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STUDIES IN HUMANS

Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial

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Abstract

There is limited evidence on the effects of prebiotics on inflammation. Therefore, the aim of this study was to evaluate the effects of inulin supplementation on inflammatory indices and metabolic endotoxemia in patients with type 2 diabetes mellitus. The participants included diabetic females ($n = 49$). They were divided into an intervention group ($n = 24$) as well as a control group ($n = 25$) and received 10 g/d inulin or maltodextrin for 8 weeks, respectively. Fasting blood sugar (FBS), HbA1c, insulin, high-sensitive C-reactive protein (hs-CRP), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), and plasma lipopolysaccharide (LPS) were measured pre and post intervention. Inulin-supplemented patients exhibited a significant decrease in FBS (8.5%), HbA1c (10.4%), fasting insulin (34.3%), homeostasis model assessment of insulin resistance (HOMA-IR) (39.5%), hs-CRP (35.6%), TNF-α (23.1%), and LPS (27.9%) compared with the maltodextrin group ($p < 0.05$). Increase in IL-10 was not significant in inulin compared with the maltodextrin group. It can be concluded that inulin supplementation seems to be able to modulate inflammation and metabolic endotoxemia in women with type 2 diabetes.

Keywords

Cytokines, lipopolysaccharide, prebiotics

History

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Introduction

Subclinical inflammation and inflammatory pathways are linked to the development of insulin resistance. Insulin resistance ultimately leads to the clinical expression of type 2 diabetes and its complications (Wellen & Hotamisligil, 2005). It has been shown that cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and high-sensitive C-reactive protein (hs-CRP) can cause the cascade of inflammation, systemic insulin resistance, and β-cell dysfunction (Goldberg, 2009).

Over the past few years, growing evidence has shown that gut microbiota has a critical role in the development of inflammation and metabolic disorders such as obesity, insulin resistance and type 2 diabetes (Cani et al., 2008). Gut microbiota is considered a target in the management of type 2 diabetes and prevention of other micro and macro vascular diseases (Burcelin et al., 2011). Gut microbiota may involve with the production of lipopolysaccharide (LPS) and metabolic endotoxemia (Suganami et al., 2007). LPS is a major component of the outer cell-wall in Gram-negative bacteria. It is known that LPS is an initiator of metabolic impairment in obesity and type 2 diabetes (Cani et al., 2008). It has been reported that the levels of circulating LPS are higher in patients with type 2 diabetes. In these patients, LPS has relationship with glycemic status and metabolic pathways (Caesar et al., 2010; Creely et al., 2007).

Prebiotics are food components which provide potential benefits to health (Cani et al., 2009). Among prebiotics, inulin-type fructans have been known to modulate gut microbiota in animals and human beings (Delzenne et al., 2011a, b). Inulin-type fructans are indigestible carbohydrates, containing fructose monomers linked by β $(1 \rightarrow 2)$ bonds. They are arranged as fermentable, soluble, and non-viscous fibers. High Performance (HP) inulin is a prebiotic with long-chain and high-molecular weight, mix of inulin-type fructans, without fructans with a degree of polymerization <10 (Kolida & Gibson, 2007). HP inulin can change composition of the gut microbiota toward *bifidobacteria* (Kolida & Gibson, 2007) as well as *Bacteroidetes* and can decrease *Firmicutes* (Dewulf et al., 2013; Everard et al., 2011, 2013). Prebiotics can restore *Akkermansia muciniphila* in obese and diabetic subjects (Everard et al., 2013). These changes are related to the improvement of glucose homeostasis and reduction of endotoxemia (Everard et al., 2013). Ingestion of 5–8 g/d HP inulin should be sufficient to exhibit a positive effect on the gut microbiota (Kolida & Gibson, 2007).

Animal studies have shown the effects of inulin-type fructans on the inflammation and metabolic endotoxemia (Cani et al., 2009; Yasuda et al., 2009). Supplementing mice with prebiotics increases the number of intestinal *bifidobacteria* and decreases the impact of metabolic endotoxemia as well as inflammatory disorders (Cani et al., 2007, 2009). Human studies investigated the effects of inulin-type fructans on the inflammatory biomarkers (Dewulf et al., 2013; Lecerf et al., 2012;

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Malaguarnera et al., 2012). Results of human studies showed that prebiotics modulate inflammatory and anti-inflammatory biomarkers' expression (Lecerf et al., 2012). Reportedly, prebiotic treatment is also correlated with LPS (Dewulf et al., 2013).

