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Effect of dietary supplementation with oat β-glucan for 3 months in subjects with type 2 diabetes: A randomized, double-blind, controlled clinical trial

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ABSTRACT

There is a growing interest in the use of functional foods. Studies indicate the contribution of β-glucans to glycemic control, but few have evaluated integrally the effect of this soluble dietary fiber on appetite-regulating hormones and microbiota in type-2 diabetes (T2D). We analyzed the effect of enriching a normal diet with oat β-glucan in thirty-seven T2D subjects. For 12 weeks, subjects consumed daily oat β-glucan or microcrystalline cellulose as control (5 g/day). We determined fasting glucose, C-peptide, insulin, HOMA, HbA1c, lipid profile, ghrelin, leptin, GLP-1, PYY, caloric intake, and intestinal microbiota. HbA1c decreased in the β-glucan group. Insulin, C-peptide and HOMA, *Lactobacillus* spp, and Butyrate-producing bacteria decreased in the β-glucan group ($p < 0.05$). Leptin ($p < 0.05$), GLP-1 ($p < 0.01$) and PYY ($p < 0.001$) were different between groups. The intake of 5 g/oat β-glucan for 12 weeks can help improve glycemic control, increase the feeling of satiety, and promote changes in the gut microbiota profile.

1. Introduction

Type-2 Diabetes (T2D) projections increase every year and recently the International Diabetes Federation (IDF) estimated that 578 million persons, 10.2% of the worlds population, would be diabetic by 2030 (Saeedi et al., 2019). This increase will generate high health expenses (Williams et al., 2020) and an increased risk of death from cardiovascular disease (Zeinalova, Kurbanov, Mirzazade, Rzayeva, & Novruzova, 2017). In this context, seeking therapeutic strategies that contribute to improving this epidemiological situation is essential. Different studies have shown the beneficial effects of dietary fiber, including improving blood glucose control (Post, Mainous, King, & Simpson, 2012), lipid profile (Surampudi, Enkhmaa, Anuurad, & Berglund, 2016), blood pressure (Aleixandre & Miguel, 2016), and decreasing cardiovascular risk (Buil-Cosiales et al., 2016).

β-glucans are a soluble dietary fiber with a polymeric structure of D-glucose units, linked by β-glucosidic bonds, the structure of which can be linear, branched or cyclic (β 1–2, 1–4, 1–3 or 1–6) depending on origin (Barsanti, Passarelli, Evangelista, Frassanito, & Gualtieri, 2011). β-glucans have shown promising effects in reducing cardiovascular risk by regulating the lipid profile (Sima, Vannucci, & Vetvicka, 2019) and glycemic control (Battilana et al., 2001), even in diabetic subjects (Tessari & Lante, 2017). There are several mechanisms that could explain these effects, with the most important being the increase in viscosity of the intestinal lumen, where β-glucans bind to glucose molecules, bile acids, monoglycerides, free fatty acids and cholesterol, decreasing absorption and increasing fecal excretion (Dong, Cai, Shen, & Liu, 2011; Sima et al., 2019). Another explanation relates to the regulation of the release of GLP-1 and PYY in entero-endocrine L cells, stimulated by short chain fatty acids (SCFAs) generated by the fermentation of β-glucans in the intestinal microbiota, increasing insulin secretion and perception of satiety (Vitaglione, Lumaga, Stanzione, Scalfi, & Fogliano, 2009; Zaremba, Gow, Drummond, McCluskey, & Steinert, 2018). Although there is contradictory information in this regard (Barone-Lumaga, Azzali, Fogliano, Scalfi, & Vitaglione, 2012), β-glucan fermentation could modulate intestinal microbiota towards a healthier profile (Arena et al., 2014).

Because of the properties of ß-glucans, the Food and Drug Administration (FDA) has authorized a health message for ß-glucans which states that consumption of 3 g/day of β-glucans from oats or barley can reduce the risk of coronary heart disease (Food and Drugs Administration, 2019). A similar recommendation was established by the European Food

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Safety Authority (EFSA) for oats and barley ß-glucans in relation to the decrease of cholesterolemia and coronary risk, but with a minimum of 4 g per serving (EFSA Panel on Dietetic Products N and A, 2011).

The effect of β-glucans depends on some of its properties, such as source of β-glucans, dietary matrix, a combination of the fiber with other nutrients, concentration, and molecular weight (Liu et al., 2016; Paquet, Turgeon, & Lemieux, 2010; Pentikäinen et al., 2014; Zhao & Cheung, 2013). To date, few studies have focused on the study of β-glucans as a therapeutic agent for the metabolic control of diabetes. In fact, in a recent meta-analysis (Shen et al., 2016) only four randomized controlled trials met stringency criteria. So there is a need for studies to investigate the effect of β-glucan enrichment over the long term.

The objective of this work is to analyze the effect of the enrichment of the normal diet for twelve weeks with 5 g/day of oats β-glucans on glycemic control (fasting glucose, C-peptide, insulin, glycated hemoglobin (HbA1c) and HOMA), lipid profile level (Total cholesterol, triglycerides, HDL-c, LDL-c, VLDL-c), appetite control peptides (ghrelin, leptin, GLP-1 and PYY), nutritional caloric intake, intestinal permeability (zonulin concentration) and Gut microbiota profile: bacterial phyla (Firmicutes, Bacteroidetes, and Verrucomicrobia), *Lactobacillus spp, Bifidobacterium spp*, *Akkermansia Muciniphila*, and butyrate-producing bacteria, among type 2 diabetic subjects, using a purified isolate of oat β-glucans.

2. Materials and methods

2.1. Study design

The present study was a parallel, placebo-controlled, randomized, double-blind (participant and care provider) clinical trial. The meta-analysis of randomized controlled trials performed by Shen et al. (2016) identified doses of β-glucans between 2.5 and 5 g/day, over a period of 3–8 weeks. Therefore, we decided to use the highest dose and increase the exposure time to 12 weeks, a time comparable with similar studies (Bao, Cai, Xu, & Li, 2014) and also sufficient to analyze changes in HbA1c, since A1C reflects the mean blood glucose for approximately 3 months (ADA, 2020). The β-glucan group then consumed 5 g/day of oat β-glucan as a supplement to their diet. Oat β-glucan had the following characteristics, bulk density 50.2 g/mL, 98.1% purified, loss on drying 3.86%, and molecular weight >1500 kDa (Xi'an Yaochang Co., Ltd., Xi'an, China). The control group was supplemented with 5 g/day of microcrystalline cellulose (MC); apparent density 0.38 g/mL, loss on drying 3.23% and degree of polymerization 100–300 units (Avicel PH101) (FMC BioPolymer Philadelphia, PA, USA). We use MC, an insoluble dietary fiber, to prevent changes in blood glucose, and analyze the metabolic influence of the type of dietary fiber in type 2 diabetics. Participants were instructed to consume the supplement daily in the morning with water or milk at room temperature. Participants were asked to not modify their lifestyle habits nor their medication unless directed to do so by their personal doctor. As a measure of protocol compliance, at the end of the intervention, the participant were asked about the supplement container and the amount consumed was reviewed. Food intake was evaluated using a 24-hour recall survey of 3 days and using a Table of Chemical Composition of Chilean Foods (Zacarías, Barrios, González, Loeff, & Vera, 2018). We measured weight and blood pressure, surveyed food intake, and collected fasting blood and stool samples at day 0 and after 12 weeks of treatment.

