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ORIGINAL ARTICLE

Effects of probiotic supplementation on semen parameters after varicocelectomy: A randomized controlled trial

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Background: The use of probiotics in the treatment of infertility is a new area of research. In this study, our objective was to examine the efficacy of probiotic supplementation on semen parameters following varicocelectomy. **Materials and Methods:** We included infertile men in our study who were the candidates for subinguinal microscopic varicocelectomy. After the surgical procedure, the patients were randomly assigned into two groups: 38 individuals received probiotic supplementation (FamiLact®), while 40 individuals received a placebo for 3 months. We compared the preoperative semen parameters with the postoperative parameters to evaluate the effects of probiotic supplementation. **Results:** A total of 78 patients were included in the study. The two groups were similar in terms of age, body mass index, infertility period, and semen parameters at baseline ($P > 0.05$). A statistically significant difference was found in sperm concentration (33.7 ± 22.5 vs. 21.1 ± 16.1 × 10^6/mL, $P = 0.046$), and the percentage of sperms with normal morphology (15.0 ± 8.9 vs. 12.0 ± 11.5, $P = 0.016$) at 3 months favoring the probiotic group. Although the probiotic group exhibited higher values for semen volume and sperm motility at 3 months, the differences were not statistically significant ($P = 0.897$ and $P = 0.177$, respectively). **Conclusion:** Our study demonstrates that the short-term use of probiotics after varicocelectomy can provide additional benefits in improving semen parameters. Probiotic supplements are cost-effective and well tolerated, making them a suitable option for enhancing the outcomes of varicocelectomy.

Key words: Fertility agents, infertility, probiotic, semen analysis, sperm, varicocelectomy

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INTRODUCTION

Varicocele is a significant reversible condition that leads to male infertility.[1] It is estimated to affect approximately 35%–40% of individuals experiencing primary infertility and around 80% of those with secondary infertility.[2] The primary factors contributing to varicocele include elevated scrotal temperature, dysplastic testicular tissue, and oxidative stress.[3-5] Research has demonstrated that individuals with varicocele exhibit considerably elevated levels of reactive oxygen species and reduced antioxidant capacity in their seminal plasma.[5-7] Furthermore, there seems to be a correlation between higher intratesticular temperatures and elevated apoptosis.[8] The primary treatment for varicocele is varicocelectomy, although surgical intervention does not appear to affect the total antioxidant capacity.[9,10]

A recent meta-analysis conducted by Wang *et al*.[11] indicated that antioxidant consumption after varicocelectomy can improve seminal parameters. Recently, the positive impact of probiotics on semen indices has been investigated and their effectiveness has been validated.[12-14] Nevertheless, the specific mechanisms through which probiotics enhance male fertility remain a topic of discussion.[13] It seems that

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probiotics exert their influence by influencing hormone secretion, facilitating the scavenging of free radicals, and improving the microenvironment of the prostate.[15]

In an unpublished study conducted by the author, probiotics were found to have a greater impact compared to antioxidants in improving the semen parameters of patients with idiopathic oligoasthenoteratozoospermia, possibly due to their effects through various pathways. In this randomized clinical trial, our aim was to investigate the effect of probiotics on semen indices after varicocelectomy. To our knowledge, this is the first study designed to achieve this objective.

METHODS

Study design

Between September 2021 and March 2023, we conducted the present double-blind randomized clinical trial. The study received approval from the institutional ethics review board (IR.BMSU.BAQ.REC.1399.049), and written consent for the use of patients' data was obtained from each participant. All the stages of the study adhered to the principles outlined in the Declaration of Helsinki or its subsequent revisions. The current study has been registered at the IRCT.ir with the registration number IRCT20150420021869N4.

Study population

This prospective study included infertile male patients, aged 18 years or older, who had a left-sided varicocele. These individuals had been unable to conceive for at least 1 year and were scheduled to undergo subinguinal microscopic varicocelectomy.

The study excluded patients who had previously undergone surgery related to the genitourinary system, had a medical condition affecting fertility, had received fertility-related treatment in the past 3 months, had idiopathic infertility, and had a history of conditions such as cryptorchidism, testis tumor, trauma to the testis, mumps after puberty, metabolic disorders, or obstructive urogenital conditions. In addition, patients who adhered to a diet specifically designed to enhance fertility consumed extreme amounts of recreational drugs or had a positive HIV test were excluded from the study.

Data collection

Convenient sampling method was used. A standardized infertility evaluation was conducted for the patients enrolled in the study. The physical examination, including the application of the Valsalva maneuver, was conducted in a warm room with the patient in a standing position, following the protocol described by Hudson.[16]

The classification of varicoceles was determined using the guidelines recommended by the World Health Organization (WHO). Grade I varicoceles were defined as those that were palpable during the Valsalva maneuver. Grade II varicoceles were characterized by palpability at rest but not visibly apparent. Grade III varicoceles were classified as those that were both palpable and visibly apparent at rest.[17]

To determine the sample size for our study, we conducted a power analysis based on the findings of Wang *et al*. for sperm concentration after 3 months of intervention.[11] The effect size observed in the previous data was 9.7, with a standard deviation of 4.4. We set a significance level (α) of 0.05 for a type one error and aimed for a power of 0.8, corresponding to a type two error rate of 0.2. Using these parameters, we calculated the required sample size to be 10. However, we included 78 participants in the final analysis, which was far beyond the calculated sample size.

The allocation of participants in the study was conducted using the simple randomization method with the assistance of Excel 2020 software (Microsoft Corporation, Washington, USA). The randomization sequence was generated by our statistician using the "RANDBETWEEN (0;1000000)" function. Odd and even numbers were assigned to the intervention and control groups, respectively. Allocation concealment was maintained through the use of sealed envelopes, which contained group numbers indicating the assigned treatment group. The enrollment of participants was conducted by two urologists who were not aware of the allocation results. Two surgeons had an equal level of experience in performing microscopic varicocelectomy.

Out of the total participants, 45 individuals were assigned to receive oral synbiotic FamiLact® (manufactured by Zist Takhmir, Iran) two times a day for 3 months. A similar number of patients were allocated to the placebo group. FamiLact capsules consist of a combination of bacterial strains, including *Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium longum*, and *Streptococcus thermophilus*, with each capsule containing 10^9 colony forming units of these strains. In addition, the capsules contain fructooligosaccharides, which serve as a prebiotic to support the growth and activity of these probiotic bacteria. The placebo drug had the same shape and color as FamiLact.

During the course of the study, a total of nine patients withdrew from the study, and three patients were lost to follow-up. The data analysis was conducted using the information from the remaining 78 patients, with 38 patients in the probiotic group and 40 patients in the placebo group.

The study flowchart illustrating the patient distribution and progress is depicted in Figure 1.

Semen analysis was performed upon the diagnosis of varicocele, and a second semen analysis was conducted 3 months after the surgery in both study groups. Computer-assisted semen analysis (CASA) using medeaLAB CASA Version 4.1 (Germany) was performed within 1 h of sperm collection to analyze the semen samples. The semen samples were collected after 2–5 days of sexual abstinence. Semen parameters were evaluated following the guidelines outlined in the 5th edition of the WHO laboratory manual for the examination and processing of human semen,[17] including semen volume (mL), sperm concentration (×10^6/mL), sperm motility (%), and normal sperm morphology (%). The semen analysis was carried out by two experienced technicians in the andrology laboratory.

All patients underwent subinguinal varicocelectomy, which was performed using the microscope at ×10 magnification. A subinguinal incision of approximately 3 cm was made. After the subcutaneous fat was exposed, the spermatic cord was carefully grasped and lifted using a Babcock clamp. It was then placed on a Penrose drain for further manipulation or examination. The veins were carefully ligated while ensuring preservation of the lymphatic and arterial vessels.

The main focus of the study was to compare the various semen parameters between the groups, including semen volume, sperm concentration, sperm motility, and morphology. These measures served as the primary outcome measures in assessing the differences between the groups.

Statistical analysis

Descriptive statistics, such as mean (standard deviation), were used to summarize the data. The normality of the distribution was assessed using the Kolmogorov–Smirnov *Z*-test. To compare the quantitative data between the groups, independent *t*-tests or Mann–Whitney *U*-tests were used. Wilcoxon test was employed to compare paired findings at baseline and after treatment within the groups. *P* value threshold of <0.05 was used to determine statistical significance in the study. All statistical analyses were conducted using the SPSS statistical software version 26.0 IBM SPSS statistics (Armonk, New York, USA).

RESULTS

A total of 78 patients were enrolled in the study. The baseline characteristics of the study participants were compared between the probiotic and placebo groups using an independent *t*-test. No statistically significant differences were found between the groups for age, body mass

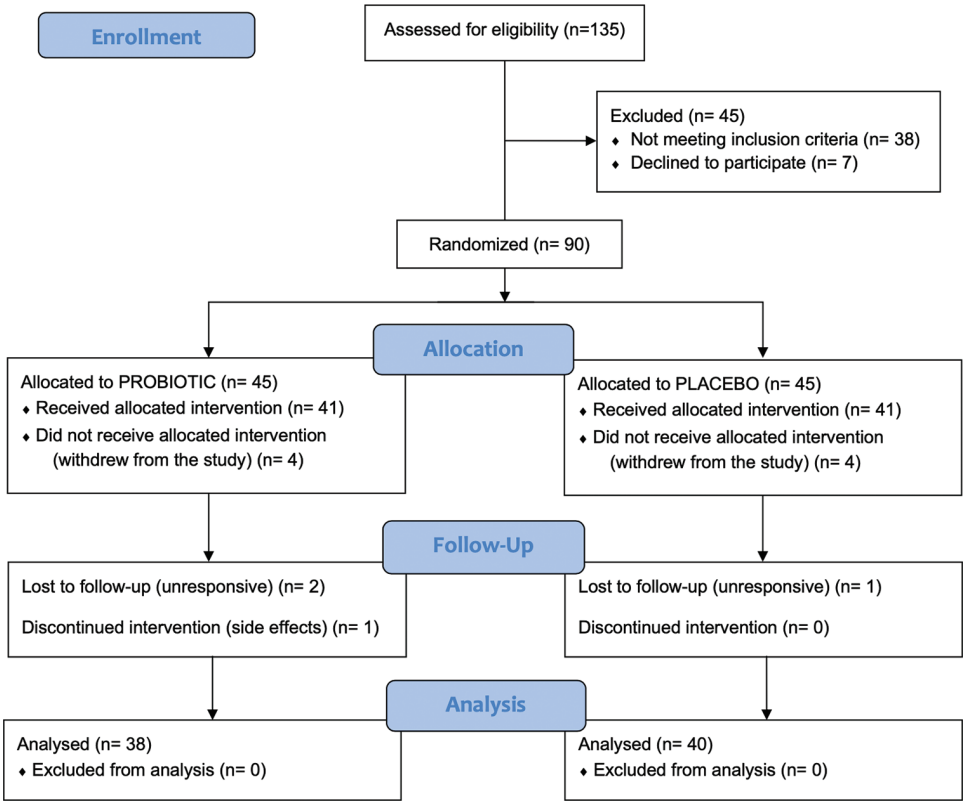

Figure 1: Study enrollment flowchart

index, and infertility period. The mean age was 31.9 (3.4) years in the probiotic group and 32.3 (4.5) years in the placebo group ($P = 0.070$). The mean body mass index was 25.1 (2.8) kg/m^2 in the probiotic group and 26.2 (2.8) kg/m^2 in the placebo group ($P = 0.174$). The mean infertility period was 28.2 (21.9) months in the probiotic group and 28.5 (22.0) months in the placebo group ($P = 0.113$).

Probiotic was well tolerated and only one patient in the case group discontinued the treatment because of flatulence. Table 1 presents the summary of semen parameters for the placebo and probiotic groups at baseline and 3 months postsurgery. Wilcoxon test and Mann–Whitney *U*-test were employed for the statistical analysis, evaluating within-group changes and between-group differences, respectively.

At baseline, there were no significant differences between the groups regarding any of the semen parameters ($P > 0.05$). Within-group analysis showed that in both groups, sperm concentration, sperm motility, and sperm with normal morphology increased statistically significantly compared to the baseline values ($P = 0.007$, $P = 0.007$, and $P = 0.026$, respectively).

At 3 months, sperm concentration was significantly different between the placebo (21.1 × 10^6/mL) and probiotic (33.7 × 10^6/mL) groups favoring the probiotic group ($P = 0.046$). Similarly, the probiotic group exhibited significantly higher sperm with normal morphology (15.0%) at 3 months compared to the control group (12.0%), ($P = 0.016$). Although the probiotic group showed higher values for semen volume and motile sperm at 3 months, the differences were not statistically significant ($P = 0.897$ and $P = 0.177$, respectively).

Moreover, the changes in semen parameters were calculated by subtracting the baseline values from the values after the intervention. Between-group analysis showed statistically significant differences between the groups regarding sperm concentration ($P = 0.049$) and normal morphology ($P = 0.038$) and nonsignificant differences regarding semen volume ($P = 0.741$) and normal sperm morphology ($P = 0.347$).

DISCUSSION

We observed that probiotic treatment had a positive impact on sperm concentration and morphology following varicocelectomy. Varicocelectomy, which is considered the standard treatment for varicocele, leads to significant improvements in semen parameters and reduced sperm DNA damage, convincing us to perform the surgery on patients.[6,18] However, it should be noted that not all patients undergoing varicocelectomy experience the same positive effects. For instance, Baazeem *et al*. conducted a study in 2011 that showed no significant increase in spontaneous pregnancy rates after varicocelectomy. Moreover, the study did find a decrease in sperm DNA fragmentation, reduced oxidative stress in the semen, and improvements in sperm concentration and motile sperm percentage.[4] A previous retrospective study involving 100 patients showed that varicocelectomy has the potential to alleviate persistent spermatic vein reflux and enhance semen indices in men with subfertility.[19] However, it remains unclear from the available data whether surgery can effectively counteract the effects of oxidation on sperm quality. Despite the improvements observed in objective parameters, the inconsistent clinical outcomes have led to the consideration of adjuvant therapies alongside varicocelectomy. In a recent meta-analysis, researchers

Table 1: Semen parameters presented as means (standard deviation) at baseline and 3 months after intervention with results of within- and between-group analyses

	Semen volume (mL)	Sperm concentration (×10^6/mL)	Motile sperm (×0.01)	Normal morphology (×0.01)
Placebo				
Baseline (a)	3.3 (1.5)	18.0 (11.0)	28.2 (24.6)	8.0 (6.9)
3 months (b)	3.4 (2.3)	21.1 (16.1)	34.2 (33.2)	12.0 (11.5)
Evolution (c)	0.1 (1.8)	3.0 (14.6)	6.1 (28.3)	3.9 (9.1)
Probiotic				
Baseline (d)	3.0 (1.4)	16.3 (11.4)	27.7 (24.3)	9.0 (5.7)
3 months (e)	3.9 (2.1)	33.7 (22.5)	43.0 (38.7)	15.0 (8.9)
Evolution (f)	0.9 (1.7)	17.3 (16.9)	15.4 (31.0)	6.1 (7.4)
Within-group analysis* (*P*)				
a versus b	0.109	0.007	0.007	0.026
d versus e	0.288	<0.001	<0.001	<0.001
Between-group analysis† (*P*)				
a versus d	0.694	0.734	0.968	0.741
b versus e	0.897	0.046	0.177	0.016
c versus f	0.741	0.049	0.347	0.038

*Wilcoxon test; †Mann–Whitney *U*-test

concluded that postoperative administration of antioxidant can effectively reduce oxidative stress and improve semen parameters.[11]

In recent years, probiotics have emerged as a potential treatment approach in various medical fields. Their minimal side effects and broad effects on different systems in the body make them an appealing option for treatment.[20] The literature suggests that probiotics have shown improvements in female fertility.[21] In 2017, Maretti and Cavallini incidentally discovered that patients with idiopathic oligoasthenoteratozoospermia who were taking probiotics for digestive issues experienced improvements in semen parameters.[15] Subsequently, further studies were conducted to investigate the effects of probiotics on semen parameters, which confirmed their effectiveness.[12-14] Nevertheless, there is ongoing debate regarding the exact mechanisms through which probiotics impact male fertility. The specific ways in which these beneficial bacteria enhance male reproductive health are not yet fully understood or agreed upon. It has been suggested that probiotics may regulate the pulsatile secretion of gonadotropins and promote fertility by interacting with kisspeptin.[22] Probiotics have the potential to reduce oxidative stress caused by free radicals.[23,24] In addition, probiotics may have a positive impact on prostatic microenvironment.[25]

In our study, the sperm concentration after 3 months of probiotic supplementation was found to be 12.6×10^6/mL higher than the control group. In a meta-analysis conducted by Wang *et al*.,[11] the sperm concentration after 3 months of antioxidant supplementation was found to be 9.7×10^6/mL higher than the control group. The difference in the percentage of motile sperm was also higher in our study compared to the reported values for antioxidants (15.4% vs. 5.4%). We assume that one of the reasons why probiotics exhibit greater efficacy than antioxidants in improving sperm concentration and motility is their ability to enhance semen parameters through multiple mechanisms, in addition to their antioxidant effects. However, the difference in the percentage of sperm with normal morphology in the present study was lower than the values reported in Wang's study for antioxidants (6% vs. 9.2%). It should be noted that without a head-to-head controlled trial, it is not possible to make a definitive statement regarding the comparative effectiveness of probiotics versus antioxidants in the postvaricocelectomy period.

To the best of our knowledge, this is the first study to examine the effects of probiotics after varicocelectomy. Probiotics are available at different price ranges and are generally affordable. Another notable characteristic is their minimal side effects, making them well tolerated by patients.

Our study had several limitations that should be considered. First, we focused solely on the impact of probiotics on semen analysis and did not assess other important factors such as hormonal profile, DNA fragmentation index, and antioxidant capacity of semen. Second, the duration of our study was relatively short, which prevented us from evaluating long-term outcomes such as fertility rates or the success of assisted reproductive methods. Third, we did not investigate the persistence of probiotic effects after discontinuation of treatment. Finally, it should be noted that the generalizability of our findings to all available probiotic products on the market may be limited, as there is variability among different products. Therefore, we recommend future research to conduct randomized controlled trials with longer follow-up periods, comparing the efficacy of probiotics with antioxidants and considering a broader range of outcomes, including fertility rates.

CONCLUSION

To summarize, our findings indicate that the short-term administration of probiotics following varicocelectomy can add extra benefit to varicocelectomy in improving sperm concentration and morphology. The affordability and favorable tolerability of probiotic supplements make them a suitable choice for enhancing the outcomes of varicocelectomy.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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ORIGINAL ARTICLE

Effect of diet low in advanced glycation end products on appetite, body composition, and brown adipose tissue markers in patients with coronary artery disease treated with angioplasty: A randomized controlled trial

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Background: Recent changes in dietary habits have resulted in increased intake of advanced glycation end products (AGEs), which are known to have a predominant contribution to the pathogenesis and complications of coronary artery disease (CAD). AGEs are also thought to induce weight gain by affecting appetite, energy expenditure, and brown adipose tissue (BAT). Here, we investigated whether the restriction of dietary AGEs could affect appetite, body composition, anthropometric indices, and BAT-derived markers in CAD patients treated with angioplasty. **Materials and Methods:** Forty-two stented CAD patients were randomly allocated into two groups that received either a low-AGEs or a control diet for 12 weeks. At baseline and postintervention, fasting blood samples were analyzed for total AGEs, nesfatin-1, and BAT-derived markers (fibroblast growth factor 21 and neuregulin 4). Subjective appetite ratings and body composition were evaluated using the Visual Analog Scale (VAS) and bioelectric impedance analysis. Anthropometric indices, including fat mass index (FMI), abdominal volume index (AVI), and body adiposity index (BAI), were calculated through the relevant formula. **Results:** Restricting dietary AGEs for 12 weeks could cause a significant reduction in weight, FMI, AVI, and BAI ($P < 0.05$) compared to the comparison group. In addition, VAS data analyses indicated a significant decrease in the sense of hunger and prospective food intake ($P < 0.05$) in the intervention group compared to the comparison group. No significant difference was seen in the measured biochemical markers between the two groups. **Conclusion:** This study indicated that the low-AGEs diet could decrease appetite, weight, and anthropometric indices in stented CAD patients.