Review of the related literature in this area shows a need for further research, especially on the human. Therefore, the present study tests the hypothesis that HP inulin changes inflammatory and anti-inflammatory biomarkers including hs-CRP, TNF-α, IFN-γ, IL-10 and metabolic endotoxemia in type 2 diabetes patients. LPS is considered an index of endotoxemia, in this study.

Materials and methods

Patients

Females patients ($n = 65$; aged 20–65 years) from Iran Diabetes Society and Endocrinology and Metabolism Clinics of Tabriz University of Medical Sciences voluntarily participated in this triple-blind randomized controlled study from December 2011 to February 2012. Inclusion criteria were having type 2 diabetes for more than 6 months, using anti-diabetic drugs, normal diet and Body Mass Index (BMI) $>25\,kg/m^2$ in the last 3 months. Type 2 diabetes was defined as a FBS level ≥ 126 mg/dL (Association AD, 2004). Patients were excluded if they had history of gastrointestinal, cardiovascular, renal, thyroid, liver, or pancreatic diseases if they were pregnant, lactating, consuming pre/probiotics' products (during and 2 weeks prior to the intervention), antibiotics, antacids, alcohol, anti-diarrheal, anti-inflammatory, lipid-lowering, or laxatives drugs; and finally if they had a typical fiber intake of >30 g. At the beginning of the trial, data including age, medication history, and diabetes duration (in years) were collected, using a questionnaire. The trial was approved by the Ethics Committee of Tabriz University of Medical Sciences and written informed consent was obtained from each patient. The trial was registered on the Iranian registry of clinical trials website (www.irct.ir/, IRCT201110293253N4).

Experimental design

Patients were randomly divided into two groups using a block randomization procedure, based on BMI and age. In every block of participants, five subjects were allocated to each arm of the trial. The allocation sequence was randomly generated by random allocation software (RAS). The intervention group received 10 g/d HP inulin supplement (Sensus, Borchwef 3, the Netherlands) and the control group received similar amounts of maltodextrin as placebo (Jiujiang Hurirong Trade CO., Ltd, China) for 8 weeks. Both maltodextrin and HP inulin had similar taste and appearance and were provided to subjects in similar opaque packages. To maintain blinding, the allocation was performed by an investigator with no clinical involvement in the study, and the main investigator remained blind until the end of analysis. The statistical analysis of data was also blind. Patients received half of the packages at the beginning and the remainder in the middle of the trial. Supplements were distributed among the volunteers in accordance with the allocation code after randomization. In order to minimize subjects' withdrawal and ensure their consumption of supplements, they received a phone call every week. Throughout the trial, the subjects were asked to have their usual physical activity and diet. All the collected data were coded for analysis.

The sample size was determined based on primary outcome of change in TNF-α, obtained from a pilot study on five patients. A minimum sample size of 22 was determined for each group by Pocock's formula (Pocock, 1990) with a confidence level of 95% and a power of 0.80. To cover an anticipated dropout of 25%, the sample size was increased to 27 in each group.

The primary outcomes of the study were hs-CRP, TNF-α, IL-10, and LPS while the secondary outcomes were weight changes, FBS, HbA1c, fasting insulin, and HOMA-IR. Dietary fiber, weight, hs-CRP, and LPS changes for glycemic parameters and dietary fiber as well as weight and LPS changes for inflammatory and anti-inflammatory markers were considered covariates in this study.

Body weight and dietary intake assessment

Anthropometric indices including body weight and height were measured at baseline and at the end of the trial. BMI was calculated as weight in kilograms divided by the squared height in meters. Dietary intakes were evaluated using a 3-day food dairy (two weekdays and one weekend) at baseline and at the end of the trial. Before the intervention, all the patients were provided with instructions on how to use a food scale and record their food intakes. The patients were informed about the food portion sizes through a food album which contained all food items typically consumed in their community. A nutritionist, as a research assistant, revised dietary records together with the patients. Dietary intakes were analyzed using the nutritionist 4 software (First data bank Inc., Hearst Corp., San Bruno, CA) containing the database from tables of content and nutritional value.

