., 2015). Mice and CD4 T cells are MHC class II dependent in humans (Nunes-Cabacet al, 2022). Importantly, the response to IL-4 during mouse CD8 T cell development and homeostasis may alter their functional response and response to pathogens (Kabaku et al., 2022).

Intensity of activity is one of the most important variables affecting the effects of exercise on the immune system. Most studies have shown that exercise leads to the renewal and improvement

of immune function (Tylutka, 2021; Papp et al, 2021). However, the mechanisms of such effects are still highly unclear. Improving exercise-induced immunity can be associated with reduced inflammation, maintenance of thymus mass, changes in memory composition and simple T lymphocytes, or increased immune monitoring. Indeed, physical activity is a powerful intervention that has great potential for improving the immune system and health outcomes in the elderly, obese, and patients with cancer and chronic viral infections (Simpson et al, 2012). However, in the present study, it was found that intense exercise activity in high-intensity intermittent exercise (HIIT) may inhibit immune responses and anti-inflammatory cytokines by increasing the production and secretion of inflammatory and proinflammatory cytokines that promote lymphocyte differentiation. They create thymus in the tissue. This was especially true in samples whose immune systems were weakened by DEX administration, and therefore exercise should be done with greater caution in order to strengthen the immune system in these individuals. Because the effect of such activities may be synergistic with the pharmacological effects of glucocorticoids in weakening the immune system and lead to a double weakening of the function of this system.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Ramadan Fasting and Melatonin Supplementation on Sleep Quality, Melatonin and Growth Hormone to Cortisol Ratio in Male Athletes

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 03 Jun 2021
Accepted: 14 Jul 2021
Published: 20 May 2022

Keywords:
Circadian rhythm
Melatonin
Sleep quality
Athletes

ABSTRACT

Introduction: Food habits and wake-sleep cycle influence circadian rhythms. Ramadan fasting (RF) changes food habits and wake-sleep cycle and causes a metabolic imbalance. Melatonin increases sleep quality and daily awareness. The purpose of this study examined the effect of four weeks Ramadan fasting and melatonin supplementation (MS) on sleep quality, melatonin levels and growth hormone (GH) to cortisol ratio in male athletes.

Methods: Thirty active men (20-25 years) were randomly divided into supplement (n=15) and placebo (n=15) groups. Body fat percentage and hormones (melatonin, growth hormone, cortisol and growth hormone-cortisol ratio) were evaluated in three times: before the month, mid fasting and post fasting. Blood samples collected at three times; before sleep, wake up time for Sahur and morning wake up time. To evaluate the intervention effect of supplement and sleep conditions on the dependent variable Repeated Measure (factorial 3*3*2) and Bonferroni post hoc tests were used.

Results: Melatonin (P=0.001), GH (P=0.001), GH-cortisol ratio (P=0.001) significantly increased in supplement group compare to placebo group. Also, Cortisol (P=0.003) and body fat percentage (P=0.001) decreased in the supplement group compare to placebo group. Sleep quality significantly improved in the supplement group (P=0.025).

Conclusion: Supplementing melatonin with improved anabolic conditions and regulated wake-sleep cycle can help the Ramadan fasting condition and prevent the decreased performance during Ramadan fasting.

► Please cite this paper as:
Rostamdokht A, Ghahremani Moghaddam M, Reiter RJ, Bijeh N. The Effect of Ramadan Fasting and Melatonin Supplementation on Sleep Quality, Melatonin and Growth Hormone to Cortisol Ratio in Male Athletes. J Nutr Fast Health. 2022; 10(2): 136-142. DOI: 10.22038/JNFH.2021.58134.1337.

Introduction

Ramadan is the holy month of the Islamic calendar. This month is observed by Muslims as a month of fasting [1]. In Ramadan, Muslims wake up in the middle of the night to eat the first meal (Sahur) and then eat nothing until sunset (Iftar). Ramadan fasting impacts behaviors such as eating [2], sleep patterns [3, 4] and hormones, especially circadian rhythm dependent hormones (Melatonin, growth hormone and cortisol) [2]. Month of Ramadan is a unique model of change in sleep patterns [1, 5], food intake [6] and habits[7, 8]. Thus, Ramadan is an excellent opportunity with unique characteristics to study these changes.

Melatonin has a myriad of effects and is used as a reliable marker of circadian rhythms [9, 10]. Studies showed significant reduction in nocturnal sleep time during Ramadan [7, 9]. During Ramadan, the peak in nightly secretion of melatonin is lower than before Ramadan, which may be due to exposure to artificial light for a longer period during Ramadan's first meal in Sahur (approximately one hour before sunrise) [11, 12]. Sleep problems cause loss of secretion of the melatonin, growth hormone, and on the other hand, increase cortisol secretion, which may not be healthy [13].

In a controlled trials study, using 2 mg of melatonin, compared to the placebo group, which was performed on 177 deducted patients, melatonin increased sleep quality and daily alertness [4]. Melatonin reduce the delay in sleep initiation, increased total sleep time and improved sleep quality [14].

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Some studies have shown that, the reduction of melatonin is associated with an increase in cortisol levels in depressed patient. There is evidence that the change in night-time light reduces synthesis of melatonin and increases cortisol secretion is associated with several diseases [2, 15, 16].

Previous studies have suggested a shift delay in the peak of melatonin during Ramadan and reported, Islamic intermittent fasting influences the circadian pattern of circulating melatonin [6, 17]. Sleep quality and duration are important variables in athlete's recovery and adaptation to exercise [18]. Also, sleep quality and duration influences athletic performance and training efficiency [18-19]. Improvement in sleep quality can improves athletic performance and reduce the risk of injury [20].

No available data are found about Ramadan fasting and melatonin supplementation on sleep quality, melatonin and growth hormone to cortisol ratio. Also, because of lunar calendar, the month of Ramadan takes place in different seasons, each year the Ramadan month occurs 11 days earlier, which creates different fasting time and day light schedule. Of the 1.5 Billion Muslims more than 500 thousands fast in Ramadan [1]. So, it is important to study and improve their sleep quality and finally health. In this study, it hypothesized that melatonin supplementation can reduce negative effects of changed circadian rhythm and catabolic effects of changes in GH and cortisol hormones in the month of Ramadan.

Materials and Methods

Study Group

This is a descriptive study with repeated measures in a random sample of active male athlete volunteers. All subjects were martial art athletes with at least three years training experience and five session training per week. The study group consisted of fasting males in Mashhad, Iran. Fifteen were supplemented with melatonin and fifteen placebo subjects were included.

The study was conducted during a week before of Ramadan, which was used as a baseline period (BL), during the second (R2) and last (LR) weeks of Ramadan 1437 Hijri (corresponding to the period from June 7, 2016 to July 7, 2016). To check the participants regular sleep patterns, they were asked to maintain sleep diaries for two weeks prior to the study. Fasting time for the participants was from before sunshine (3:30 am) to sunset (8:10 pm) during Ramadan, mean temperature was 34° and humidity was 41%. During Ramadan, participants received 3 mg melatonin capsule or matching placebo (cellulose) was taken orally 1 hour before bedtime each night for the month of Ramadan. Participants were collegiate athletes who lived in a university dormitory and respected the nutrition requirements and sleeping-awake time. Before starting the study, each participant was asked to IPAQ, PAR-Q, PSQI (Pittsburgh sleep quality index). The fat percentage measured by caliper (four point way) and calculated by Siri's formula.

Before starting the study, each participant was asked to International Physical Activity Questionnaire (IPAQ), Physical Activity Readiness Questionnaire (PAR-Q), Pittsburgh sleep quality index (PSQI). The body fat percentage measured by caliper (four point way) and calculated by Siri's formula.

Before the beginning of the study, a familiarization meeting was held for the subjects. Subjects did not have any illness that fasting would exacerbate, and all had good sleep quality. They were also asked to avoid smoking, caffeine, tryptophan and folate during the study.

Pittsburgh Sleep Quality Index (PSQI)

Sleep quality measured by Pittsburgh Sleep Quality Index (PSQI). It was developed in 1989 by Buysse et al, at the University of Pittsburgh Psychiatric clinic. The questionnaire has nine items in its original form, but since the 5th question contains 10 subfields, the entire questionnaire has 19 items that are scored in a 4-point Likert scale from 0 to 3. Closer point to 0 is better results.

