Effects of bread fortification with pomegranate peel powder on inflammation biomarkers, oxidative stress, and mood status in patients with type 2 diabetes: A randomized controlled trial

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Abstract

Background and Aims: To assess the effects of bread fortified with pomegranate peel powder (PPP) on inflammation, oxidative stress, and mood indices in patients with type 2 diabetes mellitus (T2DM).

Methods and Results: In total, 90 T2DM patients were randomized to receive either bread fortified with 3.5% pomegranate peel powder (PPP) (n=45) or PPP-free bread during 12 weeks. Dietary intake throughout the trial was assessed using food records. Laboratory parameters including total antioxidant capacity (TAC), malondialdehyde (MDA), hs-CRP-reactive-protein (hs-CRP), and psychological disorders including depression, anxiety, and stress were assessed at the beginning and end of the study. Overall, 77 diabetic patients completed the trial (39 in the PPP group and 38 in the control group) and were included in the analysis. Based on the compliance assessment, adherence to the interventions was high in the trial. PPP bread intake led to a significant reduction in hs-CRP levels (change: -0.56 ± 1.29, P=0.01) and depression score (-1.33 ± 3.66, P=0.04)), in the intervention group. Other variables including MDA, TAC, anxiety, and stress, showed no significant changes for PPP-fortified bread.

Conclusion: Our findings showed that while intake of PPP-fortified bread for 12 weeks had no significant effect on oxidative stress, hs-CRP, and mental health in T2DM patients, there might be some positive outcomes regarding inflammatory and mood states.

Trial registration Iranian Registry of Clinical Trials: Trial was registered in the Iranian Registry of Clinical Trials (available at: www.irct.ir, with ID: IRCT20191209045672N1) on 21/09/2020.

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) is increasing at an alarming rate (Khan et al., 2020). It has been shown that T2DM is prevalent among 9.3% of adults(Saeedi et al., 2019). In Iran, it is estimated that 9.2 million are affected(Saeedi et al., 2019). Patients with T2DM usually experience several complications such as foot ulcers, dental disease, and reduced resistance to infections that negatively affect patients' quality of life(Deshpande et al., 2008). Also, these patients are at higher risk of several chronic diseases including cardiovascular (CVD), nephropathy, retinopathy, and liver diseases(Martín-Timón et al., 2014). In addition, T2DM imposes a high economic burden on the healthcare system (Javanbakht et al., 2011). Therefore, finding an appropriate strategy for the management of this disease is urgently required.

Previous studies proposed several pharmacological and non-pharmacological interventions for the management of T2DM (Leite et al., 2020). Due to several complications of pharmacological methods, non-pharmacological therapies such as dietary interventions, supplementation with some nutrients, and herbal medicine have received great attention (Evans & Bahng). Recently, it has been shown that intervention with polyphenol-rich supplements or food has a beneficial effect on the clinical outcomes of diabetic patients (e.g., modulating oxidative stress and anti-inflammatory responses) (Raimundo et al., 2020). Among polyphenol-rich foods, high attention has been paid to pomegranate and its peel. In a

clinical trial, intervention with pomegranate peel extract could significantly reduce hs-CRP in diabetic patients (Haghighian et al., 2016).

Despite the presented evidence, it is not clear whether adding pomegranate peel to foods can affect the outcomes of diabetic patients or not. Bread is a good candidate for fortification because it is the main part of individuals around the world (Nikooyeh et al., 2016) as well as, the Iranian diet in which more than 60% of energy is from carbohydrates, particularly from different types of bread (Heidari et al., 2019). It should be noted that most types of bread in Iranian food culture are prepared with refined grains. Therefore, changing the composition of bread may affect health outcomes, particularly for diabetic patients. Given the current evidence of the possible beneficial effect of pomegranate peel on the outcomes of diabetic patients, we decided to fortify bread with this functional food. Therefore, the present clinical trial was aimed to assess the effects of bread enriched with pomegranate peel powder (PPP) on hs-CRP, oxidative stress, and mental health in patients with T2DM.

Materials And Methods

Study population

This investigation was a randomized, blinded, and parallel clinical trial. Details on participants, study design, and data collection were published previously(Zare et al., 2023). Participants were selected among the diabetic patients referred to the outpatient clinic of the Endocrine and Metabolism Research Center (Isfahan University Medical Sciences, Isfahan, Iran) and health centers of Isfahan City from May 2021 to January 2023. Considering the type 1 error of 5%, type 2 error of 20%, study power of 80%, and hs-CRP as a key variable, the required sample size was calculated using the relevant formula. Accordingly, we required 45 patients with T2DM in each intervention group. The study inclusion criteria were: 1) patients who were diagnosed with T2DM within the last 5 years, 2) patients with an age range of 40-60 years, 3) those who had HbA1c levels between 5.7% and 8.0% over the past 3 months, 4) patients who had a body mass index (BMI) $\leq$ 30 kg/m2. Patients with chronic kidney or liver diseases (cirrhosis and hepatitis), patients who were pregnant or lactating, those taking hormone replacement therapy (insulin or others), and those who adhered to a special diet were excluded. Also, patients who received antioxidant supplements during the last 3 months were excluded. During the trial, patients who changed the dosages and types of medications, those who were unwilling to continue the trial, and patients who reported complications due to possible side effects were excluded as well. This study was conducted according to the Helsinki Declaration and written informed consent was obtained from all participants. The protocol of the present trial was approved by the Ethical Committee at the Isfahan University of Medical Sciences (approval no. IR. MUI.RESEARCH.REC.1399.087) and registered at www.IRCT.ir (ID: IRCT20191209045672N1). Furthermore, our study was reported based on the Consolidated Standards of Reporting Trials (CONSORT) statement guidelines (Bennett, 2005).