Biochemical measurements

At baseline and at the end of trial, after an overnight fasting, 10 ml venous blood samples were collected and transferred into two vacutainer tubes, one containing EDTA for measurement of blood glycosylated hemoglobin (HbA1c) and the other containing sodium fluoride for FBS, insulin, inflammatory/anti-inflammatory biomarkers including hs-CRP, TNF-α, IL-10, and LPS. The serum samples were separated from whole blood by centrifugation at 2500 rpm for 10 min (Beckman Avanti J-25; Beckman Coulter, Brea, CA) at room temperature. FBS, HbA1c, and insulin were analyzed on the day of sampling and the remaining serum was stored at $-70\,^{\circ}C$ until assay time. FBS concentration was measured by the enzymatic method using an Abbot Model Aclyon 300, USA, autoanalyzer with a kit from Pars-Azmone (Tehran, Iran). HbA1c was determined using an automated HP liquid chromatography analyzer with commercially Bio-Rad D-10 Laboratories, Schiltigheim, France kit. Serum insulin was measured using a chemiluminescent immunoassay (CLIA) method (LIAISON analyzer (310360) Diasorin S.P.A, Verecelli, Italy). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula: [fasting insulin (μU/mL) × FBS (mg/dL)]/405 (Matthews et al., 1985).

Serum hs-CRP concentration was determined using an immunoturbidimetric assay (Pars Azmoon Co., Tehran, Iran). TNF-α and IL-10 levels were determined using an ELISA kit (eBioscience, San Diego, CA). Serum LPS concentration was determined by using a kit based on a *Limulus Amebocyte Lysate* extract (LAL kit endpoint-QCL1000; Cambrex BioScience, Walkersville, MD, Catalog Number: 50-647U, 50-648U, Lonza Group Ltd). Samples were diluted in pyrogen-free water and heated at $70\,^{\circ}C$ for 10 min to inactivate endotoxin-neutralizing agents that inhibit the activity of endotoxin in the LAL assay. Then, pyrosperse reagent (Lonza Group Ltd.), a metallo-modified polyanionic dispersant, was added to the test samples at a ratio of 1/200 (v/v) before LAL testing to minimize interference in the reaction. Internal control of recovery calculation was included in the assessment. All samples were tested in duplicate and results were accepted when the intra-assay CV was less than 10%. The endotoxin content was expressed as endotoxin units (EU) per ml. Exhaustive care was taken to avoid environmental

endotoxin contamination and all material used for sample preparation and the test was pyrogen-free.

Statistical analysis

Data were analyzed using SPSS software (version 13, SPSS Inc., Chicago, IL). The results were expressed as mean ± SD. The normality of the distribution of data was evaluated by the one-sample Kolmogorov–Smirnov test. The following analyses were performed for both primary and secondary outcomes. Unpaired *t*-test (for baseline measurements) and ANCOVA were used to compare quantitative variables. Comparison of quantitative data collected at the beginning and end of the study was performed using paired *t* test. The effects of drugs used in the two groups were compared using the Mann–Whitney U test. Covariance analyses adjusting for baseline measurements helped identify any differences between the two groups after intervention. For calculating the percentage of mean changes of markers for the beginning and end of the study, mean changes of markers from baseline were calculated in each group by [(8 weeks values − baseline values)/baseline values)] × 100. Mean changes of markers between groups were calculated by [(intervention values − control values)/control values)] × 100. Significant values of $p < 0.05$ were considered to be statistically significant.

Results

From the 65 subjects, 49 completed the trial ($n = 24$ intervention group; $n = 25$ control group, Figure 1). Patients did not report any adverse effects or symptoms with HP inulin supplementation. Table 1 shows the baseline characteristics of patients in the two groups. Initial characteristics were similar in both groups at baseline of the study. All patients were treated with metformin and glibenclamide. There was not any significant difference in drug consumption of the two groups.

Effect of HP inulin supplementation on body weight and dietary intakes

At the beginning of the study, there was not any significant difference between the two groups' body weight and BMI (Table 1). Baseline dietary intakes, except for dietary fiber which was significantly higher in control group compared with the intervention group, were similar in both groups (Table 2).