Measuring Blood Biomarkers

One week before the beginning, after the 15 days and the end of Ramadan a blood sample was collected. To minimize sleep disorder, blood samples were collected at 23:00 h (before sleep), 03:00 h (woke up for Sahur), and about 07:00 h (woke up for morning) in sitting position. Blood samples 5cc from a vein in the arm and in the sitting position analyzed to determine the levels of melatonin, growth hormone and cortisol. Blood samples were frozen and transported to laboratory.

All blood samples were stored in the laboratory and after collection all samples were analyzed in

one step by a technician. Subjects were placed in supplemental and placebo groups randomly.

Data Analysis

After collecting and entering the obtained data in SPSS software version 22, raw data was analyzed for calculating central tendency indicators, dispersion and plotting variable graphs, descriptive statistics were used. After confirming the normal distribution of data by Kolmogorov-Smirnov test and homogenization by Leven test, to compare the differences in the normal situation, the repeated measures (factorial 3*3*2) and Bonferroni post hoc test were used.

Table 1. Demographic of the study groups

Variables \ Groups	Supplement	Placebo
	Mean ± SD	Mean ± SD
Age	24.5 ± 4.13	20.72 ± 3.22
Height	1.82 ± 3.1	1.79 ± 3.4
Weight	77 ± 3.9	74 ± 5.2
BMI	24.1 ± 2.6	24.9 ± 2.3

Table 2. Sleep Quality Parameters in Supplement Group (Pittsburgh Sleep Quality Index (PSQI))

Variables	Supplement Group (Mean ± SD)		Placebo Group (Mean ± SD)
Morning Awake (Hour)	**Before**	7.6 ± 0.7	7.5 ± 0.8
	Middle	9.1 ± 2	10.9 ± 2.5
	After	8.4 ± 1.8	9.1 ± 1
Night Sleep (Hour)	**Before**	11.6 ± 0.4	11.6 ± 0.4
	Middle	12.5 ± 1.7	15.6 ± 1.3
	After	11.5 ± 0.5	13.1 ± 0.6
Reality Night Sleep (Hour)	**Before**	5.2 ± 0.3	5.2 ± 0.3
	Middle	5.8 ± 2.2	5.2 ± 3.1
	After	5.4 ± 1.1	5.3 ± 0.9
Sleep Delay (Hour)	**Before**	0.17 ± 0.11	0.14 ± 0.08
	Middle	0.12 ± 0.09	0.12 ± 0.10
	After	0.14 ± 0.10	0.11 ± 0.06
Total Score (Hour)	**Before**	3 ± 1.3	3.5 ± 1.1
	Middle	2.3 ± 1.7	8 ± 4.1
	After	3.5 ± 1	3.6 ± 2.3

Results

Comparison between variables were showed in figures 1-4. The findings of this study indicate that after one month of melatonin supplementation, serum levels of melatonin significantly increased at the beginning of the night in the supplement group compared with placebo. Also, the supplementation group had significantly higher levels of melatonin compared to the placebo group. Also, in the morning, serum levels of melatonin in the supplement and placebo group decreased after month of Ramadan compared with middle of the month of Ramadan.

Results showed higher GH levels in melatonin supplemented group compared to the placebo group. Pre-month serum levels of growth hormone in the supplement and placebo groups increased by 18% and 10.8% at 11 pm respectively. At 3 am, increased by 205% and decreased by 10% in the supplement and placebo groups respectively. At 7 am in the morning, decreased 7% and 5.7% respectively. Also, in the middle of the month, GH increased by 18.4% and 21.2% at 11 pm, 4.7% and 12% at 3 am, and 5.3% and 7.7% at 7 am, in the supplement and placebo groups respectively. Also, at the end of the month, supplementation and placebo groups increased 39.8% and 34.4% at 11 pm, 220% and 0.75% at 3 am, and 13% and 1.5% at 7 am, respectively compared to the baseline.

The findings of the present study indicate that serum cortisol levels was lower at baseline in the supplement group compared to the placebo group. Also, midnight cortisol was at its lowest level, and the supplementation group significantly decreased the proportion of placebo group. However, at the beginning of the day, the

placebo group showed a greater increase in cortisol levels than the supplement group.

The ratio of growth hormone to cortisol before the month of Ramadan in the supplement and placebo groups increased by 87.5% and decreased by 10% at 11 pm. At 3 am, increased 150% in the supplement group, and remained unchanged in placebo group. At 7 am increased by 37% and 11% in the supplement and placebo groups. Also, in the middle of the month, increased by 20% and 33% at 11 pm, in the supplementation and placebo groups respectively, at 3 am 33% and 41% increased, and at 7 am decreased by 9% in the supplement group, and remained unchanged in placebo group. Also, at the end of the month, supplementation and placebo groups increased by 125% and 20% at 11 pm, 23% and 41% at 3 am, and increased by 25% and 11% at 7 am in the morning, in the supplement and placebo groups respectively compared to the baseline.

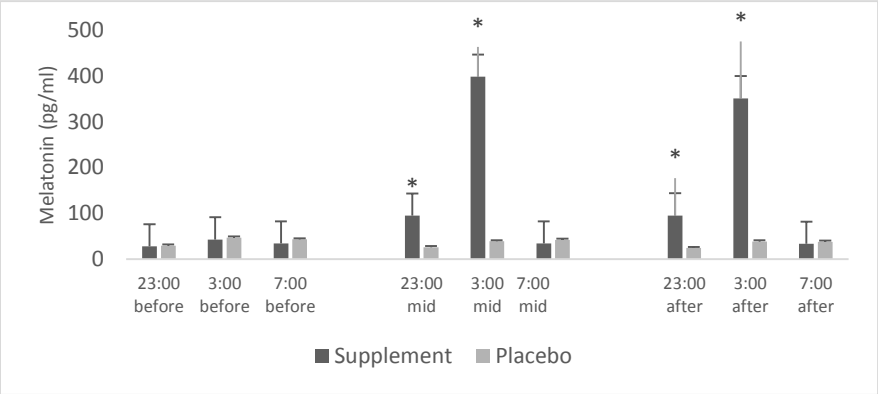

Figure 1. Effect of Ramadan fasting and melatonin supplementation on melatonin levels.

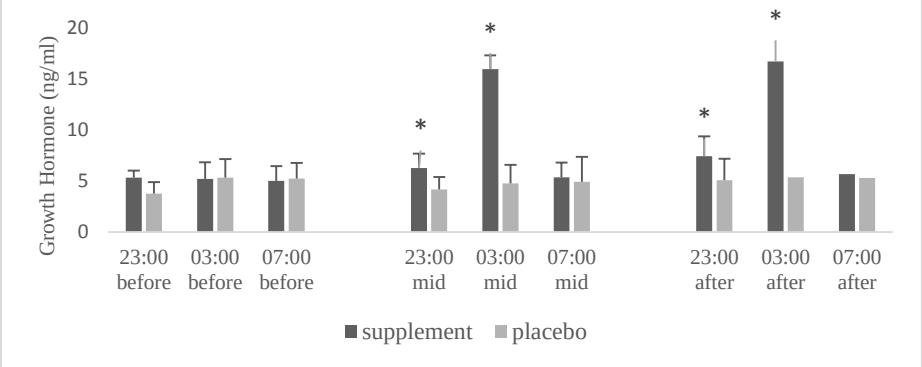

Figure 2. Effect of Ramadan fasting and melatonin supplementation on growth hormone.

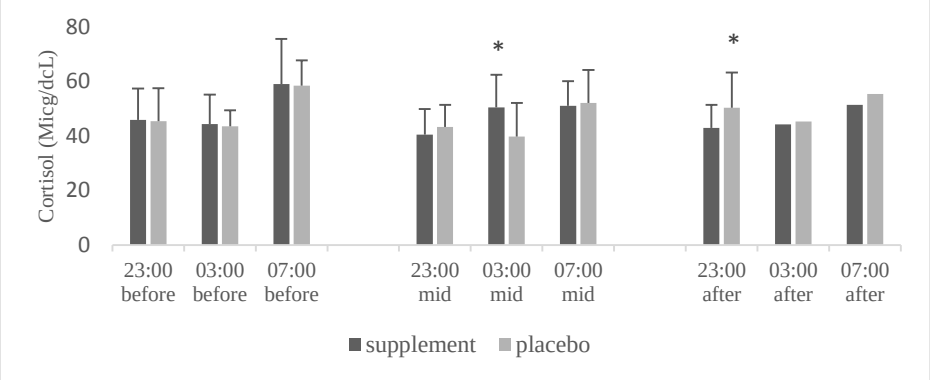

Figure 3. Effect of Ramadan fasting and melatonin supplementation on cortisol levels.

