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DM II bugging you? Probiotics may help reach your glycemic goals

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ABSTRACT:

Objective: To determine the effects of probiotics on glycated hemoglobin (HbA1c) and fasting blood glucose (FBG) in patients with Type II Diabetes (T2DM) controlled with oral medications. **Design**: Systematic Literature Review. **Methods**: Searches were conducted on PubMed using the terms: prebiotic, probiotic, type 2 diabetes, non-insulin dependent, and microbiota. Searches refined with parameters for Randomized Control Trials, written in English, and available texts. **Results**: Firouzi et al. did not show a significant change in the FBG between groups and a decrease in the HbA1c in the intervention group. Ejtahed et al. showed a significant decrease in both FBG and HbA1c from the placebo group to the intervention group. Asemi et al. showed that probiotics prevented a rise in both FBG and decreased HbA1c in the intervention group (though not significantly). **Conclusion:** FBG was found to be an inconsistent indicator of the effectiveness of probiotics for T2DM management. However, HbA1c levels were consistently lower in the intervention groups compared to control groups. While statistical significance was shown, clinical significance and extrapolation to a US population is inconclusive based on this review. The results are promising, but further studies with longer durations and a US population should be conducted.

INTRODUCTION:

Diabetes is a growing global health crisis that currently affects 463 million adults world-wide. This number is projected to continue to increase to 578 million people worldwide by 2030 and then to 700 million by 2045.[1] As of 2018, 34.2 million people in the United States live with diabetes; that's 10.5% of the population[2].

Prediction and diagnosis of type II diabetes (T2D) utilizes impaired fasting glucose, impaired glucose tolerance, and glycated hemoglobin (HbA1c) values from serum. These measures become elevated due to decreased production of insulin, or decreasing sensitivity to insulin activity.[3] For short term changes, fasting blood glucose is a better assessor of glucose metabolism. HbA1c measures are less useful in assessing recent changes in blood glucose but treatment for T2D is focused on decreasing HbA1c which is a calculation of the mean blood sugar over a period of 8-12 weeks and a better indicator of overall diabetes management.[4] Metformin is the most commonly used first line therapy which has been shown to lower HbA1c by 0.6 to 1.48%.[5,6] The CDC reports that 50% of those with diabetes have a HbA1c above 7%, and 29% have a HbA1c above 8%.[2] Thus the treatment goal of a HbA1c less than 7% may be out of reach for many patients receiving monotherapy of Metformin. Many patients

need adjunct therapies to reach their goals and with new research, manipulating the gut microbiome is a possible method to avoid more pharmaceutical therapy.

With over a thousand species of bacteria, the gut microbiome living in the intestines has long been known to be an integral part of a person's health and with the discovery of the gut-brain access and enteric nervous system, science is just learning how important a diverse ecosystem can be to managing chronic illnesses. Studies have found that manipulating the bacteria residing in the gut of those with obesity can help them lose weight.[7] One way to manipulate the gut flora is by introducing probiotics into the diet. Probiotics are "live microorganisms that confer a health benefit on the host when administered in adequate amounts."[8] The most commonly used bacteria for probiotics are various *Bifidobacterium* and *Lactobacillus* strains.

The gut microbiome is easily manipulated by the diet a person consumes, and rightly so, considering that the biome eats whatever we are eating. Poor diets can decrease the quality and the quantity of the bacteria in the intestines. Studies have shown that high fat and high fructose diets can disrupt normal gut microflora and lead to low grade, systemic and chronic inflammation.[8] This inflammation is a precursor to obesity and T2D, conditions that are also exacerbated by a high sugar diet. Patients who are obese and/or have T2D have different gut microbiome metabolism by-products which suggest a different composition of bacteria than patients not suffering from those conditions.[8] Whether the gut microbiome change contributes to the development of diabetes, or having diabetes changes the composition of bacteria in the gut is not clear, but there is a difference between the types of bacteria in a "healthy" individual's intestines versus a person with diabetes, suggesting the importance of a specific composition of gut flora.