Key words: Advanced glycation end products, appetite, brown adipose tissue, coronary artery disease, nesfatin-1

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INTRODUCTION

Overweight/obesity, a premier public health issue worldwide, is highly prevalent in patients with established coronary artery disease (CAD). Over the past two decades, the proportion of CAD patients with central obesity increased from 32.5% to 61.3%.[1] Obesity is not only an independent cardiovascular risk factor but also it is associated with other traditional cardiovascular risk factors.[2] Weight loss of around 5%–10% can lead to a clinically meaningful cardiovascular risk reduction.[3]

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Advanced glycation end products (AGEs) are a heterogeneous group of constituents with prooxidant and cytotoxic properties formed through the Maillard reaction, the nonenzymatic addition of reducing sugars to proteins, lipids, or nucleic acids. Animal-source foods, especially when prepared by high-heat cooking methods such as roasting, grilling, broiling, and frying, contain high amounts of AGEs.[4] AGEs have a fundamental role in CAD pathogenesis through receptor-dependent and independent mechanisms. Apart from AGEs-induced crosslinking with macromolecules such as collagen and elastin, which alters their structure and function, activating receptors for AGEs (RAGE) on AGEs binding triggers intracellular cascades that result in oxidative stress and inflammation.[5]

Growing evidence also suggests that AGE-RAGE signaling may contribute to weight gain and obesity, which can complicate CAD management. AGEs can enhance appetite by increasing foodstuffs' flavor, smell, and appearance.[6] In addition, RAGE is proposed as a critical regulator of weight gain and adiposity since it affects energy expenditure and the browning process, a process in which brown adipose tissue (BAT)-like phenotype is induced in white adipose tissue (WAT) in response to various stimuli.[7,8] BAT has a protective role in energy balance by dissipating energy as heat and increasing energy expenditure.[9] During BAT activation and the browning process, the secretion of BAT-derived endocrine factors (batokines), such as fibroblast growth factor 21 (FGF21) and neuregulin 4 (NRG4) is increased.[10]

Since the diet is the primary exogenous source of AGEs contributing to the total body AGEs pool,[11] dietary AGEs restriction would probably modulate different pathways involved in the progression of obesity and appears to be beneficial independently from the consumption of standard energy-restricted diets. In addition, reduced AGEs intake could be effective in CAD patients, for whom AGEs can cause more clinical outcomes. Accordingly, the present study was designed to investigate whether consuming a low-AGEs diet without calorie restriction can have beneficial effects on appetite, body composition, weight, anthropometric indices, and BAT-derived endocrine markers in CAD patients.

SUBJECTS AND METHODS

Subjects

Patients aged 50–65 years with a body mass index (BMI) of 18.5–35 kg/m^2 treated with angioplasty because of having 1 or 2 blocked arteries were assessed for eligibility. Patients were excluded from participation if they had diabetes, chronic kidney disease, cancer, thyroid, autoimmune diseases, familial hypercholesterolemia or hypertriglyceridemia, and a history of myocardial infarction, stroke, or angioplasty during the past 3 months. In addition, we excluded patients who were current smokers, consumed multivitamins, mineral or anti-oxidant supplements, or followed any weight loss diets during the past 3 months before angioplasty and women before menopause.

All patients' records that underwent angioplasty at Tehran Heart Center from September 2020 to June 2021 were prescreened, and eligible patients were invited to attend an information meeting. Patients were screened again at the first meeting, and 42 volunteers started the dietary intervention. All volunteers provided written informed consent before participation. The study protocol was approved by the Research Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1398.334) and registered at the Iranian Registry of Clinical Trials (IRCT20131125015536N10).

Study design

This study was a randomized controlled clinical trial with parallel groups. To randomly assign eligible patients to either the low-AGEs or the comparison groups, we used a computer-based generated random sequence based on sex-stratified permuted block randomization with the random block size of 2 and 4.

Data regarding anthropometric characteristics, body composition, and appetite sensation were collected at baseline and the end of the 12th week. Furthermore, 10 ml of blood was obtained from each participant after an overnight fast for biochemical analyses. All patients received their drugs and treatments during the study, and no changes were made to the health-care protocol of the hospital.

Dietary intervention and follow-up

Both groups' diets were similar in macronutrient percentage and designed to meet American Heart Association (AHA) and National Cholesterol Education Program (NCEP) dietary recommendations for CVD but differed in the AGEs content. AHA/NCEP recommendations in CVD patients include a total fat intake of 25%–30% of total energy (<20% monounsaturated fatty acid [MUFAs], <10% polyunsaturated fatty acid [PUFAs], and <7% saturated fatty acid [SFAs]), 15% protein, 50%–60% carbohydrates, and restriction of added sugars (<100 Kcal/d for women and <150 Kcal/d for men), sodium (≤2300 mg/d), and cholesterol (≤200 mg/d). Participants were instructed on the proportions and types of foods they should consume from different food groups to achieve the required macronutrient content. They were encouraged to eat to their appetite and select portion sizes that they felt were appropriate for them.

Both groups received all AHA/NCEP dietary recommendations orally and in writing. The low-AGEs group was also instructed on how to choose and prepare their foods to reduce the AGEs content of the diet. The instructions included thorough guidance on the cooking process (methods, temperature, and duration) and a food choice list. The low-AGEs group was instructed to stew, steam, boil, or poach their foods and avoid frying, baking, roasting, or grilling. The food choice list consisted of foods with high AGEs content that are not allowed and foods with lower AGEs content that are accepted for consumption. In addition, the participants were given some predefined main meals and snacks. To promote dietary compliance, telephone calls were made by the dietitian to emphasize dietary instructions every 2 weeks during the study. In addition, patients could call the dietitian whenever they had any questions about the intervention.

Measurements

Anthropometry and body composition

Body weight, height, and waist circumference were measured in fasting state using a portable digital scale (Seca, Germany), a vertical wall-mounted stadiometer (Seca, Germany), and a flexible measuring tape.

The body composition was assessed using multi-frequency (1, 5, 50, 250, 500, and 1000 kHz) bioelectric impedance analysis (InBody770, Korea). The volunteers were asked to restrain from physical activity for 8 h and avoid coffee and alcohol consumption 24 h before the test. Furthermore, they were recommended to drink 1–2 glasses of water 3 h before the test to stay hydrated.

Measurement of biochemical markers

Fasting blood samples were collected at the baseline and end of the trial, and serum was isolated. Serum concentrations of total AGEs, nesfatin-1, FGF21, and NRG4 were determined using enzyme-linked immunosorbent assay kits (Crystal Day, China).

Appetite estimation

Appetite sensation was assessed in the fasting state by Visual Analog Scale (VAS), a reliable and reproducible measure of appetite in the research setting. VAS consists of a 100 mm line anchored from "not at all" to "extremely" and evaluates the four subjective senses of hunger, fullness, desire to eat, and prospective food consumption (PFC). Participants were instructed to mark each line corresponding to their appetite level. The score of each question was quantified by measuring the distance between the mark and the beginning of the line. The composite appetite score (CAS) was calculated using the following formula:[12]

$$CAS = (\text{desire-to-eat} + \text{hunger} + [100 - \text{fullness}] + PFC)/4$$

Dietary intake and physical activity

The assessment of dietary intake was based on three 24-h dietary recalls (two working days and one weekend day) obtained from all participants in the 1st and 12th week of intervention. Then, the average daily energy and macronutrient intake based on each subject's food recalls was calculated using Nutritionist IV software modified for Iranian foods. In addition, the AGEs content of each recall was estimated using a database that lists the AGEs values of about 560 foods.[13]

Physical activity was assessed through patients' records. Subjects were educated to record the type and duration of all their activities within 24 h for 2 days (one working day and one weekend day) at weeks 1 and 12 of the intervention. Then, the mean of physical activity for each subject was calculated by metabolic equivalents of the task determined previously for each activity.[14] Furthermore, participants were required not to change their physical activity throughout the trial. The validity of this method to assess physical activity has been investigated in previous studies.[15,16]

Indices calculation

BMI was calculated as weight (kg) divided by height squared in meters. Fat mass index (FMI) was calculated by body fat mass divided by height squared. Abdominal volume index (AVI) and body adiposity index (BAI) were estimated based on the following formulas developed previously:[17,18]

$$BAI = \text{hip circumference}/\text{height}^{1.5} - 18$$

$$AVI = (2\ \text{cm} \times [\text{waist}]^2 + 0.7\ \text{cm} \times [\text{waist} - \text{hip}]^2)/1000$$

Statistical analysis

The primary outcomes were weight and waist circumference, and the secondary outcomes were anthropometric indices (FMI, AVI, and BAI), bioelectric impedance analysis variables, and serum biomarkers. Considering the type one error of 0.05 and the type 2 error of 0.20, the sample size required for each group was calculated as 21, which provides the test power of 80% for an effect size as large as 0.6, and 42 subjects entered the study.

All analyses were performed using SPSS 24.0 (SPSS, Inc., Chicago, IL, USA). The per-protocol approach was applied for data analysis. The Kolmogorov–Smirnov test was used to examine the normal distribution of variables. Except for some variables of VAS, including satiety, desire to eat, and PFC and visceral fat level that did not have a normal distribution ($P < 0.05$), the distribution of the other studied variables was normal ($P > 0.05$). For variables with nonnormal distribution, log transformation was conducted. Differences in qualitative and quantitative

variables between the low-AGEs and comparison groups were determined using Chi-square and independent sample *t*-tests, respectively. The significance of changes during the intervention within each group was detected by paired *t*-test. Multivariate analysis of covariance was used to test if the change from baseline in the outcome variable differed significantly by the group while baseline values of the outcome variable were adjusted as covariates. A two-tailed significance $P < 0.05$ was set for all analyses.

RESULTS

Forty-two volunteers started the intervention, and 39 completed the trial and were included in the final statistical analysis [Figure 1]. The baseline characteristics of the participants in both groups are depicted in Table 1. At the study initiation, there was no significant difference between the two groups regarding age, sex, weight, BMI, waist circumference, serum concentration of total AGEs, and other confounding variables ($P > 0.05$), suggesting adequate randomization.

Weight and BMI decreased in both groups during the intervention, but the reduction was more in the low-AGEs group than in the comparison group [$P = 0.02$ and $P = 0.06$ for weight and BMI, respectively; Table 2]. Although waist circumference, fat mass, and visceral fat level were decreased within both groups, and the reduction was more in the low-AGEs group, the difference between groups was not statistically significant ($P > 0.05$). Other variables of body composition did not differ between groups throughout the study. As shown in Table 2, FMI, BAI, and AVI were decreased with statistical significance in the low-AGEs group compared to the comparison group ($P = 0.04$, $P = 0.02$, and $P = 0.048$, respectively).

Dietary data analysis showed that all patients complied with AHA/NCEP recommendations. Total intakes of

Table 1: Subject characteristics at baseline

Characteristic	Groups		*P**
	Low-AGEs group (*n*=20)	Comparison group (*n*=19)	
Age (years)	58.2±1.4	56.6±1.2	0.39
Women, *n* (%)	4 (19)	4 (19)	1
Married, *n* (%)	19 (90.5)	17 (80.9)	0.38
Education, *n* (%)			0.46
Elementary	6 (28.6)	7 (33.3)	
Undergraduate	10 (47.6)	12 (57.1)	
Graduate	5 (23.8)	2 (9.5)	
Weight (kg)	81.2±2.1	82±2.3	0.82
BMI (kg/m^2)	28.5±0.7	29.3±0.8	0.43
Waist circumference (cm)	96.8±2	101.5±2	0.11
Serum total AGEs (ng/L)	648.7±144.4	581.9±124.1	0.73
SBP	12.5±0.2	12.2±0.1	0.25
DBP	7.5±0.5	7.7±0.1	0.26

Values are reported as mean±SEM. *All *P* values are calculated by independent *t*-test except for sex and education which were calculated by Chi-square test. BMI=Body mass index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; AGEs=Advanced glycation end products; SEM=Standard error of mean

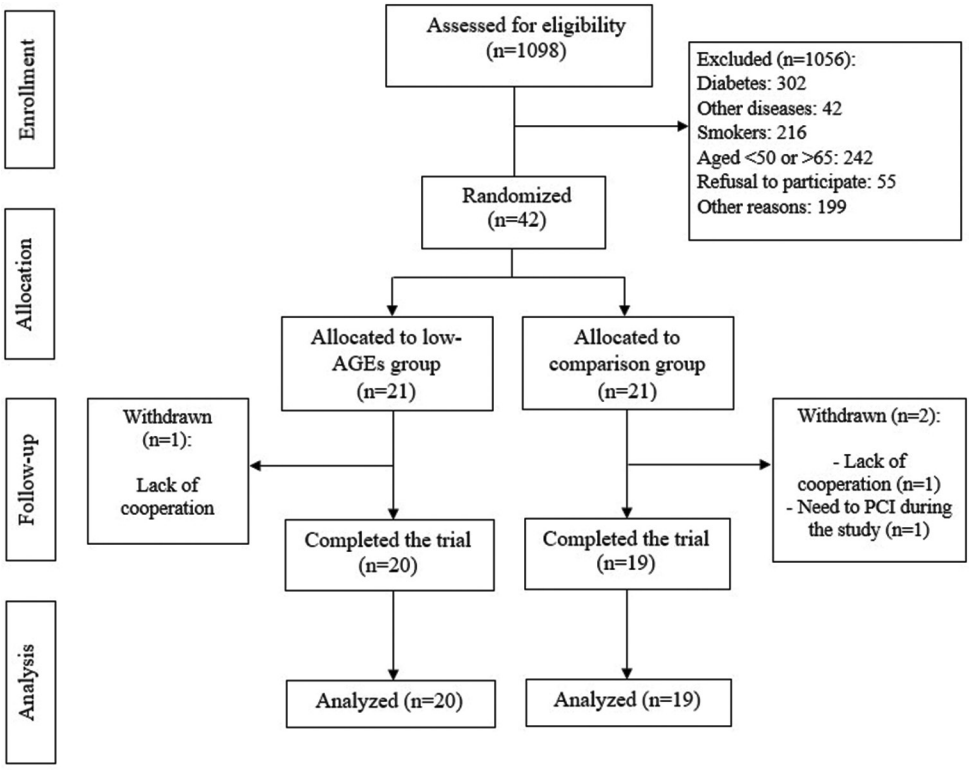

Figure 1: Flow diagram of the participants

macronutrients, the amount of fiber, added sugars, sodium, and cholesterol were all within the recommended range by AHA/NCEP. In addition, they were similar between groups throughout the dietary intervention. The total energy intake was significantly decreased in the low-AGEs group from baseline to the end of the trial ($P = 0.05$), although the difference in energy intake between the two groups was not statistically significant [$P = 0.14$; Table 3].

As expected, the two groups had a significant difference in AGEs intake [$P < 0.001$; Table 3]. Furthermore, the AGEs intake in the low-AGEs group was significantly

Table 2: Anthropometric measurements and body composition analysis of participants

Variables	Low-AGEs group (*n*=20)			Comparison group (*n*=19)			*P*†
	Baseline	Post-intervention	*P**	Baseline	Post-intervention	*P**	
Weight (kg)	81.2±2.1	78.6±2.2	<0.001	82±2.3	81±2.3	0.03	0.02
BMI (kg/m^2)	28.5±0.7	27.6±0.7	0.001	29.3±0.8	29±0.8	0.03	0.06
WC (cm)	96.8±2	94±2	0.002	101.5±2	100.1±2.3	0.06	0.22
FM (kg)	24.7±1.6	22.4±1.6	0.001	27.9±1.6	26.6±1.7	0.02	0.16
PFM (%)	30.4±1.7	28.5±1.7	0.001	33.9±1.4	32.6±1.6	0.03	0.37
FFM (kg)	56.4±1.9	56.1±1.9	0.29	54±1.6	54.3±2.1	0.16	0.1
PFFM (%)	69.6±1.7	71.5±1.7	0.001	66.1±1.4	67.4±1.6	0.03	0.37
TBW (kg)	41.5±1.4	41.3±1.4	0.32	39.8±1.2	40.1±1.3	0.16	0.12
VFL	11.2±0.9	10.1±0.9	0.001	13±0.9	12.1±1	0.004	0.53
FMI	8.8±0.7	7.9±0.7	<0.001	10.1±0.6	9.6±0.7	0.02	0.04
AVI	18.9±0.9	17.6±0.8	<0.001	20.8±0.8	20.2±0.9	0.07	0.048
BAI	29.1±1	28.4±1	0.001	29.8±0.8	29.6±0.8	0.06	0.02

*Calculated by paired *t*-test; †Calculated by ANCOVA, adjusted for the baseline values. Values are reported as mean±SEM. BMI=Body mass index; WC=Waist circumference; FM=Fat mass; PFM=Percentage of FM; FFM=Fat free mass; PFFM=Percentage of FFM; TBW=Total body water; VFL=Visceral fat level; FMI=Fat mass index; AVI=Abdominal volume index; BAI=Body adiposity index; AGEs=Advanced glycation end products; SEM=Standard error of mean; ANCOVA=Analysis of covariance

Table 3: Physical activity and nutritional intake of participants

Variables	Low-AGEs group (*n*=20)			Comparison group (*n*=19)			*P*†
	First week	12th week	*P**	First week	12th week	*P**	
Energy (Kcal/day)	1980±68	1847±82	0.05	2066±104	2029±89	0.72	0.14
Protein							
g/day	80.3±5	73.2±3.8	0.15	82.9±5.9	75.1±3.7	0.17	0.72
Percentage of energy	16.2±0.9	15.9±0.7	0.69	16.1±1	14.8±0.6	0.17	0.24
Carbohydrate							
g/day	276±15.1	264.3±16.2	0.24	290.2±21.7	301.7±18.6	0.58	0.14
Percentage of energy	55.8±2	57.2±1.8	0.28	56.2±2.3	59.5±2	0.15	0.44
Fat							
g/day	61.6±4.3	55.2±4.2	0.1	63.7±5.5	58±4.1	0.16	0.63
Percentage of energy	28±2.1	26.9±1.9	0.45	27.7±2.3	25.7±2	0.37	0.72
PUFA							
g/day	18.3±1.5	16.3±1.5	0.28	17.7±2.1	18±1.8	0.8	0.48
Percentage of energy	8.4±0.7	7.9±0.6	0.53	7.8±0.9	8.2±0.9	0.66	0.81
MUFA							
g/day	26.4±1.9	23.2±1.8	0.11	26.1±2.5	22.6±1.9	0.1	0.83
Percentage of energy	12.2±1	11.5±0.8	0.37	11.5±1.1	10.3±1	0.22	0.35
SFA							
g/day	12.5±1.3	12.1±1.3	0.71	15.2±1.5	13.2±0.9	0.15	0.49
Percentage of energy	5.7±0.6	6.1±0.7	0.52	6.5±0.5	5.9±0.3	0.18	0.77
Sugar (Kcal/day)							
Men	64.9±9.6	74.9±12.3	0.32	77.5±17.7	77.7±15.3	0.98	0.88
Women	27.5±4.8	38.2±16.1	0.63	48.5±16.9	31±10.5	0.31	0.72
Fiber (g/day)	17±1.3	19.5±1.5	0.12	15.3±1.1	17.3±0.8	0.17	0.22
Cholesterol (mg/day)	206.2±23.8	172.2±24.6	0.3	207.4±20.5	177.6±15.8	0.08	0.87
Sodium (mg/day)	1913±74	1861±99	0.52	1945±74	1820±88	0.28	0.76
Dietary AGEs (KU/day)	8378±987	6986±799	0.007	19518±2572	19399±2539	0.89	<0.001
Physical activity (MET-h/day)	33.3±1	33.2±1.1	0.87	31.5±1.1	32.1±1.1	0.5	0.84

*Calculated by paired *t*-test; †Calculated by independent *t*-test. Values are reported as mean±SEM. PUFA=Polyunsaturated fatty acid; MUFA=Monounsaturated fatty acid; SFA=Saturated fatty acid; AGEs=Advanced glycation end products; SEM=Standard error of mean

reduced at the end of the study compared to the beginning ($P = 0.007$).