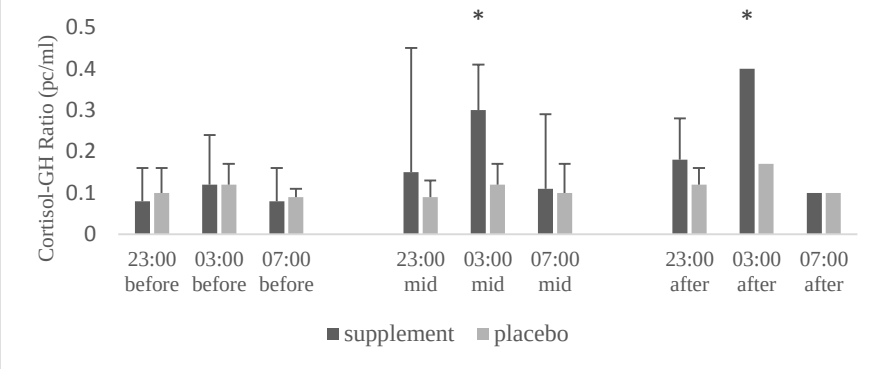

Figure 4. Effect of Ramadan fasting and melatonin supplementation on cortisol-GH ratio levels.

Discussion

During the month of Ramadan, the peak nightly secretion of melatonin was lower than before Ramadan, which may be due to exposure to artificial light for a longer period during Ramadan. The peak in melatonin is associated with lower body temperature, fatigue, decreased consciousness and physical performance.

The findings of this study indicate that after one month of melatonin supplementation, serum levels of melatonin significantly increased at the beginning of the night in the supplement group compared with placebo (245.5% in the middle of the month compared to the previous month and 261% at the end of the month Compared to the previous month). Also, mid-dark, which is near the peak in melatonin secretion, the supplementation group had significantly higher levels of melatonin compared to the placebo group (846% in the middle of the month compared with the previous one and 736.5% at the end of the month compared to the previous month), which increased the depth of sleep. Also, in the morning, serum levels of melatonin in the supplement and placebo group decreased by 1.2% and 11.9% respectively after month of Ramadan compared with middle of the month of Ramadan, suggests a possible daily increase in consciousness. During the month of Ramadan, the peak nightly secretion of melatonin is lower than before Ramadan, which may be due to exposure to artificial light for a longer period during Ramadan. This indicate that, melatonin reduces the delay in starting sleep, increases the overall sleep time and improves overall sleep quality [1].

Results related to GH showed higher GH levels in melatonin supplemented group. An important point of the study's finding is that the growth hormone coincides with the increase in melatonin, and it shows an important role of melatonin in stimulating the growth hormone secretion. Melatonin has been shown to stimulate the secretion of the growth hormone [21], and the peak time of secretion of both agents is also close, that is, they reach the peak at the same time as entering the deep sleep stage [22, 23]. By preventing the release of somatostatin, melatonin increases the secretion of the GH [24]. Melatonin increases the expression of the GH gene, a response completely blocked by somatostatin. The action of melatonin on the pituitary gland is not limited to stimulating or synthesizing the GH, but also regulates the function of other key components regulating the somatotropes [24].

The findings of the present study indicate that serum cortisol levels was lower at baseline in the supplement group compared to the placebo group (7% in the middle of the month compared with the beginning of the month and 17.3% at the end of the month compared to the beginning of the month), which possibly reduces consciousness and improves sleep status. Also, midnight cortisol was at its lowest level, and the supplementation group significantly decreased the proportion of placebo group (22.3% at the end of the month compared to the middle and 4.3% at the end of the month compared to the previous month). However, at the beginning of the day, the placebo group showed a greater increase in cortisol levels (3% in the middle of the month compared with the previous month and 8% at the end of the month) than the supplement group. In healthy individuals, levels of cortisol rise rapidly after awakening and peak at 30-45 minutes. It gradually drops throughout the day, rising again in the late afternoon, falling

by the end of the day and reaching its lowest point in the middle of the night. An 24-hour abnormal cortisol rhythm is associated with chronic fatigue syndrome[25] and insomnia[26]. Results showed positive GH/cortisol levels in melatonin supplemented group. Hormones that influenced by sleep deprivation in sports population are growth hormone and serum cortisol and these changes has been related with overtraining [27]. Many anabolic hormones, such as melatonin and GH, are secreted during sleep, and the rate of secretion of catabolic hormones such as cortisol increases during awakening. An imbalance in quality or quantity of sleep causes a reduction in the secretion of total anabolic and catabolic hormones [4, 28]. Also, GH and cortisol imbalances due to sleep deprivation related with chronic fatigue and repeated injuries coercing sportsmen to quit professional games in their career [29, 30].

Finally, this study demonstrates melatonin supplementation improves sleep quality and prevents catabolic effects of Ramadan with increases in cortisol-GH ratio. It seems that, initial days of receiving Melatonin have greater results on sleep quality, and after period of time this effects decreases. In present study, these changes in sleep quality may be because of reduced sensitivity to taking melatonin supplement or adaptations to month of Ramadan conditions in control group.

Conflict of Interest

There are no conflict of interest.

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JOURNAL OF NUTRITION FASTING AND HEALTH

Effect of Ramadan Fasting on the Blood Coagulation System in a Session Soccer Match

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 24 Feb 2021
Accepted: 08 Oct 2021
Published: 20 May 2022

Keywords:
Exercise
Acute
Coagulation
Blood
Fibrinolysis
Fasting

ABSTRACT

Introduction: Ramadan fasting is associated with modifications in athletes' metabolic, physiological, and psychological responses, which may affect their physical performance. The present study aimed to assess the changes in some of the risk factors for thrombosis in trained men after one soccer session in fasting and non-fasting states.

Methods: This study was conducted on 11 amateur soccer players with the mean age of 42±16 years and mean body mass index of 26.49±2.86 kg/m^2. The subjects played in a soccer match with the duration of ~100 minutes, including 10 minutes of warm-up and 90 minutes of a soccer match, in two sessions (one week before and on day four of Ramadan). Blood samples were collected before and after the soccer game. Fibrinogen and D-dimer were analyzed using the Clauss clotting method and the turbid metric assay, respectively. In addition, plasminogen and alpha 2-antiplasmin were analyzed via spectrophotometry, and homocysteine was examined using the ELISA assay. Data analysis was performed using the Shapiro-Wilk test and independent and dependent t-test at the significance level of P≤0.05.

Result: One session of soccer match increased homocysteine in the fasting (P=0.006) and non-fasting subjects (P=0.042). Alpha 2-antiplasmin decreased in the fasting (P=0.031) and non-fasting subjects (P=0.001), while plasminogen decreased only in the non-fasting subjects (P=0.012). One session of soccer match had no significant impact on fibrinogen and D-dimer in both states, as well as plasminogen in the fasting state (P≥0.05). Furthermore, no significant differences were observed between the fasting and non-fasting subjects in terms of homocysteine, alpha 2-antiplasmin, fibrinogen, plasminogen, and D-dimer in response to one session of soccer match (P≥0.05).

Conclusion: According to the results, one session of soccer match in the fasting state was parallel to the non-fasting state, and fasting led to no adverse consequences in the coagulation system of the subjects.

► Please cite this paper as:
Kami F, Kordi MR, Saffar Kohneh Quchan AH, Shahidi SH, Shabkhiz F. Effect of Ramadan Fasting on the Blood Coagulation System in a Session Soccer Match. J Nutr Fast Health. 2022; 10(2): 143-149. DOI: 10.22038/JNFH.2021.55933.1324.

Introduction

Each year, millions of healthy adult Muslims observe obligatory fasting from sunrise to sunset during the month of Ramadan for 29-30 days. While fasting, people refrain from eating and drinking over periods of 13-18 hours a day depending on the season (1). The fasting state induces various physiological and psychological changes (2). The different physiological responses in Ramadan probably result from disturbances in the sleep-wake cycle and changes in the timing and type of meals (3).