The presupposition that the composition of the gut microbiome has a direct relationship to diabetes allows for therapeutic application. Cell line studies indicate that metabolites of engineered E. coli and Lactobacillus species prevented expression of inflammatory cytokines and oxidative stress.[8] Inflammation and oxidative stress are implicated in the progression of T2D and present a possible mechanism for preventative measures. Furthermore, animal studies in diabetic mice show that food supplemented with certain Lactobacilli species decreased blood glucose and glycosylated hemoglobin.[8]

With the promise shown in animal trials of gut microbiome manipulation in T2D, the efficacy of probiotic therapy has not yet been routinely studied in human trials. This paper reviews the current studies on using probiotics as adjunct therapy for patients with T2D who are on oral medications in an effort to determine if probiotic administration is effective at improving diabetic control by reducing FBG compared to placebo.

METHODS:

A database search using Pubmed and the MESH terms “prebiotic”, “probiotic”, “type 2 diabetes”, “non-insulin dependent”, and “microbiota” produced 1060 results. This number was reduced using the filters to select randomized control trials (RCT), English language, and full texts. Articles were excluded if the subjects were less than 18 years old, on insulin therapy, pre-diabetic, diagnosed with type 1 diabetes, pregnant, or using endpoints that did not include fasting blood glucose and HbA1c. This search resulted in 3 studies which are used in this analysis as outlined in Figure 1.