Study design

We initially assessed and invited 520 diabetic patients who met the inclusion criteria, however, only 112 patients accepted to participate in the study. In the run-in period, we excluded 22 patients because they showed their unwillingness to participate in the trial. Distance area from their homes, the COVID-19 strike, and lack of blood sugar control were the main reasons for their exclusion. Finally, the remaining 90 patients were randomly allocated into two equal groups using the random assignment method. Computer-generated random numbers were used to implement the random allocation sequence. The patients were allocated to either PPP-enriched bread or PPP-free bread groups. Both types of bread were identical in terms of shape, size, and packaging. All the breads had the same appearance, so it was impossible to distinguish one type from another. During the run-in period, all patients received a control bread. During the run-in period, two sessions were held for all patients. In each session, adherence to bread intake and possible complications related to bread were assessed to select patients with high compliance. After the run-in period, all patients (n=90) were followed for 12 weeks. The investigator and patients were in touch through text messaging and phone calls during the trial. Also, they were asked not to change their usual dietary intake and physical activity throughout the study. At baseline and end of the trial, a fasting blood sample and data on mental health were collected from each participant. In the current study, we did not measure any indicator to determine an individual's compliance. However, by assessing the results obtained from the dietary records, we could determine the adherence to the intervention. Also, compliance with intake of the bread was monitored through routine visits and phone interviews once a week.

Physical activity was assessed using two 3-day physical activity records at baseline and end of the trial. In addition to physical activity, usual dietary intakes were assessed using two 3-day food records pre- and post-intervention period. A trained nutritionist described how to fill out the food and physical activity records. The records were checked by a nutritionist. After collecting the dietary records, the serving sizes were converted to grams per day. Then, the average intakes of energy and nutrients for each 3-day record were calculated using a customized version of Nutritionist IV software (First Data Bank; Hearst Corp, San Bruno, CA, USA).

Interventions

Pomegranate peel powder:

Pomegranate fruits were purchased from the Fars province, Iran. The pomegranate peel was separated, and then, dried and ground in powder. The PPP were stored at − 20 °C until the preparation of the bread. For the preparation of PPP-rich bread, 3.5 grams of PPP per 100 grams was added to wheat flour. This dosage was safe and was selected based on previous studies (Sayed-Ahmed, 2014). Other constituents that were used for bread preparation included sourdough, rye flour, salt, sugar, yeast, and water. The only difference between pomegranate and control bread was PPP. Both breads were produced by Simin Bread Company, Iran.

MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) test

MTT colorimetric measure of pure PPP against fibroblast L929 cells was conducted according to the Badano JA method (Badano et al., 2019). Briefly, 100 µL of cell suspension in RPMI-1640 medium (1 × 104 cells/well) was seeded into each well in 96-well microplates and then incubated for 24 h, (37 ◦C and, 5 % CO_2 air humidified). After that, the old medium was deleted and 100 µL of the different concentrations of PPP were added to each well, and incubated for another 24, 48, and 72 h in similar conditions. Consequently, 10 µL of MTT solution (5 mg/mL PBS) was added to each well of the plates in the dark and incubated at 37 ◦C for 2–3 h. Then, to dissolve the formed formazan crystals, the old media containing MTT was removed, and DMSO (100 µL) was gently added to each well. The optical density of each well was measured by an ELISA plate reader (Startfix-2100, Awareness, and USA) at 570 nm. DMSO 1 % and doxorubicin were used as negative and positive controls, respectively. The concentrations that inhibit half of the cell population (IC50) were obtained by modeling the percentage of cytotoxicity versus the concentration of PPP. The assay was done in triplicate and results were reported as mean ± standard deviation (SD). The cell viability (%) was determined according to eq (2).

$$\%\text{Cell viability} = \frac{\text{Absorbance of treated cells} - \text{background absorbance(b)}}{\text{Absorbance of untreated cells(c)} - \text{background absorbance(b)}} \times 100 \qquad (2)$$

Where b = blank, and c = control.

Assessment of variables

Data on age, gender, education, medication, supplement use, and history of diseases were collected using an interview-based questionnaire. Anthropometric measurements including weight and height were measured using standard techniques. All measurements were done by M Z in the morning while the participants wearing underwear. The height was measured to the nearest 0.1 cm without footwear using a stadiometer (Seca, Hamburg, Germany). Weight was measured to the nearest 0.1 kg with light clothing using a Seca Beam Balance (Seca). BMI was calculated using the following formula: weight (kg)/height (m) ^{2} (kg/m^2). Data on outcome variables including laboratory parameters and psychological factors were collected at baseline and after 3 months post-intervention.

Laboratory measurements

Ten mL blood samples were taken after 12 hours of overnight fasting at the beginning and end of the intervention period. Then, were separated serums and stored at − 80 ◦C until further analysis. HbA1c, total antioxidant capacity (TAC), malondialdehyde (MDA), and hs-CRP were measured as primary outcomes. Latex Immunoturbidimetric assay was used to measure hs-CRP (aptec, Belgium). The Cupric Reducing Antioxidant Capacity method was used to measure the serum TAC using a standard kit (Kiazist Life Sciences, Iran). Assessment of MDA was based on thiobarbituric acid reactive substance (TBARS) measured by standard kit (Kiazist Life Sciences, Iran). HbA1c levels were measured by BT1500 device by turbidimetric immunoassay. All kits had an intra-assay CV□10% and inter-assay CV □ of 8%.

Mental health

Depression, anxiety, and stress scale-21(DASS-21) were employed to measure the mood status at baseline and 3 months after the intervention (Henry & Crawford, 2005). DASS-21 is a self-report questionnaire with three subscales that consists of seven questions related to each subscale. Mood status is evaluated as the endpoint. Each question is on a four-point (0-3) Likert scale to identify the severity of depression, anxiety, and stress. Samani et al. reported that the scale had an appropriate validity and reliability among Iranian people (Sahebi et al., 2005).

Statistical analysis

Statistical analyses were conducted based on the per-protocol approach. The per-protocol analysis included data from those individuals who completed the intervention. The normality of continuous variables was assessed by skewness and Kolmogorov–Smirnov tests. All outcome variables in the current study were normally distributed. To assess the differences between the intervention and control groups in terms of continuous variables, an independent samples t-test was used. Chi-square test was used to assess the distribution of categorical variables among the 2 groups. To do a within-subject comparison in each group, we used a paired sample t-test. Also, changes in outcome variables were compared between the two groups using the independent samples t-test. To assess the effects of PPP-rich bread on inflammation and mental health, we used analysis of covariance (ANCOVA and MANCOVA) considering age, gender and weight as covariates. A P-value less than 0.05 was considered significant. Statistical analysis was conducted using SPSS version 23 (SPSS Inc., Chicago, IL, USA).