After intervention, body weight and BMI remained unchanged in the maltodextrin group (70.5 ± 11.0 to 70.7 ± 10.9 kg, 29.9 ± 4.2 to 29.9 ± 4.1 kg/m^2, respectively; $p < 0.05$), while they decreased significantly in HP inulin group (75.4 ± 11.3 to 72.8 ± 11.2 kg, 31.6 ± 4.1 to 30.5 ± 4.0 kg/m^2, respectively;

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

Variables	Maltodextrin group ($n = 25$)	HP Inulin group ($n = 24$)
Age (y)	48.7 (9.7)	47.8 (10.1)
Weight (kg)	70.5 (11.5)	75.4 (11.3)
Height (cm)	153.5 (6.0)	154.4 (5.8)
BMI (kg/m^2)	29.9 (4.2)	31.6 (4.1)
Diabetes duration (y)	5.3 (4.6)	7.3 (5.4)
Metformin 500 mg (tablets/d)	2.7 (0.9)	2.8 (1.1)
Glibenclamide 5 mg (tablets/d)	1.9 (1.2)	2.3 (0.9)

BMI = body mass index. For all characteristics, there were no significant differences between Maltodextrin and Inulin groups (all $p > 0.05$, based on independent samples *t* tests).

[a]Results are presented as the mean ± standard deviation.

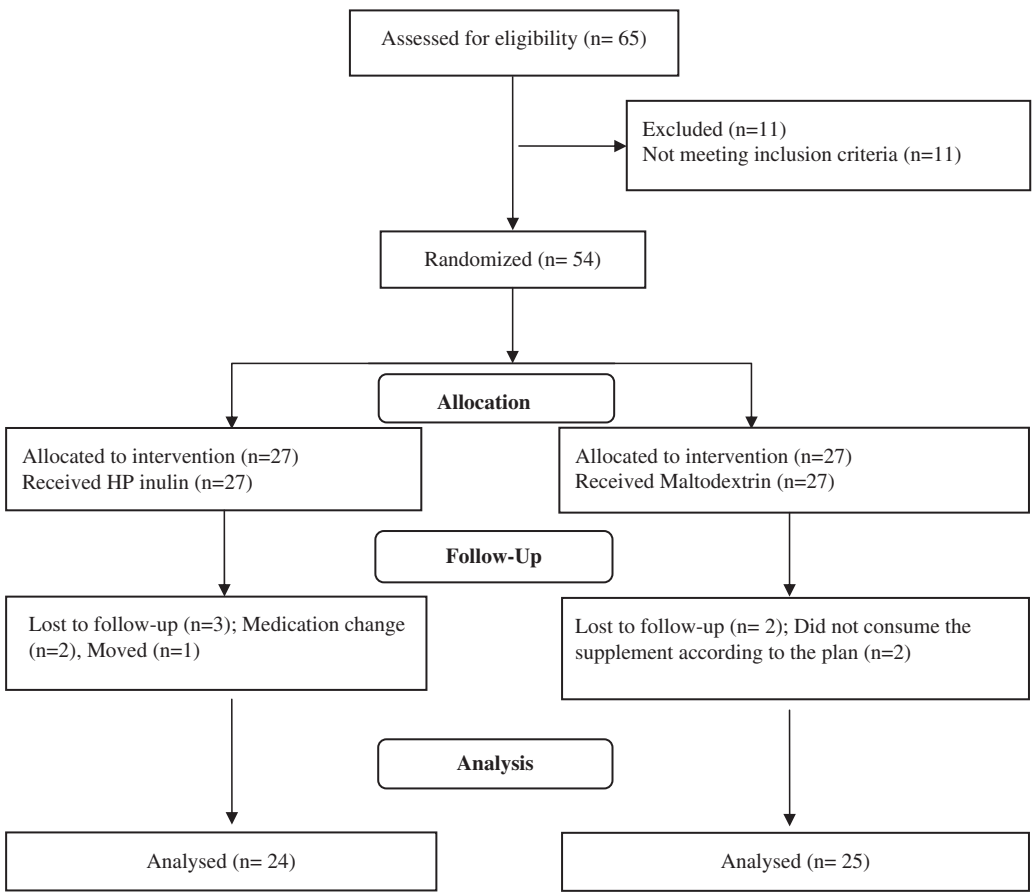

Figure 1. Flow chart of the study.