2.2. Participants

Patients with type-2 diabetes were recruited from a health center in Talca, Maule Region, Chile. They were then contacted and visited by the research team. Each participant received verbal and written information about the study protocol and an informed consent form was signed. The study was approved by the Institute of Nutrition and Food Technology of the University of Chile, by the scientific ethics committee of the Maule Health Service and the Talca Community Health Service. The protocol was registered at www.clinicaltrial.gov (ID NCT04299763). The inclusion criteria were, type-2 diabetic male and female patients, between 30 and 60 years, BMI between 30–35 kg/m^2, HbA1c < 10%, <10 years with diagnosed diabetes and use of oral hypoglycemic drug metformin. Pregnant or breastfeeding women, subjects with intestinal complications, organic insufficiencies or immunodeficiencies, treatment with drugs that influence the intestinal microbiota and regular use of insulin were excluded.

The recruited patients were randomly assigned by an independent computer procedure, to one of the groups (control or β-glucan). Volunteers and nurses did not know the code or the content of the supplements. The supplements were only identified with a code number.

2.3. Methods

Subjects were contacted and a home visit was coordinated during the morning at the beginning (week 0: W0) and at the end of the intervention (week 12: W12). Subjects fasted (for at least 12 hrs) until the arrival of the researchers. Body weight was measured using a digital scale (Omron HBF-516, Lake Forest, IL) calibrated after each use (1 kg). Blood pressure was measured with a digital sphygmomanometer (Omron HEM-CR24, Bannockburn, IL). Blood samples were centrifuged at 1600*g* for 10 min, and the plasma was aliquoted and stored at −20 °C until analysis. HbA1c levels were determined by HPLC (Biorad, Marnes la Coquette, France). Fasting glycemia and lipid profile (total cholesterol, HDL-Cholesterol, LDL-cholesterol, VLDL-cholesterol and Triglycerides - TAG) were determined with enzyme kit and automatic analyzer (Roche Diagnostics; Hitachi, Tokyo). Hormones related to appetite control (ghrelin, leptin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), insulin, and c-peptide were determined using the Magnetic Luminex assay (R&D Systems Inc., Minneapolis, MN) and the MAGPIX reader (Luminex Corporation, Austin, TX), we used 25 μL of undiluted sample on a 6 plex plate according to manufacturer instructions. We also calculated the homeostatic model assessment of insulin resistance (HOMA).

Fecal samples were requested from each subject. Intestinal microbiota DNA was extracted using the commercial QIAamp DNA Stool Mini Kit following manufacturer instructions (QIAGEN Canada, Mississauga, ON, Canada). DNA was then quantified and stored at −20 °C until analysis. Specific primers were used for each phylum or bacterial population of interest (Table 1). qPCR was performed using the AriaMx Real-time PCR System (Agilent, Santa Clara, CA) and Brilliant II SYBR® Green QPCR Master Mix Kit (Agilent Technologies, Santa Clara, CA). Each microtube contained: 10 μL of Brilliant II SYBR; 1.25 μL each primer; 2 μL of DNA sample and 8.5 μL of water, for a total volume of 23 μL, an amount sufficient according to the equipment manual. The program used was an initial denaturing cycle of 95 °C for 10 min, 40 annealing cycles of 30 s at 95 °C, 60 s at: (a) 60 °C for *Bifidobacteria spp, Bacteroidetes, Akkermancia Muciniphila, Verrucomicrobia,* and *Firmicutes*; (b) 58 °C for *Lactobacillus spp*; (c) 55 °C for Total Bacteria and (d) 53 °C for Butyrate-Producing Bacteria. Populations were quantified using the semi-quantitative method.

2.4. Statistical analysis

Results are expressed as mean and standard deviation. The differences between initial and final times of each group were analyzed using the paired sample *t*-test, for variables with a non-normal distribution, the Wilcoxon test was used. Differences between groups were assessed using an unpaired *t*-test. In the event of non-normal distribution, the Mann Whitney non-parametric test was used. A two way ANOVA was used to determine the effect of treatment and time. We calculated a sample of 17 subjects per group, with a statistical power of 80%, confidence of 95%, and variance of 0.22 to detect a decrease in HbA1c of 0.4%. After considering 20% potential loss, we recruited 21 individuals

Table 1
Sequences used to identify phyla and bacterial family.

Primers	Sequences	Concentration	Reference
Total Bacteria	5′ CTCCTACGGGAGGCAGCAGT 3′ 5′ GGACTACCAGGGTATCTAA 3′	1.0 μM	Magne et al. (2006)
Lactobacillus spp	5′ AGCAGTAGGGAATCTTCCA 3′ 5′ CACCGCTACACATGGAG 3′	1.0 μM	Rinttila, Kassinen, Malinen, Krogius, and Palva (2004)
Bifidobacterium spp	5′ CACCCGTTTCCAGGAGCTATT 3′ 5′ GCGTGCTTAACACATGCAAGTC 3′	1.0 μM	Penders et al. (2005)
Butyrate Producing Bacteria	5′ GCIGAICATTTCACITGGAAYWSITGGCAYATG 3′ 5′ CCTGCCTTTGACATRTCIACRAANGC 3′	20 μM	Louis and Flint (2007)
Akkermansia muciniphila	5′ CAGCACGTGAAGGTGGGGAC 3′ 5′CCTTGCGGTTGGCTTCAGAT 3′	0.35 μM	Derrien, Collado, Ben-Amor, Salminen, and de Vos (2008)
Bacteroidetes	5′ GGARCATGTGGTTTAATTCGATGAT 3′ ŚAGCTGACGACAACCATGCAG 3′	5.0 μM	Guo et al. (2008)
Firmicutes	5′ GGAGYATGTGGTTTAATTCGAAGCA 3′ 5′ AGCTGACGACAACCATGCAC 3′	1.0 μM	Guo et al. (2008)
Verrucomicrobia	5′ TGGCGGCGTGGWTAAGA 3′ 5′-ATTACCGCGGCTGCTGG-3′	25 μM	Navarrete et al. (2015)

per group. Statistical significance was a $p < 0.05$. All analyses were performed with SPSS version 19 (IBM, Chicago, IL).

3. Results

3.1. Characteristics of the study sample

Forty-four obese subjects with type 2 diabetes were randomly assigned to the control or β-glucan group. 37 subjects completed the trial (17 control and 20 β-glucan) and were included in the analysis. Dropouts were not due to intolerance or side effects, but to personal reasons. The population was composed up of 28 women and 9 men. Compliance with the protocol was adequate and the general perception of the groups was good: 72% of the control group and 92% in the β-glucan group indicated that the consumption of the supplement had a positive effect. Some secondary effects were observed: gurgling (bowel sounds) ($n = 8$ control group and $n = 9$ β-glucan group) and flatulence ($n = 6$ control group and $n = 5$ β-glucan group). Weekly stool frequency improved significantly ($p = 0.011$) in the β-glucan group (data not shown).

Dietary intake at day 0 and after 12 weeks of the intervention was determined. The control consumed: 1829 ± 306 Kcal/day, 16.2 ± 4.7% of energy as protein, 32.7 ± 8.6% of energy as fat, 50.4 ± 9.0% of energy as carbohydrates and 21.4 ± 6.9 g dietary fiber. The β-glucan group had an intake of 2035 ± 407 kcal/day, 15.9 ± 2.8% of the energy as protein, 29.2 ± 8.9% of the energy as fat, 55.3 ± 8.7% of the energy as carbohydrates and 22.1 ± 11.8 g of dietary fiber when starting the intervention. Nutrient and energy intake remained stable during the study period and no significant differences were observed between groups.