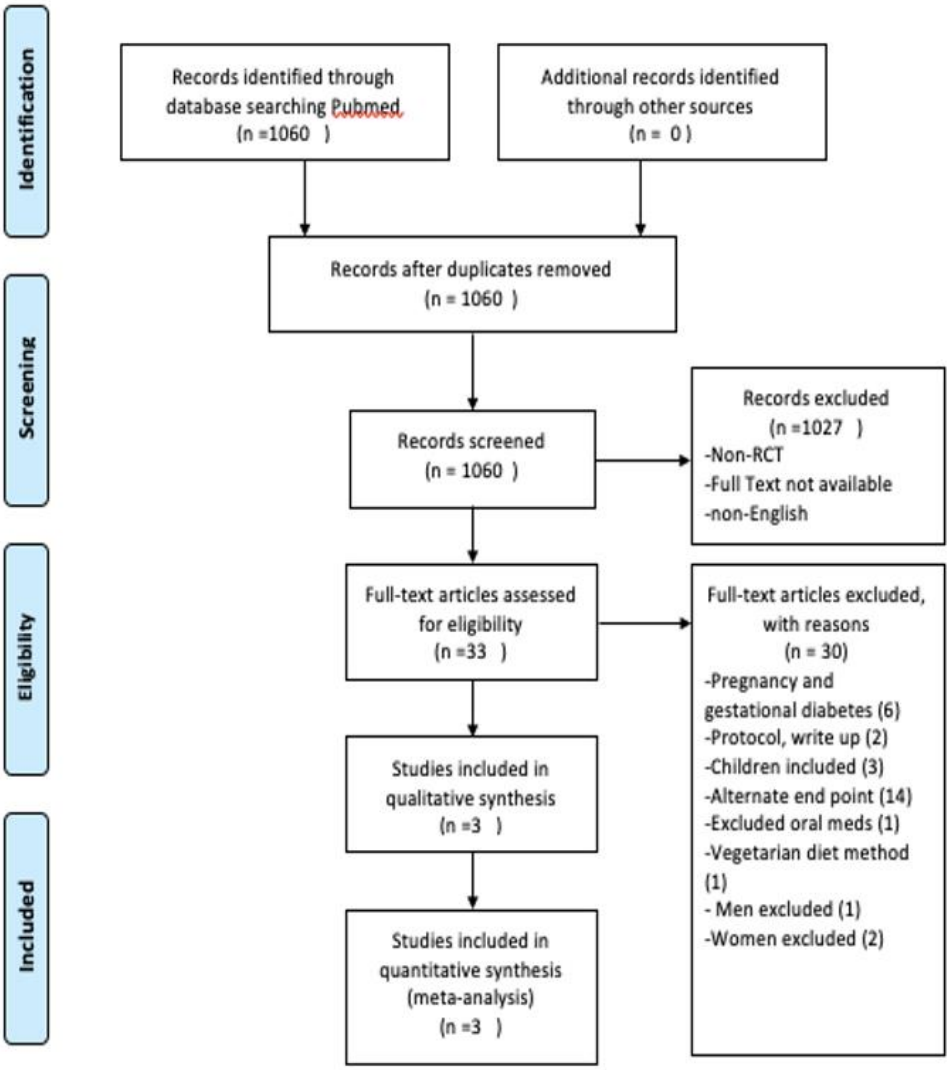

Figure 1. PRISMA algorithm for the selection of appropriate studies concerning the use of probiotics as adjunct therapy in adults with type II diabetes.

RESULTS:

Study 1

Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized control trial. Firouzi et al. 2016.[9]

Objective

Investigate the effects of multi-strain probiotics on many diabetes related outcomes in patients with type II diabetes including: glycemic control, lipid profile, blood pressure, and high-sensitivity C-reactive protein.

Design

This study is a randomized, double-blinded, parallel-group, controlled clinical trial performed at a diabetes clinic in Kuala Lumpur, Malaysia. A sum of 136 patients were selected based on a diagnosis of type II diabetes for at least 6 months, no use of insulin therapy or antibiotics, HbA1c between 6.5 and 12%, FBG less than 15mmol/L, BMI between 18.5 and 40kg/m^2, 3 months of stabile dose of medication, and age 30-70 years. These participants were split into groups of 68 and assigned to receive either a probiotic or placebo for a period of 12 weeks. All participants received the same dietary recommendations in order to reduce variation apart from the intervention. All participants were instructed to stop the use of any probiotic containing food and maintain their fiber intake two weeks prior to the start of the study and throughout the duration. Each patient fasted for 10-12 hrs prior to every evaluation and laboratory draw. The intervention consisted a powder containing six bacterial strains: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium longum, and Bifidobacterium infantis.* The powder was mixed into a glass of water and consumed morning and evening each day. Compliance was enforced by asking patients to bring their package of powder to each assessment for evaluation.

Glycemic control, anthropometry, BMI, lipid profile, blood pressure, high sensitivity CRP, and fecal samples were collected at baseline, 6 weeks, and 12 weeks. Statistical analysis of the data included intention to treat and per protocol formats.

Results

The study screened 6976 patients and selected 456 as eligible after which 136 agreed to participate. The participants were 52.2% male and groups were comparable in BMI (p=0.419) and oral antidiabetic medication (p=0.144). After 12 weeks, 20.6% dropped out of the study and 25.7% of the participants were classified as non-compliant, but were included in the study. According to per protocol analysis 48 participants from the treatment group completed the study and 53 from the placebo group.

The HbA1c slightly increased in the placebo group (-0.02± 0.56) and decreased (0.14 ± 0.41%) in the probiotic group. Additionally the mean insulin levels were significantly different between the two groups. These results were significant between

the two groups as seen in Table 1 below, but FBG had no significant changes throughout the duration of the study. The results for FBG varied widely as both the probiotic (-0.1± 1.5) and placebo (0.3±2.1) groups had unclear trends with large margins for error.