$p < 0.05$). There was significant difference in intake of energy, carbohydrate and total fat between the two groups at the end of the trial. Intake of energy and total fat decreased significantly in the intervention group, while in the control group they remained unchanged.

Effects of HP inulin supplementation on glycemic status, inflammatory, anti-inflammatory biomarkers, and metabolic endotoxemia

At the beginning of the trial, we did not observe any significant difference between the glycemic indices of the intervention and control groups (Table 3). At the end of the trial, there was a significant decrease in FBS (15.1 mg/dL, 8.5%), HbA1c (1.1% (6.82 mmol/mol), 10.4%), fasting insulin (4.1 μU/mL, 34.3%), and HOMA-IR (1.9, 39.5%) in the intervention compared with the control group ($p < 0.05$, analysis of covariance adjusted for dietary fiber, weight, hs-CRP, LPS changes, and baseline values).

The two groups did not show significant difference in baseline inflammatory biomarkers, except for hs-CRP and LPS (Table 4). After 8 weeks, significant decrease in levels of hs-CRP (3.8 ng/mL, 35.61%), TNF-α (2.9 pg/mL, 23.0%), and LPS (4.2 EU/mL, 27.9%) and a non-significant increase of IL-10 (1.9 pg/mL, 15.4%) were observed in the intervention compared with the control group ($p < 0.05$, analysis of covariance adjusted for dietary fiber, weight, hs-CRP, LPS changes, and baseline values). Inflammatory and anti-inflammatory biomarkers and LPS did not change in the control group. The levels of inflammatory biomarkers and LPS decreased, while IL-10 increased in the intervention group ($p < 0.05$, paired t test).

Table 2. Dietary intakes in the studied groups at baseline and at the end of study.

Variables	Period	Maltodextrin group ($n = 25$)	HP Inulin group ($n = 24$)
Energy (kcal/d)	Initial	1770.2 (205.6)	1693.6 (250.6)
	End	1798.2 (238.9)	1417.9 (236.7)[a,c]
Carbohydrate (g/d)	Initial	224.7 (47.9)	203.0 (64.3)
	End	223.3 (37.4)	175.9 (50.3)[c]
Protein (g/d)	Initial	54.8 (11.9)	51.2 (15.2)
	End	55.3 (14.7)	54.2 (12.2)
Total fat (g/d)	Initial	52.9 (13.3)	54.3 (12.1)
	End	51.8 (14.9)	45.6 (8.7)[a,c]
Dietary fiber (g/d)	Initial	18.3 (6.6)	10.6 (4.6)[b]
	End	14.9 (3.9)	12.9 (4.3)

Values are presented as mean (SD).
[a] $p < 0.05$, paired t test.
[b] $p < 0.05$, independent t test.
[c] $p < 0.05$ analysis of covariance adjusted for dietary fiber and baseline values.

Discussion

Our findings provided evidence for modulation of glycemic, inflammatory/anti-inflammatory biomarkers, and metabolic endotoxemia status in type 2 diabetic patients with HP inulin supplementation. Our results showed that HP inulin supplementation decreased body weight and BMI significantly. For considering the effects of weight change and other confounding factors, ANCOVA was used. FBS, HbA1c, fasting insulin, HOMA-IR, hs-CRP, TNF-α, and LPS decreased significantly in the intervention group compared with the control group. Increase in IL-10 was not significant.

The observed decreases in body weight, BMI, and energy intake are in agreement with earlier results (Parnell & Reimer, 2009). Parnell & Reimer (2009) reported that supplementation of healthy adults with oligofructose – at a dose of 21 g/day for 12 weeks – leads to reduction in body weight.

In our trial, energy intake of the intervention group significantly decreased (1693.6 ± 250.6 to 1417.9 ± 236.7 Kcal/day, $p < 0.05$). Verhoef et al. (2011) reported that effects of oligofructose on appetite, gut satiety hormones and energy intake are dependent on the dose of oligofructose. They showed that an intake of 16 g/d and not 10 g/d oligofructose for 13 days in men and women with BMI = 24.8 was able to reduce energy intake. Pedersen et al. (2013) showed that supplementation of non-obese humans with oligofructose at a dose of 35 g/day for 5 weeks decreased pancreatic polypeptide and ghrelin, increased PYY, but made no significant changes to energy intake and appetite, or glucagon-like peptide1 (GLP-1) and glycemic status. Dewulf et al. (2013) did not report significant impact of inulin-type fructans on BMI. The results of these studies are inconsistent with our results. Differences in pathologic state, BMI, basal levels of hormones, which are involved in control of appetite as well as type and duration of supplementation, can be proofs for the diversity of results obtained in various studies. The exact

Table 3. Changes in glycemic status of patients at baseline and at the end of study.