Results

Cytotoxicity and IC50

The results of the cytotoxicity of PPP against the fibroblast L929 cell line are presented in Fig 1-3. As can be seen in Fig.1 by increasing the concentration of PPP from 125 to 1900, the cytotoxicity against the fibroblast cells increased (Fig.1). There was not a significant difference between the IC50 of PPP after 24 h and 48h and this confirmed that the toxicity of PPP was not time dependent until 48 h while after 72 h the cytotoxicity increased and there was a significantly different ($P<0.05$) between the cytotoxicity after 24h and after 72h that can be attributed to the release of antioxidant compounds from PPP to the medium (Fig. 2). Moreover, the cell viability for different concentrations was decreased by increasing the concentration from 125 to 1900 (µg/mL) (Fig.3).

Baseline characteristics and dietary intake

Of the 90 T2DM patients (35 men and 55 women) at baseline, seven in the control group and six in the intervention group were excluded due to side effects related to the intervention (n=4), very low compliance (n=4), covid-19 infection (n=2), and unwillingness to continue the study (n=1), and having a severe disease or surgery (n=2). At last, 77 subjects (39 in the intervention and 38 in the control group) completed the intervention period. The flow diagram of this study is shown in **Fig. 4**.

The baseline characteristics of T2DM patients in the PPP-rich bread and control groups are displayed in **Table 1**. We found no significant differences between the two groups in terms of demographic variables, past medical history, drug and supplement use, and anthropometric measures. Also, there were no significant differences regarding physical activity between the groups throughout the trial. The results of 3-day food records showed no significant changes between the two groups in terms of energy, macronutrient, and micronutrient intakes (**Table 2**).

The effects of PPP-rich bread on serum concentrations of hs-CRP, TAC, MDA, and mental health are presented in **Table 3**. We found a significant reducing effect of PPP-rich bread on hs-CRP levels (2.75 ± 1.56 mg/dL at baseline vs. 2.19 ± 2.03 mg/dL at the end of the trial, P<0.001) and depression scores (6.66 ± 4.60 at baseline vs. 5.33 ± 4.36 at the end of trial, P: 0.04), however, this reduction was not significant between the two groups. For others including TAC, MDA, stress, and anxiety, no significant effect was seen either in the within- or between the group comparison. When we controlled for age, gender and weight in the ANCOVA and MANCOVA test, no changes were observed in the findings.

Side effects

During the study, the only reported adverse event was gastrointestinal complications including constipation and stomach discomfort (3 patients in the intervention group and 1 patient in the control group).

Discussion

In the current study, we found that PPP-rich bread had no significant effect on hs-CRP, oxidative stress, and mental health compared with the control bread. To the best of our knowledge, this is the first study that assessed the effects of PPP-rich bread on the clinical outcomes of T2DM patients.

Diabetes is one of the major challenging issues of public health (Amiri, 2016). Inflammatory biomarkers are found higher in diabetic patients compared with non-diabetic ones. Moreover, as Inflammatory biomarkers are connected with endothelial dysfunction, they can increase the risk of cardiovascular diseases (CVDs) among diabetic individuals (Garcia et al., 2010). In addition to CVDs, patients with T2DM have an increased risk for psychological symptoms such as depression, anxiety, and stress, which may exert additional negative effects on their quality of life (Eren et al., 2008). Pharmacological treatments along with lifestyle modifications (diet and exercise) are suggested to meet glycemic targets in these patients. However, it is not clear that adding PPP in the formulation of the main food items such as bread induces the health properties of PPP.

Our study revealed that bread fortified with PPP for 12 weeks ameliorated the hs-CRP marker within the group in T2DM patients. However, compared to the control bread, its effect was not significant. Previous studies examined the effects of PPP supplements or extracts on inflammation and no study was available on PPP-fortified foods. In a clinical trial, Haghighian et al. showed that PPP extract for 8 weeks and Grabež M et al. study for 6 weeks significantly decreased the levels of inflammatory biomarkers in

obese women with dyslipidemia, and in patients with type 2 diabetes mellitus, respectively (Grabež et al., 2022; Haghighian et al., 2016). Moreover, a significant decrease in hs-CRP concentrations (32%) was observed after 12 weeks of administration of 250 mL/day pomegranate juice in diabetic patients (Sohrab et al., 2014). There are mechanisms indicating the beneficial effect of PPP on inflammation. PPP contains punicalagin and ellagic acid that have a reducing effect on pro-inflammatory cytokines (Abbaspoor et al., 2020). This effect is medicated by the inhibitory effect of punicalagin and ellagic acid on the MAPKs (Mitogen-activated protein kinases) pathway involved in inflammatory conditions. Moreover, PPP is a rich source of polyphenols that induce anti-inflammatory effects through inhibition of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and attenuation of oxidative stress (Vitale et al., 2017).

In this study, we also did not find any significant changes in serum MDA and TAC levels. In contrast, Bagheri et al. showed that 200 mg/kg consumption of pomegranate peel extract in rats decreased MDA compared with a control group (Bagheri et al., 2023). Jafari et al. showed that daily consumption of peel pomegranate extract (450 mg/d) for 8 weeks, ameliorated MDA levels in hemodialysis patients (Jafari et al., 2020). The beneficial effect of 1 g pomegranate peel hydro-alcoholic extract on MDA levels in Osteoarthritis patients was also reported in another clinical trial (Mahdavi & Javadivala, 2021). In terms of TAC, we found one study investigating the effect of pomegranate peel. Haghighian et al. Showed that PPP extracts for 8 weeks increased TAC levels. In the current study, although PPP-fortified bread had a higher amount of polyphenols compared with the control bread, it did not have any effects on MDA and TAC levels. Further studies are needed to illustrate the facts in this regard.