In the low-AGEs group, the sense of hunger ($P = 0.04$), desire to eat ($P = 0.03$), and CAS ($P = 0.04$) were decreased, and the satiety score ($P = 0.01$) increased significantly after the intervention compared to the baseline [Table 4]. No changes in appetite scores occurred in the comparison group. Between-group analysis revealed that the low-AGEs diet could significantly decrease the sense of hunger ($P = 0.03$) and PFC ($P = 0.01$) and also caused a notable reduction in CAS ($P = 0.06$).

The results of biochemical markers are indicated in Table 5. The serum concentration of nesfatin-1, NRG4, and FGF21 was not significantly changed within or between groups after the intervention.

DISCUSSION

In this study, restriction of dietary AGEs for 12 weeks significantly decreased weight and caused a notable reduction in BMI postintervention. Many clinical trials have assessed the effect of a low-AGEs diet on weight, BMI, and WC, and their findings are controversial. While some studies have shown the reducing effect of the low-AGEs diet on weight and BMI,[19,20] others found no effect.[21,22] Meta-analysis of prior studies has depicted that consumption of the low-AGEs diet can significantly reduce weight and BMI compared to the high-AGEs diet, with a more pronounced effect in studies with a duration of more than 8 weeks,[23] which is consistent with our findings. In our study, despite the two-fold decrease in WC, the difference between groups was not significant. Similarly, the mentioned meta-analysis found no significant difference in WC between the low and high-AGEs diets.[23]

It has recently been hypothesized that AGEs play a putative role in the pathogenesis of obesity by their ability to increase appetite and energy intake through enhancing sensory-stimulating properties of foodstuffs.[6,24] Furthermore, a growing body of evidence highlighted the role of AGEs in promoting insulin resistance and activating pro-inflammatory pathways.[25,26] Considering the central role of insulin in regulating energy balance and the implication of pro-inflammatory cascades in mediating hypothalamic dysregulation of energy balance, insulin resistance, and inflammation may represent further potential mechanisms supporting the ability of AGEs to disrupt hypothalamic control of energy balance leading to body weight gain.[27] Several studies have indicated the effect of dietary AGEs limitation on improving insulin resistance and reducing inflammatory markers.[21,28] Therefore, the reducing effects of the low-AGEs diet on weight and BMI might be attributed to its beneficial impacts on insulin resistance and inflammation, which is more notable in overweight and obesity.

In the present study, we also evaluated the effects of the low-AGEs diet on anthropometric indices (FMI, AVI, and BAI). Most previous studies used BMI as the primary outcome because CDC/WHO currently recommends it for classifying overweight and obesity. However, epidemiological studies have questioned the capacity of BMI to predict cardiovascular risk due to its limitation in distinguishing excess adipose tissue from lean mass.[29,30] BMI calculation does not consider intra-abdominal or visceral adipose tissue, which its accumulation is closely associated with increased CVD risk.[31] Therefore, simple-to-use anthropometric indices have been recently developed as a surrogate or complementary measure to estimate central obesity more accurately. FMI is a potential indicator of

Table 4: Effect of dietary intervention on subjective appetite scores

Variables	Low-AGEs group (*n*=20)			Comparison group (*n*=19)			*P*†
	Baseline	Post-intervention	*P**	Baseline	Post-intervention	*P**	
Hunger	40.2±5.7	29.1±4.8	0.04	32.9±5	40.5±5.3	0.24	0.03
Satiety	41.4±4.1	52.3±4.3	0.01	45.2±5.8	49±5	0.46	0.37
Desire to eat	61.5±4.4	52.4±4.5	0.03	64.3±5.1	64.8±3.3	0.43	0.12
PFC	67.9±4.7	65.7±4.9	0.48	71±7	80.2±5.2	0.07	0.01
CAS	58.9±4.4	50.8±3.9	0.04	53.4±4.2	57.4±3.6	0.4	0.06

*Calculated by paired *t*-test; †Calculated by ANCOVA, adjusted for the baseline values. Values are reported are mean±SEM. ANCOVA=Analysis of covariance; PFC=Prospective food consumption; CAS=Composite appetite score; SEM=Standard error of mean

Table 5: Biochemical markers of participants at baseline and postintervention

Variables	Low-AGEs group (*n*=20)			Comparison group (*n*=19)			*P*†
	Baseline	Post-intervention	*P**	Baseline	Post-intervention	*P**	
Total AGEs (ng/L)	648.7±144.4	618±122.8	0.65	581.9±124.1	632.1±121.9	0.34	0.34
Nesfatin-1 (ng/mL)	13.6±4.4	14±3.7	0.71	7.7±2.3	8.8±2.2	0.1	0.83
NRG4 (ng/mL)	3.1±0.8	3±0.7	0.64	2.1±0.4	1.9±0.4	0.35	0.33
FGF21 (pg/mL)	318.8±68.6	297.3±66.3	0.39	246.8±47.4	231±40.1	0.55	0.88

*Calculated by paired *t*-test; †Calculated by ANCOVA, adjusted for the baseline values. Values are reported as mean±SEM. AGEs=Advanced glycation end products; NRG4=Neuregulin 4; FGF21=Fibroblast growth factor 21; SEM=Standard error of mean; ANCOVA=Analysis of covariance

body adiposity superior to BMI and PBF because of taking fat mass and height into account, which reduces the bias associated with BMI and PBF.[32,33] Previous research has highlighted the capability of FMI to predict metabolic syndrome and cardiovascular risk in young adults.[34] AVI, a reliable anthropometric tool that reflects the total volume of the abdomen by including WC and HC, has been used by researchers to indirectly estimate the visceral fat volume.[35] AVI sensitivity to evaluate fat deposition in viscera and associated metabolic abnormalities have been confirmed in prior studies.[36,37] Also, BAI is reported as another index that could be a valid predictor of body fat.[17] Despite no significant decrease in fat mass, the reduction in FMI, which adjusts fat mass for height, was significant between the two groups in our study. Furthermore, a significant decrease in AVI and BAI was observed in the low-AGEs group compared to the comparison group. Regarding these indices being a better indicator of visceral adipose tissue, restriction of dietary AGEs might improve metabolic disturbances associated with CAD through reduced visceral fat. Few prior trials have focused on changes in abdominal obesity, and most studies have assessed the relationship between indices and risk factors cross-sectionally based on one static measurement. But when it comes to chronic diseases like CAD, due to the impact of long-term accumulation of the risk factors, there is a need to evaluate the dynamic change of risk factors such as anthropometric indicators over time, which we tried to achieve in this research.

The percentage of macronutrient intake and the essential recommendations of AHA/NCEP guidelines (levels of SFA, MUFA, PUFA, cholesterol, added sugar, and sodium) were not different between the studied groups. However, dietary AGEs content was significantly lower in the low-AGEs group over the intervention period. Despite a falling trend of serum total AGEs concentration in the low-AGEs group throughout the study, this trend was not significant. Our results are consistent with those of other studies, which have either shown no changes in serum AGEs levels following intake of the low-AGE diet or have found decreases in plasma carboxy methyl lysine (CML) concentrations after a high-AGEs diet administration.[38,39] Interestingly, AGEs calculated from recalls and urinary AGEs had shown the expected changes in the mentioned studies. It has been suggested that measuring a combination of circulating, tissue, and excreted AGEs concentrations might better represent the total AGEs burden in the body since each measurement has its limitations.[19,39] AGEs are also characterized by complex structural and molecular heterogeneity, making it difficult to quantify them. Although various instrumental and immunochemical methods are used to measure AGEs, there is currently no gold standard method for AGEs quantification.[40]

In the present study, the low-AGEs diet decreased the sense of hunger, PFC, and CAS compared to the control diet. A recent animal study reported that an AGEs-rich diet could activate neuronal and hormonal signaling engaged in appetite regulation and energy homeostasis.[41] However, a human study found no changes in VAS appetite scores after consuming a high or low-AGEs meal.[42] The difference might be attributed to the different design of the mentioned study in which the acute response to dietary AGEs was assessed, whereas we evaluated the longer-term effect of dietary AGEs on subjective appetite sensations.

Our findings showed no changes in the serum concentration of nesfatin-1 by restriction of dietary AGEs. One of the mechanisms of appetite regulation that AGEs affect is hormones. Among appetite-regulating hormones, the effect of dietary AGEs on ghrelin has previously been investigated in a single-meal study which observed increased ghrelin response after a high-AGEs meal compared to a low-AGEs meal.[42] Until now, no study has assessed the relationship between AGEs and nesfatin-1 secretion. Appetite regulation and energy hemostasis are controlled by a very complex neuro-humoral system, which includes short-term and long-term signals, and many peripheral and central peptides are involved in this system.[43] Therefore, the lack of change in nesfatin-1 might be due to the compensatory effects of other peptides involved in this system, which were not investigated here.

One of our hypotheses was that the effects of AGEs restriction on weight loss might occur through increased energy expenditure by BAT. Our findings showed no effect of the low-AGEs diet, an influential factor in reducing RAGE signaling, on BAT-derived markers. Until now, no human trial has tested the relationship between RAGE and BAT. Animal evidence suggests a link between RAGE and high-fat diet (HFD)-induced obesity and subsequent metabolic dysfunction due to enhanced concentration of RAGE ligands such as CML and methylglyoxal which are known AGEs.[7] A recent study in mice showed that RAGE deletion increased the expression of uncoupling protein-1 (UCP-1), usually only expressed in BAT, in WAT of RAGE knockout mice. In addition, transplantation of adipocyte-RAGE-deleted adipose tissue protected the recipient mice from HFD-induced obesity through upregulation of thermogenic programs and UCP-1 expression in the recipients' native BAT or WAT.[8] Hence, the protective mechanism of RAGE antagonism might be partially due to the induction of browning in WAT, which may have potential therapeutic implications for obesity treatment. The studies conducted in this field are of the animal type in which the RAGE gene is knocked out, and adipose tissue gene expression is used to track the changes in BAT activity. Here, we studied the effects of more subtle dietary AGEs restriction-induced changes in serum levels

of BAT-derived markers, and this may partially explain why we did not observe any associations. In human trials, the most well-established method to measure BAT activity is ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography. Since this method is expensive and exposes the individuals to harmful radiation,[44] we measured serum levels of NRG4 and FGF21 as BAT markers that their secretion is increased during browning or BAT activity enhancement.

The analysis of dietary recalls indicated no significant difference in energy intake between the two groups. However, energy intake decreased remarkably in the low-AGEs group at the end of the study compared to the beginning, which partly justifies the weight loss in the low-AGEs group. On the other hand, considering the nonsignificant difference in energy intake between the two groups and the reported relationship between the AGE-RAGE pathway and energy expenditure and the browning process in animal and human studies,[7,8,45] it may be said that the significant weight difference between the two groups is at least in part due to the increase in BAT activity and energy expenditure in the intervention group, which might have been detected by measuring more specific BAT markers and indirect calorimetry. Future prospective trials are recommended to investigate the contribution of AGEs and the potential role of RAGE in this regard.

This study was the first to assess the long-term effects of AGEs restriction on appetite, anthropometric indices, and BAT-derived markers. However, our study had some limitations. Due to the COVID-19 pandemic, we could not measure the participants' energy expenditure by indirect calorimetry. In addition, blinding was not practically possible because of the dietary intervention, and the open-label design increases the risk of biased results. Furthermore, it would be much better if the participants in both groups were provided with their foods as ready-to-eat items or packed food portions throughout the study.

CONCLUSION

Our results showed that dietary AGEs restriction decreased weight and anthropometric indices reflecting visceral adipose tissue in CAD patients. It may also be appropriate for controlling appetite. Since this was possible without a substantial modification in energy intake, the low-AGEs diet may offer a feasible treatment goal of risk reduction in overweight and obese CAD patients.

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Conflicts of interest

There are no conflicts of interest.

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Review Article

The denervation or activation of renal sympathetic nerve and renal blood flow

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The denervation or activation of the sympathetic nerve in the kidney can affect renal hemodynamics. The sympathetic nervous system regulates the physiological functions of the kidneys. Stimulation of sympathetic efferent nerves affects various parameters related to renal hemodynamics, including sodium excretion, renin secretion, and renal blood flow (RBF). Hence, renal sympathetic fibers may also play an essential role in regulating systemic vascular resistance and controlling blood pressure. In the absence of renal nerves, the hemodynamics response to stimuli is negligible or absent. The effect of renal sympathetic denervation on RBF is dependent on several factors such as interspecies differences, the basic level of nerve activity in the vessels or local density of adrenergic receptor in the vascular bed. The role of renal denervation has been investigated therapeutically in hypertension and related disorders. Hence, the dynamic impact of renal nerves on RBF enables using RBF dynamic criteria as a marker for renal denervation therapy.

Key words: Renal blood flow, renal sympathetic denervation, renal sympathetic nerve activity

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INTRODUCTION

The sympathetic nervous system regulates a wide range of physiological functions within the body. The sympathetic nervous system innervates the kidneys through the vasculature, tubules, and juxtaglomerular apparatus. Since, the kidneys play an important role in adjusting blood pressure, the neural control of the kidneys is critical for regulating the body's fluid volume, sodium homeostasis, and renin release.[1] It has been suggested that animals' basal renal sympathetic nerve activity (RSNA) is at a minimum level under normal conditions. However, this activity is raised in pathological conditions, such as hypertension.[2] In addition, the RSNA fluctuations affect sodium reabsorption from renal tubular cells and renin release from juxtaglomerular cells. Due to the involvement of renal adrenergic nerves in regulating renal vascular resistance (RVR) and renal hemodynamics such as renal blood flow (RBF), the kidneys can adapt to both physiologic and pathologic stimulants.[3] The activity of sympathetic nerves of afferent and efferent renal arteries affects RBF and glomerular filtration rate (GFR).[4] Furthermore, stimulating renal efferent nerves change renal hemodynamics by increasing renin secretion, enhancing tubular fluid and electrolyte absorption, and reducing water and sodium excretion.[5] The renal nerves are inactive under normal conditions and based on the steady state measurement of RBF. However, they respond to experimental stimuli or several diseases where the RSNA exceeds the physiological level. In general, the dynamic measurement of RBF indicates that renal nerves are incessantly regulating RBF.[6]

Central sympathetic signals from the kidneys target various organs, such as the heart and lead the peripheral arteries to constrict and increasing of

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blood pressure.[7] The role of renal denervation has been investigated therapeutically in hypertension, chronic kidney insufficiency, and chronic heart failure (HF) conditions.[8] This review intends to evaluate the effects of renal nerve sympathetic activity or renal sympathetic denervation (RSDN) on RBF in physiological and pathological conditions based on basic and clinical evidences.

SYMPATHETIC RENAL INNERVATION

There are sympathetic inputs and outputs in the kidneys; the efferent sympathetic nerves from the central nervous system (CNS) and the afferent sympathetic nerves from the kidneys to the CNS constitute the sympathetic innervation of the kidneys. The sympathetic nerves innervate the kidneys through a dense network of postganglion neurons. Along the renal artery, preganglionic nerves enter the kidney from the hilus[9] and the branches of the renal sympathetic efferent nerves innervate glomerular arterioles, proximal tubules, and the juxtaglomerular system.[10] Activation of the sympathetic nerve increases the production of noradrenaline (NA) from the nerve terminals and denervation of the kidney causes a significant reduction in NA (by 95%).[11] The release of increased NA has three primary outcomes as follows:

1. NA stimulates beta-adrenergic receptors (β1-ARs) of juxtaglomerular granular cells, which in turn release renin and increase the activity of the renin-angiotensin-aldosterone system (RAAS)
2. NA reduces sodium and water excretion by increasing tubular reabsorption
3. NA reduces RBF and GFR by contracting renal arteries.[11][Figure 1].

The activation of distinct adrenoceptor (ARs) subtypes found on the renal vasculature by the renal sympathetic nervous system mediates adrenergic control of the kidneys. ARs support renal hemodynamic and tubular functions and are found on the renal vasculature, nephrons, and proximal tubules in the kidneys. The α-ARs are the most important regulators of renal vascular tone among the different types of ARs.[12] During an adrenergic response, NA released into the circulation binds to the smooth muscle cells' α1 receptors, causing the smooth muscle to contract. By mediating catecholamine-induced effects on the ARs type α1 found on the renal vasculature, the renal sympathetic nervous system significantly affects the renal hemodynamics.[12]

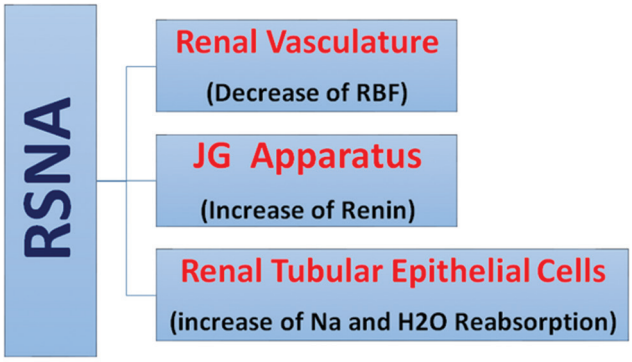

Figure 1: Schematic image of the effect of increased renal sympathetic nerve activity on different parts of the kidney. RSNA = Renal sympathetic nerve activity

Activation of the sympathetic efferent nerves of the kidney can occur in response to reinforced afferent signaling of the sensory nerve fibers of the kidney, which can be induced by various effectors such as renal hypoxia, ischemia, and oxidative stress.[8]

The pelvic area is the primary location of the afferent renal sensory nerves and the pressure in this area defines the activity of the nerves. Thus, as a reno-renal reflex response, enhancement in the urine flow rate raises the firing rate of renal afferent fibers, decreasing efferent RSNA and increasing sodium excretion from urine.[13] The renal afferent fibers are either chemo-sensitive and respond to nociceptive stimuli (such as inflammation, ischemia, acidosis, oxidative stress, adenosine, and angiotensin (Ang) (II)) or are mechano-sensitive (more common in the renal cortex) and respond to stretch.[14] The nervous system centers that received these signals include the nucleus tractus solitaries, paraventricular nucleus (PVN) of the hypothalamus, rostral ventrolateral medulla (RVLM), and subfornical organ.[15-17]

The neuronal activity in sympathetic premotor nuclei in the brain stem and hypothalamus, including RVLM and PVN, determines the degree of RSNA. Preganglionic neurons in the intermediolateral cell column of the spinal cord get input from the neurons in the RVLM; these neurons then project to postganglionic neurons, which in turn project to peripheral organs such the heart, arteries, and kidneys.[18] Figure 2 summarizes the central and peripheral pathways of sympathetic control of the kidney.