The characteristics of the subjects are presented in Table 2. High blood pressure was the most prevalent secondary pathology (75.7%, $n = 28$) alone or combined with other non-communicable diseases (NCDs), followed by dyslipidemia (35%, $n = 7$) and hypothyroidism (19%, $n = 5$). Thirteen subjects (14%) presented only type 2 diabetes. Weight and BMI did not change significantly during the intervention or between groups, in both groups a non-significant decrease in systolic pressure was observed, while diastolic blood pressure remained almost constant, without significant differences between the groups (Table 3). Systolic blood pressure was reduced in both groups, but not significantly, while diastolic pressure remained stable during the intervention period (Table 3).

3.2. Glycemic control

At the end of the intervention, HbA1c there was a decrease in the β-glucan group ($p < 0.001$) while in the control group it increased, but not significantly. Between the groups, the change was not different ($p = 0.074$), with a non-significant trend. Fasting glycemia and insulin remained stable during the period. An increase was observed in the control group and a decrease in the β-glucan group, but these changes were not different. Peptide C decreased in the β-glucan group ($p = 0.023$). Between the groups, there was a difference ($p = 0.03$). A significant change between groups was found in HOMA ($p = 0.013$), with a 28% reduction in the β-glucan group.

Table 2
Demographic and clinical characteristics of the study subjects by group at the beginning of intervention.

	Control	β-glucan	p-value*
n (woman/man)	17(13/4)	20 (15/5)	
Age (years)	52.8 ± 3.45	49.3 ± 6.75	0.058
Weight (Kg)	81.7 ± 14.9	87.3 ± 15.3	0.264
Height (cm)	155 ± 9.20	159 ± 9.3	0.202
BMI (Kg/mt2)	34.2 ± 7.04	33.2 ± 5.16	0.781
SBP (mmHg)	143 ± 19.9	141 ± 20.1	0.850
DBP (mmHg)	82.7 ± 13.3	81.9 ± 11.0	0.832
Time since diabetes diagnosis (years)	6.88 ± 3.84	7.55 ± 5.87	0.681
HbA1c (%)	8.78 ± 1.73	8.91 ± 1.57	0.823
Fasting glucose (mg/ml)	210 ± 62.3	170 ± 46.6	**0.045**
Insulin (pmol/l)	146 ± 17.0	158 ± 43.1	0.522
HOMA	63.4 ± 24.8	46.5 ± 18.0	0.360
PEP-C (ng/ml)	0.54 ± 0.16	0.99 ± 0.86	**0.032**
CT (mg/dl)	145 ± 63.9	147 ± 48.7	0.920
TAG (mg/dl)	258 ± 150	269 ± 130	0.823
HDL-C (mg/dl)	25.1 ± 10.9	27.1 ± 9.34	0.419
LDL-C (mg/dl)	53.3 ± 21.5	59.2 ± 27.8	0.548
VLDL-C (mg/dl)	36.3 ± 18.4	40.0 ± 15.8	0.588
Ghrelin (ng/ml)	0.99 ± 0.19	0.94 ± 0.25	0.970
Leptin (ng/ml)	0.22 ± 0.12	0.52 ± 0.29	**0.004**
GLP-1 (μg/ml)	15.0 ± 6.1	18.7 ± 2.23	0.068
PYY (pg/ml)	199 ± 13.4	160 ± 19.0	**0.000**
Zonulin (ng/ml)	2.10 ± 0.62	1.70 ± 0.47	0.373

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: Glycosylated hemoglobin A1c; HOMA: homeostatic model assessment of insulin resistance; CT: Total Cholesterol; TAG: Triglycerides. Data show mean ± SD. *T-test.

3.3. Lipid profile

We analyzing the lipid profile, and no significant changes were observed between the groups. However, in the control group, a non-significant increase in CT, HDLc, VLDLc, and TAG was found, while in the β-glucan group CT, VLDLc, and TAG decreased, this latter was significantly different ($p = 0.041$) (Table 3).

3.4. Hormones

The groups were comparable in most of the parameters, except for

Table 3
Changes in clinical, hormonal and dietary factors at the end intervention between groups.

	Control	β-glucan	p-value*
Weight (Kg)	−0.15 ± 2.13	−0.80 ± 3.51	0.786
BMI (Kg/mt2)	−0.05 ± 0.86	−0.31 ± 1.34	0.833
SBP (mmHg)	−8.73 ± 16.4	−7.83 ± 15.2	0.880
DBP (mmHg)	1.18 ± 10.2	−2.00 ± 7.72	0.347
HbA1c (%)	0.36 ± 1.28	−0.68 ± 1.89*	0.074
Fasting glucose (mg/ml)	16.6 ± 110	−30.8 ± 77.2	0.140
Insulin (pmol/l)	0.08 ± 0.91	−1.31 ± 1.85	**0.022**
HOMA	9.86 ± 38.2	−26.9 ± 33.2	**0.013**
PEP-C (ng/ml)	0.07 ± 0.36	−0.73 ± 0.82*	**0.030**
CT (mg/dl)	0.12 ± 64.7	−9.6 ± 57.1	0.326
TAG (mg/dl)	22.1 ± 138	−75.1 ± 152*	0.056
HDL-C (mg/dl)	2.29 ± 10.3	1.75 ± 9.03	0.998
LDL-C (mg/dl)	−1.16 ± 23.7	5.44 ± 37.4	0.666
VLDL-C (mg/dl)	11.2 ± 16.4	−2.80 ± 23.5	0.108
Ghrelin (ng/ml)	0.04 ± 0.22	0.15 ± 0.26	0.363
Leptin (ng/ml)	0.02 ± 0.14	−0.24 ± 0.35	**0.033**
GLP-1 (μg/ml)	0.31 ± 5.66	−8.51 ± 0.60*	**0.001**
PYY (pg/ml)	−10.2 ± 21.4	46.0 ± 28.9*	**0.000**
Zonulin (ng/ml)	0.16 ± 0.37	−0.04 ± 0.41	0.128
Energy (Kcal/day)	−56.7 ± 453	−83.9 ± 667	0.695
Proteins (g/day)	−5.15 ± 21.2	−8.89 ± 29.4	0.880
Fat (g/day)	−2.91 ± 17.4	−0.21 ± 29.8	0.928
Carbohydrates (g/day)	−1.14 ± 83.2	−8.29 ± 100	0.833
Dietary fiber (g/day)	−0.13 ± 12.13	−1.17 ± 14.3	0.928

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: Glycosylated hemoglobin A1c; HOMA: homeostatic model assessment of insulin resistance; CT: Total Cholesterol; TAG: Triglycerides.
Data show mean ± SD. * T-test.

fasting blood glucose and YY peptide, which were higher in the control group. C peptide and leptin were higher in the β-glucan group, so results should be interpreted with caution (Table 2). Ghrelin concentrations were stable during the intervention period. Leptin concentration changed significantly between the groups ($p = 0.033$). In the β-glucan group, the concentration decreased, while in the control group no important changes were observed. Inverse situations were observed in GLP-1 and PYY. In the control group, we observed non-significant increases in GLP-1 and decreases in PYY. On the other hand, in the β-glucan group, while GLP-1 decreased significantly ($p = 0.001$), PYY increased remarkably ($p = 0.001$). When comparing the groups, the effect was significant.