	n	Mean ± SD			Within-group *p* value	Week 6 − baseline	Week 12 − baseline	Interaction *p* values	
		Baseline	Week 6	Week 12				PP	ITT
Glycemic control parameters and inflammatory marker									
FBG (mmol/L)[a]								0.667	0.955
Probiotics group	48	7.3 ± 1.1	7.0 ± 1.2	7.2 ± 1.2	0.814	−0.3 ± 1.7	−0.1 ± 1.5		
Placebo group	53	7.9 ± 2.2	8.0 ± 2.3	8.2 ± 2.3	0.577	0.1 ± 2.2	0.3 ± 2.1		
HbA1c (%)[a]								<0.05	0.582
Probiotics group	48	7.46 ± 1.2	7.33 ± 1.2	7.32 ± 1.4	0.061	−0.14 ± 0.41	−0.14 ± 0.62		
Placebo group	53	7.29 ± 1.6	7.28 ± 1.6	7.31 ± 1.7	0.094	−0.01 ± 0.41	0.02 ± 0.56		
Insulin (μU/mL)[a]								<0.05	<0.05
Probiotics group	48	13.1 ± 8.6	10.8 ± 6.8	10.2 ± 6.3	0.050	−2.3 ± 6.8	−2.9 ± 8.5		
Placebo group	53	12.0 ± 6.7	12.5 ± 6.8	13.8 ± 6.3	0.231	0.5 ± 12.2	1.8 ± 9.0		

Table 1. Change in glycemic variables.

Critique

The randomized, double blind design and inclusion of both per-protocol and intention to treat analyses are strengths of this study. However, the randomization of participants was performed by the lead researchers and not a third person, which draws some concern. The total number of participants was the highest among the selected studies lending more statistical power to its results. Of note, this study focuses on the per-protocol results as these showed statistical significance. This is noted in Table 1 as the change in HbA1c is only significant in the per-protocol analysis, and not in the intention to treat analysis. In the discussion of the results the researchers neglect to mention that the most appropriate analysis of results should focus on intention to treat results and not per-protocol as the researchers take more liberty in deciding which participants or data points are included in the assessment.

Despite the design elements seeking to reduce variability between groups the researchers note that FBG is difficult to alter in well controlled patients with diabetes due to the variables introduced by uncontrolled factors such as different levels of physical activity and duration of fasting.

Finally, the study length is of concern when glycemic measurements such as HgA1c are used. These laboratory values are known to take months to fully adjust to changes in treatment or medication and a 12-week study may be insufficient to fully realize the impact of probiotics.

Study 2

Probiotic yogurt improves antioxidant status in type 2 diabetic patients

Ejtahed et al. 2011[10]

Objective

To determine the effect of probiotic-laced yogurt on blood glucose and antioxidant status in patients with type 2 diabetes and on oral medication compared to conventional yogurt.

Design

This study was a six week, randomized, double-blind, controlled clinical trial of 64 patients 30-60 years-old with at least a year-old diagnosis of T2DM recruited from an endocrinology clinic in Iran. Exclusion criteria included patients using insulin, cholesterol lowering medications, diuretics, or exogenous hormones, pregnant or breastfeeding patients, or patients with kidney, liver or inflammatory disease, thyroid disorders, immunodeficiency, or lactose intolerance. Patients were matched on sex and age and randomized into two groups containing 32 participants each. The control group received conventional yogurt with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures (present in standard yogurts), and the interventional group received an identical container of yogurt which contained *L. Bulgaricus* and *S. Thermophilus* as well as *B Lactis* Bb12 and *L. acidophilus* La5 cultures. There was a one-week run-in period where participants were instructed to not eat yogurt or other fermented foods. Participants were then given a week's supply of yogurt at a time and instructed to keep it refrigerated. Consumption compliance was monitored by a weekly telephone call. Three-day food diaries were conducted at the beginning and end of the study. Anthropometric measurement and 12 hour fasting blood samples were taken at the beginning and end of the trial.