Activating of renal afferent sensory nerve (by modulation of posterior hypothalamic activity and secretion of oxytocin and vasopressin) affects the sympathetic outflow to highly innervated organs such as the kidneys, heart, and peripheral blood vessels.[19,20] Stimulation of the afferent system activates the cardiovascular regulatory centers in the CNS. The destruction of these nerves (in some diseases) reduces the central sympathetic flow to major organs regulating blood pressure, especially the kidneys, heart, and peripheral arteries.[14]

Renal denervation is believed to be effective in treating numerous diseases that are accompanied by increased

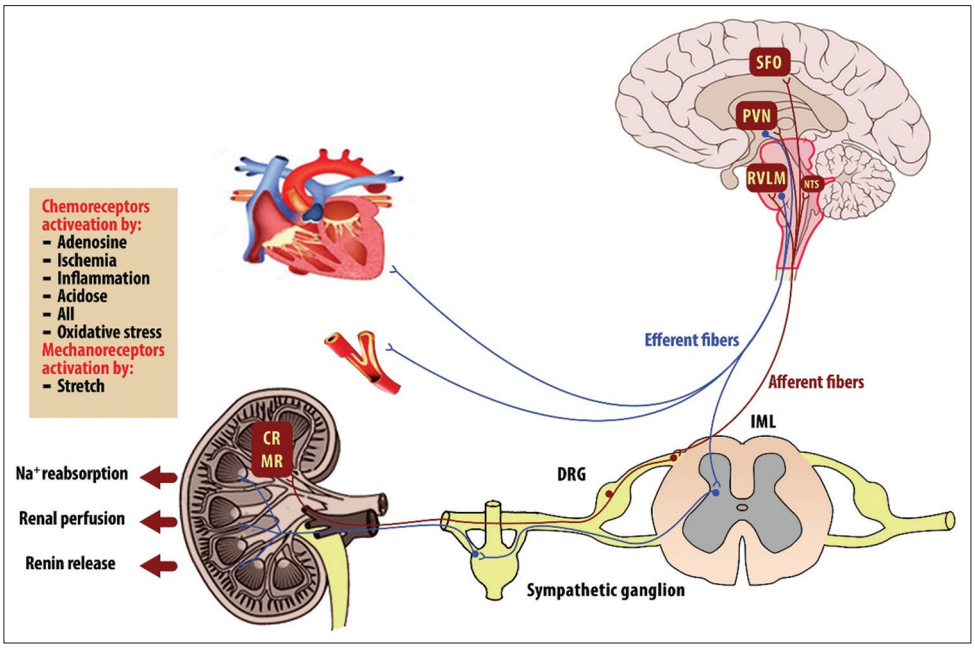

Figure 2: Schematic image of the connections between renal afferent sensory signaling and renal efferent sympathetic outflow on the kidney and other cardiovascular organs, which regulate blood pressure. Renal mechano and chemoreceptor reflexes, which are carried out by renal afferent nerves, regulate the activity of premotor neurons in the rostral ventrolateral medulla and paraventricular nucleus. CR = Renal chemoreceptors; DRG = Dorsal root ganglion; IML = Intermediolateral cell column; MR = Renal mechanoreceptors; NTS = Nucleus tractus solitarius; PVN = Paraventricular nucleus of the hypothalamus; RVLM = Rostral ventrolateral medulla; SFO = Subfornical organ

sympathetic renal activity, such as chronic and end-stage renal disease, hypertension, cardiac-renal syndrome, left ventricular hypertrophy, and improper fluid retention in HF.[19] In general, afferent sympathetic fibers may also play an essential role in regulating systemic vascular resistance and controlling blood pressure.[20]

RENAL SYMPATHETIC NERVE ACTIVITY AND RENAL BLOOD FLOW

The share of RBF from cardiac output is about 20% at rest, so its regulation plays a vital role in controlling blood pressure.[21] The kidneys have two robust auto-regulation mechanisms for regulating blood pressure, tubule-glomerular feedback, and myogenic response.[22] However, the importance of RSNA in the physiological regulation of RBF is still controversial based on the two findings. The first finding indicated that electrically stimulated renal nerves at different frequencies affect RBF differently, and in the pathophysiological range of RSNA, a significant decrease in RBF was observed.[6] Other findings violate the influence of renal nerves in the physiological regulation of RBF since renal denervation is not affecting basal RBF. However, both of these findings had significant drawbacks.[23] The electrical stimuli inherently cannot distinguish between physiological, pathophysiological, and supraphysiological effects. RSNA recruits special renal postganglionic fibers in response to specific stimuli with different effects.[23] In addition, the particular axons can electively innervate the vessels, juxtaglomerular cells, or tubules, and even axons that innervate juxtaglomerular arteries can be differentiated from those that innervate other renal vessels.[24] Eventually, by changing RSNA, which occurs through either stimulation or denervation, it must overcome powerful autoregulatory mechanisms to affect the steady state of RBF.[23]

It is stated that the vascular system is insensitive to slight changes in RSNA. In experimental models, RSNA was increased progressively by electrical stimulation of the renal nerves in anesthetized cats or dogs[25-27] or reflex activation in conscious dogs.[28,29] At low RSNA levels, only renin release occurred, and then, slightly increased levels have resulted in changes in sodium excretion, and still, RBF alteration was obtained only at much higher levels,[30] indicating that in daily life, changes in RSNA at near resting levels have minimal impact on RBF.[30]

Grady and Bullivant measured RBF during the daily activity in conscious rats, demonstrating that RBF decreased with increasing activity levels; however, this result was not obtained when RSNA was previously blocked with local anesthetics.[31] In alert rabbits, a moderate increase or decrease in RSNA affected RBF. However, sound stress, air-jet stress, and hypoxia increased RSNA by 12%–31%, reducing RBF by 8%–12% compared to controls.[32] In addition, an increase in blood volume, which reduces RSNA by 25%, leads to a 17% increase in RBF.[33] It is also reported that rapid and physiological changes in sympathetic output affect RBF during normal daily activities.[6]

Routine activities such as sleeping or grooming have increased RSNA and concomitantly decreased RBF.[34] Furthermore, a small increase in heart rate and RSNA in unilaterally renal denervated rabbits showed considerable differences in the RBF of innervated and denervated kidneys. These findings suggested that RSNA changes in the physiological range affect RBF, so further research is needed to elucidate the role of renal nerves in the dynamic regulation of resting RBF.[23]

Sympathetic activity has two components: frequency and amplitude. The frequency shows baroreceptor modulation and central generation and the amplitude indicates the number of recruited nerves. Since various afferent stimuli can change these components, changes in the frequency or number of recruited nerves or multiple activation patterns can affect kidney function.[35,36] It is shown that dilatation of a pig's uterus reduced RBF by sympathetic nerves without altering blood pressure.[37] In Mancia *et al.*'s experiments, RBF decreased by 8%, 15%, and 19% in the three states of confrontation; without movement, forelimb movement, and hind limb and forelimb movement, respectively.[38] An experiment on conscious baboons demonstrated that RBF decreased in response to psychological stress.[39] Another study found that acute psychological stress in conscious monkeys reduced RBF by increasing RSNA.[40] Furthermore, RSNA increases and RBF decreases in moderate heat stress.[41-43]

RBF decreases in response to a slight increase in RSNA, but whether RBF increases in response to a slight decrease in RSNA is ambiguous. In the alert rabbits, an increase in plasma volume caused a moderate reduction in RSNA (by 25%) and a significant rise in RBF. In contrast, this response was not obtained in the renal denervated animals.[33]

RBF in the cortex and medulla was also decreased after the electrical stimulation of the sympathetic nerves.[44] Stimulation of the renal sympathetic nerve creates a different pattern in medullary perfusion and renal cortex, attributed to the less sensitive medulla in the anesthetized rat.[45] In rabbits, activation of the renal sympathetic nerves resulted in a greater increase in RBF and cortical perfusion than in medullary perfusion.[46,47] They were similar at each stimulation level of perfusion changes in the inner and outer medulla.[48]

In humans, renal function is measured in response to stimuli related to RSNA change instead of direct RSNA assay, while it is impossible to measure RSNA directly.[35] Psychological stress increases the activity of the sympathetic muscle nerve by up to 30% and decreases cortical blood flow by up to 36%.[49] Submerging in water and neck suction increases RBF due to decreased RSNA levels.[50,51] To sum up, it is clear that the stimulation of the sympathetic nerves of the kidney reduces RBF, and many studies proposed that the alterations in RSNA induced by natural behavioral activities had a remarkable effect on RBF [Table 1].

RENAL SYMPATHETIC DENERVATION AND RENAL BLOOD FLOW

The RSDN is performed to determine the nonneurological effects on the kidney. In this case, either the response is weak and difficult to measure or there is no response at all. Studies indicated that RBF increases in alert and resting animals after renal denervation, so RSNA is responsible for supplying the tonic level of renal vasoconstriction.[31,32] Furthermore, GFR was increased in patients with refractory hypertension with bilateral renal denervation.[53] In contrast, there was no difference in RBF between innervated and denervated kidneys in alert and resting rats.[34] Similarly, in anesthetized rats during the 1st h after unilateral renal denervation, no difference in RBF was observed in the denervated and innervated kidneys.[3] Such findings were also detected in rabbits on days 14–21[54] or after 7 weeks.[30] Similarly, there was no change in RBF after administrating an adrenergic blocker (dibenamine) to relaxed and stress-free state patients.[55] In general, the effect of RSNA on RBF differs in anxiety and pathophysiological conditions from calm and restful conditions. Anxiety and pathophysiological conditions reduce RBF, but in calm conditions, there is a slight tonic effect on RBF.[48] The tonic result of basal RSNA on RBF seems to be negligible, and acute surgical denervation has little impact on renal hemodynamics.[48] Overall, the basal renal nerve activity does not affect renal hemodynamics; for example, it is specified that in alert dogs and humans, renal denervation with medication or surgery does not affect RBF,[56,57] and in nondiuretic rats after acute unilateral denervation, renal plasma flow (RPF) remains unchanged in the kidneys.[58]

Table 1: The effect of renal sympathetic nerve activity on renal blood flow

RSNA in animal or human	RBF	Reference
Anesthetized cat	Decrease	[25]
Anesthetized dog	Decrease	[26]
Conscious dog	Decrease	[28,29]
Conscious rat	Decrease	[31,34]
Conscious rabbit	Decrease	[32]
Anesthetized pig	Decrease	[37]
Conscious cat	Decrease	[38]
Conscious baboon	Decrease	[39,42]
Anesthetized rat	Decrease	[43,52]
Conscious monkey	Decrease	[40]
Human	Decrease	[49]

RSNA=Renal sympathetic nerve activity; RBF=Renal blood flow

All stimuli that significantly reduced RBF in renal innervated rabbits, such as air-jet stress, hypoxia, or noise stress, failed to elicit an RBF response after renal denervation.[32] Similarly, following baroreflex alteration of RSNA, the response of RBF was significantly altered in response to change in arterial pressure after administering a calcium antagonist or an Ang II antagonist following renal denervation in rats.[59] In the same way, in conscious cats, RBF responses to confrontation following renal denervation were eliminated.[38] Other studies have shown that acute denervation causes diuresis and natriuresis in anesthetized dogs and rats without significantly affecting renal hemodynamics parameters.[58,60,61] No alteration in RBF was reported with renal denervation performed on unconscious pigs[62] and cats[63,64] and no difference was observed in anesthetized monkeys in renal excretory function after renal denervation.[65] However, in conscious baboons, RBF responses to psychological stress following renal denervation were persisted.[39] The impact of RSDN on RBF at different times after RSDN in patients with resistant hypertension indicated a 20% increase in total blood flow per cardiac cycle and a significant decrease in blood pressure, without any changes in RBF.[66] It is also stated that under normal sympathetic tone, the sympathetic nerve fibers of the kidney have little effect on the dynamic auto-regulation of renal vascular tone and, consequently, on RBF.[3] In a study on a pig model, RBF increased acutely after RSDN and remained at the same acute peak even after a month, while RBF reserve remained lower, and based on these observations, it can be concluded that such changes in RBF parameters can be a valuable biomarker for successful denervation.[67] Hemodynamic measurements in renal arteries of healthy pigs after RSDN, immediately, 3 weeks, and 3 months after RSDN indicated that RBF at rest propends to increment.[68] This results agree with relative increase in RBF after renal denervation in dogs.[69] However, as contradictory results in this regard, in anesthetized nondiuretic rats, RBF and GFR remained unchanged after denervation.[58] Furthermore, some studies have reported that renal basal sympathetic nerve resection in normal dogs and rats does not affect RBF.[70,71] A study on rats determined the regional blood flow in the cortex and medulla of the left kidney, and they did not observe a significant effect on intracortical blood distribution after renal denervation,[72] However, acute unilateral renal denervation increased RBF and RPF without altering GFR. In general, renal denervation did not affect intracortical blood flow distribution and renal hemodynamics.[72] Otherwise, it is suggested that renal denervation causes a rapid (approximately 25%) increase in cortical perfusion in anesthetized rats.[73] In hypertension and congestive HF (CHF) rat model, RSDN increased basal RBF.[70] However, in Sprague Dawley rats (SD), RSDN did not affect RBF.[3] These disagreements may be due to differences among animal species or the RSDN method.[30]

It has been reported that renal denervation does not significantly alter arterial pressure in spontaneously hypertensive rats (SHR) over a short period of 1 h, despite interfering with intrarenal function (such as increasing RBF, dynamic autoregulation of RBF, and variability of RBF).[74] Meanwhile, despite causing systemic hypotension, RSDN does not affect perfusion and renal function at various intervals (directly and after 3 months) and does not alter RBF in patients with hypertension.[75] Hence, it can be deduced that the effect of RSDN is negligible on acute or chronic renal perfusion.[75] However, a case report indicated that RSDN was associated with increased RPF.[76]

In Wistar Kyoto (WKY) and SHR, acute renal denervation under genetic control resulted in continuous diuresis and natriuresis in SHR and not in WKY, and there was no significant change in RBF.[77] Also, in SD and Munich-Wistar (MW) rats, similar to SHR, renal hemodynamics remained unchanged.[77] Acute denervation studies have shown a negligible tonic effect of renal efferent nerves on renal arteries in SHR, WKY, and SD-MW rats.[77] Strain differences have been identified between SHR and WKY in renal excretory response to acute unilateral renal RSDN.[77] Also, the effect of acute renal RSDN on RBF or GFR is not noticeable in normal adult rats in hydroponic, euvolemic, or volume-expanded conditions.[58,78] Table 2 shows the effect of RSDN on RBF in some studies models.

Overall, there is a degree of uncertainty in these studies. The reasons for the above inconsistent results are not specific, because the studies were performed either under anesthesia or consciously. Factors such as differences between animal species, the method of RSDN, the degree of RSNA required to impact on RBF, final evaluation of renal hemodynamics, and validation of renal denervation are factors that can be involved in these differences.[82] Studies in normal animals presented where basal RSNA was sub-vasoconstrictor, basal RBF and dynamic RBF auto-regulation were not altered by the elimination of basal RSNA by renal denervation.[70] Also, under a number of physiological and pathological circumstances, there may be a change in the functional participation of a1– ARs.[12] In the deoxycorticosterone acetate-salt (DOCA)-salt-hypertensive rats, a local change in the density of α1-ARs may be responsible for the increased responsiveness of the mesenteric vascular bed to α1-AR agonists, and Suzuki *et al*. discovered that the mesenteric vasculature of DOCA-salt hypertensive rats had increased α1-AR density and affinity.[83] Compared to normotensive WKY rats, SHR rats showed enhanced affinity of the small mesenteric artery α1–AR.[84] Both Dahl salt-sensitive rats and SHR rats showed higher renal densities of α1-AR and α2–AR.[85] Additional research in various salt-related hypertension animal models has shown that a local change in the α1-AR density may be the cause of the increased

Table 2: he effect of renal sympathetic denervation on renal blood flow

RSDN	Model	RBF	References
Transmission blocking drug (xylocaine)	Conscious rat	Increase	[31]
Bilateral	Conscious rabbit	Increase	[32]
Bilateral	Conscious sheep	Increase	[79]
Acute and chronic	Anesthetized rat	No change	[48]
-	Anesthetized rat	Increase (cortical RBF)	[73]
Chronic (14–21 days)	Rabbit	No change	[54]
Chronic (7 weeks)	Rabbit	No change	[30]
-	Conscious rat	No change	[34]
Adrenergic blocking drug (dibenamine)	Human unstressed	No change	[55]
Adrenergic blocking drug (dibenamine)	Anxious human	Increase	[55]
Surgical or pharmacological	Conscious dogs and humans	No change	[56,57]
Acute unilateral	Nondiuretic rats	No change	[58]
-	Rats	No change	[59]
-	Conscious cats	No change	[38]
Unilateral	Anesthetized rats and dogs	No change	[58,60,61]
Acute	Anesthetized pigs	No change	[62]
-	Cat	No change	[63,64]
Chronic bilateral	Anesthetized monkeys	No change	[65]
-	Conscious baboons	No change	[39]
Acute unilateral	Rat	Increase	[72]
-	Hypertensive patients	No change	[66]
Acute	Rat	No change	[3,77]
-	Porcine model	Increase	[67]
Chronic	Pig	Increase	[68]
-	Normal dog	No change	[70,71]
Acute	Hypertensive rats	Increase	[70,74]
Acute	Congestive heart failure rat	Increase	[70]
Acute	Spontaneously hypertensive rats	No change	[77]
Acute	Wistar-Kyoto genetic control rats	No change	[77]
Chronic	Normotensive rats (Sprague–Dawley strains)	No change	[80]
Acute	Volume-expanded Rat	No change	[78]
Acute	Hydropenic, euvolemic rat	No change	[58]
-	Pacing-induced heart failure rabbit	Increase	[81]
Chronic	Resistant hypertension patient	No change	[75]

RSDN=Renal sympathetic denervation; RBF=Renal blood flow

reactivity of the vasculature to catecholamine.[86] The neurovascular transduction mechanisms may vary as a result of these variations in vascular beds' sensitivity.[86] Aging modifies the distribution of the vascular α1-AR subtype in humans, which differs from animal models, changes with vessel bed.[87] These discoveries provide possible new therapeutic targets that might be used in a variety of clinical scenarios.