The lack of significant effect of PPP-fortified bread on hs-CRP and oxidative stress might be explained by the interaction of available nutrients in bread with polyphenols of PPP. Fiber and other nutrients in the bread might decrease the absorption of polyphenols. Also, Iranians usually consume bread with other foods such as cheese, legumes, nuts, dairy products, and other grains. These foods contain high amounts of fiber, vitamins, and minerals. Some of them may interact with the effects of bread polyphenols as well as their absorption. It should be kept in mind that PPP-fortified bread could decrease hs-CRP by 0.56 mg/dL. Although this reduction was not significant between the groups, this was of clinical importance. Therefore, the effect of long-term or usual intake of PPP-fortified bread on inflammatory biomarkers and oxidative stress is not clear. Further studies are needed to examine this effect.

The results of our study showed a reducing trend in depression scores in both groups; however, this reduction was not significant in the between-group comparison. The reduction in both groups other than extra-attention paid, might be due to the effect of whole-grain bread used for the fortification. Previous studies have shown a significant inverse association between whole grains and depression among Iranian adults(Sadeghi et al., 2020). Therefore, it seems that PPP-fortified bread has no significant effect on psychological status. In contrast with our findings, Barghchi et al. showed that PPP supplement along with a weight loss diet for 8 weeks decreased depression and stress scores compared with weight loss

alone (Barghchi et al., 2023). Therefore, the administration of PPP supplements might be more effective than PPP fortification regarding its effects on mental health.

To the best of our knowledge, this investigation is the first randomized clinical trial to assess the effects of bread fortified with PPP on the clinical outcomes of patients with T2DM. Moreover, the present trial was performed in a homogenous population through strict inclusion–exclusion criteria. Furthermore, dietary intake and physical activity were monitored throughout the study. We also recruited both genders, hence, the findings could apply to both. Some limitations in our study should be considered when interpreting our findings. Because of funding limitations, we couldn't use the ELISA kit for oxidative stress measurement. We could not measure specific biomarkers for compliance assessment; however, adherence to the intervention was assessed using dietary records. In the current study, only patients with type 2 diabetes were included and therefore, the generalizability of our findings to T1DM patients should be done with caution. We provided a part of bread a person consumed throughout the day (100 gr/day). Therefore, the effect of non-fortified bread cannot be excluded.

In conclusion, dietary intake of PPP-fortified bread for 12 weeks had no significant effect on hs-CRP, oxidative stress, and psychological indices among individuals with T2DM compared with a control bread. However, the reducing effect of PPP-fortified bread on hs-CRP levels in the within-group comparison might be clinically important. It should be noted that the dosage of PPP administered in the current study had no side effects. Further investigations should be conducted to assess the effect of PPP fortification using other foods such as dairy products. According to the results of the MTT assay, it can be concluded that the administered dosage of PPP in bread (3.5 g/100g bread was safe and did not show significant toxicity.

Declarations

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Authorship:

Maryam Zare: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Writing – reviewing and editing draft.

Mohammad Javad Tarrahi: Developed the statistical design and analysis, Writing – reviewing and editing draft.

Omid Sadeghi: Formal analysis and Interpretation of Data, Writing – reviewing and editing draft. **Mozhgan Karimifar:** Investigation, Interpretation of Data, writing – reviewing and editing draft. **Sayed Amir Hossein Goli**: Methodology, Writing – reviewing and editing draft, **Reza Amani:** Project supervision, Investigation, Methodology, Writing - review & editing. All authors have seen and approved the final version of the manuscript.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

References

1. Abbaspoor, Z., Amani, A., Afshari, P., & Jafarirad, S. (2020). The effect of education through mobile phone short message service on promoting self-care in pre-diabetic pregnant women: A randomized controlled trial. *J Telemed Telecare*, *26*(4), 200-206. https://doi.org/10.1177/1357633x18791419
2. Amiri, M. (2016). Diabetes mellitus type 2; an international challenge. *Ann Res Dial*, *1*(1), e04.
3. Badano, J. A., Braber, N. V., Rossi, Y., Vergara, L. D., Bohl, L., Porporatto, C., Falcone, R. D., & Montenegro, M. (2019). Physicochemical, in vitro antioxidant and cytotoxic properties of water-soluble chitosan-lactose derivatives. *Carbohydrate polymers*, *224*, 115158.
4. Bagheri, S., Khorramabadi, R. M., Assadollahi, V., Khosravi, P., Cheraghi Venol, A., Veiskerami, S., & Ahmadvand, H. (2023). The effects of pomegranate peel extract on the gene expressions of antioxidant enzymes in a rat model of alloxan-induced diabetes. *Archives of Physiology and Biochemistry*, *129*(4), 870-878.
5. Barghchi, H., Milkarizi, N., Dehnavi, Z., Askari, V. R., Rajabzade, F., Ostad, A. N., Jarahi, L., Goshayeshi, L., Sobhani, S. R., & Nematy, M. (2023). The Effects of pomegranate Peel Supplementation on Depression, Anxiety, and Stress Symptoms of Patients with Non-alcoholic Fatty Liver: A Randomized Clinical Trial. *Journal of Nutrition, Fasting & Health*, *11*(2).
6. Bennett, J. A. (2005). The consolidated standards of reporting trials (CONSORT): Guidelines for reporting randomized trials. *Nursing Research*, *54*(2), 128-132.
7. Deshpande, A. D., Harris-Hayes, M., & Schootman, M. (2008). Epidemiology of diabetes and diabetes-related complications. *Physical therapy*, *88*(11), 1254-1264.