Lifestyle changes for one month may influence and modify human health. For instance, dehydration is a significant challenge associated with Ramadan fasting. Signs of dehydration have been categorized by an increased hematocrit rate or hemoglobin concentration and plasma osmolality, which increase blood viscosity. High blood viscosity is a secondary effect of dehydration, which may increase the risk of thrombosis (4). Moreover, dehydration increases the level of coagulation factors (clot formation) and reduces fibrinolysis (clot degradation) (5).
Blood coagulation and fibrinolysis contain two primary physiological systems that are

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monitored by a balance between activators and inhibitors (6). These factors are strongly associated with the risk of thrombosis. Thrombosis is a hemostatic barrier, which blocks the arteries (7). The fibrinolytic system in the blood is responsible for eliminating and destroying the clot, and fibrinolysis is activated upon the activation of the coagulation system (8). Hemostasis is a physiological process associated with the functional balance between the blood coagulation and fibrinolytic systems (9). These systems play a pivotal role in the human inflammatory response, which also reflects blood viscosity and the risk of thrombosis (8).

It is widely acknowledged that fasting, exercise training, and dehydration cause various consequences in the coagulation and fibrinolytic systems (10). In a study in this regard, Darzabi et al. (2020) reported that Ramadan fasting increases osmolarity and serum electrolytes (11). In addition, Javanmardi et al (2018) stated that cerebral venous sinus thrombosis increases during Ramadan due to dehydration (12). Some studies have also proposed that a small degree of dehydration induced by acute exercise and fluid restriction increases homocysteine and significantly impairs the endothelial function (13).

Many fasting individuals and athletes continue their exercise training during Ramadan. Furthermore, important world competitions may be scheduled during this month, such as the FIFA World Cup 2014 and 2018 and the Olympic Games in London in 2012. Therefore, researchers and coaches are concerned about the fact that prolonged fasting and exercising while fasting harms the health and performance of athletes (14, 15). To date, no exercise training studies have been focused on the impact of acute exercise training on the function of the coagulation system while fasting.

The present study aimed to investigate the impact of one soccer training session in the fasting and non-fasting states on some of the risk factors for thrombosis, including fibrinogen, homocysteine, D-dimer, alpha 2-antiplasmin, and plasminogen, in fasting men.

Materials and Methods

This study was conducted on 11 amateur soccer players with the mean age of 42±16 years and mean body mass index of 26.49±2.86 kg/m^2. The subjects played a soccer matched in two sessions one week before and on day four of Ramadan. All the participants trained at least two days per week for an average of 90 minutes per day. The match sessions were scheduled at 17:30-19:30 during the control period (before fast breaking in Ramadan). In both periods, the match sessions lasted for ~100 minutes, including 10 minutes of warm-up and 90 minutes of a soccer match (45+45 minutes).

The physical performance parameters of the match were recorded using a global positioning system (GPS). The study was conducted in Tehran, Iran in 2019. In this period, Ramadan started on May 6th and ended on June 4th. The length of each daytime fast was approximately 15 hours, starting at ~03:50 and ending at ~19:50. Written informed consent was obtained from the participants, and data were collected using the physical activity readiness questionnaire. The inclusion criteria of the study were no history of cardiovascular diseases, coagulation disorders, and chronic disorders and no smoking habits. The study protocol was approved by the Ethics Committee of the Department of Physical Education at Tehran University (IR.UT.SPORT.REC.1398.042).

The current research was performed in three stages. At the first stage, blood samples were collected before starting the soccer game. The second stage was playing the soccer game, and the third stage involved blood sample collection after the intervention. Due to the sensitivity of the tests, the blood samples were collected before and after the intervention on the football field and immediately centrifuged. The plasma was separated and transferred to a freezer.

For the analysis of homocysteine, the blood samples were poured in tubes containing lyophilized EDTA powder for the removal of water from the materials to prolong the life of moisture-sensitive materials. The samples were placed in ice immediately after blood sampling, and the plasma was rapidly separated via centrifugation (Eppendorf centrifuge 5702; made in Germany). In addition, coagulation test samples were collected in plastic or silicone tubes containing 3.2% sodium citrate at the ratio of 1:9 (1: volume of sodium citrate, 9: volume of blood samples) and separated via centrifugation at 2500-2000 grams for 15 minutes in order to isolate the plasma samples. On the day of the measurements, the samples were taken out of the freezer and allowed to thaw. Each parameter

was analyzed in accordance with the instructions of the kits' manufacturer.

The D-dimer assay was implemented based on the change in the turbidity of a microparticle suspension, which was measured via photometry using the STA®-Liatest® D-Di kit and the STA compact coagulation analyzer (made in France). Before performing the test with two different levels of control (N & P), the accuracy and reproducibility of the results were assessed (i.e., quality control). Moreover, the D-dimer assay of the plasma samples was automatically carried out using the analyzer at 540 nanometers immediately after the samples were loaded.

The alpha 2-antiplasmin and plasminogen assays were implemented based on a colorimetric (chromogenic) assay, which was measured via photometry using the Stachrom Antiplasmin kit (made in France) and the STA compact coagulation analyzer. Before running the test with two different levels of control (N & P), the accuracy and reproducibility of the results were evaluated (i.e., quality control). The alpha 2-antiplasmin assay of the plasma was automatically carried out using the analyzer at 405 nanometers immediately after the samples were loaded.

In the present study, the fibrinogen assay was performed based on the Clauss clotting method. Clot detection by the STA compact coagulation analyzer involves an electromagnetic-mechanical system and monitors the oscillation of a steel ball within the cuvette with the thrombin and diluted plasma. When the oscillation of the steel ball is stopped by clot formation, the sensor registers the time in seconds. In our study, the time was translated into fibrinogen concentration from a standard fibrinogen curve and stored on the STA compact. Before using the samples with two different levels of control (N and P), the accuracy and reproducibility of the results were assessed (i.e., quality control).

The concentration of homocysteine was determined using the ELISA assay (Axis® Homocysteine EIA kit; made in Germany). Following that, the samples were analyzed using the awareness technology INC reader (made in USA). Before performing the test with three levels of controls (low, normal, and high), the accuracy and reproducibility of the results were evaluated (i.e., quality control).

Data analysis was performed in SPSS version 21 using the Shapiro-Wilk test to assess the normality of data distribution and Levene test to evaluate the homogeneity of the obtained data. Since the ANCOVA defaults were not met, dependent t-test was employed for the intragroup comparison, and independent t-test was used for the intergroup comparison. In all the statistical analyses, the P-value of less than 0.05 was considered significant.

Table 1. Anthropometric Characteristics of subjects

Conditions	n	Sex	Mean age ± SD	Body mass index (BMI)
Fasting	11	Male	16 ± 0.42	25.92 ± 1.64
Non-fasting	11	Male	16 ± 0.42	26.49 ± 2.86

Table 2. Physical performance values recorded by GPS

Variables	Fasting	Non-Fasting
Duration of the match (min)	100.18	101
The amount of calories consumed (Kcal)	586	688
The average distance traveled (m)	6500	7700
Maximum speed (Km/h)	25.7	25.9
Maximum heart rate (beats/min)	199	190

Table 3. Independent t-test to compare the changes in plasma level factors between non-fasting and fasting groups

Variables	conditions	Timing		Mean ± SD (95% CI)	P-Value
Homocysteine	Non-Fasting	Pre-Test	Post-Post	1.45 ± 0.62 (0.06 – 2.84)	0.042*
Fibrinogen	Non-Fasting	Pre-Test	Post-Post	-8 ± 12.30 (-35.42 – 19.42)	0.53
D-dimer	Non-Fasting	Pre-Test	Post-Post	0.11 ± 0.06 (-0.03 – 0.26)	0.111
Plasminogen	Non-Fasting	Pre-Test	Post-Post	-10.8 ± 3.53 (-18.68 – -2.95)	0.012*
Alpha 2-antiplasmin	Non-Fasting	Pre-Test	Post-Post	-16.27 ± 3.67 (-24.45 – -8.09)	0.001*
Homocysteine	Fasting	Pre-Test	Post-Post	2.18 ± 0.64 (0.74 – 3.61)	0.006*
Fibrinogen	Fasting	Pre-Test	Post-Post	-9 ± 13.47 (-39.01 – 21.01)	0.519
D-dimer	Fasting	Pre-Test	Post-Post	0.08 ± 0.04 (-0.02 – 0.19)	0.131
Plasminogen	Fasting	Pre-Test	Post-Post	-7.27 ± 4.38 (-17.04 – 2.49)	0.128
Alpha 2-antiplasmin	Fasting	Pre-Test	Post-Post	-10.54 ± 4.20 (-19.91 – -1.17)	0.031*