Variables	Period	Maltodextrin group ($n = 25$)	HP Inulin group ($n = 24$)	MD (95%CI) between groups
FBS (mg/dL)	Initial	157.8 (10.6)	161.7 (15.1)	3.9 (−4.8 to 12.7)
	End	156.1 (14.2)	146.6 (19.9)[a,b]	−15.1 (−27.1 to −3.0)*
	MD (95% CI) within groups	−1.7 (−6.6 to 3.3)	−15.1 (−10.3 to 19.9)	
HbA1c (%) (mmol/mol)	Initial	8.2 (0.9) (66.0 (7.2))	8.4 (0.9) (68.0 (7.2))	0.2 (1.2) (−0.4 (2.4) to 0.8(4.9))
	End	8.3 (1.1) (67.0 (8.9))	7.7 (0.7) (61.0 (5.6))[a,b]	−1.1(6.8) (−1.7(10.5) to −0.6 (3.7))
	MD (95% CI) within groups	0.1(0.6) (−0.1(0.6) to 0.4 (2.5))	−0.7 (4.3) (−1.0 (6.2) to −0.5 (3.1))	
Fasting insulin (μU/mL)	Initial	13.2 (3.8)	14.0 (4.3)	0.8 (−1.8 to 3.5)
	End	13.4 (3.8)	9.2 (3.2)[a,b]	−4.1 (−7.3 to −1.0)
	MD (95% CI) within groups	0.2 (0.1 to 0.5)	−4.8 (−6.7 to −2.8)	
HOMA-IR	Initial	5.1 (1.6)	5.6 (2.0)	0.5 (−0.7 to 1.7)
	End	5.2 (1.6)	3.4 (1.4)[b]	−1.9 (−3.2 to −0.5)

FBS, fasting blood sugar; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; MD, mean difference; CI, confidence interval. Values are presented as mean (SD).
[a] $p < 0.05$, paired t test.
[b] $p < 0.05$ analysis of covariance adjusted for dietary fiber, energy intake, hs-CRP, LPS changes, and baselines values.
*Adjusted for dietary fiber, energy intake, hs-CRP, LPS changes, and baselines values with ANCOVA.

Table 4. Changes in inflammatory biomarkers, IL-10 and LPS of patients at baseline and at the end of study.

Variables	Period	Maltodextrin group ($n = 25$)	HP Inulin group ($n = 24$)	MD (95%CI) between groups
hs-CRP (ng/mL)	Initial	13.0 (8.9)	8.0 (3.0)[b]	−5.0 (−1.0 to −9.2)
	End	11.9 (6.5)	5.3 (3.0)[a,c]	−3.8 (−7.9 to −0.1)*
	MD (95% CI) within groups	−1.1 (−4.6 to 2.3)	−2.7 (−3.3 to −2.0)	
TNF-α (pg/mL)	Initial	17.4 (3.9)	16.3 (4.9)	−1.1 (−4.1 to 1.8)
	End	18.0 (3.8)	13.3 (4.4)[a,c]	−2.9 (−4.7 to −1.2)
	MD (95% CI) within groups	0.6 (−0.3 to 1.5)	−3.0 (−3.8 to −2.3)	
IL-10 (pg/mL)	Initial	12.6 (3.3)	13.6 (3.9)	1.0 (−1.4 to 3.4)
	End	12.5 (3.2)	15.0 (3.3)[a]	1.9 (0.1 to 4.0)
	MD (95% CI) within groups	−0.1 (−0.7 to 0.6)	1.4 (0.3 to 2.5)	
LPS (EU/mL)	Initial	25.5 (5.7)	21.4 (6.6)[b]	−4.1 (−8.2 to −0.1)
	End	25.6 (6.2)	16.0 (7.5)[a,c]	−4.2 (−7.6 to −0.9)
	MD (95% CI) within groups	0.1 (−1.1 to 1.3)	−5.4 (−7.2 to −3.7)	

hs-CRP, high-sensitive C reactive protein; TNF-α, tumor necrosis factor-alpha; IL-10, interleukin-10; LPS, plasma lipopolysaccharide; MD, mean difference; CI, confidence interval. Values are presented as mean (SD).