8. Eren, I., Erdi, Ö., & Şahin, M. (2008). The effect of depression on quality of life of patients with type II diabetes mellitus. *Depression and anxiety*, *25*(2), 98-106.
9. Evans, J. L., & Bahng, M.-K. NON-PHARMACEUTICAL INTERVENTION OPTIONS FOR TYPE 2 DIABETES.
10. Garcia, C., Feve, B., Ferre, P., Halimi, S., Baizri, H., Bordier, L., Guiu, G., Dupuy, O., Bauduceau, B., & Mayaudon, H. (2010). Diabetes and inflammation: fundamental aspects and clinical implications. *Diabetes & metabolism*, *36*(5), 327-338.
11. Grabež, M., Škrbić, R., Stojiljković, M. P., Vučić, V. M., Grujić, V. R., Jakovljević, V., Đurić, D. M., Suručić, R., Šavikin, K., & Bigović, D. (2022). A prospective, randomized, double-blind, placebo-controlled trial of polyphenols on the outcomes of inflammatory factors and oxidative stress in patients with type 2 diabetes mellitus. *Reviews in Cardiovascular Medicine*, *23*(2), 57.
12. Haghighian, M. K., Rafraf, M., Moghaddam, A., Hemmati, S., Jafarabadi, M. A., & Gargari, B. P. (2016). Pomegranate (Punica granatum L.) peel hydro alcoholic extract ameliorates cardiovascular risk factors in obese women with dyslipidemia: A double blind, randomized, placebo controlled pilot study. *European Journal of Integrative Medicine*, *8*(5), 676-682.
13. Heidari, Z., Feizi, A., Azadbakht, L., Mohammadifard, N., Maghroun, M., & Sarrafzadegan, N. (2019). Usual energy and macronutrient intakes in a large sample of Iranian middle-aged and elderly populations. *Nutrition & Dietetics*, *76*(2), 174-183.
14. Henry, J. D., & Crawford, J. R. (2005). The short-form version of the Depression Anxiety Stress Scales (DASS-21): Construct validity and normative data in a large non-clinical sample. *British journal of clinical psychology*, *44*(2), 227-239.
15. Jafari, T., Fallah, A. A., Bahrami, M., & Lorigooini, Z. (2020). Effects of pomegranate peel extract and vitamin E on oxidative stress and antioxidative capacity of hemodialysis patients: a randomized controlled clinical trial. *Journal of Functional Foods*, *72*, 104069.
16. Javanbakht, M., Baradaran, H. R., Mashayekhi, A., Haghdoost, A. A., Khamseh, M. E., Kharazmi, E., & Sadeghi, A. (2011). Cost-of-illness analysis of type 2 diabetes mellitus in Iran. *PLoS One*, *6*(10), e26864.
17. Khan, M. A. B., Hashim, M. J., King, J. K., Govender, R. D., Mustafa, H., & Al Kaabi, J. (2020). Epidemiology of Type 2 Diabetes - Global Burden of Disease and Forecasted Trends. *J Epidemiol Glob Health*, *10*(1), 107-111. https://doi.org/10.2991/jegh.k.191028.001
18. Leite, R. G. O. F., Banzato, L. R., Galendi, J. S. C., Mendes, A. L., Bolfi, F., Veroniki, A. A., Thabane, L., & dos Santos Nunes-Nogueira, V. (2020). Effectiveness of non-pharmacological strategies in the management of type 2 diabetes in primary care: a protocol for a systematic review and network meta-analysis. *BMJ Open*, *10*(1), e034481.
19. Mahdavi, A. M., & Javadivala, Z. (2021). Systematic review of the effects of pomegranate (Punica granatum) on osteoarthritis. *Health Promotion Perspectives*, *11*(4), 411.
20. Martín-Timón, I., Sevillano-Collantes, C., Segura-Galindo, A., & del Cañizo-Gómez, F. J. (2014). Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World journal of*

diabetes, *5*(4), 444.

21. Nikooyeh, B., Neyestani, T. R., Zahedirad, M., Mohammadi, M., Hosseini, S. H., Abdollahi, Z., Salehi, F., Mirzay Razaz, J., Shariatzadeh, N., & Kalayi, A. (2016). Vitamin D-fortified bread is as effective as supplement in improving vitamin D status: a randomized clinical trial. *The Journal of Clinical Endocrinology & Metabolism*, *101*(6), 2511-2519.
22. Raimundo, A. F., Félix, F., Andrade, R., Garcia-Conesa, M.-T., Gonzalez-Sarrias, A., Gilsa-Lopes, J., do Ó, D., Raimundo, A., Ribeiro, R., & Rodriguez-Mateos, A. (2020). Combined effect of interventions with pure or enriched mixtures of (poly) phenols and anti-diabetic medication in type 2 diabetes management: a meta-analysis of randomized controlled human trials. *European journal of nutrition*, *59*, 1329-1343.
23. Sadeghi, O., Sadeghian, M., Rahmani, S., Maleki, V., Larijani, B., & Esmaillzadeh, A. (2020). Whole-grain consumption does not affect obesity measures: an updated systematic review and meta-analysis of randomized clinical trials. *Advances in Nutrition*, *11*(2), 280-292.
24. Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., & Ogurtsova, K. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes research and clinical practice*, *157*, 107843.
25. Sahebi, A., Asghari, M. J., & Salari, R. S. (2005). Validation of depression anxiety and stress scale (DASS-21) for an Iranian population.
26. Sayed-Ahmed, E. (2014). Evaluation of pomegranate peel fortified pan bread on body weight loss. *International Journal of Nutrition and food sciences*, *3*(5), 411-420.
27. Sohrab, G., Nasrollahzadeh, J., Zand, H., Amiri, Z., Tohidi, M., & Kimiagar, M. (2014). Effects of pomegranate juice consumption on inflammatory markers in patients with type 2 diabetes: a randomized, placebo-controlled trial. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, *19*(3), 215.
28. Vitale, M., Vaccaro, O., Masulli, M., Bonora, E., Del Prato, S., Giorda, C. B., Nicolucci, A., Squatrito, S., Auciello, S., & Babini, A. C. (2017). Polyphenol intake and cardiovascular risk factors in a population with type 2 diabetes: The TOSCA. IT study. *Clinical Nutrition*, *36*(6), 1686-1692.
29. Zare, M., Goli, A. H., Karimifar, M., Tarrahi, M. J., Rezaei, A., & Amani, R. (2023). Effect of bread fortification with pomegranate peel powder on glycemic indicators, antioxidant status, inflammation and mood in patients with type 2 diabetes: Study protocol for a randomized, triple-blind, and placebo-controlled trial. *Journal of Diabetes & Metabolic Disorders*, *22*(1), 921-929.

Tables

Table 1: Baseline characteristics of the study participants[1].

Variable	Bread with PPP	Control	P-value[2]
sex			0.13
Female (%)	53.3 (24)	68.9 (31)	
Age	51.88 ± 5.10	55.02 ± 4.97	0.004
Body weight (kg)	72.51 ± 8.41	69.29 ± 9.67	0.09
HbA_1C (%)	6.51 ± 0.96	6.49 ± 0.97	0.92
Physical activity (MET/min/day)	32.33 ± 4.48	32.55 ± 4.91	0.84
Education level n (%)			
Diploma and university degree	77.8 (35)	80 (36)	0.79
Medication use			
Anti-diabetic (%)	88.9 (40)	77.8 (35)	0.16
Statins (%)	66.7 (30)	60 (27)	0.51
Anti-depressants (%)	13.3 (6)	4.4 (2)	0.26

[1]Values are presented as mean ± SD and percent for quantitative and qualitative variables, respectively.