THE SYMPATHETIC NERVOUS ACTIVITY IN PATHOLOGICAL CONDITIONS

Overactive sympathetic nerves are linked to hypertension and numerous cardiometabolic disorders, but the underlying mechanisms are poorly understood.[88] Sympathetic hyperactivity is associated with decreased GFR, RBF, and salt excretion, and this might affect systemic blood pressure. Renal denervation has been demonstrated to be an effective therapeutic method for lowering blood pressure. The relationship between renal sympathetic nerves and the pathophysiology of hypertension, HF, and chronic kidney disease has been highlighted.[89] Based on these phenomena, renal denervation helps lower blood pressure and may be used to treat insulin resistance,[90] obesity-related hypertension,[91] HF,[92] chronic kidney disease,[93] metabolic syndrome,[94] diabetes,[95] and obstructive sleep apnea.[96]

Hypertension

Sympathetic hyperactivity is a common trait in both human and animal models of hypertension. When compared to normotensive people, RSNA in hypertension patients is twice.[97] However, Gattone *et al*. demonstrated that renal damage is mitigated by sympathetic function suppression irrespective of systemic hypertension.[98] Antiadrenergic, diuretics, Ang-converting enzyme inhibitors (ACEi), AngII

receptor blockers (ARBs), calcium-channel blockers, and anti-renin medicines are just a few of the many efficient anti-hypertensive medications that are now on the market. However a significant portion of individuals with essential hypertension are drug-resistant, meaning they are unable to lower their blood pressure despite taking three separate antihypertensive medications at the recommended dose.[99] Renal denervation is a therapeutic option for severe resistant hypertension patients.[100,101] The rise in blood pressure was reduced in the DOCA-salt rat model of hypertension by surgically ablate both efferent and afferent renal neurons.[102] The afferent renal nerve activity in the clipped kidney was increased in the two-kidney-one-clip (2K1C) mouse and rat models, while afferent renal denervation (ARDN) and total renal denervation (TRDN) attenuated the increase in blood pressure.[103,104] The expression of Ang II receptors was assessed in both kidneys of the 2K1C rat model, and the results revealed a significant up-regulation of Ang II receptor mRNA in the clipped kidney; while, renal denervation led to a normalization of their expression in the ischemic kidneys.[105] TRDN reduced the rise of blood pressure during the emerging stage of hypertension in stroke-prone SHR (SHRSP), but such finding was not seen by ARDN.[106] It seems that the suppression of the development of hypertension in SHRSP is a result of the denervation of efferent renal nerves.[106] RSDN is helpful in the pathophysiological circumstances of sympathetically driven hypertension, such as obesity-related hypertension.[107] RSDN, lowered renin production and enhanced RBF in individuals with essential hypertension, indicating that the efferent renal nerves had been successfully targeted.[76] RSDN does not necessarily have antihypertensive effects in several animal models, such as Ang II salt-induced hypertensive rats, Wistar rats, and dogs whose hypertension was brought on by chronic nitric oxide (NO) synthase suppression.[108-110] Both ARDN and TRDN were unable to reduce blood pressure elevation in Ang II or high salt diet-induced hypertensive rats (AngII-salt rats).[111] AngII-salt rats show continually high blood AngII levels despite sympathetic nerve activity and vascular disorders such as arteriosclerosis, endothelial dysfunction, and impairment of vasodilator response to sympathetic suppression.[111] It seems that, RSDN may not lower blood pressure even if it lowers the sympathetic outflow from the brain. In addition, RSDN may be inefficient in lowering blood pressure in the presence of pathophysiological factors linked to the development of vascular diseases, such as advanced age and isolated systolic hypertension.[111] RSDN may be useful in treating certain types of hypertension and offers the potential for more individualized disease management.[112]

Heart failure

Sympathoexcitation is a feature of chronic HF, especially in the heart and kidneys.[113] Renal vasoconstriction, reduced RBF, increased water and salt reabsorption, and renal fibrosis are all brought on by increased RSNA.[114] Following stimulation of the sympathetic nerves that innervate the vasculature, the vasculature (macro-and microcirculations) is susceptible to endothelial cell malfunction, smooth muscle cell hypertrophy, and vasoconstriction. The release of renin from the kidneys, activation of the RAAS, and renal damage are all further enhanced by increased RSNA. RSNA causes pathological changes in the kidneys, which increase blood volume, cause tissue edema, and cause systemic vasoconstriction through Ang II to considerably worsen HF.[115] The success of neuro-hormonal modulators, including beta-blockers, ACEi, ARBs, aldosterone antagonists, diuretics, and neprilysin inhibition, as standards of care to treat CHF is a testimony to the substantial role the SNS plays in worsening HF severity.[116-119] Despite the fact that these pharmacotherapies have been effective in lowering morbidity and early death, pharmacotherapy resistance, unintended side effects, and patient nonadherence to medication regimens[120,121] continue to aggravate HF symptoms over time. Therefore, there is still a clinical unmet need for supplemental or alternative therapy approaches to treat HF. In animal model studies of the CHF, it was found that acute renal denervation in anesthetized rats, increased RBF,[70] so it can be concluded that the renal nerves may apply a tonic vasoconstrictive function in CHF.[122]

DiBona and Sawin investigated the tonic effect of basal RSNA on dynamic autoregulation of RBF in rats, and found that, RSDN increased basal RBF in CHF and SHR but not in SD and WKY rats[70] and notably ameliorated auto-regulation of RBF.[70] In the pacing-induced HF model in rabbits, decreased RBF, increased RVR, increased expression of Ang II receptor type 1 (AT1), and decreased expression of Ang II type II receptor (AT2) in renal cortical arteries, was specified.[81] These alterations were stopped by RSDN before induction of HF. Principally, the results of these animal studies cleared that the activity of renal sympathetic nerves has a deleterious effect on RBF and can be associated with changes in the expression of Ang II receptors so that renal denervation may be effective in the treatment of CHF.[92]

HF is linked to sleep apnea,[123] and RSDN counteracted the decrease of renal hypoperfusion during apnea and the activation of the RAAS in the kidney.[124] An improvement in sodium excretion, an increase in cardiac output, and an improvement in RBF mediating unfavorable responses were all seen in animal models of RSDN after myocardial infarction.[125,126]

Kidney diseases

Another research used an ovine model of hypertensive chronic kidney disease to show the efficacy of RSDN. In

comparison to sham controls animal, the hypertensive CKD accompanied with RSDN showed larger improvements in GFR, RBF, and albuminuria 5 months after the ablation.[127] Furthermore, RSDN recovered estimated GFR (eGFR) by changes of intrarenal hemodynamics in CKD patients.[128,129] The eGFR assessments could help to evaluate the exact renal functions.[130]

It has been shown that ischemic acute kidney injury changes renal hemodynamics and is associated with endothelial cell dysfunction brought on by an increase in the formation of reactive oxygen species, which reduces NO availability.[131] Numerous physiological functions of NO in the kidney include the control of RSNA.[132] By reducing NO synthesis may directly increase sympathetic nervous system activity in CKD patients.[133] The glomerular microvasculature becomes more constricted as a result of NO production inhibition and proximal tubular reabsorption decreases.[133] RSDN treatment has stopped these effects.[134] However, RSDN may not be suitable for lowering blood pressure in patients with polycystic kidney disease.[135]

Renal denervation and future challenge

Despite new data demonstrating the large benefits of RSDN, there are still numerous unsolved problems, including responder identification, procedural guidance, effects persistence, and the applicability of clinical outcomes. The identification of responders is a particularly important subject matter. The hypertensive patients who had a baseline plasma renin activity > 0.65 ng/ml/h or a baseline heart rate > 73.5 bpm were more sensitive to RSDN.[136,137] The preference of patients for RSDN is another crucial feature that has to be taken into account in addition to the identification of responders. A nationwide web survey in Japan revealed that the presence of side effects while taking antihypertensive medications, younger patient age, male sex, higher systolic blood pressure (at home or at the office), and poor antihypertensive drug adherence were all significant predictors of preference for RSDN.[138] This should be considered while deciding on a course of antihypertensive treatment. Finally, it is debatable whether renal nerve regeneration impacts the long-term responses to renal denervation. The re-innervation of the renal nerves may start in humans as early as 28 days.[139] Similar events were seen in dogs where, 3–6 months after transplantation, renal autografts were re-innervated.[139] On the basis of enough data, it is envisaged that the therapeutic use of RSDN would proceed completely.

CONCLUSION

Several afferent and central pathways are involved in inducing an increase in RSNA, all of which result in a significant reduction in RBF that is proportional to the increase in RSNA. Without renal nerves, the response to stimuli is minimal or absent. Based on experiments, the effect of RSDN on RBF varies. The dynamic impact of renal nerves on RBF enables using RBF dynamic criteria as a biomarker in renal denervation therapy.

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Conflicts of interest

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REVIEW ARTICLE

Diabetes and diabesity in the view of proteomics, drug, and plant-derived remedies

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Diabetes and obesity are highly prevalent in the world. Proteomics is a promising approach to better understanding enzymes, proteins, and signaling molecules involved in diabetes processes which help recognize the basis of the disease better and find suitable new treatments. This study aimed to summarize the molecular mechanisms from the beginning of insulin secretion in response to stimuli to the pathology of the insulin signaling pathway and, finally, the mechanisms of drugs/chemicals remedies that affect this process. The titles and subtitles of this process were determined, and then for each of them, the articles searched in PubMed and ScienceDirect were used. This review article starts the discussion with the molecular basis of insulin biosynthesis, secretion, insulin's mechanism of action, and molecular aspect of diabetes and diabesity (a new term showing the relation between diabetes and obesity) and ends with the drug and plant-derived intervention for hyperglycemia.

Key words: Diabesity, diabetes, metabolomics, signal transduction

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METABOLOMICS IN INSULIN SECRETION AND EFFECT

Insulin biosynthesis

In the pancreas, β-cells are the only cells committed to transcribing the insulin gene that may be replaced during β-cells injury by γ-cells.[1] In contrast, the insulin receptors are widely distributed even on cells that are not known as insulin responsive.[2] Human insulin is synthesized as a preproinsulin peptide, which is processed to proinsulin and then to insulin (consisting of A and B chains with a total of 51 amino acids) by the effect of endopeptidases. Insulin gene expression is regulated by some nutrients and insulin itself. Several transcription factors bind to numerous sequences in the promoter region of the insulin gene for regulating the expression of insulin, among them pancreatic and duodenal homeobox-1(PDX-1), MafA, (Mast cell function-associated antigen), and B-2/neurogenic differentiation 1 are the famous ones.[3]

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Molecular mechanism of insulin secretion

The blood glucose level is regulated by the opposite action of insulin and glucagon within a narrow range.[4] Elevation of blood glucose after a meal stimulates β-cells to increase insulin secretion. In contrast, α-cells secrete glucagon when the blood glucose is low, thereby increasing gluconeogenesis, glycogenolysis, and blood glucose. Between meals, the reduction of blood glucose triggers the release of norepinephrine and neuropeptide galanin from the sympathetic nerves resulting in increasing glucagon secretion and inhibiting insulin secretion.[5] During a meal, the secretion of acetylcholine and the pituitary adenylate cyclase-activating polypeptide, vasoactive intestinal polypeptide, glucagons like peptide 1 (GLP-1), and gastric insulinotropic polypeptide (GIP) which potentiate glucose-induced insulin secretion.[6]

The effectors that modulate insulin secretion are categorized as initiators, potentiators, and inhibitors.

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Glucose, some amino acids, and fatty acids are the most famous initiators.[7] As an initiator of insulin secretion, arginine increases intracellular calcium [Ca2+]i through a ATP-sensitive K + channel-independent pathway. Only when there is an initiator, do the potentiators enhance insulin secretion.[8]

Glucose transporter 2 (GLUT2) and glucokinase (GK) are two glucose sensors in β-cells. When glucose enters the β-cell via GLUT2, it is phosphorylated by GK and trapped inside the cells. GLUT2 gene expression is increased in diabetes, indicating the importance of β-cell responses. The kinetics properties of GK (like low Km) make it a delicate sensor of glucose.[9,10] Another biochemical property of β-cells is the low levels of lactate dehydrogenase (LDH), which causes levels of NADH to remain high and ultimately increases insulin secretion. That is why pharmacological inhibition of LDH increases NADH levels and stimulates mitochondrial shuttles which ultimately leads to insulin secretion.[11,12]

K + ATP-independent pathways of insulin secretion involve Krebs cycle intermediates (anaplerosis), perhaps via malonyl-CoA. Moreover, insulin release is correlated with citrate and malate.[13] Elevated citrate and a-ketoglutarate trigger the release of calcium-independent insulin secretion, indicating the importance of anaplerosis on the stimulation of β-cells.[13,14] The β-cell resting membrane potential is largely defined by ATP-sensitive K + channels (KATP). As ATP/ADP ratio increases due to glucose metabolism, KATP is closed which leads to depolarization of the cell membrane and the opening of the voltage-dependent L-type Ca2+ channels. This leads to the elevation of [Ca2+]i and the movement of insulin-containing granules toward the plasma membrane [Figure 1].[15,16] Calcium activates calmodulin-dependent protein kinases, which phosphorylate a series of proteins such as myosin light chain and result in insulin secretion.[17]

β-cells express N-, P/Q-, and L-type Ca2+ channels. The earlier one plays a significant role in Ca2+ influx. The L-type channels open if there is a depolarization signal and then inactivate slowly. Inside the cell, calcium ions as a feedback effector can close L-type channels and prevent further calcium entry.[15,18]

The time required for exocytosis of insulin-containing granules is much less than the time required for calcium distribution in the cytoplasm after the opening of calcium channels, indicating that the granules are close to calcium channels and are sensitive to local changes in calcium concentration. Beta cells may contain thousands of secretory granules, but only a tiny number is available for immediate release which is known as the readily releasable pool (RRP). The rest of the granules that are known as reserve pools must be moved to RRP before discharge. The RRP is absent in type 2 diabetes.[19-21] The number of released granules is dependent on the activation of protein kinase C, which phosphorylates the exocytotic proteins such as Mammalian uncoordinated protein (Munc), a protein associated with secretory granules. Any decrease

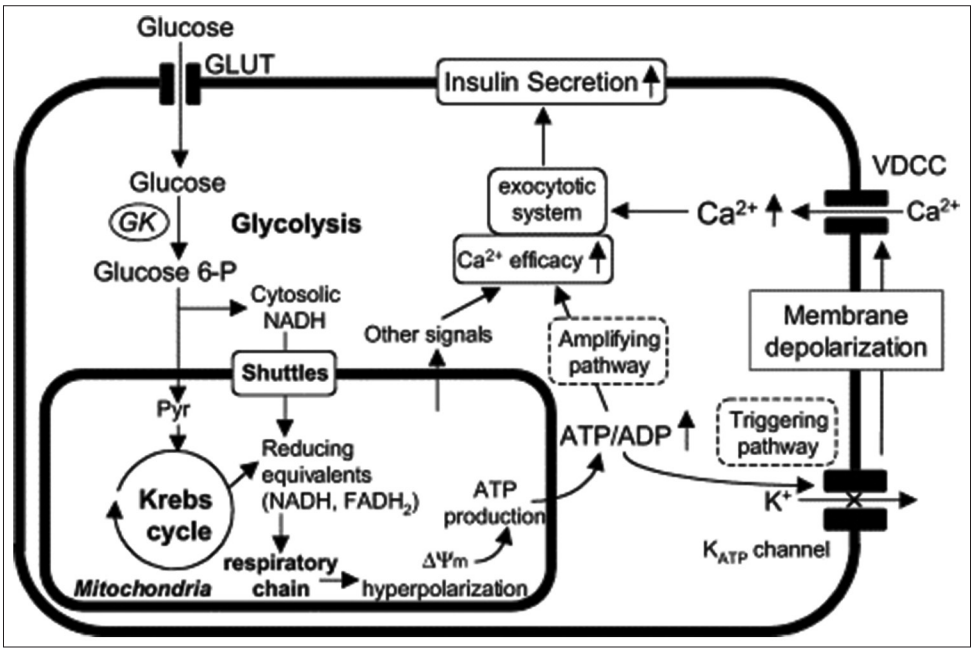

Figure 1: Mechanism of insulin secretion by the cytosolic ATP/ADP ratio (adapted from reference 16). Insulin secretin processes start from entering glucose into β-cells which results in increasing ATP production. As ATP/ADP ratio increases due to glucose metabolism, ATP-sensitive K+ channels are closed which leads to the depolarization of the cell membrane and the opening of the voltage-dependent Ca2+ channels, leading to the elevation of intracellular calcium and movement of insulin-containing granules toward the plasma membrane. GLUT: Glucose transporter 2, VDCC: voltage-gated calcium channel

in Munc production in the cells results in decreased insulin secretion.[17,21,22]

Therefore, glucose-stimulated insulin secretion is biphasic. In the first phase, previously synthesized insulin-containing membrane-docked granules are released from the RRP store triggered by Ca2+ influx, and reach a maximum level after 5-10 min, and is followed by a developing second phase consisting of the release of granules from the reserved pool. Type 2 diabetes patients have problems mainly with first-phase insulin secretion, but second-phase insulin secretion is also affected.[23,24] Although the exact mechanism by which vesicles are transported to the membrane is unclear, kinesin appears to be involved as a protein motor.[25] While inhibition of class IA PI3K (Class IA phosphatidylinositol-3-kinase) decreases insulin secretion,[26] others reported acute inhibition of class IA PI3K enhances glucose-induced insulin secretion.[27]

Insulin's mechanism of action

The main function of insulin is to regulate blood sugar. Insulin is transported through the portal vein to the liver where it reduces glucose release, increases glucose storage and lipogenesis,[28,29] intensifies the transport of amino acids into the cell, and inhibits lipolysis. Insulin affects the expression of several genes and stimulates DNA replication, causing cell proliferation and growth. Glucose enters the cell through glucose transporters (GLUTs) in the cell membrane. GLUT1 is found in most cells. GLUT2 is located in the liver and beta cells, GLUT3 in the brain, and GLUT4 in skeletal muscle, heart, and adipose tissue.[30] In hepatocytes, glucose uptake is greatly increased by activation of glycolytic enzymes (GK, phosphofructokinase 1, and pyruvate kinase) through activation of protein phosphatase and inhibition of protein kinase A. Glucose 6-phosphatase activity is also reduced. The final result of these processes is a decrease in blood sugar and an increase in the glucose content of the liver.[31] In addition, activation of phosphatase and reduction of cAMP levels leads to increased glycogen synthase activity and decreased glycogen phosphorylase activity, with a net consequence of increased glycogen synthesis.[32] Insulin emerges all of the effects through binding to its receptor and consequent activation of several signal molecules. Activation of insulin receptor substrates (IRSs) results in the activation of PI3K which in turn, phosphorylates membrane phospholipids (phosphatidylinositol 4,5 phosphate, PIP2), and produce phosphatidylinositol 3,4,5 triphosphate (PIP3) which activates protein kinase B (PKB, also called Akt), PIP3-dependent kinase (PDK), PKC (principally PKC-λ), and small ribosomal subunit protein 6 kinase (S6K).[32,33]

Second, activation of PKB and PKC-λ leads to displacement of GLUT4 to the cell membrane.[34] Furthermore, activated PKB results in the phosphorylation of glycogen synthase kinase-3 (GSK3), which is a pivotal regulatory molecule of glycogen metabolism.[35] Insulin also exerts its effects by regulating gene expression, mainly through sterol-regulated element-binding protein (SREBP).[36] SREBP increases GK, pyruvate kinase, lipoprotein lipase (LPL), fatty acid synthase, and acetyl-CoA carboxylase and decreases G6Pase, F1,6Pase, and PEPCK activity.[31,37,38]

METABOLOMICS IN DIABETES

Diabetes classification

Diabetes mellitus is a syndrome with numerous symptoms and causes. Based on recently provided guidelines by the American Diabetes Association, four main forms of diabetes mellitus exist, type 1 diabetes (autoimmune diabetes), formerly known as insulin-dependent or juvenile-onset diabetes, type 2 diabetes (due to insulin resistance), formerly known as noninsulin-dependent diabetes, gestational diabetes mellitus, other types of diabetes due to various causes (i.e., monogenic and drug or chemical induced diabetes). Despite previous perceptions, type 1 and type 2 diabetes are seen in both children and adults. Nowadays, the traditional classification of diabetes is no longer valid because diabetes type 1 and 2 are found in both adults and children.[39] Another rarely found diabetes is Brittle diabetes. It is defined by unexplained fluctuation between hyperglycemia and hypoglycemia and recurrent diabetic ketoacidosis.[40]