Results

Per protocol results are based on 60 participants as four patients were excluded for changing medication during the trial or for not following protocol (30 participants for each group). The only statistically significant difference between the control and intervention group pre-study was the time from diagnosis. The intervention group on average had diabetes for 5.82 years (± 4.95 years) compared to the control group that had diabetes on average for 4.08 years (± 4.28 years) (P=0.039). There were no significant differences in dietary intake before or during the trial between the two groups. Fasting blood glucose and HbA1c significantly decreased in the intervention group compared to the control group (P=0.009 and P=0.019,respectively). However, based on the statistics, HbA1c did not significantly change for the intervention group (P=0.230), but the control group's HbA1c significantly increased (P=0.003) which

possibly led to the significant difference between the groups. See Table 2 for values.

Variables	Conventional yogurt ($n = 30$)	Probiotic yogurt ($n = 30$)
Glucose (mmol/L)		
Baseline	7.35 ± 1.28	8.06 ± 2.49
After intervention	7.53 ± 1.32	7.36 ± 2.41†,‡
HbA1c (%)		
Baseline	6.87 ± 0.81	7.29 ± 1.21
After intervention	7.17 ± 0.66‡	7.17 ± 1.24†

Table 2- Effects of 6 weeks of probiotic and conventional yogurt consumption on blood glucose, HbA1c, insulin, and oxidative stress markers

Critique

A strength of this study includes the RCT design and testing the activity of the cultures after a week to ensure that the participants are always consuming live cultures. Some concerns for this study include the per protocol analysis which, in a small sample size, can overestimate the effectiveness of the treatment. Also, there might possibly be a recall bias for the dietary consumption data; however, since the participants weren't initially matched based on diet or nutritional status before sorting, and the data wasn't stratified based on dietary information after the study, the only utility for the dietary information was to prove the similarities of the two groups and a recall bias might not have that much of a negative effect. There was no disclosure of conflicts of interest (one author is associated with the Iranian dairy industry as stated on the title page though in what capacity is unknown).

Study 3

Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes.

Asemi et al 2013[3]

Objective

To determine how multispecies probiotics affect the metabolic status and oxidative stress of patients with T2DM.

Design

This study was an 8-week, double-blind, randomized control trial of 60 patients 35-70 years-old with a T2DM diagnosis recruited from a diabetes clinic in Kashan, Iran. Exclusion criteria included usage of insulin or vitamin supplements, pregnancy, co-condition including chronic kidney, liver, lung or inflammatory diseases, heart valve disease, or allergies. Patients were matched on age, sex, BMI and oral hypoglycemic medications (type and dosage) and were assigned to the intervention or control group with 30 participants each with a makeup of 9 males and 21 females for each group.

Patients were required to complete a 2-week run-in period prior to starting where they were instructed to refrain from taking probiotic foods and complete a 3, nonconsecutive day food diary. After the run-in period, participants were either given probiotic supplement capsules or placebo capsules to take each day. Participants were to continue their normal activity and dietary habits while occasionally completing 3-day food diaries. The probiotic capsules contained *L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, Bifidobacterium breve, B. longum, Streptococcus thermophilus*, and 100 mg fructo-oligosaccharide with lactose as carrier substances. The placebo contained the same substances besides the bacteria and was packed in identical capsules. Consumption compliance was monitored by a once-weekly phone interview and the food diaries. Anthropometric measurements and overnight fasting blood samples were taken at the beginning and end of the study.

Results

Per protocol results are based on 54 participants after three patients from each group were excluded for use of antibiotics, supplements, or insulin or a diagnosis of chronic kidney disease. No significant differences were found between the intervention and control groups at the beginning of the study for dietary consumption or biochemical measures except for the HbA1c which was higher in the intervention group compared to the control group ($p = 0.007$). At the end of the study, there was a statistically significant difference between the intervention and control group for mean changes in FBG ($P=0.01$). However, both FBGs increased. The FBG for the intervention group increased by 1.6 (±6) mg/dL, however the FBG for the control group increased by 28.8 (±8.5) mg/dL. In this study, the probiotic appeared to prevent a rise in FBG instead of lowering. HbA1c was decreased in the intervention group and increased in the control group but neither change was statistically significant nor were the changes between groups significant. See Table 3 for results.