[2] Resulted from the Chi-squared test for qualitative variables and independent t-test for quantitative variables.

Table 2: Dietary intakes at baseline and 12 weeks of intervention[1].

Nutrients	Intervention (n=45)	Control (n=45)	P-value[2]
Energy (kcal/day)			
Week 0	1998.98 ± 468.98	1979.65 ± 444.51	
Change	-61.17.80 ± 383.92	-116.98 ± 318.76	0.57
Carbohydrate(g/day)			
Week 0	300.16 ± 79.44	306.15 ± 78.60	
Change	-10.59 ± 63.03	-21.15 ± 49.56	0.50
Protein(g/day)			
Week 0	69.39 ± 19.51	70.82 ± 14.97	
Change	-2.11 ± 21.70	-3.28 ± 16.73	0.82
Fat (g/day)			
Week 0	62.26 ± 20.67	55.83 ±19.48	
Change	-1.87 ± 21.50	-2.00 ± 21.67	0.98
Vitamin C (mg/d)			
Week 0	109.50 ±55.32	117.82 ± 65.58	
Change	9.86 ±84.54	-1.08 ± 70.23	0.61
Vitamin E (mg/d)			
Week 0	20.97 ± 8.40	20.59 ± 11.66	
Change	-0.58 ± 11.53	-1.20 ± 13.78	0.85
Selenium (mcg/d)			
Week 0	0.08 ± 0.03	0.08 ± 0.03	
Change	0.00 ± 0.05	-0.00 ± 0.04	0.60
Zinc (mg/d)			
Week 0	7.2 ± 2.3	6.98 ± 1.96	
Change	0.24 ± 2.93	0.11 ± 2.79	0.87
Vit A(mcg/d)			
Week 0	983.58 ± 784.04	798.69 ± 454.00	
Change	-58.20 ±1169.33	-210.45 ± 920	0.60
Beta-carotene(mcg/d)			

Week 0	397.35 ± 716.74	275.25 ± 311.68	
Change	82.19 ± 648.73	-61.23 ±362.13	0.33

[1]Values are reported as mean ± SD

[2]Obtained from independent t-test.

Table 3: Effect of treatment bread with PPP on inflammation and oxidative stress markers and mood scores[1]

Variables	Intervention		P value[2]	Control		p-value[2]	p-value[3]	p-value[4]
	pre	post		Pre	Post			
Hs-CRP (mg/dL)	2.75 ± 1.56	2.19 ± 2.03	0.01	2.45 ± 1.38	1.64 ± 1.19	<0.001		
Change	-0.56 ± 1.29			-0.81 ± 1.16			0.37	0.30
MDA (nmol/mL)	232.51 ± 104.87	217.67 ± 70.47	0.447	236.09 ± 76.84	213.24 ± 53.03	0.07		
Change	-14.84 ± 117.36			-22.85 ± 75.33			0.72	0.87
TAC (nmol/mL)	1467.92 ± 309.77	1513.87 ± 395.99	0.34	1606.35 ± 303.60	1608.16 ±324.34	0.96		
Change	45.94 ± 298.39			1.81 ± 274.49			0.50	0.49
Depression	6.66 ± 4.60	5.33 ± 4.36	0.04	5.89 ± 4.32	4.44 ± 4.28	0.01		
Change	-1.33 ± 3.66			-1.44 ± 2.83			0.89	0.65
Anxiety	5.30 ± 4.11	6.03 ±3.80	0.17	4.72 ± 3.89	5.62 ± 3.25	0.08		
Change	0.72 ± 3.00			0.89 ± 2.73			0.81	0.92
Stress	9.27 ± 6.09	8.15 ± 5.48	0.24	7.13 ± 5.24	6.86 ± 5.73	0.66		
Change	-1.12 ± 5.42			-0.27 ± 3.42			0.47	0.45

[1]Variables are expressed as mean ± SE

[2]Calculated by Paired-Samples T-test

[3]Calculated by independent t-test

[4]Obtained from multivariable analysis of covariance (MANCOVA), adjusted for age, sex, and baseline weight.

MDA: Malondialdehyde; TAC: Total Antioxidant Capacity; hs-CRP: high-sensitive C-reactive protein