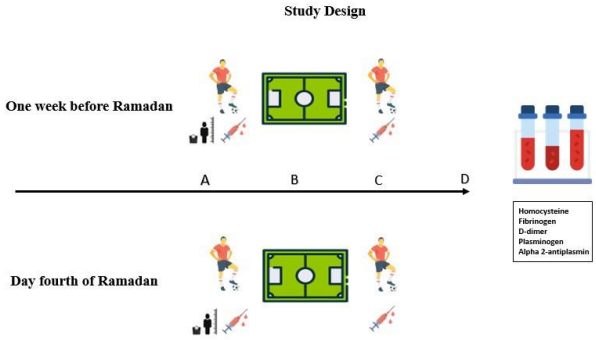

Figure 1. Note. A = Before starting the soccer match all participant measured blood samples and body mass index; B = Starting soccer match and it took about 100 minutes; C = Immediately after soccer match all participant blood sample got and froze; D = The data insert to the laboratory to analyze the blood sample biomarkers

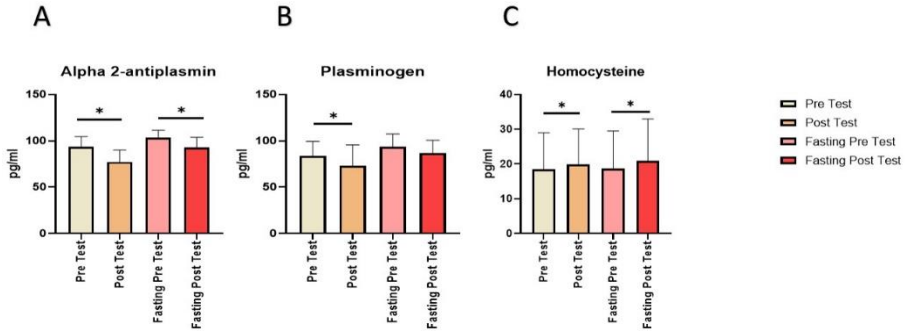

Figure 2. Impact of one session soccer match during non-fasting and fasting on blood biomarkers. ✱ *P* at least < 0.05 from the analysis by indented t-test. Note. Pre-test and post-test refer to the non-fasting state.

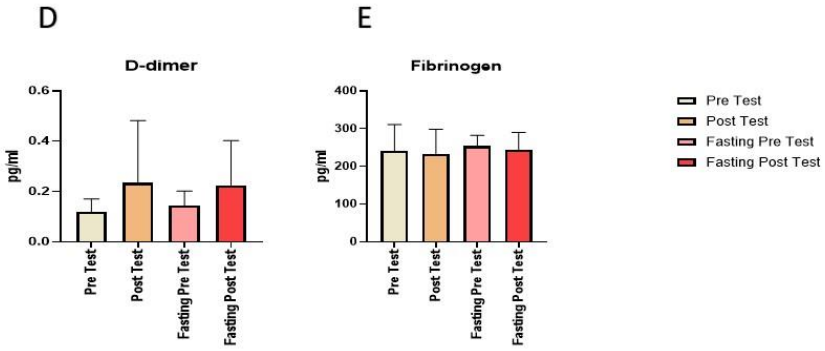

Figure 3. Impact of one session soccer match throughout non-fasting and fasting on blood biomarkers. Note. Pre-test and post-test refers to the non-fasting state

Results

Tables 1 and 2 show the demographic characteristics of the participants and the football match recorded by GPS, respectively. Compared to the pretest and posttest in the fasting and non-fasting states, the mean level of plasma homocysteine increased significantly (non-fasting: P=0.042; fasting: P=0.006) (Figure 2A), the mean level of alpha 2-antiplasmin significantly decreased (non-fasting: P=0.001; fasting: P=0.031) (Figure 2B), and the mean concentration of plasminogen decreased as well (non-fasting: P=0.012) (Figure 2C) (Table 3).

Overall, data analysis indicated that the levels of the risk factors had no significant differences after one session of the soccer match in the fasting and non-fasting groups (P>0.05) (Figure 3). By examining the percentage of the plasma volume, changes in the non-fasting and fasting groups were evaluated using the Dill and Costill equation (16), it was observed that these changes were not significant, and the effect of fasting on the number of thrombosis factors was not significant either (non-fasting: -6.52%; fasting: -5.6%; P>0.05).

Discussion

The most important findings of the current research indicated that partaking in a soccer match while fasting is Similar to a non-fasting state. Interestingly, the number of the calories expended, the mean distance moved, maximum speed, and heart rate were nearly equal in both states. Fasting does not change the metabolic, coagulation, or fibrinolytic process, thereby leading to thrombosis in response to the elimination of a meal as long as there is proper and healthy nutrition throughout the year, and fasting is accompanied with exercise training. Furthermore, fasting modulates the inflammatory factors that influence thrombosis, and it is possible that the duration of fasting and the intensity and duration of exercise training affect these factors (10).

Ramadan fasting is a unique metabolic pattern in which an individual must avoid eating and drinking from dawn to dusk. It seems that changes in the number, type, and timing of the meals and reduced fat intake, adipose tissue loss, sleep patterns, and wakefulness during Ramadan have varied metabolic effects (10). Homocysteine is a common amino acid, which is produced during the metabolism of methionine (17). Elevated total homocysteine could increase the risk of neurodegenerative and cardiovascular diseases. Some of the main factors that affect homocysteine concentrations include age, gender, nutritional status, and physical activity. Our findings indicated that one soccer session match could increase homocysteine in the fasting and non-fasting states.

In line with our findings, the results obtained by Herrmann et al. (2003b), Konig et al. (2003), Real et al. (2005), Gelecek et al. (2007), Venta et al. (2009), Bizheh and Jaafari (2011), Deminice et al. (2011, 2013), and Iglesias-Gutierrez et al. (2012) demonstrated elevated total homocysteine after acute exercise, along with a continued increase reaching the maximum values within six hours post-exercise (18-26). During acute exercise, skeletal muscles increase protein and amino acid catabolism (27). This cortisol-dependent regulation results in the simultaneous uptake of amino acids into the liver to induce glucose synthesis (27, 28). Therefore, exercise increases plasma and muscle-free amino acids, which contribute to homocysteine formation from methionine (29). In addition, exercise while fasting leads to dehydration and haemoconcentration, which also increase homocysteine concentration. In this regard, Sánchez et al. (2019) reported that increased homocysteine following acute exercise was due to haemoconcentration, while rehydration during exercise prevents the increase in homocysteine concentrations (28).

Our findings demonstrated that the levels of plasminogen (insignificant decrease in the fasting state) and alpha 2-antiplasmin decreased following the soccer match. To date, no studies have examined plasminogen and alpha 2-antiplasmin levels during exercise training in the fasting state. Alpha 2-antiplasmin is the major plasma inhibitor of plasmin with a crucial function in diminishing plasmin activity and inhibiting fibrinolysis (30). The duration of fasting and exercise training are reported to alter its rate possibly due to the moderating effect of fasting on the immune system and the stimulation of the synthesis of this factor by the inflammatory cells in the liver, which modulate the production of alpha 2-antiplasmin. However, physical activity has been shown to be directly associated with TPA level of the tissue plasma activators. A study conducted by Ibrahim et al. (2011) showed that Ramadan fasting significantly reduced the inhibitory concentrations of type I plasminogen activator (PAI-1) (31). Based on these findings regarding simultaneous fasting with sports activity, it could be suggested that by increasing the level of TPA activator following exercise and reducing the inhibitory effect of PAI-1 on TPA due to fasting in response to plasminogen conversion, plasminogen conversion into plasmin is expected to increase, which ultimately reduces the plasminogen level.