3.5. Gut microbiota

Intestinal patency was determined by zonulin concentration which remained stable during intervention period. A slight non-significant increase in the control group and a slight non-significant decrease in β-glucan group was observed. No changes between the groups were observed. Total bacteria decreased in the β-glucan group ($p < 0.05$). Also, there was a treatment and interaction effect (Two-way ANOVA, $p < 0.037$ and $p < 0.024$, respectively). Firmicutes, Bacteroidetes and Verrucomicrobia phyla decreased in β-glucan group ($p < 0.05$, T-test), However, only in Verrucomicrobia phyla showed an interaction effect ($p < 0.001$, Two way Anova) (Fig. 1).

Populations of *Lactobacillus spp* decreased significantly in the β-glucan group ($p = 0.020$) and there was a time effect ($p < 0.016$, Two way Anova). Populations of *Bifidobacteruim spp* decreased in β-glucan group ($p < 0.042$, T-test), however, no differences were observed for the interaction. *Akkermansia M.* did not change in the β-glucan group. There was an interaction effect (0.031, Two-way ANOVA). Populations of butyrate-producing bacteria in the β-glucan group decreased significantly ($p = 0.019$), while in the control group bacteria increased. There was an interaction effect ($p < 0.005$, Two-way ANOVA) (Fig. 2).

4. Discussion

The present study shows that long-term intake of 5 g/day of a supplement of oat β-glucan consumed daily for 12 weeks as part of a regular diet, has a beneficial effect on the metabolic control of T2D subjects, improving glycemic control, TAG, intestinal habit (stool frequency), regulating hormones and modifying the intestinal microbiota.

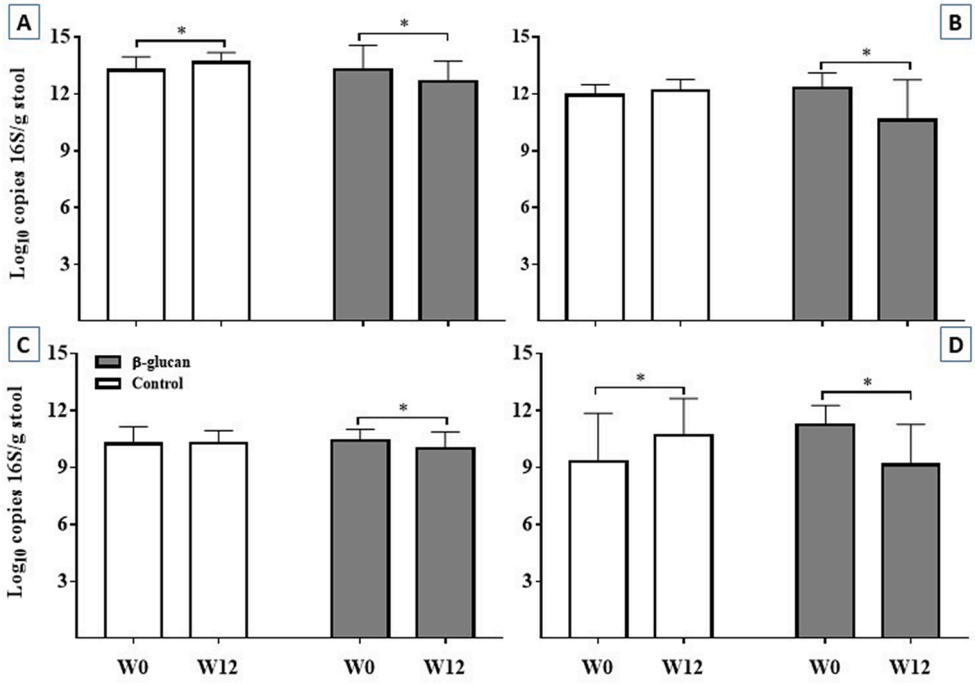

Fig. 1. Change in the gut microbiota profile and bacterial family during the intervention and between groups. A: Total bacteria; B: Firmicutes; C: Bacteroidetes and D: Verrucomicrobia. The figures show Log10 of copies of genes of interest per g/stool at day 0 and after 12 weeks of intervention. *$p < 0.05$. T-Test or Wilcoxon rank test for paired samples for each group.

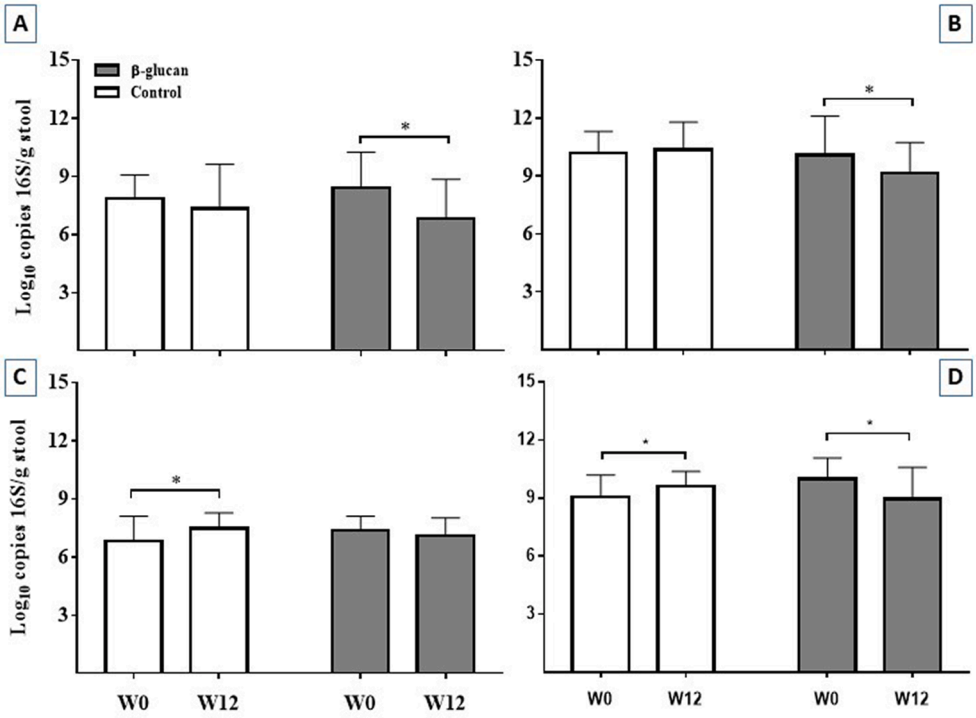

Fig. 2. Change in the gut microbiota profile and bacterial family during the intervention and between groups. A: *Lactobacillus spp*; B: *Bifidobacterium spp*; C: *Akkermansia Muciniphila* and D: butyrate-producing bacteria. The figures show Log10 of copies of genes of interest per g/stool initial and end of intervention. *$p < 0.05$. T-Test or Wilcoxon rank test for paired samples for each group.