Figures

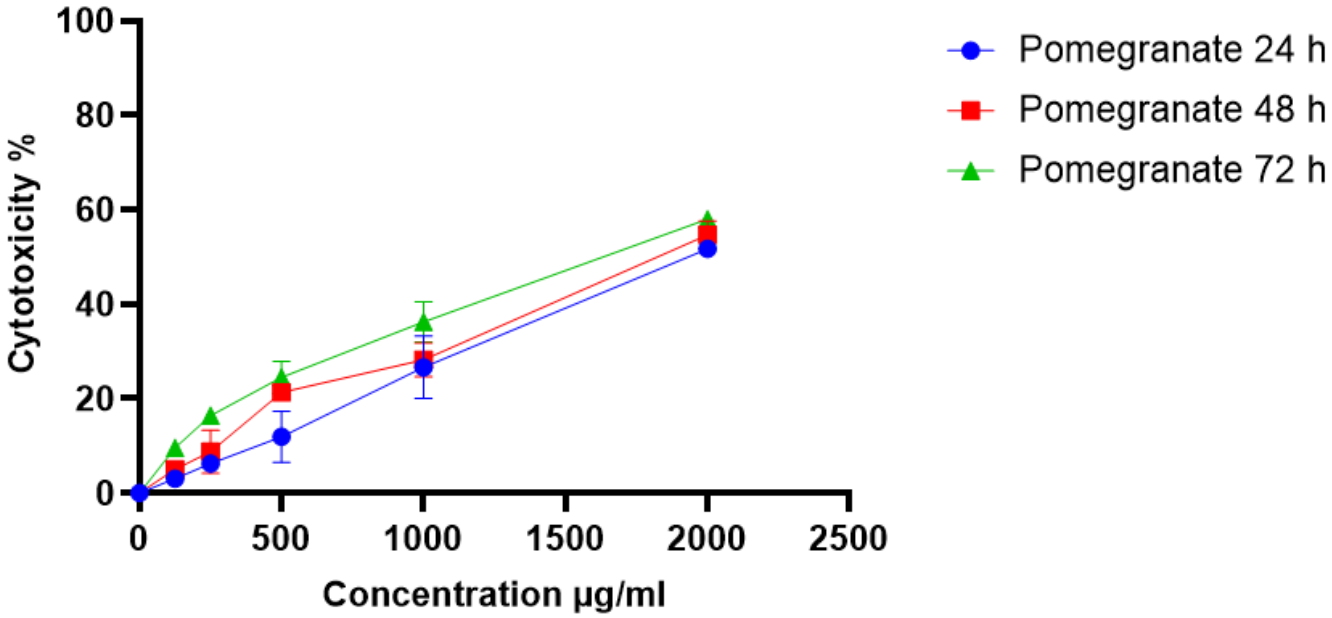

Figure 1

Cytotoxicity (%) effect of peel pomegranate powder fibroblast L929 cells.

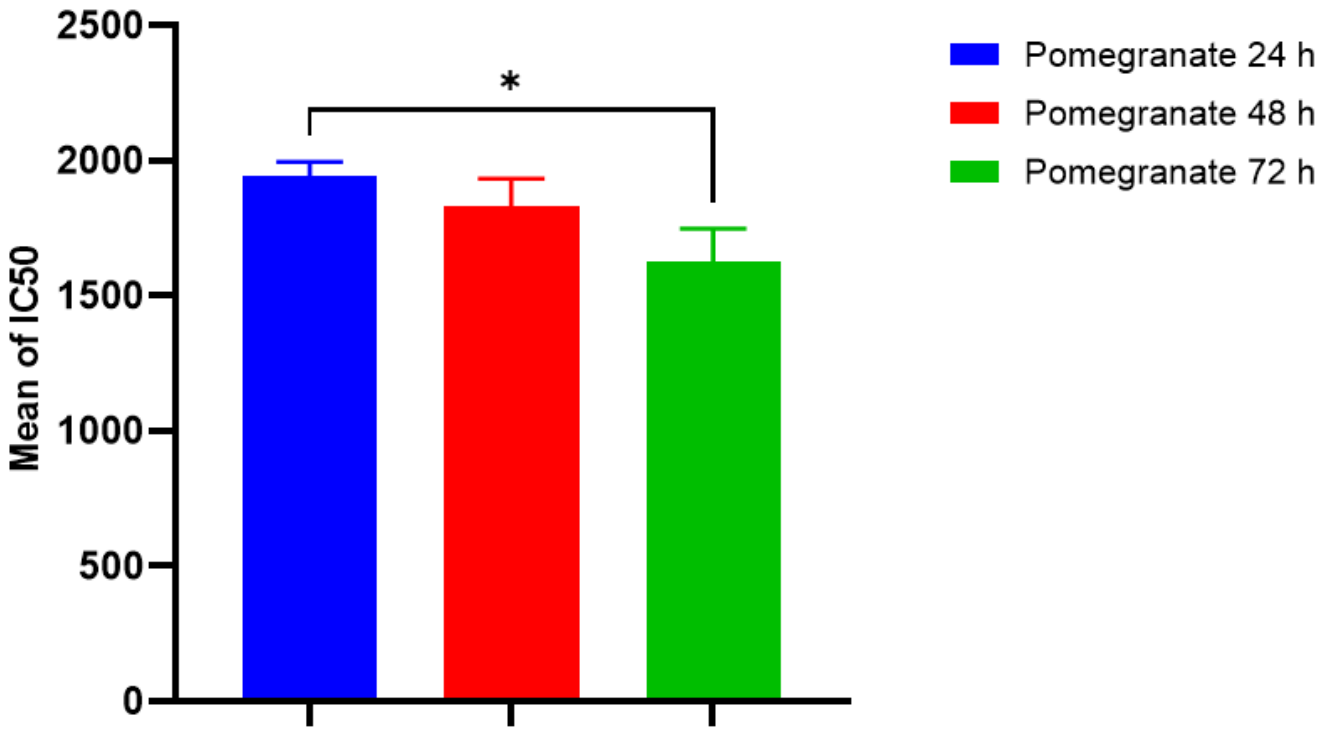

Figure 2

Comparison of IC50 of Pomegranate compound at 24, 48, and 72 hours on fibroblast cell line using Tukey's multiple comparisons test.