Our findings indicated that one session of soccer match had no effect on the fibrinogen

concentration in the fasting and non-fasting states. Fibrinogen is a plasma glycoprotein synthesized by the liver and plays a key role in fibrin thrombus formation, while also increasing plasma viscosity (32). Research has yielded contradictory results regarding the acute effects of acute exercise on the levels of fibrinogen. For instance, Bizheh et al. (2011) observed that a single bout of circuit resistance exercise (intensity: 35% 1RM) caused no significant changes in the fibrinogen levels of sedentary middle-aged men (23). In another study, Mahmoodinezhad et al. (2016) reported the significant elevation of fibrinogen after an acute exhausting aerobic training session in female athletes (33). The findings of Kahraman et al. (2011) showed that an acute submaximal aerobic exercise caused no significant changes in the fibrinogen levels of sedentary young men (34). The differences in fibrinogen in response to acute exercise may be due to the differences in the training type, intensity, and duration (33). It is quite established that moderate exercise enhances blood fibrinolytic activity without the concomitant activation of blood coagulation mechanisms. In contrast, intense exercise induces the simultaneous activation of blood fibrinolysis and coagulation. Therefore, it seems that the intensity of the soccer match in the present study (not very intense) helped increase fibrinogen levels (23, 33, 34).

D-dimer is the end-product of the fibrinolysis pathway and the result of fibrin degradation. According to the literature, changes in the fibrinogen factor and d-dimer have an inverse correlation. In the activities that decrease the fibrinogen factor, the d-dimer factor will increase (35). Since the levels of fibrinogen (constituent of fibrin) did not change in the current research, D-dimer was not altered significantly.

One of the limitations of the present study was implementing a single soccer match without variations in terms of the mode of the exercise and physical fitness assessment. Therefore, further investigations are required to assess the long-term effects of fasting and the variations in the intensity of exercise training on physical fitness measurements, coagulation, and the fibrinolytic system.

Conclusion

According to the results, one session of a soccer match exerted similar effects on the blood coagulation factors in the fasting and non-fasting states. Therefore, proper nutrition and hydration strategies could be adopted to prevent the detrimental effects of fasting on thrombotic factors.

Acknowledgments

Hereby, we extend our gratitude to the participants for assisting us in this research project.

Conflicts of Interest

None declared.

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JOURNAL OF NUTRITION FASTING AND HEALTH

The Effect of Combined Exercise and Propolis Supplementation on Glycemic Index in Women with Type 2 Diabetes

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ARTICLEINFO

Article type:
Research Paper

Article History:
Received: 13 Mar 2022
Accepted: 03 Jul 2022
Published: 20 May 2022

Keywords:
Exercise
Propolis
Insulin resistance
Type 2 diabetes

ABSTRACT

Introduction: Diabetes is a metabolic disorder characterized by long-term hyperglycemia. However, nutrition and exercise can both help to lower blood sugarlevels. This study aimed to investigates the effect of eight weeks of combined exercise and propolis supplements on glycemic indicators in women with type 2 diabetes.

Method: This applied research was conducted on 60 women with type 2 diabetes, who were selected from Shiraz Medical Center and divided into four groups of 15, including 1) placebo, 2) exercise with placebo, 3) exercise with propolis, and 4) propolis. Groups 3 and 4 received propolis in the form of 500 mg capsules three times a day (morning, noon, and night) after each meal for eight weeks. Groups 2 and 3 also performed three sessions of combined training (resistance-aerobic) per week.

Combined training was resistance training with an intensity of 60-85% of a maximum repetition and aerobic training with an intensity of 50-70% of the maximum heart rate.

Results: Exercise, propolis consumption and exercise led to a significant reduction in fasting blood glucose, insulin, insulin resistance and glycosylated hemoglobin simultaneously with propolis consumption (P≤0.05). In addition, exercise with propolis consumption compared to exercise and propolis had a more significant effect on lowering fasting blood glucose, insulin, IR, and glycosylated hemoglobin (P≤0.05).

Conclusion(s): According to the results, exercise and propolis could positively affect the glycemic indicators of diabetic patients. Nevertheless, the combination of exercise and propolis had more favorable effects on improving glycemic indicators than each one alone in Non-insulin-dependent diabetes.

► Please cite this paper as:
Moayedi F, Taghian F, Jalali Dehkordi Kh, Hosseini SA. The Effect of Combined Exercise and Propolis Supplementation on Glycemic Index in Women with Type 2 Diabetes. J Nutr Fast Health. 2022; 10(2): 150-157. DOI: 10.22038/JNFH.2022.64400.1386.

Introduction

Diabetes Mellitus Type 2 (DMT2) is the most common type of chronic diabetes, which is characterized by elevated plasma glucose levels due to insulin resistance (IR) and impairment of insulin secretion or insulin receptors (1). As a result of this factor, glucose is excreted from the blood in the kidneys and then excreted in the urine. According to studies conducted from 2005 to 2011, 6.8% of the world's population without diabetes were diagnosed with diabetes. Iranian studies indicate that 7.7% of the population aged 25 to 64, or two million people have diabetes and 16.8% have impaired glucose tolerance (2). This disease is the most common cause of kidney problems, amputation without trauma and blindness (3). Chronic hyperglycemia is a symptom of diabetes, as is difficulty metabolizing carbohydrates, fats, and proteins. This disease resulted from the interaction between IR and decreased pancreatic beta-cell function caused by poor motility and stress. Diabetes is a chronic endocrine disorder with long-term hyperglycemia facing by a lack of permanent or low secretion of insulin or IR (4). Based on research conducted by the International Illegal Federation, 382 million children and adults worldwide were affected by the disease in 2003, and projections show that number will surpass 592 million by 2025 (5). Studies have shown that nutrition essential in improving and controlling diabetes.

Recently, honey and other substances associated with bees, such as propolis, have gained more attention. Pollen, enzymes, pollen, and wax are combined with propolis, a resin-like substance

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collected from buds of poplar and cone trees. Propolis is also combined with bee enzymes, pollen, and wax. "Propolis" refers to a substance used in hives where pro means defense and polis refers to a city, which is this hive here, and its general meaning is defense of the hive. Propolis is used by bees to soften and seal their hives' interior walls and cavities (6). Propolis is rarely available in pure form, and the primary active materials in propolis are flavonoids such as chrysinas potent antioxidants that bees take from plant and flower parts. In recent decades, propolis has been the subject of extensive research around the world, in which its chemical composition and biological properties have been extensively studied.

There has been some recent research into the mechanisms and functions of propolis, and its therapeutic applications have been reviewed to illustrate its importance, but limited evidence suggests it could be useful for diabetics (7). On the other hand, studies have shown that exercise helps control blood sugar (BS) in DMT2 patients (8). In addition, physical activity can increase the response of skeletal muscles to plasma insulin levels and insulin signaling and metabolism. Therefore, physical activity increases glucose transporter expression and glycogen synthesis function (9). Regular exercise improves glucose metabolism by increasing insulin. The stimulation of insulin by muscle tissue causes more than 75% of glucose to be absorbed by the body due to long-term exercise increasing glucose transporters to muscle cells, and insulin receptor bases, which prevents obesity and complications of DMT2 in winter (10). Exercise can reduce BS and glycosylated hemoglobin (HbA1C) in patients with type 2 diabetes, as well as prevent long-term complications (11). Patients with DMT2 may benefit from endurance and resistance exercise, including blood glucose control study aimed to investigate a course of combined exercise and propolis supplementation on glycemic indicators in women with DMT2 considering the therapeutic role of sports activities and consumption of propolis, as well as the small amount of research in this field.

As shown in the above studies, there is insufficient research concerned with the simultaneous effects of aerobic and resistance training with propolis. A Synergistic placement of exercises and propolis was conducted to examine the effects of eight weeks of combined exercise with and without propolis on glycemic index and insulin resistancein women with DMT2.