Several studies and meta-analyses have demonstrated the positive effect of oat β-glucans on glycemic control parameters in type 2 diabetic subjects when incorporated into the diet (Andrade et al., 2014; Hou et al., 2015; Shen et al., 2016). Our study showed similar effects, with a 0.68% decrease in glycated hemoglobin A1c. This effect may be related to various properties and effects of oat β-glucans. First, its gelling capacity, which occurs at low concentrations (1%) and at an optimal temperature of 37 °C (body temperature), which does not occur with barley β-glucan (57 °C) (Mäkelä, Maina, Vikgren, & Sontag-Strohm, 2017). This property would decrease the glycemic effect of food (Ekström & Henningsson-Bok, 2017). In addition, a decrease in the expression of glucose transporters SGLT1 and GLUT2 has been described (Abbasi, Purslow, Tosh, & Bakovic, 2016), and an inhibition of the enzyme α-glycosidase (Dong et al., 2011); effects that would keep blood glucose lower. These properties regulate the absorption of carbohydrates, but there is another effect that could participate in this control: the increase of GLP-1. This incretin stimulates release of insulin, even more intensely than GIP (Jones, Bloom, Buenaventura, Tomas, & Rutter, 2018). The increase of this peptide would be related to the generation of short chain fatty acids, acetate, propionate and butyrate, a product of β-glucan fermentation by the intestinal microbiota, which would stimulate endocrine L cells of the intestine and increase production (Burcelin, 2017).

Similar interventions in asymptomatic individuals with barley β-glucan (Aoe et al., 2017) or in overweight women supplemented with oat β-glucan (Beck, Tapsell, Batterham, Tosh, & Huang, 2010), have described no changes in lipid profile. Some trials in subjects with T2D have shown no changes (Cugnet-Anceau et al., 2010), but there are other investigations that show significant changes in LDL-cholesterol, non-HDL-cholesterol and Apo-B (Ho et al., 2016), so there is conflicting information. In the present study, it was possible to identify a significant decrease in triglycerides (TAG), without changes in the rest of the lipid profile similar to that described by Tessari and Lante (2017). These authors evaluated the effect of a functional bread with beta-glucans in diabetic subjects and found a significant decrease in TAG, without changes in the rest of the lipid profile. This decrease in TAG levels could be related to the action of SCFA, since they could modulate lipolysis in adipocytes; while butyrate increases lipolytic action by inhibiting histone deacetylase enzymes (HDAC) (Rumberger, Arch, & Green, 2014), acetate attenuates lipolysis by decreasing hormone-sensitive lipase (HSL) phosphorylation (Jocken et al., 2018). This modification therefore would have no effect on enteroendocrine peptides, as it has been described that infusions of GLP-1 and PYY separately or in combination does not increase serum lipase levels (Schmidt et al., 2016).

In short-term studies, it has been possible to observe an important effect of oat β-glucans in transforming diet, modulating levels of ghrelin, PYY, GLP-1, GIP and leptin, among others (Barone-Lumaga et al., 2012; Hartvigsen et al., 2014; Vitaglione et al., 2009). At least one long-term study has also demonstrated similar effects (Richter et al., 2019). In this study, no significant differences were observed in leptin and ghrelin, while GLP-1 and PYY showed significant changes. Incretin decreased 48%, while YY peptide increased 26%. In this regard, the evidence is contradictory, studies in murine models indicate a significant increase of more than 2 times in both peptides (Adam et al., 2014). While human studies using solid and liquid food matrices enriched with β-glucans have not shown significant effects on GLP-1 (Barone-Lumaga et al., 2012; Hartvigsen et al., 2014). Other studies show a decrease in this incretin 90 min after consuming a breakfast containing β-glucan versus a control. ($p = 0.021$) (Zaremba et al., 2018). On the other hand, other studies have reported a 16% ($p < 0.005$) increase in AUC for PYY (Vitaglione et al., 2009). So studies are still lacking in this regard.

A possible explanation for this finding may be fermentation of β-glucans by intestinal microbiota. This process, with the consequent release of SCFA, would stimulate FFAR2 receptors, along with the activity and number of colonic endocrine L cells, increasing the release of basal PYY but not from GLP-1(Brooks et al., 2016). GLP-1 secretion is regulated by a combination of pathways activated by nutrients, neural and hormonal signals, with nutrient intake being the main regulator of its expression in enteroendocrine L cells. Therefore, the lower levels of GLP-1 compared with the control could be related to a homeostatic

effect produced by the formation of gels by β-glucans, producing a lower absorption and availability of glucose monomers (Abbasi et al., 2016; Dong et al., 2011).

Intestinal microbiota was analyzed using a semi-quantitative method previously described (Kralik & Ricchi, 2017; Liu & Saint, 2002). Bacterial 16S rRNA genes from variable regions 3 and 4 were used as reference (housekeeping) (Magne et al., 2006). While some studies report an increase in *Lactobacillus* and *Bifidobacterium* populations due to the intake of barley β-glucans (Arena et al., 2014) and in-vitro studies show that oat β-glucans promote the growth of *Lactobacillus* (Dong, Yu, Dong, & Shen, 2017), other investigations do not report significant changes for the same bacterial populations (Hughes, Shewry, Gibson, McCleary, & Rastall, 2008). In this investigation, a significant decrease in the relative quantity of *Lactobacillus* and *Bifidobacterium* was identified in the group supplemented with oat β-glucans, a result that has not been described in other similar studies. However, like effects have been described with other dietary fibers (Pedersen et al., 2016). In vitro studies suggest that stimulation of bacterial populations of gut microbiota are related to the degree of polymerization of dietary fibers. In this sense, the *Lactobacillus* and *Bifidobacterium* populations are negatively correlated (Chen et al., 2020), which could explain the effect of oat β-glucan.

In T2D subjects, an increase in *Lactobacillus* populations mediated by the intake of metformin has been described (Wu et al., 2017). Metformin has a close relationship with the microbiota, in fact, since there is a feeble microbiota with little diversity, the anti-hyperglycemic and anti-hyperlipidemic effect of metformin is weakened (Wu et al., 2019). In this study, oat β-glucan could be modulating bacterial populations. One interesting finding is a significant decrease in butyrate-producing bacteria. Ingestion of oat β-glucans has previously been described to increase butyrate concentrations (Queenan et al., 2007), both in the cecum and the colon (Adam et al., 2014) and that it is more effective than barley beta-glucans (Shen, Dang, Dong, & Hu, 2012). Therefore, this effect could be responding to better functioning of the butyrate-producing bacteria and not to the increase in population size. This decrease is also related to the significant decrease in the Firmicutes phylum, where most of these bacterial groups are classified. The increase in these bacterial populations could be mediated by factors such as diet and medications in T2D subjects. The dietary fiber could reduce this overgrowth. With our results, it is not clear why the abundances of these phyla and bacterial groups decrease. We could attribute them to the characteristics of oat beta-glucans, but it is necessary to carry out more studies, perhaps in vitro studies, to identify the causes and mechanisms involved.

An unexpected and intriguing finding was the significant increase in gut microbiota populations, especially Verrucomicrobia phylum, including *Akkermansia Muciniphila*, and an increase in butyrate-producing bacterial populations in the group supplemented with microcrystalline cellulose (control). These findings could suggest that microcrystalline cellulose could regulate these populations collaborating with a better metabolic profile in subjects with type 2 diabetes, as has been described with other compounds and food matrices (Jayachandran, Chung, & Xu, 2020).