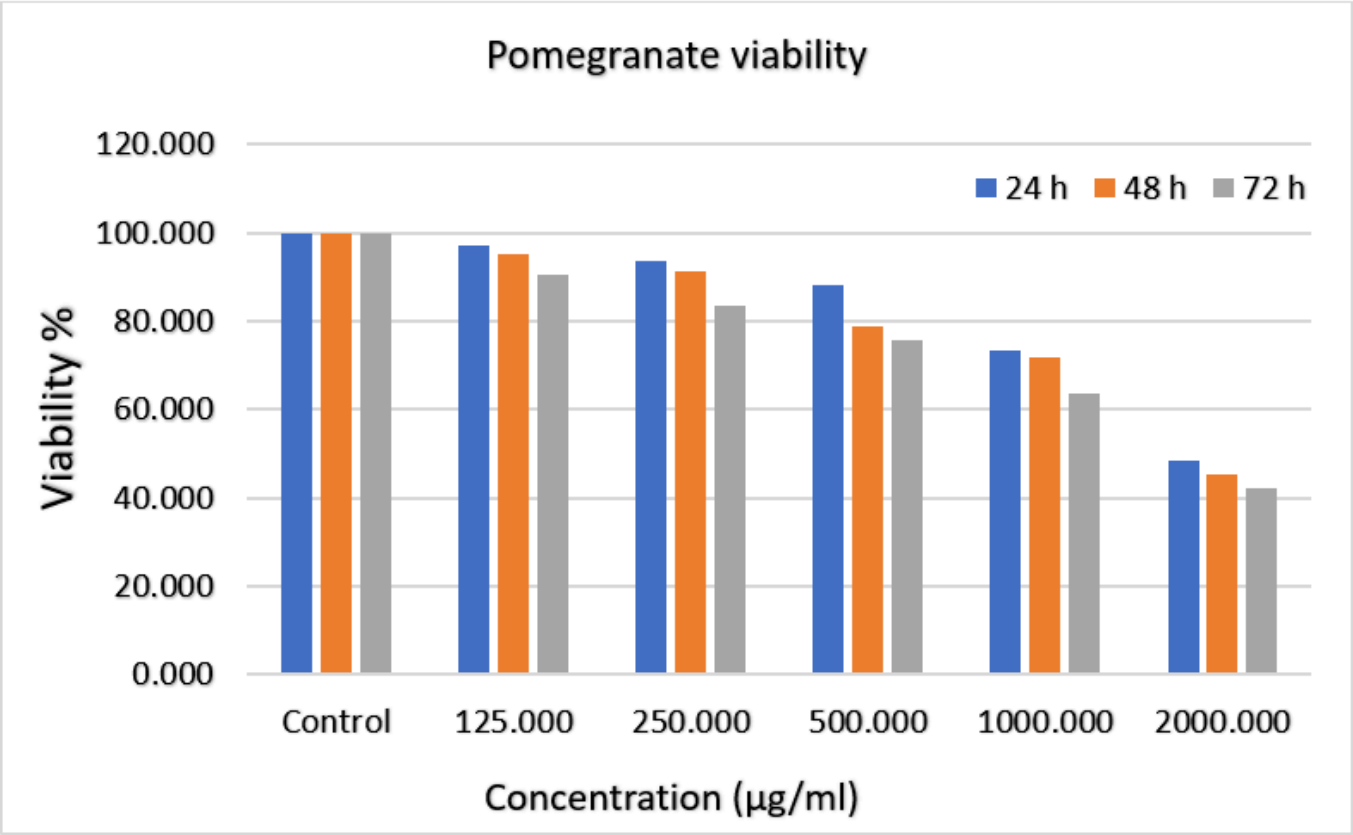

Figure 3

Cell viability in different concentrations of PPP

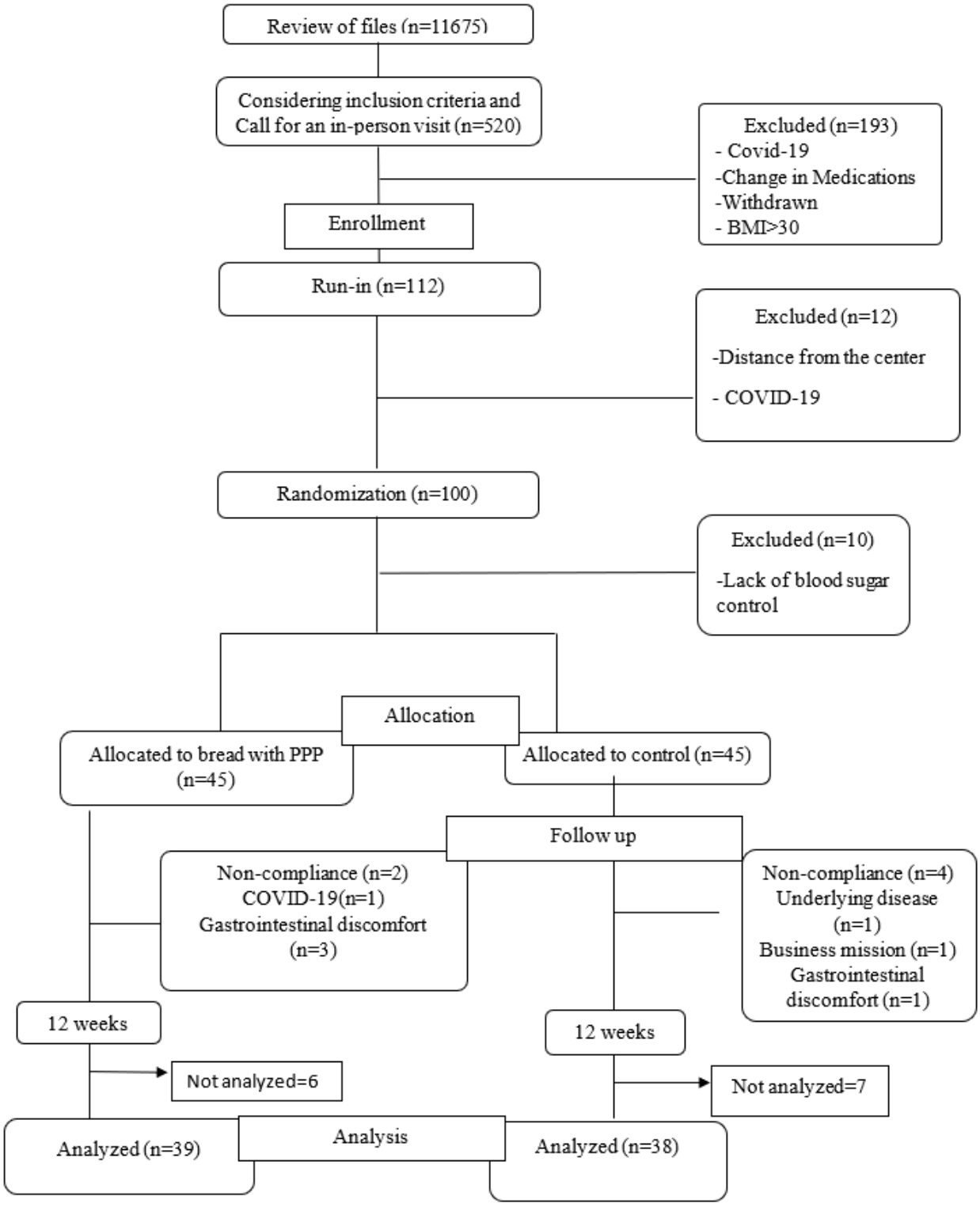

Figure 4

Study flow diagram based on CONSORT statement.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2010Checklist.doc
- protocol.pdf
- protocolframe.docx