Materials and Methods

This appliedstudy was conducted on 60 women with DMT2, who referred to Shiraz Medical Center and selected as a statistical sample. The inclusion criteria included being between 40 and 60 years old, lack of having a history of cardiovascular disease, having a history of diabetes longer than six months, smoking, no supplements, just food. The exclusion criteria included having regular exercise in the last six months and missing more than three sessions of exercise. Upon completion of the informed consent process, subjects were randomly divided into four groups: a placebo, exercise with placebo, exercise with propolis supplement. The present study was registered in the Ethics Committee of the Islamic Azad University of Khorasgan, Iran (with the code IR.IAU.KHUISF.REC.1400.265) and the Iranian clinical trial database (with the number IRCT20211229053561N1). Supplemental propolis capsules of 500 mg were given to subjects three times a day after each meal for eight weeks (12). Exercise groups performed three sessions per week of selected exercise. The supplement and placebo groups did not participate in any sports activities during the study period. In this research, two different types of training programs were used: an aerobic training program and a resistance training program for eight weeks (24 sessions). The aerobic exercise program includes: pedaling using a stationary bike and each session 35-50 minutes with an intensity of 50-70 % was the maximum heart rate. The training program consisted of three warm-up sections, including static and dynamic stretching exercises (5 minutes), cycling on a stationary bike (25-40 minutes), and cooling down (5 minutes), including stretching exercises. The training program in the first and second week was It was performed for 35 minutes with an intensity of 50-60% of the reserve heart rate and in the seventh and eighth week for 50 minutes with an intensity of 60-70% of the reserve heart rate. The maximum heart rate was calculated with the formula age-220 to determine the training intensity (13). The exercise program was performed in the first and second week for 35

minutes with an intensity of 50-60% of the reserve heart rate and in the seventh and eighth week for 50 minutes with an intensity of 60-70% of the reserve heart rate. The training intensity was controlled through a Polar watch heart rate monitor during the training. The resistance training program was designed similar to the study of de Valens et al. (2017). The resistance training program included upper body and lower body exercises, in a progressive manner, using 6 exercise machines, and the duration of each session was about 40-50 minutes. Warming up and cooling down at the beginning and end of each exercise were done for 5 minutes by stretching movements. To perform upper body exercises in each session, special devices for chest vertical press, back arm extension, and open front bending were used, and for lower body exercises, leg bending and thigh bending and extension devices were used. The training intensity was increased every two weeks, so that the first and second week, 60%, the third and fourth week, 70%, the fifth and sixth week, 80%, and the seventh and eighth week, 85% was a maximum repetition. Subjects were encouraged to complete 8-12 repetitions until they were able to complete the repetitions. 30 seconds rest between each set and 1 minute between devices. The range of load and repetitions was in accordance with the hypertrophy exercise set by the American College of Sports Medicine regarding resistance exercise for health in the adult population (14). A sample of 5 cc of blood was taken from subjects' arms before the pre-test and 48 hours after the last training session. After separating the serum at a temperature of two to eight, the blood was centrifuged (Behdad brand made in Iran) for ten minutes and transferred to the laboratory to measure sugar indicators. Serum HbA1C level was measured by ELISA method to measure fever and fasting blood glucose (FBG) using the Iranian Pars kit. Plasma insulin level was measured using the Diaplus kit by ELISA method made in America. Fasting insulin × (mg/dL) FBG ÷22/5 was used to calculate the IR of the formula HOMA IR= (MU/ml, (15). The Kolmogorov-Smirnov test was used to determine the normality of the data distribution, and a one-way analysis of variance was used to analyze the data using Tukey's post hoc test (P≤0.05).

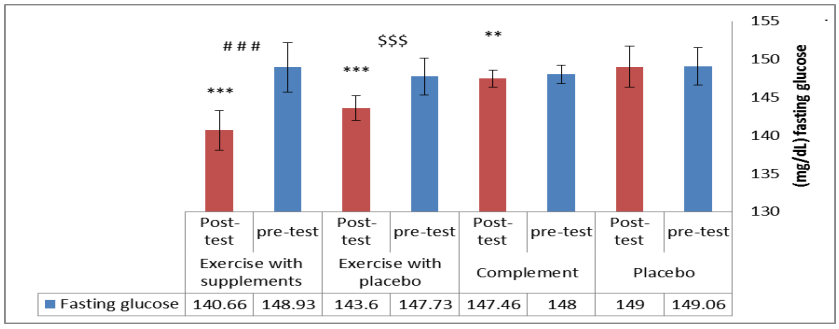
Table 1. Demographic characteristics of the subjects in the four groups of research (mean ± standard deviation)

Practice with Supplement	Placebo with Exercise	Complement	Placebo	Measurement Time	Subject Specifications
54.07±2.86	51.67±4.67	52.53±5.90	53.67±5.01	pre-test	Age (years)
164.20±2.56	164.93±1.83	164.66±2.76	165.40±1.91	pre-test	Height (cm)
75.53±2.56	76.11±2.92	75.80±2.42	74.87±1.80	pre-test	Weight (kg)
72.67±2.64	74.33±2.84	75.40±2.53	74.80±1.74	Post-test	
28.04±1.56	27.94±1.21	27.99±1.63	27.37±0.76	pre-test	Body mass index
26.98±1.62	27.33±1.20	27.84±1.66	27.34±0.72	Post-test	(kg / m2)

Results

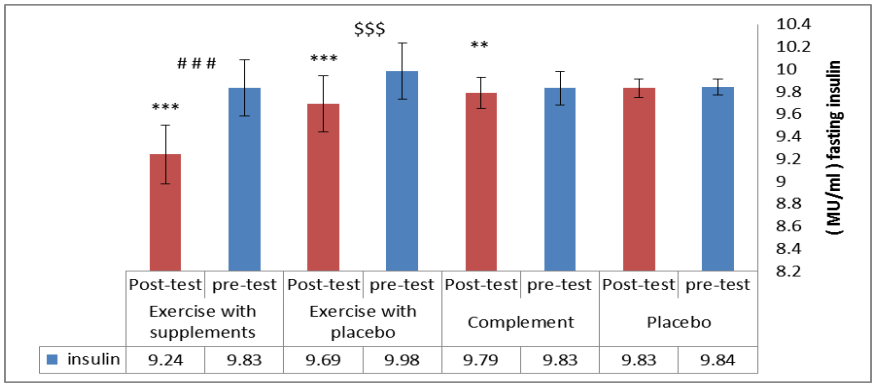
Table 1 shows the demographic characteristics of the subjects the levels of FBG, insulin, IR, and Hb A1C for the four research groups are shown in Figures 1 to 4, respectively. There was IR (P=0.001 and F=80.02) and Hb A1C (P= 0.001 and F=41.90) among the research groups according to the analysis of one-way variance (SiD) in changes in FBG (P=0.001 and F=72.08), insulin (P=0.001 and F=36.44)Tukey's post-hoc test showed that FBG levels in the exercise group with propolis were significantly lower than the exercise with placebo, propolis, and placebo groups (P=0.001 The exercise group with placebo was significantly lower than in propolis and placebo groups (P=0.001). However, there was no SiD in FBG changes between propolis and placebo groups (P= 0.83) (Figure 1) as well as insulin levels (Figure 2). IR (Figure 3) and Hb A1C (Figure 4) in the exercise group with propolis were significantly lower than in the exercise with placebo, propolis, and placebo groups (P= 0.001). In addition, the exercise in the placebo group was significantly lower than in the placebo and propolis groups (P=0.001). The results of the paired-sample t-test showed that the FBG, insulin, IR, and Hb A1C in the post-test were significantly reduced compared to the pre-test (P=0.001) in training with placebo and propolis groups FBG (P=0.001), insulin (P=0.001), IR (P=0.001), and Hb A1C (P=0.001) in the post-test were significantly reduced compared to the pre-test in the exercise with the placebo group. In the propolis group, the FBG (P=0.006), insulin (P=0.003), and IR (P=0.001) reduced compared to the pre-test in the post-test significantly.

However, no significant difference in Hb A1C level (P=0.21) was observed in the post-test compared to the pre-test. Furthermore, in the placebo group FBG (P=0.33), Hb A1C (P=0.58), IR (P=0.17), and insulin (P=0.23) did not change in the post-test compared to the pre-test.