Molecular aspects of type 2 diabetes

Recent research revealed some genetics and epigenetics factors involved in the pathogenesis of type 2 diabetes. Some monogenic loci are known to be associated with type 2 diabetes, but none of them are the main cause of the disease (i.e., >50% in all cases). The most important genes that are involved in the progression of diabetes type 2 are GLUT-2, HNF4α,[41] pancreatic GK (MODY 2), preproinsulin gene (INS), and peroxisome proliferator-activated receptor γ (PPARγ).[42-44] Recent evidence has proposed a role for a ligand-gated transcription factor named PPARγ in the etiology of type 2 diabetes.[45] When activated, PPARγ binds to another transcription factor, retinoid X receptor. After dimerization, a specific set of insulin-sensitive genes in adipose tissue such as LPL, fatty acid transporter protein (FATP), fatty acyl CoA synthase, and glucose transporter 4 (GLUT4), become activated [Figure 2]. Thiazolidinediones (TZDs) as hypoglycemic agents and PPARγ ligand increase the sensitivity of the body to insulin. Thus, TZDs provide a new way of treating insulin resistance.[46,47] Mutations in the PPARγ gene seem to be related to insulin resistance,[43] adipocyte hypertrophy, and hepatic steatosis.[48]

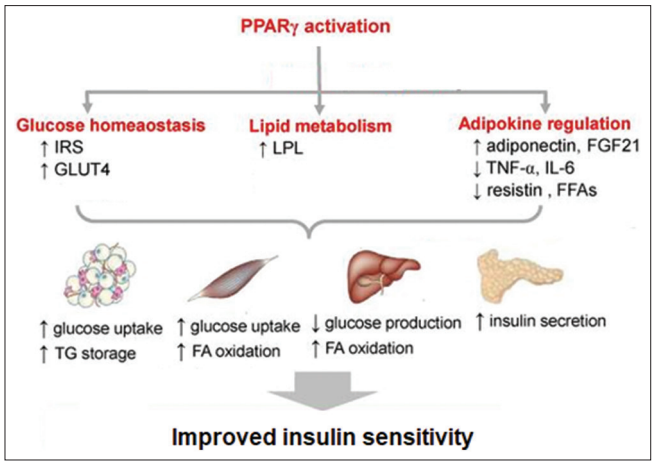

Figure 2: Mechanisms of actions of peroxisome proliferator-activated receptor (PPAR) γ ligands in glucose and lipid metabolism resulting in improved insulin sensitivity, adapted from reference 49. The activation of PPAR affects the gene expression of three different pathways. It increases IRS and glucose transporter 4 in glucose metabolism, increases lipoprotein lipase in fat metabolism and increases adiponectin, and decreases tumor necrosis factor-alpha. The set of these effects in important metabolic tissues such as fat, muscle, and liver leads to an increase in glucose uptake and consumption and glucose tolerance. PPAR: Proliferator-activated receptor, IRS: insulin receptor substrate, GLUT4: Glucose transporter 4, LPL: Lipoprotein lipase, FGF21: Fibroblast growth factor 21, TNF: Tumor necrosis factor, IL-6: Interleukin-6

Another important molecule involved in the regulation of lipid metabolism in the liver is PPARα, which regulates the expressions of enzymes of fatty acid metabolism such as fatty acid transport proteins (FATPs), carnitine palmitoyl transferases, acyl-CoA oxidase, and apolipoprotein A-I.[49] Therefore, PPARα agonists (pemafibrate) improve hyperlipidemia (hypertriglyceridemia) in high fructose-fed rats.[50] Furthermore, it has been postulated that activation of PPARα can improve insulin resistance.[51]

In pancreatic β-cells, the glucose-sensing system consists of GLUT2 and GK.[52] The GK gene contains two different promoters for the expression of tissue-specific GKs in the liver and β-cells. Both GLUT2 and GK sense the oscillation of blood glucose levels. When glucose enters the cells via GLUT2 is phosphorylated by GK and trapped in the cells. GK is a key enzyme in glycolysis, and GLUT2 plays an important role in the equilibration of glucose inside and outside the cells.[53]

Epigenetics as a new molecular approach helps scientists to link genetics, environmental factors, and diseases. Epigenetics processes such as DNA methylations, histone modifications, and microRNAs make changes in gene functions, not necessarily changes in the nucleotide sequence, that may be inherited by the next generation. For example, infants born from mothers with gestational diabetes represent hypermethylation and epigenetic downregulation of IGF2 gene, which affects insulin sensitivity. Epigenetic mechanisms were found to affect genes involved in insulin resistance such as GLP-1 receptor. However, much more studies are necessary to fully understand epigenetic mechanisms in the pathogenesis of type 2 diabetes.[41]

Diabesity (diabetes + obesity)

The simultaneous increase in the prevalence of obesity and insulin resistance as a major component of metabolic syndrome and diabetes type 2 encouraged the scientists to coin a new term expressing the relationship between diabetes and obesity, diabesity. Obesity and type 2 diabetes are spreading epidemically, and the number of people diagnosed with diabetes has increased by about six times in the last 40 years. Type 2 diabetes is complex because it is a multifactorial disease related to several pathological factors such as high blood levels of triglycerides, obesity, impaired glucose tolerance, and insulin resistance, all of which are referred to as metabolic syndrome (insulin resistance syndrome).[54-56] However, although most individuals with type 2 diabetes are obese, obesity alone does not always provide a route to insulin resistance because some obese persons do not have insulin resistance and vice versa, suggesting the role of other factors in insulin resistance.[55] The hallmarks of almost all metabolic syndromes include obesity, insulin resistance, low high-density lipoprotein cholesterol (HDL-C), dyslipidemia, and high blood pressure. Evidence suggests that metabolic syndrome starts in the early years of life and spreads from childhood to adulthood, leading to type 2 diabetes. Inflammatory processes are believed to link obesity and insulin resistance, known as the inflammation hypothesis. For example, chemokines and interleukin 6 (IL-6) production released from adipose tissues trigger insulin resistance.[55] Furthermore, elevated plasma fatty acids reduce activation of IRS-1-linked PI-3K activity by insulin in skeletal muscle. Lipid-induced insulin resistance is linked to defects in the transport of GLUT4. Saturated fatty acids initiate metabolic inflammation through toll-like receptors and inflammasomes that lead eventually to increased production of pro-inflammatory cytokines. It is now believed that pro-inflammatory cytokines interfere with insulin signaling and insulin action in adipocytes and hepatocytes by activating numerous kinases.[56] The main factor increasing the prevalence of insulin resistance is diet and the resulting obesity. Nutrition, along with other factors such as physical activity, sleep, and mental health, should be considered in diabesity prevention.[57] It has previously been shown that saturated fats cause weight gain, hyperlipidemia, and insulin resistance. However, a low carbohydrate-high fat diet is more effective in comparison to a low-fat diet in reducing central fat,[58] indicating that focusing on fat alone is not enough. Recent studies suggest that consumption of refined carbohydrates especially fructose may increase the risk of insulin resistance.[59-61]

Fructose in diabetes and metabolic syndrome

Fructose consumption (in many food products), the prevalence of obesity, and related metabolic syndrome have simultaneously increased in the past four decades, indicating the causal effect of fructose on insulin resistance.[62] Fructose leads to several metabolic derangements, most importantly insulin resistance.[63] Fructose reduces the expression of GLUT4 gene, significantly increases hepatic triglyceride synthesis, impairs insulin signaling, and subsequently reduces insulin sensitivity.[59,64] Fructose reduces hepatic expression of IRS-2, increases plasma insulin levels, and causes an abnormal glucose tolerance test indicating disturbed hepatic insulin signaling.[65] Furthermore, phosphorylation of some members of the insulin signaling pathway (IRS1 and Akt) is reduced after feeding a fructose-rich diet presumably through increased activation of protein-tyrosine phosphatase 1B which leads to insulin resistance.[59,66] Moreover, increased free fatty acids in fructose-fed animals contribute to insulin resistance. If free fatty acids are not removed effectively, it can lead to increased triglyceride production.[65,66] Therefore, high fructose intake leads to visceral adiposity and weight gain. Fructose as a palatable food additive encourages overeating. Further, it is essential to know that fructose cannot efficiently suppress appetite, but instead increases ghrelin, known as the hunger hormone.[67]

The effect of chronic fructose consumption in adipogenesis performed by activating sterol regulatory element-binding protein 1c (SREBP1c), a potentiator of lipid synthesis. Fructose activates SREBP1c indirectly by induction of hyperinsulinemia.[68] Fructose also reduces PPARα expression in the liver cells.[69] Hence, decreased PPARα expression can result in reduced β-oxidation which was seen in insulin resistance.[70] There is also a close relationship between a high fructose diet and impaired vascular relaxation through induction of oxidative stress that may be the underlying mechanism for blood pressure.[71,72]

Liver in diabetes and insulin resistance status

Among several diabetic-related organ complications, the liver plays a major role in insulin resistance. Several epidemiological studies have reported an association between elevated aspartate transaminase (AST) and alanine aminotransferase (ALT) levels and diabetes type 2 and insulin resistance status.[73-75] AST and especially ALT may be valuable tools for diagnosis and prediction of diabetes type 2 and insulin resistance,[76-78] especially when considered along with gamma-glutamyltransferase (GGT) to improve the prediction of impaired fasting glucose.[79] It has been reported that changes in the ALT/AST ratio are parallel with changes in β-cell function and insulin sensitivity, providing a pathologic basis for the association of the aminotransferases with a higher risk of developing type 2 diabetes.[80] On the other hand, Liu *et al*. reported elevated ALT, AST, and GGT levels in nondiabetic but insulin-resistant adults, especially those who were obese, indicating the impact of obesity in this relationship.[81] Increased risk of diabetes incidence is correlated to nonalcoholic fatty liver disease (NAFLD) and circulating liver enzymes (AST, ALT, GGT, and alkaline phosphatase).[82] The relation between NAFLD and its advanced form of nonalcoholic steatohepatitis (NASH) can be explained by the lipotoxic state, which results in the necroinflammation of hepatocytes.[83]

Increased ALT activity even within the reference intervals correlates with increasing hepatic fat. Elevated hepatic aminotransferases indicate fat accumulation in the liver, as seen in NAFLD, a characteristic feature of insulin resistance syndrome.[84] NAFLD is defined as high lipid deposition in the liver parenchymal cells in patients without a history of high alcohol consumption.[85] There is a vicious circle between insulin resistance and inflammation, so that each condition accelerates the other to develop NAFLD. Regarding inflammatory processes, nuclear factor-kappa B (NF-κB) plays a transcriptional regulator in the expression of IL-6 and tumor necrosis factor-alpha (TNF-α), known as pro-inflammatory cytokines.[86] Inhibition of TNF-α receptor improves insulin resistance and ameliorates NAFLD.[87] Furthermore, as previously described, high fructose diet could lead to metabolic syndrome and insulin resistance. One possible mechanism may be triggering an inflammatory response by fructose feeding through stimulation of TNF-α production.[61] Mazzoli *et al*. showed that inflammation reversed after removing fructose from the diet,[88] indicating fructose-induced inflammatory processes that lead to liver injury and increasing circulating liver markers.

THERAPEUTIC INTERVENTION FOR HYPERGLYCEMIA

Persistent hyperglycemia is the major concern in insulin resistance and diabetes. For this reason, all treatment strategies aim to lower blood glucose. Many pharmacologic agents act through different mechanisms to normalize blood sugar. In this section, conventional drugs along with new hypoglycemic drug candidates, some of which with no risk of hypoglycemic shock, and plant-derived drugs will be discussed. Therapeutic agents and their proved/proposed mechanisms of action are summarized in Table 1.

Amino acid derivatives

Some amino acid derivatives have been studied in recent years with promising outcomes as new treatments for type 2 diabetes. Nateglinide, an o-phenylalanine derivative, is the most famous hypoglycemic agent with an amino acid backbone. Nateglinide increases blood insulin levels after a few minutes of oral administration.

Table 1: Therapeutic agents and their proved/proposed mechanisms of action

	Drug/chemical	Proposed mechanism (s)	Reference
Amino acid derivatives	Nateglinide	↑ glucose-dependent insulin secretion	[89]
	Agmatine	↑insulin secretion through secretion of endorphins	[91]
	4-hydroxyisoleucine	Insulinotropic↑GLUT4, expression of IRS-1, activates PI3-kinase, ↓ TNFα expression	[93-96]
PPARγ activators	TZDs	↑ expression of GLUT4, LPL, GK, fatty acyl-CoA synthase, and adiponectin	[97]
GLP-1 receptor agonists	TZDs	↑ insulin secretion, through upregulation of AMP-activated protein kinase	[102]
	Pioglitazone	↓ PEPCK, and G6Pase	[103,104]
	Lobeglitazone	↑ β-cell viability	[108]
SGLT2 inhibitors	Ertugliflozin, dapagliflozin, canagliflozin, and Empagliflozin	↓ glucose reabsorption, GLP-1	[99,110]
Dipeptidyl peptidase-IV inhibitors	Vildagliptin, sitagliptin, linagliptin, saxagliptin, alogliptin	↓ GLP-1 and GIP degradation	[114,115]
α-glucosidase inhibitors	Miglitol, acarbose, nicotinic acid, hydroxyproline	Pancreatic α-glucosidase competitive inhibition	[117-119]
Biguanides	Metformin	↓ gluconeogenesis through inhibition of glycerol-3-phosphate dehydrogenase, ↓ cyclic AMP downregulation of gluconeogenic genes, ↓ glucose uptake, ↑ expression and translocation of GLUT4	[123-125]
GLP-1 receptor agonists	Albiglutide, dulaglutide, exenatide, liraglutide, lixisenatide, dayexenatide	Improve glycemic control through activation of GLP-1	[128]
Drug candidates	Bromocriptine	through CNS, reduces insulin resistance and hepatic gluconeogenesis, ↓ IL-6 and leptin, ↑ PPAR-γ/adiponectin, and GLP-1	[130,131]
	Vanadium compounds	↑ GK activity, inhibition of PEPCK, in part, by nonselective inhibition of phosphotyrosine phosphatase	[99,133]
	Colesevelam (bile acid sequestrants)	↑ secretion of incretin	[134]

PPARγ=Peroxisome proliferator-activated receptor gamma; GULT 4=Glucose transporter 4; IRS-1=Insulin receptor substrate-1; PI3=Phosphatidylinositol-3; TNFα=Tumor necrosis factor-alpha; LPL=Lipoprotein lipase; GK=Glucokinase; AMP=Activated protein kinase; PEPCK=Phosphoenolpyruvate carboxykinase; GLP-1=Glucagons like peptide 1; IL-6=Interleukin 6; TZDs=Thiazolidinediones ;↑=Means increase; ↓=Mean decrease

Nateglinide binds to the sulfonylurea receptor in β-cells and increases insulin secretion by closing the K-ATP channels. Unlike sulfonylureas, nateglinide does not inhibit the opposite activity of glucagon, so its effect is without risk of hypoglycemia. It is essential to know that action of nateglinide is glucose-dependent. KATP channels' response to nateglinide is lower in euglycemia in comparison to hyperglycemia. Therefore nateglinide does not cause prolonged insulin release. This impedes the continuous secretion of insulin and protects β-cells from exhaustion. Recent research has shown that nateglinide affects the exocytosis of insulin-containing granules. This function is independent of its effect on the K-ATP channels. Therefore, nateglinide is effective not only in the first but also in the second phase of insulin secretion showing its great benefits in treating type 2 diabetic patients.[89]

Agmatine, a decarboxylated form of arginine, is another amino acid derivative that is under investigation for its hypoglycemic effect. It reduces blood sugar by increasing insulin secretion and glucose uptake through increased secretion of endorphins from the adrenal glands. This effect may be performed via activation of the imidazoline I2 receptor.[90] It also impedes the reduction of insulin signaling members in a high-fat diet, streptozotocin (STZ)-induced diabetic mice.[91]

Another amino acid derivative with hypoglycemic effects comes from the fenugreek seeds. In 1973 for the first time, Fowden *et al*. isolated and reported an unusual amino acid in the defatted seeds and identified it as 4-hydroxyisoleucine (4-OH-Ile).[92] Glucose-dependent insulinotropic effect of 4-OH-Ile was approved using isolated β-cells.[93] More importantly, it has been reported that the hypoglycemic effect of 4-OH-Ile is not limited to its insulinotropic effect. Haeri *et al*. showed that in multiple injected diabetic type 1 rats, 4-OH-Ile still is having a hypoglycemic impact without any increase in insulin recreation, indicating that 4-OH-Ile potentiates insulin signaling.[94] This possibility was reinforced by the provision of molecular evidence. It has been shown that 4-OH-Ile increases the number of GLUT4, downregulates the expression of TNF-α, stimulates the expression of IRS-1,[95] and activates PI3-kinase in the muscles of diabetic rats.[96] These pieces of evidence show that 4-OH-Ile has multiple mechanisms from insulinotropic to insulinomimetic actions.

Peroxisome proliferator-activated receptor γ activators

Activators of PPARγ exert their clinical benefits by activating several genes involved in fat and glucose metabolism. PPARγ responsive genes are present in three major tissues, adipose tissue, liver, and muscle which are involved in glucose regulation and fatty acid storage. PPARγ agonists

increase the expression of several genes including, GLUT4, LPL, GK, fatty acyl-CoA synthase, and adiponectin, thereby increasing glucose uptake and fatty acid oxidation, leading to improve insulin sensitivity.[97] Treating patients with pioglitazone, a PPARγ activator maintains β-cell function, increases HDL-C cholesterol, improves insulin sensitivity, and decreases glucose levels with no enhancement of endogenous insulin secretion.[98,99]

It has been reported that TZDs protect the β-cells from apoptosis through activation of AMP-activated protein kinase (AMPK) independent of PPARγ[100] and improve the glucose-sensing ability of β-cells via upregulation of GLUT2 and GK gene.[101] Furthermore, TZDs potentiate insulin secretion, mediated through upregulation of AMP-activated protein kinase,[102] indicating multiple sites of actions of TZDs. In the liver cell line, pioglitazone decreases PEPCK, and glucose-6-phosphatase and increases GK expressions, thereby reducing gluconeogenesis and increasing glycolysis.[103,104]

Besides the crucial beneficial effect of TZDs, there have been reports of their severe several side effects such as fractures, water retention, and weight gain.[105] Troglitazone, the first generation of TZDs, has been withdrawn from the market because of its potential hepatotoxicity.[106] Recently, some new PPARγ agonists have been introduced or are under investigation. Lobeglitazone as a new member of the TZDs family of antidiabetic drugs activates both PPARα and PPARγ with a lower effective dose and acceptable safety. In fat cells, it works as an insulin sensitizer to improve cell response to insulin.[107] In β-cell line (INS-1), lobeglitazone increases cell viability and improves hyperglycemia.[108] Reglitazar (also known as Reglixane) is the newest non-thiazolidinedione dual PPAR agonist (PPARα/γ) developed by Pfizer. It shows a potent capacity to lower triglycerides and blood glucose besides its ameliorating effect on diabetic complications, such as cataracts, nephropathy, and neuropathy.[109]

Sodium-glucose co-transporter type 2 inhibitors

Sodium-glucose co-transporter type 2 (SGLT2) is the predominant transporter of glucose found in the kidney, responsible for the reabsorption of glucose, whereas SGLT1 is expressed in the kidney and small intestine to pass glucose or galactose across the epithelial cells.[110] Recently discovered SGLT2 inhibitors (ertugliflozin, dapagliflozin, canagliflozin, and empagliflozin) through blocking glucose reabsorption lower the kidney threshold and increase excretion of glucose in the urine with a lower risk of hypoglycemia in comparison to other hypoglycemic agents. Desirable bioavailability and the need to use only one dose per day introduced them as a suitable choice to control hyperglycemia. However, these inhibitors are less effective in people with reduced kidney function (104 and 115). In addition, since SGLT1 is also expressed in the intestine, a dual-action inhibitor that inhibits both types 1 and 2 can be more effective. Comparing sotagliflozin as the first dual SGLT1/SGLT2 inhibitor to SGLT2 inhibitors showed greater glucosuria and glycemic control.[110] Sotagliflozin also increases GLP-1 which can help to reduce hyperglycemia.[99] Metformin has long been used for treating polycystic ovary syndrome.[111] Interestingly, other members of the dual SGLT1/SGLT2 inhibitors, licogliflozin, attenuate hyperinsulinemia, and androgen production in women with polycystic ovary syndrome.[112,113] These two hypoglycemic agents with different mechanisms of action but with similar effect on PCOS initiates some new hypothesis on the pathological basis of the disease.