There are various studies that associate glycemic control with the relative abundance of some bacterial species or compounds derived from their metabolism. For example, Akkermansia muciniphila, this bacterium has the function of regulating intestinal permeability by maintaining the mucous layer and it has been associated with glycemic control, regulation of insulin resistance and decrease in plasma cholesterol (Depommier et al., 2019). On the other hand, metabolites derived from fermentation in the intestinal microbiota (such as SCFA) have been related to development of DM1 (Kim, 2018) and the control of T2D (Mandaliya & Seshadri, 2019).

The results observed in the glycemic control could be associated with the gelling effect of β-glucans. This property would decrease or delay the release of glucose in the intestinal lumen, reducing the pick of glucose and in turn of insulin. These results also could be associated with this property of glucans rather than to the change in the intestinal microbiota.

We have exhaustively evaluated the effect of oat b-glucans on metabolic control in subjects with type-2 diabetes. Our results showed the effect of this dietary fiber on the regulation of appetite hormones and fecal microbiota in T2D. Some of our results were unexpected and raise new research questions to be answered in future investigations. In conclusion, this 3-month intervention trial in T2D subjects shows that a daily single intake of 5 g with a supplement of oat β-glucan (higher than the official recommendation of 3 g) improves, in general terms, metabolic control. Oat β-glucan may be useful as a supplement for regular consumption among persons with T2D.

Ethical approval

Human experiments were performed in accordance with human protection regulations of the Institute of Nutrition and Food Technology of the University of Chile. The protocol was approved by the Ethics Committee of the Maule Health Service, Chile and the Talca Community Health Service.

Author contributions

JP, VM and MA were responsible for study conception and design. JP performed experiments. JP and MA analyzed data. JP, VM and MA interpreted experiment results. JP prepared figures. JP and MA drafted manuscript. JP, VM and MA edited, revised and approved the final version of manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abbasi, N. N., Purslow, P. P., Tosh, S. M., & Bakovic, M. (2016). Oat β-glucan depresses SGLT1- and GLUT2-mediated glucose transport in intestinal epithelial cells (IEC-6). *Nutrition Research, 36*(6), 541–552. https://doi.org/10.1016/j.nutres.2016.02.004.

Adam, C. L., Williams, P. A., Dalby, M. J., Garden, K., Thomson, L. M., Richardson, A. J., , … Ross, A. W., et al. (2014). Different types of soluble fermentable dietary fibre decrease food intake, body weight gain and adiposity in young adult male rats. *Nutrition & Metabolism (Lond), 11*(1), 36. https://doi.org/10.1186/1743-7075-11-36.

Administration USF and D (2019). Title 21 - Food and Drugs Chapther I - Ffood and Drug Administration Department of Health and Human Services Subchapter b - Food for Human Consumption. [Internet]. Available from: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=101.81.

Aleixandre, A., & Miguel, M. (2016). Dietary fiber and blood pressure control. *Food and Function, 7*(4), 1864–1871. https://doi.org/10.1039/c5fo00950b.

American Diabetes Associatio-ADA (2020), Glycemic Targets: Standards of Medical Care in Diabetes—2020, Diabetes Care 2020 Jan; 43(Supplement 1): S66-S76. https://doi.org/10.2337/dc20-S006.

Andrade, E. F., Lobato, R. V., De Araújo, T. V., Zangerônimo, M. G., De Sousa, R. V., & Pereira, L. J. (2014). Effect of Beta-Glucans in the control of blood glucose levels of diabetic patients: A systematic review. *Nutrición Hospitalaria, 31*(1), 170–177. https://doi.org/10.3305/nh.2015.31.1.7597.

Aoe, S., Ichinose, Y., Kohyama, N., Komae, K., Takahashi, A., Abe, D., … Yanagisawa, T. (2017). Effects of high β-glucan barley on visceral fat obesity in Japanese individuals: A randomized, double-blind study. *Nutrition, 42*, 1–6. https://doi.org/10.1016/j.nut.2017.05.002.

Arena, M. P., Caggianiello, G., Fiocco, D., Russo, P., Torelli, M., Spano, G., & Capozzi, V. (2014). Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria. *International Journal of Molecular Sciences, 15*(2), 3025.3039. https://doi.org/10.3390/ijms15023025.

Bao, L., Cai, X., Xu, M., & Li, Y. (2014). Effect of oat intake on glycaemic control and insulin sensitivity: A meta-analysis of randomized controlled trials. *British Journal of Nutrition, 112*(3), 457–466. https://doi.org/10.1017/S0007114514000889.

Barone-Lumaga, R., Azzali, D., Fogliano, V., Scalfi, L., & Vitaglione, P. (2012). Sugar and dietary fibre composition influence, by different hormonal response, the satiating capacity of a fruit-based and a β-glucan-enriched beverage. *Food and Function, 3*(1), 67–75. https://doi.org/10.1039/c1fo10065c.

Barsanti, L., Passarelli, V., Evangelista, V., Frassanito, A. M., & Gualtieri, P. (2011). Chemistry, physico-chemistry and applications linked to biological activities of β-glucans. *Natural Product Reports, 28*(3), 457–466. https://doi.org/10.1039/c0np00018c.

Battilana, P., Ornstein, K., Minehira, K., Schwarz, J. M., Acheson, K., Schneiter, P., … Tappy, L. (2001). Mechanisms of action of beta-glucan in postprandial glucose metabolism in healthy men. *European Journal of Clinical Nutrition, 55*(5), 327–333. https://doi.org/10.1038/sj.ejcn.1601160.

Beck, E. J., Tapsell, L. C., Batterham, M. J., Tosh, S. M., & Huang, X. F. (2010). Oat β-glucan supplementation does not enhance the effectiveness of an energy-restricted diet in overweight women. *British Journal of Nutrition, 103*(8), 1212–1222. https://doi.org/10.1017/S0007114509992856.

Brooks, L., Viardot, A., Tsakmaki, A., Stolarczyk, E., Howard, J. K., Cani, P. D., … Bewick, G. A. (2016). Fermentable carbohydrate stimulates FFAR2-dependent colonic PYY cell expansion to increase satiety. *Molecular Metabolism, 6*(1), 48–60. https://doi.org/10.1016/j.molmet.2016.10.011.

Buil-Cosiales, P., Toledo, E., Salas-Salvadó, J., Zazpe, I., Farràs, M., Basterra-Gortari, F. J., … Martínez-González, M. A. (2016). Association between dietary fibre intake and fruit, vegetable or whole-grain consumption and the risk of CVD: Results from the PREvención con DIeta MEDiterránea (PREDIMED) trial. *British Journal of Nutrition, 116*(3), 534–546. https://doi.org/10.1017/S0007114516002099.

Burcelin, R. (2017). When gut fermentation controls satiety: A PYY story. *Molecular Metabolism, 6*(1), 10–11. https://doi.org/10.1016/j.molmet.2016.11.005.

Chen, M., Fan, B., Liu, S., Imam, K. M. S. U., Xie, Y., Wen, B., & Xin, F. (2020). The in vitro effect of fibers with different degrees of polymerization on human gut bacteria. *Frontiers in Microbiology, 11*, 819. https://doi.org/10.3389/fmicb.2020.00819.

Cugnet-Anceau, C., Nazare, J. A., Biorklund, M., Le Coquil, E., Sassolas, A., Sothier, M., … Moulin, P. (2010). A controlled study of consumption of β-glucan-enriched soups for 2 months by type 2 diabetic free-living subjects. *British Journal of Nutrition, 103*(3), 422–428. https://doi.org/10.1017/S0007114509991875.