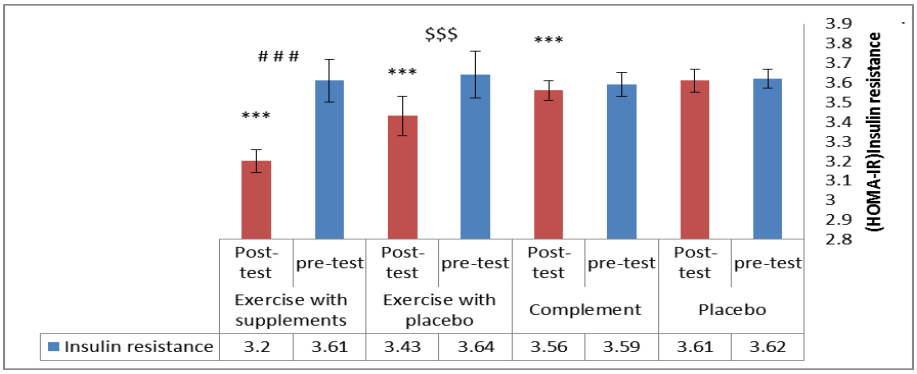
*** P = 0.001 Significant decrease compared to pre-test
** P = 0.006 Significant decrease compared to pre-test
P=0/001 Significant decrease compared to exercise groups with placebo and propolis and placebo
$$$ P= 0/001 Significant decrease compared to placebo and propolis groups

Figure 1. FBG levels in pre-test and post-test in four research groups



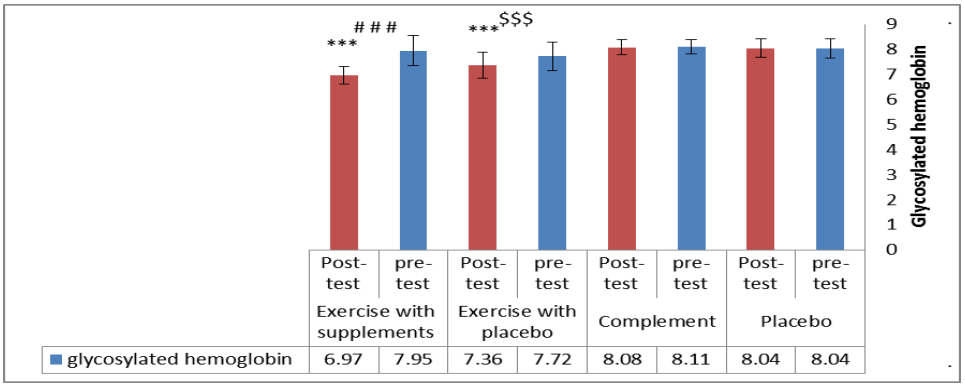
*** P = 0.001 and ** P = 0.003 Significant decrease compared to pre-test
P=0.001 Significant decrease compared to exercise groups with placebo, propolis and placebo
$$$ P= 0.001 Significant decrease compared to placebo and propolis groups

Figure 2. Insulin levels in pre-test and post-test in four research groups



*** P = 0.001 Significant decrease compared to pre-test
P=0.001 Significant decrease compared to exercise groups with placebo, propolis and placebo
$$$ P=0.001 Significant decrease compared to placebo and propolis groups

Figure 3. IR levels in pre-test and post-test in four research groups



*** P = 0.001 Significant decrease compared to pre-test
P= 0.001 Significant decrease compared to exercise groups with placebo, propolis and placebo
$$$ P= 0.001 Significant decrease compared to placebo and propolis groups

Figure 4. Hb A1C levels in pre-test and post-test in four research groups

Discussion

The results had a SiD on the consumption of propolis supplements for eight weeks to reduce FBG, insulin, IR, and Hb A1C levels in diabetic women. Clinical studies have reported the effects of propolis hypoglycemia Consistent with the results of the present study, Samadi et al. (2018) reported that 12 weeks of using 900 mg of propolis supplement improved FBG in diabetic patients (16). BS levels are regulated with supplementation by network pharmacology and Zardine bio antioxidants, which suppress free radical production (17). Bioflavonoids are highly reactive, and their interactions with free radicals trap them, whereas hydroxyl bioflavonoids are oxidized by radicals and then turned into low-reactive and more stable radicals (18). BS can be controlled by peripheral tissues that stimulate glucose uptake by using propolis, and preventing them from entering the bloodstream and reducing glucose uptake into the gut (19). In addition, propolis treatment can control BS by stimulating glucose uptake by peripheral tissues, deterring its release into the bloodstream, or decreasing intestinal glucose (20). According to studies, propolis reduced plasma insulin with its antioxidant properties, and IR. Ropolis in mice also reduced plasma insulin and IR in Hong et al. (2015). This result was also shown in Kitamura's research. The intervention also improved insulin function in the Zamami (2014). Experimental and clinical research may be affected by propolis supplementation, extracts and phenolic compounds, such as phenyl ester of caffeic acid in propolis.This research reduces IR, combats oxidative stress, creates inflammatory factors, decreases the level of adiponectin and transfer of glucose to the tissues, digestive enzymes of carbohydrates, especially alpha-amylase and alpha-glycosidase (23). The authors of Elisa et al. (2015) significantly reduced TNF* levels, followed by a significant reduction in IR and a decrease in FBG.Studies have shown thatpropolis enhances glucose transport via GLUT4 by reducing IR (24). Enhancing propolisleads to alpha-amylase, which can delay the hydrolysis of polysaccharides and reduce glucose uptake (25). According to a study by Zhou et al. (2016), reports of propolis use reduced Hb A1C (26). In this study, the glossy hemoglobin group in propolis-treated control mice was reduced by 8.4% compared to the control. In addition, Cain Clark et al. (2018) examined the use of propolis on the glycemic index in patients and stated that propolis improved FBG and Hb A1C levels, but no changes were observed in insulin levels and IR (27). Afsharpour et al. (2020) found that three servings of 500 mg of propolis per day reduced FBG, plasma insulin, Hb A1C and IR in patients at eight weeks (28). Despite similar studies, some studies do not show positive results. For example, 230 mg propolis supplement for 60 days did not affect FBG or acidic antioxidant status, which only prevented the increase of blood uric acid and reduced glomerular filtration (29). Moreover, Samadi et al (2017) observed no SiD in serum insulin level and IR between propolis and placebo after 12 weeks. Elisa et al.

(2015) reduced TNF* levels significantly, followed by significant declines in IR and FBG levels. (16). Different doses of propolis may explain the differences studies. Hyperglycemia, which causes oxidative stress in diabetic patients, is a major cause of the imbalance between antioxidants and oxidative agents (30). Reactive oxygen species (ROS) are produced as a result of increased glucose oxidation ROS are produced as a result of increased glucose oxidation Oxidative stress and IR are worsened by increased ROS, which causes lipid oxidation, particularly in the cell membrane (31). Moreover, the research findings showed that eight weeks of combined exercise significantly reduced FBG, insulin, IR, and Hb A1C in women with DMT2. Regarding the effect of combined exercise on the level of glycemic index and IR, the results of the study were consistent with the results of the study of Enteshary et al. (32) and Esmaili et al.(33), and Mirzandeh et al. (34) showing a significant decrease in IR index after combined training reported with DMT2The use of combined exercise in different order did not cause significant changes in Hb A1C, IR and functional factors. Different research results can probably be attributed to differences in training intensity and duration as well as differences in age and gender of the research samples. As a basis for explaining the mechanisms involved, chronic hyperglycemia may impair beta-cell function and worsen IR under diabetic conditions. Physical activity without insulin and two to three hours after eating with insulin cause muscles to consume large amounts of glucose. The repeated contractions of muscles during exercise have an insulin-like effect, releasing large amounts of glucose into the cells to expend energy. These frequent contractions enhance the number of insulin-dependent glucose transportersin the long run and enhance the membrane's permeability to glucose. Furthermore, muscle fibers have a low glycogen concentration for a long period, and muscle cells rebuild their glycogen reserves after exercise, and the blood glucose concentration decreases for several hours. Both aerobic and resistance training enhance the frequency of GLUT-4 and glucose uptake even in DMT2 (35). Aerobic and resistance training is the most effective type of exercise in controlling glucose and insulin activity. The combined exercise was proposed as the most effective for regulating blood glucose and plasma insulin activity (36). Exercise increases plasma insulin sensitivity by increasing mRNA and glucose transporter proteins, reducing release, and enhancing the clearance of acids. Free fat, insulin receptor signaling, glycogen synthesis, hexokinase, and glucose release from the blood to the muscle due to increased capillaries, as well as the uptake of glucose change the composition of the muscle (37).

The results showed that simultaneous use of propolis supplement and combined exercise reduced glycemic index and IR in patients with DMT2 compared to exercise and consumption of propolis alone. Therefore, the simultaneous interaction of this supplement and exercise is more effective in reducing IR, and combination exercise with propolis has interactive effects in improving glycemic index in DMT2 patients.

Conclusion

According to the results, exercise and propolis alone could affect IR and **glycemic indicators** in diabetic patients due to the results of this study However, combined exercise (aerobic-resistance) with propolis supplement ratioalone had more favorable effects on improving the glycemic index of DMT2 patients. However, more studies are needed to examine the mechanisms affecting them in more depth.

Acknowledgments

Hereby, the author would like to express their appreciation to all the volunteers participating in the present study are appreciated and thanked. Researchers covered all costs of the present study.

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