Dipeptidyl-peptidase-4 inhibitors

Dipeptidyl-peptidase-4 (DPP4), a transmembrane peptidase, inactivates GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). Several DPP-4 inhibitors (vildagliptin, sitagliptin, linagliptin, and saxagliptin) and a new generation, alogliptin, are clinically available to treat diabetes type 2. DPP4 inhibitors reduce hyperglycemia by impeding GLP-1 and GIP degradation. This results in increased insulin secretion, delayed gastric emptying, and decreased glucagon secretion, thereby reducing blood sugar.[114,115] Recent researches show a pathological role for DPP4 in lung diseases, especially COVID-19, which is believed to have a role in disease progression. Therefore, DPP4 inhibitors may have a beneficial effect in treating DPP4-related lung diseases.[116]

Alpha-glucosidase inhibitors

Miglitol and acarbose are the most known α-glucosidase competitive inhibitors that impede hyperglycemia by inhibiting pancreatic α-glucosidase in the intestine. By inhibiting α-glucosidase, glucose production in the intestine is reduced, leading to glycemic control.[117] Nowadays, many studies are performed to find more potent and tolerable α-glucosidase inhibitors. New α-glucosidase inhibitors come from microbial metabolites such as nicotinic acid and hydroxyproline, which inhibit α-glucosidase, equal or stronger than acarbose.[118,119]

Biguanides

Biguanides are a class of antihyperglycemic drugs that are used for treating diabetes, prediabetes, and polycystic ovary syndrome. Phenformin and buformin have been excluded from the market because of their toxic effect (lactic acidosis). However, metformin is still globally used as a safe hypoglycemic agent for treating type 2 diabetes.[120,121] Two different forms of the drug include immediate-release (metformin IR), known under the commercial name, Glucophage, and slow-release (metformin SR). Reports suggest that although

metformin SR is famous for more tolerability, metformin IR lowers HbA1c (but not blood sugar) more effectively than the other.[122] After years of research on the action mechanism of metformin, several modes of action have been proposed, some of which are achieved by a concentration of metformin beyond pharmacological doses that is not achievable in clinical practice. Decreased liver gluconeogenesis through inhibition of glycerol-3-phosphate dehydrogenase remains the main mechanism of the hypoglycemic effect of metformin. Inhibition of glycerol-3-phosphate dehydrogenase leads to an increment of NADH/NAD+ ratio and a subsequent decrease in gluconeogenesis from glycerol and lactate. It is worth knowing that gluconeogenesis from other sources (alanine) is not mainly affected by metformin, explaining why metformin rarely causes hypoglycemia. However, other mechanisms should also be considered. Metformin regulates gluconeogenesis in the liver by decreasing the levels of cyclic AMP. Low levels of cAMP inhibit activation of cAMP-responsive element-binding protein 1 leading to reduced expression of key gluconeogenic enzymes; phosphoenolpyruvate carboxykinase (PEPCK), and glucose-6-phosphatase (G6Pase). In addition, metformin downregulates gluconeogenic gene expression by activating AMPK.[123]

Metformin also decreases the transport of glucose from the intestine into the blood. This is the earliest hypoglycemic effect of oral consumption of metformin.[124] Furthermore, metformin has been considered for decades to reduce insulin resistance. This property has led to its clinical use in the treatment of obesity and polycystic ovary syndrome, in addition to the treatment of diabetes. This effect is achieved by inducing the expression and translocation of GLUT4 to the membrane. Epigenetic modifications are believed to be implicated in this phenomenon. After activation of AMPK by metformin, transcriptional repressor histone deacetylase 5 is decreased which leads to a subsequent increase in GLUT4 expression.[125] Through activation of AMPK, metformin exerts anti-inflammatory properties by reducing NF-κB p65 phosphorylation, leading to the reduction of inflammatory cytokines (TNF-α, IL-6, and C-reactive protein).[126] The multifunctional properties of metformin make it a suitable candidate for treating COVID-19, probably as an addictive drug. It reduces entry of the virus to host cells, virus assembly, and maturation.[127]

Glucagons like peptide 1 receptor agonists

Response to food ingestion that mediates by incretin (like GLP-1) is impaired in diabetes type 2 patients. The application of GLP-1 receptor agonists solves this problem and improves glycemic control. GLP-1 receptor agonists consist of many members, albiglutide, dulaglutide, exenatide extended-release (which are prescribed once weekly), liraglutide, lixisenatide is administered once, and dayexenatide is taken twice a day.[128] GLP-1 receptor agonists increase insulin sensitivity, suppress appetite, decrease glucagon, HbA1C, and free fatty acid levels and decrease body weight. Furthermore, liraglutide reduces hyperglycemia-induced atherosclerosis by suppressing PI3K/AKT signaling pathway that thereby the reduction of abnormal proliferation of vascular smooth muscle cells. Interestingly, GLP-1 receptor agonists increase nerve cell survival and differentiation and therefore have a beneficial effect on the treatment of Alzheimer's disease, Parkinson's disease, and stroke.[129]

Drug candidates need further investigation

Bromocriptine, a dopamine agonist, has long been used to treat hyperprolactinemia and prolactinoma. Bromocriptine shows a moderate antihyperglycemic effect in type 2 diabetes. It may be helpful in the treatment of diabetic individuals that respond poorly to conventional drugs. The exact mechanism of action is poorly understood. Bromocriptine is different from other hypoglycemic agents because by acting through CNS, it reduces insulin resistance and hepatic gluconeogenesis and improves glucose tolerance.[130] In diabetic rat models, bromocriptine reduced IL-6 and leptin, increased PPAR-γ/adiponectin, and GLP-1 altogether ameliorated hyperglycemia.[131]

The biological activity of vanadium compounds, including the hypoglycemic effect, has been studied for years. However, their clinical use is limited due to low bioavailability and difficulty in crossing the biological membrane.[99] The binding of vanadium to organic compounds (such as glycine and EDTA) facilitates its passage through bacterial membranes and increases its effectiveness.[132] Furthermore, an organic vanadium complex (Bis [α-furancarboxylato] oxovanadium [IV]) increases insulin sensitivity, and GK activity, and inhibits PEPCK, a key enzyme in gluconeogenesis. These effects may be exerted, at least in part, by nonselective inhibition of phosphotyrosine phosphatase.[99,133]

Bile acid sequestrants like cholestyramine and colesevelam are resins that bind to cholesterol in the intestine and reduce the enterohepatic circulation of bile acid, and then serum cholesterol levels. Colesevelam, the new generation, enhances glycemic control by increasing the secretion of incretin and improving the function of beta cells.[134] The clinical benefits of bile acid sequestrants and their exact mechanism of action are under investigation.

Plant-derived remedies

Before the invention of oral hypoglycemic drugs, the major remedies came from medicinal plants. Plants are a massive source of phytochemicals with several biological activities. The isolation, purification, and identification

of their active ingredients with antidiabetic activity have drawn the attention of many researchers for decades. One of the most famous medicinal plants is fenugreek. Fenugreek (*Trigonella foenum graecum* L.) is cultivated in the Middle East and Mediterranean region. Fenugreek is used for its hypolipidemic, antihypercholesterolemic, and antidiabetic properties.[99,135] Feeding STZ-injected diabetic rats with powdered fenugreek seeds significantly reduced blood sugar. Moreover, creatinine, AST, ALT, and triglycerides levels reduced while HDL-C levels increased after oral administration of fenugreek seeds, showing that it may protect liver and kidney tissues.[136] The antidiabetic, and insulin-sensitizing effect of fenugreek was also confirmed by human studies.[137,138]

Chemical analysis of fenugreek indicates that the seeds consist of high dietary fiber, mucilaginous fiber, steroidal saponins (diosgenin, gitogenin, and tigogenin), fenugreekine (a sapogenin peptide ester), and trigonelline (a major important alkaloidal found in the seeds). The seeds also contain coumarins, galactomannan (a specific type of soluble fiber consisting of mannose and galactose), and 4-OH-Ile a hydroxyl derivative of isoleucine.[138] Trigonelline, the major alkaloid of fenugreek, has been reported as a hypoglycemic agent.[139] Li *et al*. reported that trigonelline ameliorates diabetic nephropathy and insulin resistance by increasing protein levels of PPARγ. Moreover, it simultaneously decreased the protein levels of TNF-α and leptin in type 2 diabetes mellitus rats.[140] Trigonelline also suppresses inflammation, regulates the secretion of adipocytokines, and increases β-cell mass.[141] Another molecular study suggested that trigonelline increases insulin sensitivity by promoting insulin receptor autophosphorylation and GLUT4.[142] 4-OH-Ile is another constituent found in the seeds responsible for the antidiabetic activity of fenugreek (review in section 3-1). Other ingredients found in fenugreek are coumarin (and its derivatives like scopoletin) and fenugreekine. It has been reported that coumarins and relative derivatives are involved in the suppression of gluconeogenesis, α-glucosidase, protein tyrosine phosphatase, and increasing cellular uptake of glucose, insulin levels, insulin sensitivity, and the half-life of GLP-1, which all contribute to help glycemic control.[143] Coumarins upregulate or stimulate PPARγ, GLUT4, adiponectin, GK, and glucose 6-phosphate dehydrogenase.[144] There is no valuable report about the hypoglycemic effect of fenugreekine. Fenugreek seeds have a high content of soluble fiber that regulates blood sugar by delaying gastric emptying and interfering with the intestinal absorption of glucose.[145] This evidence suggests that fibers might be responsible for the antihyperglycemic of fenugreek instead of a hypoglycemic activity. Fenugreek may affect intestinal glucose uptake by directly acting on α-amylase activity.[146] Because fenugreek increases insulin receptors in red blood cell membranes, a possibility was strengthened that in addition to its antihyperglycemic effect in the digestive system, it also has a hypoglycemic effect by increasing glucose uptake into peripheral tissues.[147]

Capparis spinosa (Caper), is another edible medicinal plant widely used as a food additive. It has long been used as diuretics, analgesic, antihemorrhoid, and antirheumatic. Furthermore, roots and bark are effective against fever, rheumatism, paralysis, coughs, asthma, and inflammation. Antidiabetic properties of caper have been attributed to the bioactive components found in different parts of the plant.[148] Several bioactive components are present in caper, including alkaloids, glucosinolate (glucocapperin), and sitosterol derivatives.[149]

Different parts of Capparis spinose show valuable antihyperglycemic activity. In our previous study, oral administration of caper root extract to diabetic rats significantly reduced plasma glucose without increasing insulin levels, indicating its insulinomimetic property.[150] Moreover, other studies have shown that fruit extract could potentiate insulin sensitivity and reduce gluconeogenesis in STZ-induced diabetic mice, confirming previous results.[151] These results were confirmed by a human study in Iran showing a hypoglycemic and hypolipidemic effect of the fruit extract.[152] Several mechanisms have been proposed for the hypoglycemic effect of caper. Caper can reduce the absorption of carbohydrates in the intestine, inhibit gluconeogenesis, and increase cellular uptake of glucose. It also shows antihypercholesterolemic and hypolipidemic properties that make it suitable for treating metabolic syndrome and fatty liver.[149] It has been proposed that it may alleviate steatohepatitis through up-regulation of fibroblast growth factor 21.[153] At the molecular level, Capparis spinose decreases PEPCK, a key enzyme in gluconeogenesis, presumably through reduction of hepatic nuclear factor-4α (HNF-4α) and subsequent decrease in PEPCK gene expression.[154]

Many other herbs with various bioactive compounds have been used to treat diabetes. Bitter melon is one of the most frequently used medicinal herbs that contains an insulin-like polypeptide (polypeptide-p or p-insulin). Subcutaneous injection of the plant extract reduces blood sugar in type 1 diabetic patients. Recombinant p-insulin has been produced with a similar hypoglycemic property.[155] Gymnemic acids extracted from Gymnema sylvestre have a similar atomic structure to that of glucose, so they inhibit the absorption of glucose in the gastrointestinal tract and thus prevent glucose increase after a meal. It activates insulin-dependent enzymes such as glycogen synthetase, glucose 6-phosphate dehydrogenase, and hexokinase. In addition, Gymnema sylvestre extract regenerates beta cells and therefore increases the level of insulin in the

blood of diabetic patients.[156] Ginkgo biloba (Ginkgo) has high levels of flavoglucoside, and its administration of the leaf extract prevents diabetic nephropathy by suppressing tissue transglutaminase.[157] It protects β-cell cells and improves insulin expression in diabetic type 2 rat models.[158] Additionally, flavonoid compounds in Silybum marianum (milk thistle) such as silybin may reduce insulin resistance and improve glucose metabolism in high-fat-fed mice. It may show its effects at least in part through activating the Farnesoid X receptor.[159] Silymarin can recover pancreatic function, regulate IRS-1/PI3K/Akt signaling pathway, and increase GLUT4 expression, and glucose uptake.[160] Ameliorating effect of milk thistle on the fatty liver has been noted in a diabetic model.[161] At the molecular level, the expression of transcription factors involved in lipid metabolism, such as PPARγ, and PPARα in the liver, has been postulated by Silymarin, suggesting its beneficial effects in the treatment of fatty liver.[162]

Securigera securidaca is used in traditional Iranian medicine for various purposes. The seed extract of the plant significantly reduces blood sugar and lipids levels in diabetic rats.[163] Green tea (Camellia sinensis) contains catechins (mainly epicatechin, epicatechin gallate, and epigallocatechin), flavanols, and flavandiols.[164] Administration of green tea extract to laboratory animals increases glucose tolerance, and insulin secretion and decreases DPP-IV activity, and starch digestion.[165] Moreover, flavonoids found in Camellia sinensis seeds ameliorate insulin resistance induced by TNF-α.[166]

Diallyl disulfide is an organosulfur distilled oil from garlic composed of two allyl groups connected by two sulfur atoms, which is hydrophobic and has a strong garlic odor. There are several reports regarding the antitumoral activity of allyl disulfide in different types of cancer.[167] Allyl disulfide inhibits glucose metabolism in breast cancer stem cells through inhibition of CD44/pyruvate kinase M2/AMPK pathway. Inhibition of glucose metabolism which is more active in cancer cells than normal cells may be the underling mechanism of its antitumor activity. However, the antidiabetic activity of allyl disulfide should be further studied *in vitro* and *in vivo* due to conflicting reports.[168]

CONCLUSIONS

Diabetes and insulin resistance are becoming a problem for health systems worldwide. Therefore, from the human point of view and the budget that it imposes on health systems, diabetes, and its related disorders should be considered a special worldwide issue. It is clear that to find a way to reduce the incidence of the disease or to effective treatment of existing patients, the physiological pathways and underlying pathological mechanisms of the disease must be identified. Therefore, it is necessary to know the signaling pathways, proteins and enzymes, and effective metabolic substances involved in this pathway. This study tried to review from the beginning of this pathway, i.e., the mechanisms of insulin secretion to the factors affecting its impact on the target tissues in the view of proteomics. Ultimately, the mechanism of medications and drug candidates on different parts of this long signaling pathway was discussed. An exciting field of study in the future is the investigation of chemicals that reduce the incidence or severity of diseases such as Covid-19 by lowering insulin resistance.

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ORIGINAL ARTICLE

Evaluating the effect of cow's milk fortified with albumin powder on malnutrition and anthropometric indices in primary-school children with mild-to-moderate underweight: A randomized double-blinded clinical trial

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Background: A proper diet plan is one of the necessary conditions for maintaining the children's health. The aim of this study was to evaluate the effect of consumption of pasteurized cow's milk fortified with albumin protein in primary-school children, in Yasuj, Iran. **Materials and Methods:** In this double-blind randomized clinical trial with 12 weeks of duration, 60 children aged 7–13 years, mild to moderate underweight (−1≥ weight-for-age *z*-score ≥−3), were randomly assigned to control and albumin groups. The albumin group and the control group received 200 cc of milk with 10 g of albumin powder and 200 cc of milk with 10 g of cornstarch powder, respectively. At the beginning and end of the study, food intake and anthropometric indices were measured. **Results:** After 12 weeks of intervention, none of the anthropometric indices (weight, weight-for-age *z*-score, body mass index (BMI), BMI-for-age *z*-score, and waist circumference) showed significant changes as compared to baseline in the control group, but weight-for-age *z*-score and BMI-for-age *z*-score showed significant increase as compared to baseline in the albumin group (before: −2.25 ± 0.40, after: −1.98 ± 0.35, $P = 0.001$ and before: −3.48 ± 0.86, after: −3.06 ± 0.71, $P = 0.009$, respectively). The comparison of the mean changes between the two groups showed significant difference regarding weight-for-age *z*-score (control group: −1.70 ± 0.31 in comparison with albumin group: −1.98 ± 0.35, $P = 0.002$), BMI (control group: 12.08 ± 1.96 in comparison with albumin group: 12.13 ± 1.49, $P = 0.03$), and BMI-for-age *z*-score (control group: −3.11 ± 0.91 in comparison with albumin group: −3.06 ± 0.71, $P = 0.02$). **Conclusion:** The consumption of albumin powder with milk can improve weight-for-age *z*-score and BMI-for-age *z*-score indices in children with mild-to-moderate underweight. Larger controlled interventional studies with longer duration are recommended.

Key words: Albumins, malnutrition, milk, primary-school children

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INTRODUCTION

Protein energy malnutrition (PEM) has been recognized as a major risk factor of global health, leading to remarkable deaths in children.[1] Pediatric malnutrition is described as an imbalance between nutrient body demand and food supply giving rise to energy, protein, and micronutrients deficiencies which may affect children growth, development, and incidence of chronic diseases.[2]

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Children are more likely to be malnourished due to their special nutritional requirements for their physical and mental growth and development.[3,4] Evidences suggest that malnutrition in the early years of life commonly leads to reduced body growth, mental disorders like mental retardation, lack of academic achievement, and reduced work efficiency.[5-7] Moreover, they may encounter physical and mental disorders in later life.[5-7] Therefore, an appropriate dietary plan is essential for maintaining children's health.[7,8] Primary school children are an important target group in terms of social, health and nutritional vulnerability, since they enter the new school environment, and this often causes alterations in some habits and methods of life, especially their dietary habits.[9-11]

PEM is the most common type of malnutrition among Iranian children,[12-14] and usually the median protein intake among Iranian children is less than recommended for their age group.[12-14] Previous studies have found insufficient food intake as the main reason for this finding.[12-14] Moreover, behavioral problems related to eating and lack of proper education of parents are the next influential factors in this deficiency.[12-14] Lack of adequate protein intake impairs height weight growth throughout affecting musculoskeletal growth. On the other hand, lack of protein intake by affecting the process of carbohydrate metabolism (glycogen storage of liver and muscle) leads to insufficient supply of substrate for the anabolic process participating in growth.[11,15,16] Low quality of protein intake is also determined as the main factor affecting the process of growth and repair.