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., , … Cani, P. D., et al. (2019). Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: A proof-of-concept exploratory study. *Nature Medicine, 25*(7), 1096–1103. https://doi.org/10.1038/s41591-019-0495-2.

Derrien, M., Collado, M. C., Ben-Amor, K., Salminen, S., & de Vos, W. M. (2008). The Mucin Degrader Akkermansia muciniphila is an abundant resident of the human intestinal tract. *Applied and Environmental Microbiology, 74*(5), 1646–1648. https://doi.org/10.1128/AEM.01226-07.

Dong, J., Cai, F., Shen, R., & Liu, Y. (2011). Hypoglycaemic effects and inhibitory effect on intestinal disaccharidases of oat beta-glucan in streptozotocin-induced diabetic mice. *Food Chemistry, 129*(3), 1066–1071. https://doi.org/10.1016/j.foodchem.2011.05.076.

Dong, J., Yu, X., Dong, L., & Shen, R. (2017). In vitro fermentation of oat β -glucan and hydrolysates by fecal microbiota and selected probiotic strains. *Journal of the Science of Food and Agriculture, 97*(12), 4198–4203. https://doi.org/10.1002/jsf.8292.

EFSA Panel on Dietetic Products N and A (NDA). (2011). Scientific Opinion on the substantiation of health claims related to beta-glucans from oats and barley and maintenance of normal blood LDL-cholesterol concentrations (ID 1236, 1299), increase in satiety leading to a reduction in energy intake (ID 851, 852. European Food Safety Authority Journal, 9(6), 2207. https://doi.org/10.2903/j.efsa.2011.2207.

Ekström, L. M. N. K., Henningsson-Bok, E. A. E-, Sjöö, M. E. & Östman, E. M. (2017). Oat β-glucan containing bread increases the glycaemic profile. Journal of Functional Foods, 32, 106–111. https://doi.org/10.1016/j.jff.2017.02.027.

Guo, X., Xia, X., Tang, R., Zhou, J., Zhao, H., & Wang, K. (2008). Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. *Letters in Applied Microbiology, 47*(5), 367–373. https://doi.org/10.1111/j.1472-765X.2008.02408.x.

Hartvigsen, M. L., Gregersen, S., Lærke, H. N., Holst, J. J., Bach-Knudsen, K. E., & Hermansen, K. (2014). Effects of concentrated arabinoxylan and β-glucan compared with refined wheat and whole grain rye on glucose and appetite in subjects with the metabolic syndrome: A randomized study. *European Journal of Clinical Nutrition, 68*(1), 84–90. https://doi.org/10.1038/ejcn.2013.236.

Ho, H. V. T., Sievenpiper, J. L., Zurbau, A., Blanco-Mejia, S., Jovanovski, E., Au-Yeung, F., … Vuksan, V. (2016). The effect of oat β -glucan on LDL-cholesterol, non-HDL-cholesterol and apoB for CVD risk reduction: A systematic review and meta-analysis of randomised-controlled trials. *British Journal of Nutrition, 116*(8), 1369–1382. https://doi.org/10.1017/S000711451600341X.

Hou, Q., Li, Y., Li, L., Cheng, G., Sun, X., Li, S., & Tian, H. (2015). The metabolic effects of oats intake in patients with type 2 diabetes: A systematic review and meta-analysis. *Nutrients, 7*(12), 10369–10387. https://doi.org/10.3390/nu7125536.

Hughes, S. A., Shewry, P. R., Gibson, G. R., McCleary, B. V., & Rastall, R. A. (2008). In vitro fermentation of oat and barley derived β-glucans by human faecal microbiota. *FEMS Microbiology Ecology, 64*(3), 482–493. https://doi.org/10.1111/j.1574-6941.2008.00478.x.

Jayachandran, M., Chung, S. S. M., & Xu, B. (2020). A critical review of the relationship between dietary components, the gut microbe Akkermansia muciniphila and human health. *Critical Reviews in Food Science and Nutrition, 60*(13), 2265–2276. https://doi.org/10.1080/10408398.2019.1632789.

Jocken, J. W. E., González-Hernández, M. A., Hoebers, N. T. H., van der Beek, C. M., Essers, Y. P. G., Blaak, E. E., & Canfora, E. E. (2018). Short-chain fatty acids differentially affect intracellular lipolysis in a human white adipocyte model. *Frontiers in Endocrinology (Lausanne), 8*, 372. https://doi.org/10.3389/fendo.2017.00372.

Jones, B., Bloom, S. R., Buenaventura, T., Tomas, A., & Rutter, G. A. (2018). Control of insulin secretion by GLP-1. *Peptides, 100*, 75–84. https://doi.org/10.1016/j.peptides.2017.12.013.

Kim, C. H. (2018). Microbiota or short-chain fatty acids: Which regulates diabetes? *Cellular & Molecular Immunology, 15*(2), 88–91. https://doi.org/10.1038/cmi.2017.57.

Kralik, P., & Ricchi, M. (2017). A basic guide to real time PCR in microbial diagnostics: Definitions, parameters, and everything. *Frontiers in Microbiology, 8*, 108. https://doi.org/10.3389/fmicb.2017.00108.

Liu, M., Zhang, Y., Zhang, H., Hu, B., Wang, L., Qian, H., & Qi, X. (2016). The anti-diabetic activity of oat β-d-glucan in streptozotocin-nicotinamide induced diabetic mice. *International Journal of Biological Macromolecules, 91*, 1170–1176. https://doi.org/10.1016/j.ijbiomac.2016.06.083.

Liu, W., & Saint, D. A. (2002). A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. *Analytical Biochemistry, 302*(1), 52–59. https://doi.org/10.1006/abio.2001.5530.

Louis, P., & Flint, H. J. (2007). Development of a semiquantitative degenerate real-time PCR-based assay for estimation of numbers of butyryl-coenzyme A (CoA) CoA transferase genes in complex bacterial samples. *Applied and Environmental Microbiology, 73*(6), 2009–2012. https://doi.org/10.1128/AEM.02561-06.

Magne, F., Abély, M., Boyer, F., Morville, P., Pochart, P., & Suau, A. (2006). Low species diversity and high interindividual variability in faeces of preterm infants as revealed by sequences of 16S rRNA genes and PCR-temporal temperature gradient gel electrophoresis profiles. *FEMS Microbiology Ecology, 57*(1), 128–138. https://doi.org/10.1111/j.1574-6941.2006.00097.x.

Mäkelä, N., Maina, N. H., Vikgren, P., & Sontag-Strohm, T. (2017). Gelation of cereal β-glucan at low concentrations. *Food Hydrocolloids, 73*, 60–66. https://doi.org/10.1016/j.foodhyd.2017.06.026.

Mandaliya, D. K., & Seshadri, S. (2019). Short Chain Fatty Acids, pancreatic dysfunction and type 2 diabetes. *Pancreatology, 19*(2), 280–284. https://doi.org/10.1016/j.pan.2019.01.021.

Navarrete, A. A., Diniz, T. R., Braga, L. P. P., Silva, G. G. Z., Franchini, J. C., Rossetto, R., … Tsai, S. M. (2015). Multi-analytical approach reveals potential microbial indicators in soil for sugarcane model systems. Lehman RM, editor. *PLoS One, 10*(6), Article e0129765. https://doi.org/10.1371/journal.pone.0129765.