	Placebo (n = 27)				Probiotic supplement (n = 27)				p^b
	week 0	week 8	change	p^a	week 0	week 8	change	p^a	
FPG, mg/dl	134.5±9.6	163.3±12	28.8±8.5	0.002	143.8±10.7	145.4±9.5	1.6±6	0.80	0.01
$HbA1_C$, %	6.35±0.3	6.53±0.28	0.18±0.31	0.55	7.71±0.37	7.41±0.41	−0.3±0.37	0.42	0.32

Table 3-Within-group and between-group comparisons of metabolic profiles, hs-CRP, and biomarkers of oxidative stress after supplementation

Critiques

The strengths of this study include the two-week run-in period with no probiotic foods and the matching for multiple factors of the participants before randomization ensures that there is less difference between the two groups. Some concerns include the per protocol analysis leading to an overestimation of effect and the lack of disclosures of conflicts of interest. The study states that a company who sells

probiotics provided the probiotics for the trial, but it is unclear whether this was a "donation" or if they were purchased.

DISCUSSION:

In summary, FBG was found to be a variable indicator of improved glycemic control with the use of probiotics. Firouzi et al. found no significant difference, Ejtahed et al. found significant differences within the intervention group, and Asemi et al. stated that it stabilized FBG compared to the increase seen in the control group. This may indicate that probiotics are an ineffective tool in the management of T2DM as results are inconclusive. The alternative is that FBG is a poor endpoint for the measurement of glycemic control with the use of this intervention. FBG is highly variable based on the duration of fasting, activity level, and many homeostatic metabolic functions. These may be less impacted by probiotics whereas the postprandial glucose levels may be more influential. A better method of measurement may be HbA1c, which is defined as a mean blood sugar indicator over a period of about 120 days.[11] FBG contributes only about 30% to the changes in HbA1c while postprandial contributes close to 70%.[12] Thus probiotics could have minimal effect on FBG but if they significantly decrease postprandial glucose the HbA1c could be decreased and glycemic control improved.

All three studies demonstrated consensus on statistically significant differences in HbA1c after the use of probiotics, but clinically the results are questionable in clinical significance when possible error is considered. The change was a decrease of less than once percentage point in each of the studies. Most patients with diabetes are looking for more significant drops to reach their HbA1c goals. While a promising start, in order to fully explore the possibility of probiotic mediated reduction in HbA1c, longer duration studies must be performed to determine if this will be a clinically significant adjunct therapy. Firouzi et al. was the longest study which was carried out across 84 days, but the HbA1c is a measure across a period of 120 days. Thus, a study lasting at least 120 days would more accurately reflect the impact probiotics may have on glycemic control.

Furthermore, studies are needed to investigate the target population of this clinical query. These studies were completed in Malaysia and Iran and in order to apply this research into clinical practice in the United States, which is the goal of this review, it would be better supported with results in a similar population. While treatment guidelines and therapy are comparable between Iran, Malaysia, and the United States, activity level, diet, and many other aspects of glycemic control vary widely across different populations and thus the application of probiotics may look different in the US compared to that of Iran or Malaysia.

While the results of the effects of probiotic usage on HbA1c are promising, there needs to be further studies to determine if they would be a useful adjunct treatment for patients with T2DM on oral medications. Some other considerations for future studies

include larger sample sizes, longer study durations, evaluating post-prandial blood glucose levels as an endpoint, and the utilization of probiotics in fermented foods vs. probiotic capsules.

CONCLUSION:

FBG is an inconclusive measure of improved glycemic control in the treatment of T2DM with probiotics, but cannot be ruled out as a possible benefit as HbA1c uniformly improved. Postprandial glucose levels may be significantly impacted by the use of probiotic supplements and is a target for future research. Research with a longer duration is also needed to fully unveil the effect of probiotics on HbA1c. The benefits offered in the use of probiotic supplements include improved glycemic control with little side effects and ease of administration.

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