[a] $p < 0.05$, paired t test.

[b] $p < 0.05$, independent t test.

[c] $p < 0.05$ analysis of covariance adjusted for dietary fiber, energy intake changes, and baseline values (also for LPS change in hs-CRP, TNF-α and IL-10).

*Adjusted for dietary fiber, energy intake, hs-CRP, LPS changes, and baseline values with ANCOVA.

mechanism(s) of weight reduction by inulin remain(s) unclear. Some gut satiety hormones, especially those that are responsive to diet composition, including GLP-1, PYY, and ghrelin, are proposed for weight reduction (Parnell & Reimer, 2009; Verhoef et al., 2011). Probably, short-chain fatty acids (SCFA) which are produced by the fermentation of inulin leads to increased GLP-1 and PYY concentration (Verhoef et al., 2011).

The results obtained from several studies on the effects of fructooligosaccharides on glycemic status in diabetic patients are inconsistent. One study showed positive effects of oligofructose on glycemic status in these patients (Yamashita & Kawai, 1984). In contrast, in another study no effects were reported for inulin-type fructans in diabetic patients (Alles et al., 1999). Dewulf et al. (2013) did not observe significant change in HbA1c, FBS, insulin, and HOMA-IR of obese women treated with inulin-type fructans. According to a new systematic review, 31% of clinical trials showed a decrease in serum glucose after supplementation with fructans (Bonsu et al., 2011).

The reduction in glycemic status following HP inulin can be explained via several mechanisms. Prebiotics can delay gastric emptying by increasing the secretion of gut hormones like GLP-1 and production of SCFA (Cani & Delzenne, 2009; Cherbut, 2003). Inulin may improve glycemic status by increasing hepatic AMP-activated protein kinase and decreasing gastric inhibitory polypeptide (GIP), involved in insulin resistance (Pyra et al., 2012). Decreasing the level of LPS (Cani et al., 2007) and gut microbiota alteration (Cani et al., 2007; Dewulf et al., 2013) are additional proposed mechanisms.

Another outcome of inulin supplementation was improving inflammatory/anti-inflammatory biomarkers and metabolic endotoxemia. To the best of our knowledge, no previous study has evaluated the impact of HP inulin on inflammatory/anti-inflammatory biomarkers, and metabolic endotoxemia in diabetic patients. In animal studies, the effects of inulin-type fructans on inflammatory biomarkers and metabolic endotoxemia have been investigated (Cani et al., 2009; Serino et al., 2012; Yasuda et al., 2009). Yasuda et al. (2009) reported that supplementation of young pigs with prebiotic (50:50, short: long-chain inulin) can decrease inflammation-related genes, including TNF-α, Transferrin receptor (TFRC) and SLC11A1 (solute carrier family 11 or NRAMP1). It has been shown that supplementation of diabetic mice with oligofructose or glucooligosaccharide reduces LPS (Everard et al., 2013; Serino et al., 2012). Cani et al. (2007) observed oligofructose decreases LPS and pro-inflammatory cytokines such as IL-1α, IL-1β, and IL-6 in mice.