Meats, eggs, dairy products, and legumes are the valuable food sources of protein with high biologic value, yet their consumption is less in the age group of children.[1,17,18] Issues such as family economy, individual taste, and increasing consumption of ready-to-eat foods are among the factors limiting the consumption of this valuable source of protein.[19-22]

For many years, various egg components including albumin have been available as ready-to-eat supplements for therapeutic purposes. Albumin is an egg white protein with very high nutritional value. According to some previous findings, the consumption of this protein can improve many indicators of malnutrition and therefore has been considered by researchers as a supplement of protein therapy.[19,21]

Given that children are more likely to be malnourished due to their special nutritional requirements for their physical and mental growth, finding a safe and workable nutritional solution to improve growth-related indicators seems necessary. Furthermore, because of positive effects of albumin protein have been shown in improving the malnutrition's indicators in some age groups and also lack of study on the age group of primary school children; we conducted this study with the aim at investigating the effect of consuming pasteurized cow's milk fortified with albumin protein in underweight 7–13 years-old primary school children in Yasuj, Iran.

METHODS

Study design and patients' characteristics

This is a randomized controlled, parallel, double-blind trial conducted on July 5, 2020 to November 10, 2020 and conformed to the Declaration of Helsinki Guidelines. The study protocol was reviewed and approved by the ethics committee of Shiraz University of Medical Sciences, Shiraz, Iran, (approval number.IR.SUMS.REC.1399.483), and enrolled in the Iranian Registry of Clinical Trials (IRCT20210109049971N1).

The inclusion criteria were as follows: Elementary students, with lack of underlying diseases affecting student development such as diabetes, hypothyroidism, liver and kidney problems, seizures, and mental retardation, do not take any dietary supplements or have a special diet 6 months before the study, mild to moderate underweight (as defined by the World Health Organization − 1≥*z*-score≥−3). Children who did not consume prescribed beverage, hospitalized during the intervention, and those who were lactose intolerant or had allergy to cow milk were excluded.

Sample size

The sample size of 60 was calculated according to weight for age in a previous similar study Graham *et al*.[23] with considering a type 1 error of 5%, power of 80%, and a drop-out rate of 10%.

Sampling

Participants aged 7–13 years old were selected by two-stage cluster sampling from the list of all primary schools in Yasuj, Iran. In this way, at first, the list of all primary schools in Yasuj was prepared and several schools were selected as the main cluster by simple random method. After coordination with the school principal, students were selected from each school. For all students whose parents had given their final consent, the anthropometric parameters (height and weight) were measured and entered into the Anthro WHO software to calculate the standard score (*Z* score). In a *Z* score system, weight-for-age is expressed as the number of standard deviations (SDs) or *Z* scores below or above the reference mean.[24] According to the definition of the World Health Organization, −1≥ weight-for-age *z*-score≥−3 was considered as mild to moderate underweight.[25] This stage continued until the number of eligible children reached to 60.

Randomization

Participants were randomly allocated in a 1:1 ratio to the albumin and control groups. Randomization was conducted by the random allocation software[26] to allocate patients using blocked randomization with a fixed block size of two. The allocation was performed according to this order and continued until all participants are specified to an arm. Randomization was done by an investigator who had no clinical involvement in the trial. Furthermore, other procedures including enrollment, sequence generation, allocation concealment, and randomization process were all performed by the principal investigators.

Blinding

To blind patients to the samples, the interventions in the both groups were identical in appearance and color. The interventions were coded differently in each group to blind the investigator.

Intervention

Participants in the albumin group received 200cc cow's milk (3% fat, Pegah-e Fars Dairy Company, Iran) with 10 g albumin powder (Golpoodr Golestan Company, Iran) per day and in the control group received 200 cc cow's milk (3% fat, Pegah-e Fars Dairy Company, Iran) with 10 g cornstarch powder (Golpoodr Golestan Company, Iran) per day for 12 weeks. In this way, every 2 weeks, 14 packages 200 cc of cow's milk with 140 g of albumin powder in the albumin group and also, 14 packages 200 cc of cow's milk with 140 g of cornstarch powder in the control group were delivered to the children's parents and they were instructed to combine 10 g of albumin powder with 200 cc of lukewarm milk daily and be given with the child's usual breakfast in the albumin group and 10 g of cornstarch powder with 200 cc of lukewarm milk in the control group as the same.

Parents of children were asked to come every 2 weeks for delivery of interventions (cow's milk + albumin powder/cow's milk + cornstarch powder) and adherence checklist. Adherence to the study was measured using a designed daily checklist. Parents determined daily consumption or nonconsumption of their children by marking this checklist. If participants consumed < 80% of the prescribed, subjects were excluded from the study analysis. Moreover, consuming the assigned intervention was reminded to the parents of the children by a text message every week and they were asked not to change their children's diet and normal physical activity and to refrain from taking any kind of food supplement during the study period.

Outcomes and measurements

Before the study, the demographic questionnaires were filled through face-to-face interview by the main investigator. To assess dietary intake and monitor dietary compliance, 3-day dietary recalls (including 2-week days and 1 weekend day) were collected from subjects at baseline and at the end of the study phase. Nutrient composition was determined by Nutritionist IV version 3.5.2 (Hearst Corp., San Bruno, CA). Height was measured using a wall-fixed tape to the nearest 0.1 cm. Before and after intervention, body weight was measured to the nearest 0.1 kg using (SECA) scale while participants were in light clothes. In addition, waist circumference was measured by a nonstretchable measure tape according to standard methods based on either bony landmarks (iliac crest, last rib, or midpoint) or external landmarks (minimal waist, largest abdominal circumference, umbilicus, 1 cm above umbilicus, or 1 inch above umbilicus).[27] Body composition indices including body fat percent, lean body mass percent, and present of total water were determined by BIA (Bodystat QuadScan 4000 device, England) at baseline and at the end of the study. Physical activity was measured using the Children's Physical Activity Questionnaire.[28]

Statistical methods

Statistical analysis of the data was performed using the SPSS software version 16 (IBM, Armonk, USA). Normality of data was assessed by Kolmogorove–Smirnov test. The Chi-square test was used for qualitative statistical data. Furthermore, to compare the changes between the start and end of the intervention in each group, paired *t*-test was used for the data with normal distribution and Wilcoxon signed-rank test was used for skewed data. Independent *t*-test was used to compare the two groups with normal distribution. The analysis of covariance was used to adjust energy and physical activity. Mann–Whitney test was used in the case of abnormal distribution. $P < 0.05$ was considered statistically significant.

RESULTS

Figure 1 shows the general study process. During the intervention phase of the study, three patients left the study due to different reasons. The mean ± SD of age of participant was 9.14 ± 2.11 and 9.87 ± 2.36 years in the control and albumin group, respectively. In our study, about 25% of the participants were boys. In the baseline, none of the measured parameters had a significant difference between the two groups.

The changes in the measured parameters are shown in Table 1. In the control group, none of the measured anthropometric indices including weight, body mass index (BMI), weight-for-age *Z*-Score, BMI-for-age *Z*-Score, and waist circumference (cm) could not show a significant change after 12 weeks of intervention compared to baseline ($P > 0.05$ for all cases). While in the albumin group, *z*-score weight for age (before:

Table 1: Effect of interventions on the levels of measured parameters in participants after 12 weeks

Valuables	Control group (*n*=29)			Albumin group (*n*=28)			*P***	*P****
	Before	After	*P**	Before	After	*P**		
Weight (kg)	21.88±4.32	22.10±5.69	0.83	20.46±4.15	21.22±4.54	0.11	0.27	0.38
BMI (kg/m^2)	11.90±1.87	12.08±1.96	0.61	11.64±1.35	12.13±1.49	0.07	0.04	0.03
Weight-for-age *Z*-score	−1.77±0.27	−1.70±0.31	0.21	−2.25±0.40	−1.98±0.35	0.001	0.002	0.01
BMI-for-age *Z*-score	−3.22±0.77	−3.11±0.91	0.46	−3.48±0.86	−3.06±0.71	0.009	0.001	0.02
Waist circumference (cm)	52.86±3.14	52.75±4.55	0.63	51.23±3.77	52.45±4.21	0.38	0.32	0.45
Adipose tissue (%)	15.62±4.01	16.33±4.67	0.34	15.40±3.98	15.78±4.44	0.61	0.39	0.55
Lean tissue (%)	49.34±12.53	50.53±15.17	0.65	48.73±13.55	49.42±15.17	0.79	0.28	0.62
Total water (%)	64.45±16.78	63.59±15.23	0.77	64.35±16.91	64.71±16.36	0.90	0.76	0.9
Physical activity (MET/min/day)	31.65±7.88	29.86±9.35	0.43	35.17±8.45	33.28±7.89	0.39	0.14	0.19

*Paired *t*-test has been used for changes within groups; **Independent t-test was used to compare the mean of changes between the groups; ***P-value was adjusted for energy and physical activity as confounders. Numbers are expressed as mean±SD. SD=Standard deviation; BMI=Body mass index

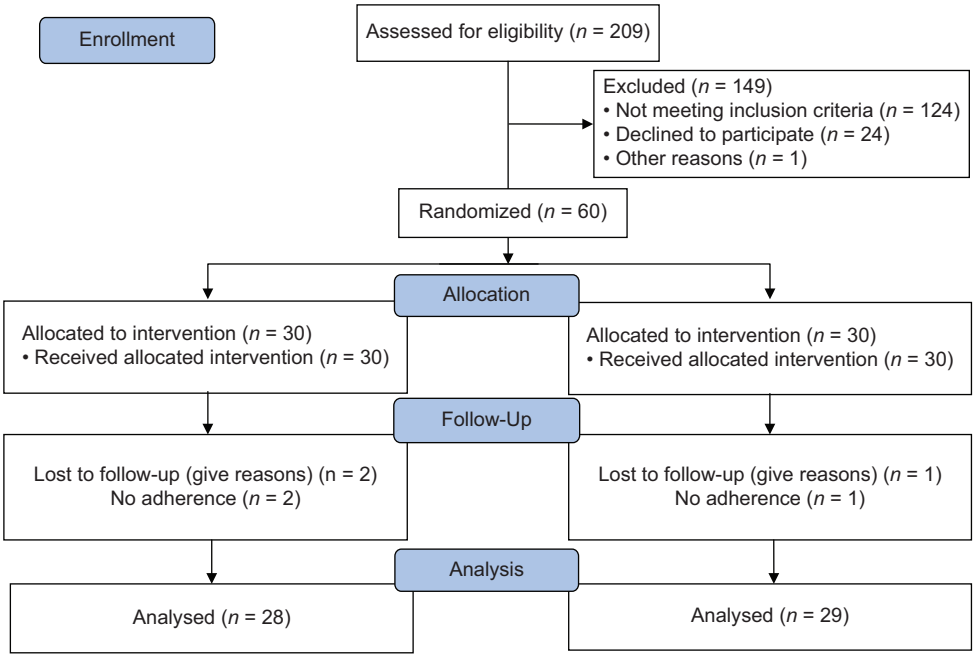

Figure 1: Flow diagram of the trial

−2.25 ± 0.40, after: −1.98 ± 0.35, P = 0.001) and *z*-score BMI for age (before: −3.48 ± 0.86, after: −3.06 ± 0.71, P = 0.009), were able to show a significant improvement compared to baseline. The changes were significant in comparison with the control group for BMI (control group: 12.08 ± 1.96 in comparison with albumin group: 12.13 ± 1.49, P = 0.03), weight-for-age *Z*-Score (control group: −1.70 ± 0.31 in comparison with albumin group: −1.98 ± 0.35, P = 0.002), [Figure 2a], and BMI-for-age *Z*-Score (control group: −3.11 ± 0.91 in comparison with albumin group: −3.06 ± 0.71, P = 0.02), [Figure 2b].

After 12 weeks' intervention, body composition (adipose tissue [%], lean tissue [%], and total water [%]) did not change significantly compared to baseline in any of the studied groups (P > 0.05 for all cases). Furthermore, the comparison of the mean changes of these indices between the study groups did not indicate any significant change (P > 0.05 for all cases). In term of physical activity, none of within and between the group changes was significant.

Table 2 shows the changes in food intake. In both groups, the amount of received energy showed a significant increase after the intervention, but after comparing the mean changes between the two groups, these changes were not significant (P = 0.35). Similarly, the amount of dietary fat intake in both groups showed a significant increase when compared to the baseline, while changes between the two groups could not show a significant difference (P = 0.4). The mean protein intake in both groups was significantly increased and this mean increase was significant in the albumin group compared to the control group (P = 0.02). In terms of carbohydrate and fiber, there was not seen a significant change between groups.

There was no change in the results after adjusting the effect of energy intake and physical activity.

Table 2: Effect of interventions on food intake in participants after 12 weeks

Valuables	Control group (*n*=29)			Albumin group (*n*=28)			*P***	*P****
	Before	After	*P**	Before	After	*P**		
Energy (kcal/kg)	81.38±113.29	96.98±107.11	0.04	88.62±108.93	105.32±122.99	0.03	0.17	0.35
Carbohydrate (g/day)	269.77±83.09	298.15±95.30	0.07	272.81±72.25	286.43±60.31	0.06	0.10	0.25
Protein (g/kg)	2.31±2.83	2.88±2.49	0.02	2.56±3.08	3.72±1.74	0.001	0.04	0.02
Fat (g/day)	64.99±5.65	73.17±7.36	0.03	64.80±6.31	70.78±11.19	0.001	0.10	0.4
Fiber (g/day)	5.66±2.61	5.81±3.03	0.23	4.92±3.88	5.26±2.43	0.03	0.52	0.8
Percentage calorie of carbohydrate out of total calorie	60.59±67.9	55.64±62.54	0.77	60.18±63.92	51.26±43.20	0.53	0.76	0.41
Percentage calorie of protein out of total calorie	11.37±10.01	11.91±9.31	0.83	11.57±11.31	14.15±5.65	0.27	0.27	0.11
Percentage calorie of fat out of total calorie	32.84±10.38	30.72±10.86	0.45	32.16±12.56	28.50±18.03	0.37	0.57	0.16

*Paired *t*-test has been used for changes within groups; **Independent t-test was used to compare the mean of changes between the groups; ****P*-value was adjusted for energy and physical activity as confounders. Numbers are expressed as mean±SD. SD=Standard deviation; BMI=Body mass index

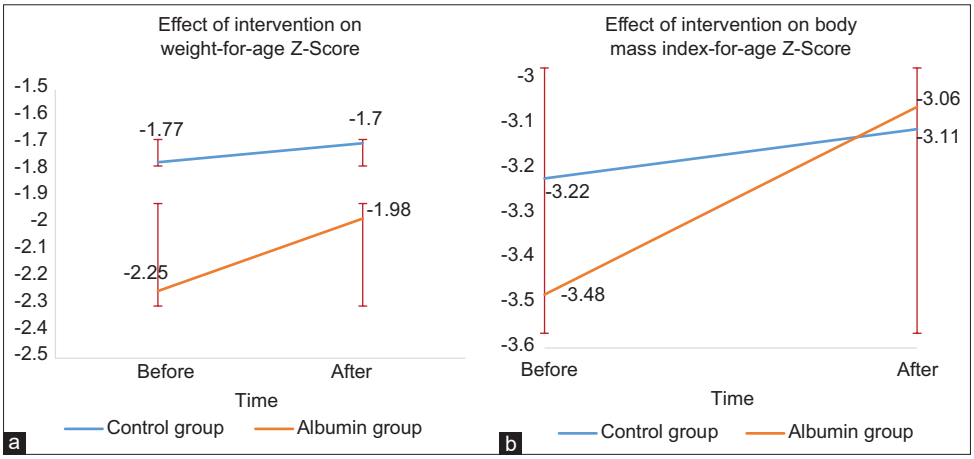

Figure 2: (a) Changes in weight-for-age *Z*-Score and (b) changes in body mass index-for-age *Z*-Score

DISCUSSION

To the best of the authors' knowledge, this is the first study that examined the effect of 12 weeks consumption of 200 cc cow's milk fortified with 10 g albumin powder on malnutrition indices in children with mild and moderate malnutrition. Our findings showed a significant improvement was seen in *Z*-score of weight-for-age, BMI, and *Z*-score of BMI-for-age after 12 weeks' intervention. The body mass composition of the participants did not change significantly neither in the group receiving albumin protein powder with milk nor in the group receiving cornstarch powder with milk.

In this study, consumption of cow's milk fortified with albumin powder for 12 weeks could not significantly change weight in participants. Contrary to our finding, the result of the school milk program on children's nutritional status in Malaysia showed a significant reduction in the prevalence of malnutrition and being underweight (6.8%–3.15% reduction);[29] however, it should be noted that this information was for a period of 2 years and it is possible to attribute this significant results to a longer study period. An interventional study examining the effect of school milk schemes on Chinese girls reported that milk consumption led to weight gain over 2 years.[30] Another interventional study of 92 Japanese children demonstrated an increase in weight among children who drank more milk over a 3-year period.[31] Again, in both previous studies, the longer duration of the intervention can be considered as a prominent point in these studies leading to significant weight gain. Berkey *et al*.[32] confirmed the positive relationship between milk consumption and weight gain, affirming that children who drink more than three glasses of milk per day have a higher BMI than children who drink 1–2 glasses or 0–0.5 glasses of milk per day. Drinking more milk by providing extra energy for underweight children can lead to more weight gain. The amount of milk intake in our study was not as much as mentioned by Berkey *et al*. Therefore, in addition to the short study time, the low amount of milk intake may be another reason for nonsignificant results in our study.

According to our findings, a significant improvement in *Z*-score of weight-for-age, *Z*-score of BMI-for-age, and BMI in the intervention group compared to the control group was observed. Although the 12-week intervention did not significantly alter weight directly, the weight-dependent

indices improved significantly compared with the control group, which can be attributed to the albumin protein used in the study. However, measuring waist circumference did not show significant changes in this study, which short time of intervention may lead to this result.

None of the groups showed significant changes in body mass composition including adipose tissue, lean body mass, and total body water. The interpretation of this result is very difficult due to the lack of a similar study that measures the effect of albumin powder consumption on children's body mass composition. The lack of significance of changes in body mass composition in both groups, despite significant changes in some weight-dependent indicators, may be due to the fact that the overall impact on weight-dependent indicators changes was low. On the other hand, it should not be forgotten that the measurement of body mass composition with BIA can be affected by the measurement error or the required conditions that may lead to nonsignificant results.

After 12 weeks of intervention, calorie, fat, and protein intake increased significantly in both groups. However, the significant changes between groups were only observed in protein intake, which indicates the accuracy of the study design and acceptable adherence of participants.

Reporting no side effects by the participants is one of the noteworthy points of this study. In addition, this study is the first and only study to investigate the effect of consuming albumin powder with a beneficial food source such as milk on the anthropometric indices on underweight children, which could show the positive effect of this intervention in this important age group. One of the limitations of this study, in addition to the small sample size, is the short duration of the intervention. Furthermore, we did not measure and discuss the effects of our interventions on other nutrients intake. Due to the unclear effect of long-term use of albumin powder with rich and beneficial nutrients such as milk in underweight and malnutrition children, it is recommended to conduct a stronger controlled intervention study over a longer period of time.

CONCLUSION

According to the available results, it seems that the consumption of albumin powder with milk can improve some anthropometric indices in children with mild to moderate underweight without any side effects. Although body mass composition could not change significantly, it seems that better results could be achieved by prolonging the intervention time. It should be mentioned that although providing energy and protein intake are the main components of interventions to improve underweight status, micronutrients are of great significance in healthy growth and development, as well. Therefore, pure protein fortification may not substitute whole food consumption in growing children and all food groups should be consumed.

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Conflicts of interest

There are no conflicts of interest.

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