Paquet, E., Turgeon, S. L., & Lemieux, S. (2010). Effect of xanthan gum on the degradation of cereal β-glucan by ascorbic acid. *Journal of Cereal Sciences, 52*(2), 260–262. https://doi.org/10.1016/j.jcs.2010.06.003.

Pedersen, C., Gallagher, E., Horton, F., Ellis, R. J., Ijaz, U. Z., Wu, H., … Robertson, M. D. (2016). Host–microbiome interactions in human type 2 diabetes following prebiotic fibre (galacto-oligosaccharide) intake. *British Journal of Nutrition, 116*(11), 1869–1877. https://doi.org/10.1017/S0007114516004086.

Penders, J., Vink, C., Driessen, C., London, N., Thijs, C., & Stobberingh, E. (2005). Quantification of Bifidobacterium spp., Escherichia coli and Clostridium difficile in faecal samples of breast-fed and formula-fed infants by real-time PCR. *FEMS Microbiology Letters, 243*(1), 141.147. https://doi.org/10.1016/j.femsle.2004.11.052.

Pentikäinen, S., Karhunen, L., Flander, L., Katina, K., Meynier, A., Aymard, P., … Poutanen, K. (2014). Enrichment of biscuits and juice with oat β-glucan enhances postprandial satiety. *Appetite, 75*, 150–156. https://doi.org/10.1016/j.appet.2014.01.002.

Post, R. E., Mainous, A. G., 3rd., King, D. E., & Simpson, K. N. (2012). Dietary fiber for the treatment of type 2 diabetes mellitus: A meta-analysis. *Journal of the American Board of Family Medicine, 25*(1), 16–23. https://doi.org/10.3122/jabfm.2012.01.110148.

Queenan, K. M., Stewart, M. L., Smith, K. N., Thomas, W., Fulcher, R. G., & Slavin, J. L. (2007). Concentrated oat β-glucan, a fermentable fiber, lowers serum cholesterol in hypercholesterolemic adults in a randomized controlled trial. *Nutrition Journal, 6*(1), 6. https://doi.org/10.1186/1475-2891-6-6.

Richter, J., Závorková, M., Vetvicka, V., Liehneová, I., Kral, V., & Rajnohova-Dobiasova, L. (2019). Effects of β-glucan and Vitamin D supplementation on inflammatory parameters in patients with diabetic retinopathy. *Journal of Dietary Supplements, 16*(4), 369–378. https://doi.org/10.1080/19390211.2018.1458769.

Rinttila, T., Kassinen, A., Malinen, E., Krogius, L., & Palva, A. (2004). Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *Journal of Applied Microbiology, 97*(6), 1166–1177. https://doi.org/10.1111/j.1365-2672.2004.02409.x.

Rumberger, J. M., Arch, J. R. S., & Green, A. (2014). Butyrate and other short-chain fatty acids increase the rate of lipolysis in 3T3-L1 adipocytes. *PeerJ, 2*, Article e611. https://doi.org/10.7717/peerj.611.

Saeedi, P., Petersohn, I., Salpea, P., Malanda B., Karuranga, S., Unwin, N., … & IDF Diabetes Atlas Committee (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Research and Clinical Practice 157, 107843. https://doi.org/10.1016/j.diabres.2019.107843.

Schmidt, J. B., Sjödin, A., Stevner, L. S., Ritz, C., Michaelsen, N. B., Thomsen, A. B., … Astrup, A. (2016). Serum lipase activity and concentration during intravenous infusions of GLP-1 and PYY 3–36 and after ad libitum meal ingestion in overweight men. *Physiological Reports, 4*(18), Article e12980.

Shen, R.-L., Dang, X.-Y., Dong, J.-L., & Hu, X.-Z. (2012). Effects of oat β-glucan and barley β-glucan on fecal characteristics, intestinal microflora, and intestinal bacterial metabolites in rats. *Journal of Agricultural and Food Chemistry, 60*(45), 11301–11308. https://doi.org/10.1021/jf302824h.

Shen, X., Zhao, T., Zhou, Y., Shi, X., Zou, Y., & Zhao, G. (2016). Effect of oat β-glucan intake on glycaemic control and insulin sensitivity of diabetic patients: A meta-analysis of randomized controlled trials. *Nutrients, 8*(1), 39. https://doi.org/10.3390/nu8010039.

Sima, P., Vannucci, L., & Vetvicka, V. (2019). β-glucans and cholesterol (Review). *International Journal of Molecular Medicine, 41*(4), 1799–1808. https://doi.org/10.3892/ijmm.2018.3411.

Surampudi, P., Enkhmaa, B., Anuurad, E., & Berglund, L. (2016). Lipid lowering with soluble dietary fiber. *Current Atherosclerosis Reports, 18*(12), 75. https://doi.org/10.1007/s11883-016-0624-z.

Tessari, P., & Lante, A. (2017). A multifunctional bread rich in beta glucans and low in starch improves metabolic control in type 2 diabetes: A controlled trial. *Nutrients, 9*(3), 297. https://doi.org/10.3390/nu9030297.

Vitaglione, P., Lumaga, R. B., Stanzione, A., Scalfi, L., & Fogliano, V. (2009). Beta-Glucan-enriched bread reduces energy intake and modifies plasma ghrelin and peptide YY concentrations in the short term. *Appetite, 53*(3), 338–344. https://doi.org/10.1016/j.appet.2009.07.013.

Williams, R., Karuranga, S., Malanda, B., Saeedi, P., Basit, A., Besançon, S., … Colagiuri, S. (2020). Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Research and Clinical Practice, 162*, Article 108072. https://doi.org/10.1016/j.diabres.2020.108072.

Wu, B., Chen, M., Gao, Y., Hu, J., Liu, M., Zhang, W., & Huang, W. (2019). In vivo pharmacodynamic and pharmacokinetic effects of metformin mediated by the gut microbiota in rats. *Life Sciences, 226*, 185–192. https://doi.org/10.1016/j.lfs.2019.04.009.

Wu, H., Esteve, E., Tremaroli, V., Khan, M. T., Caesar, R., Mannerås-Holm, L., … Bäckhed, F. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Natural Medicine, 23*(7), 850–858. https://doi.org/10.1038/nm.4345.

Zacarías, I., Barrios, L., González, C. G., Loeff, T., & Vera, G. (2018). *Chilean table of food composition*. Santiago, Chile: Institute of Nutrition and Food Technology (INTA), University of Chile.

Zaremba, S. M. M., Gow, I. F., Drummond, S., McCluskey, J. T., & Steinert, R. E. (2018). Effects of oat β-glucan consumption at breakfast on ad libitum eating, appetite, glycemia, insulinemia and GLP-1 concentrations in healthy subjects. *Appetite, 128*, 197–204. https://doi.org/10.1016/j.appet.2018.06.019.

Zeinalova, N. V., Kurbanov, Y. Z., Mirzazade, V. A., Rzayeva, R. A., & Novruzova, M. S. (2017). The risk of cardiovascular death in type 2 diabetes. *Klinicheskaia meditsina (Mosk), 95*(1), 57–59.

Zhao, J., & Cheung, P. C. K. (2013). Comparative proteome analysis of Bifidobacterium longum subsp. infantis grown on β-glucans from different sources and a model for their utilization. *Journal of Agricultural and Food Chemistry, 61*(18), 4360–4370. https://doi.org/10.1021/jf400792j.