In some human studies, positive effects of prebiotics on the inflammatory/anti-inflammatory biomarkers, and metabolic endotoxemia were reported (Dewulf et al., 2013; Lecerf et al., 2012; Malaguarnera et al., 2012). It has been shown that supplementation of healthy volunteers with inulin and Xylo-oligosaccharide (XOS) (3 g inulin + 1 g XOS) for 4 weeks significantly reduced expression of inflammatory cytokins such as IL-1β or TNF-α and increased anti-inflammatory cytokins such as IL-13 and IL-10 expression in blood (Lecerf et al., 2012). It has been reported that supplementation of obese women with oligofructose-enriched inulin and increased the levels of *Bifidobacterium* and *Fprausnitzii*. Both bacteria negatively correlated with serum LPS (Dewulf et al., 2013). Fructo-oligosaccharides (FOS) with probiotics in patients with non alcoholic steatohepatitis showed significant reduction in TNF-α, hs-CRP, HOMA-IR, serum endotoxins, and steatosis (Malaguarnera et al., 2012). These results are in agreement with ours. In contrast to our results, Dewulf et al. (2013) reported insignificant results for the treatment effect of oligofructose-enriched inulin on plasma CRP concentration. Anderson et al. (2004) and Jain et al. (2004) reported that supplementation with oligofructose plus probiotics did not affect systemic inflammation in adult patients admitted to intensive care unit. The diversity of results obtained in various studies may be due to the basal levels of measured parameters, differences in ethnicity, genotype, dose, type and time of supplementation, pathologic state as well as, basal levels of gut microbiota and inflammatory/anti-inflammatory status of the subjects.

The underlying mechanism(s) of inulin effects on inflammation and metabolic endotoxemia are not yet known. Some proposed mechanism(s) are:

(i) Change in gut microbiota: It has been shown that prebiotics increase the levels of *Bifidobacterium* and *Faecalibacterium prausnitzii* that are negatively correlated with the level of serum LPS (Dewulf et al., 2013). *F. prausnitzii* as the major bacterium of *Firmicutes* group exhibits anti-inflammatory effects (Sokol et al., 2008). It decreases in obese diabetic patients and has negative relationship with inflammatory biomarkers in obese patients (Furet et al., 2010). It has been reported that inulin-type fructans increase the levels of

F. prausnitzii (Dewulf et al., 2013). Prebiotics decrease gut permeability through improving gut epithelial barrier function that ultimately decreases the level of endotoxemia and inflammatory biomarkers (Everard et al., 2013; Spindler-Vesel et al., 2007). Prebiotics increase L-cell activity and ultimately GLP-2 secretion that may strengthen the gut barrier and improve gut tight junctions via increasing *A. muciniphila*. Also, prebiotics by raising *A. muciniphila*, can increase 2-arachidonoylglycerol (2-AG) endogenous levels (one lipid in endocannabinoid system) and can reduce metabolic endotoxemia as well as systemic inflammation (Everard et al., 2013).

(ii) Short-chain fatty acids (SCFA) from inulin fermentation in the colon: Beneficial effects of inulin on inflammation can be mediated by SCFA that can reduce the level of LPS through decreasing gut permeability (Cani et al., 2007). Butyrate controls inflammation via preventing inhibitor of kappa B (IκB) degradation and production of nuclear factor kappa B (NF-kB) (Place et al., 2005), controlling the macrophage as well as neutrophil activators and chemo-attractants (Fusunyan et al., 1999), and increasing the expression of suppressor of cytokine signaling 3 (SOCS3) (Weber & Kerr, 2006).

(iii) Oxidative stress reduction by decreasing hyperglycemia and free fatty acid (FFA): Hyperglycemia and probably increased levels of FFA induce high concentrations of ROS (King & Loeken, 2004). Immobilization stress also increases intestinal permeability and consequent metabolic endotoxemia (Cani et al., 2008). Our previous findings have indicated that HP inulin can decrease oxidative stress in diabetic patients (Pourghassem et al., 2013).

(iv) Falls in serum insulin: Falls in insulin levels may suppress inflammation via improvement of Kupffer cell function and LPS clearance (Cornell, 1980).

(v) Weight loss: It is known that obesity results in inflammatory state. Weight loss and fat mass reduction can help in reduction of inflammatory biomarkers (Sharman & Volek, 2004).

There were some limitations in our trail, including its rather sample size, fairly short duration of its intervention as well as no assessment of serum SCFA, serum FFA and other inflammatory/anti-inflammatory biomarkers. We also did not evaluate gut and fecal microbial composition. Despite these limitations, this is the first triple blind study to investigate the effect of inulin on inflammation in patients with type 2 diabetes.

Conclusion

Based on the results of this trial, inulin supplementation may improve glycemic status, biomarkers of inflammation/anti-inflammation, and LPS levels in type 2 diabetic patients. Further investigations are needed to confirm positive effects of HP inulin on inflammatory/anti-inflammatory indices in type 2 diabetic patients.

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Declaration of interest

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