**The Effect of Microbiome-modulating Probiotics, Prebiotics, and Synbiotics on Glucose Homeostasis in Type 2 Diabetes: A Systematic Review, Meta-Analysis, and Meta-Regression of Clinical Trials**

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# Supplementary Table ST1. Further Review of the T2D pathophysiology in relation with the gut microbiome.

|  |  |
| --- | --- |
| Topic | Review |
| T2D pathology | T2D reflects a dysfunction in the pancreatic islet β-cells that secrete insulin. As the initial compensation of the high blood glucose levels remain ineffective, these β cells change in function and structure to compensate. Thus, homeostasis is difficult as the secreted levels of insulin is unable to reduce blood glucose levels, resulting in chronic hyperglycemia. Apart from this, adipocytes also stimulate insulin resistance. This is done by dysregulation of adipokines, a higher release of free fatty acids (FFAs) and other inflammatory pathways. These, along with a decrease in the number of incretins released (for example, glucagon-like peptide-1 or GLP-I) or resistance to incretins, hyperglucagonemia, increased reabsorption of glucose by the kidneys and abnormal gut microbiome may also contribute to the development of T2D [1]. |
| Gut Microbiome and T2D pathophysiology | The gut microbiome is an important factor that must be considered in T2D pathogenesis. The human gut harbors mainly six phyla: *Bacteroidetes*, *Verrucomicrobia*, *Actinobacteria*, *Fusobacteria*, *Bacteroidetes,* and *Proteobacteria*. *Bacteroidetes* consists of gram-negative bacteria while *Firmicutes* consists of mainly gram-positive bacteria. Gut dysbiosis may increase risk of developing T2D. A correlation between a decrease of diversity of the microbiome and insulin resistance was also found [2]. With respect to T2D, *Bifidobacterium*, *Akkermansia*, *Roseburia* and *Faecalibacterium* showed a negative association, while *Fusobacterium*, *Blautia* and *Ruminococcus* were positively associated [3]. Additionally, the movement of gram-negative bacteria like *Proteobacteria* from the intestine into the tissues also signals to the development of T2D [2]. The gut microbiota not only affects the pancreas, but also the liver, muscle, and other metabolic organs. *Bifidobacterium lactis* increases the synthesis of glycogen and represses the genes associated with gluconeogenesis in the liver. It also enhances the movement of GLUT-4 (glucose transporter-4) to the surface of the cell, and thereby, increases glucose uptake. *Lactobacillus gasseri* BNR17 also does the same in muscles. *Akkermansia muciniphila* and *Lactobacillus plantarum* were seen to prevent hyperglycemia in mice that were resistant to insulin by repressing the hepatic flavin monooxygenase 3. *A. muciniphila* also prevents carbohydrate breakdown, thus decreasing postprandial hyperglycemia. *L. rhamnosus*, that is a lactobacillus, improves sensitivity to insulin by increasing levels of adiponectin, an adipokine [3].  The gut microbiome also produces various products that also help in glucose homeostasis. For example, butyrate can bind to G-protein coupled receptors, GPCR41 and GPCR43 and stimulates the release of GLP-1 and GLP-2 [3]. Short chain fatty acids (SCFA), another product also has the same effect as butyrate. The release of GLP-1 enhanced the secretion of insulin, along with inhibiting the secreting of glucagon. GPCR43 activated due to SCFA binding, inhibits the insulin signaling in adipocytes. However, excessive activation of the receptor resulted in increase in systemic insulin sensitivity. Additionally, SCFA itself activates gluconeogenesis in the intestine. Also, butyrate inhibits the enzyme histone deacetylase. This is seen to stimulate the growth and differentiation and enhance the function, along with inhibit cell death, of pancreatic β-cells [2]. *Bifidobacterium* and *Lactobacillus* can convert primary conjugated bile salts to bile acids that are deconjugated by producing bile salt hydrolases [3]. These bile acids bind to the nuclear farnesoid X receptor and G-protein coupled bile acid receptor-1 to release GLP-1 [2,3]. Bile acids also increase glycogen synthesis while reducing the process of gluconeogenesis in the liver, along with increasing the secretion of insulin. On the other hand, the gut microbiome also synthesizes amino acids with branched chains (BCAA) like valine, leucine, and isoleucine. An increase in BCAA levels is correlated with insulin resistance, increasing the risk of developing T2D. there is also a positive association between BCAA levels in the plasma after fasting and HOMA-IR measures, which may help to diagnose a patient with T2D [2]. Therefore, it is essential to maintain a balance of the gut microbiome. |

# Supplementary Table ST2. Review of the various biomarkers of glycemia and insulinemia investigated in the study.

|  |  |
| --- | --- |
| Biomarker | Function/Explanation |
| Fasting plasma glucose (FPG) | In order to diagnose, treat, manage, and prevent T2D, various biomarkers are used. An important biomarker is the level of glucose in the blood. High blood glucose or hyperglycemia is an indication of insulin resistance or decreased secretion of insulin, which is seen in patients with T2D. Fasting plasma glucose (FPG) is the direct measure of glucose from the plasma/blood after a period of fasting for at least 8 hours [4,5]. A high FPG indicates that the body is not able to lower the blood glucose levels, which could either be due to insulin resistance or decreased insulin secretion. Insulin also regulates basal hepatic glucose production or gluconeogenesis by reducing free fatty acids in the hepatic portal system [6]. Insulin resistance leads to twice as much production of glucose as compared to that of healthy individuals. This results in hyperglycemia in the fasting state in T2D patients [7]. |
| Glycated Hemoglobin A1c (HbA1c) | Glycated Hemoglobin A1c (HbA1c) tests the amount of glucose that coats hemoglobin, i.e., it becomes glycated. All of the blood glucose will attach onto the hemoglobin protein and will be measured. HbA1c test value below 5.7% is normal. A value from 5.7% to 6.4% indicates prediabetes, while a value above 6.5% indicates diabetes [8]. Hence, it is an indirect measure of blood glucose [4]. Chronically high glucose levels, as seen in T2D, will manifest on the surface of the protein, resulting in a high A1c levels [8]. |
| Homeostasis model for insulin resistance (HOMA-IR) | Homeostasis model for insulin resistance (HOMA-IR) is used to assess insulin resistance [9]. Insulin resistance occurs when there is decreased uptake of glucose by the cells in the body. This leads to an imbalance in glucose homeostasis, and leads to T2D [10]. HOMA-IR is usually calculated using the formula: HOMA-IR = (fasting blood glucose (mg ∕dL) × fasting insulin (μIU∕ L)) ∕405 [9]. |
| Quantitative insulin sensitivity check index (QUICKI) | Quantitative insulin sensitivity check index (QUICKI) assesses the sensitivity to insulin, a decrease in which leads to hyperglycemia. QUICKI can be calculated using the formula: QUICKI = 1∕[log (insulin (μIU∕mL)) + log (glucose (mg∕dL))] [9]. |
| C-peptide | The C-peptide is the proinsulin connecting peptide. It is a cleavage product formed when proinsulin is cleaved to synthesize insulin, containing the A and B chains only [11]. It is secreted along with insulin [11]; therefore, a low level of C-peptide will indicate a decreased secretion of insulin. The mature insulin is the necessary hormone that lowers the blood glucose levels by increasing glucose uptake by cells. A low level of insulin measured may be due to decreased secretion. However, elevated insulin levels or hyperinsulinemia is strongly correlated with T2D onset. The dysregulation of insulin secretion without hypoglycemia is called hyperinsulinemia and may indicate insulin resistance. Hyperinsulinemia also results due to lack of insulin clearance and may indicate T2D. A lower fasting C-peptide measurement is also associated to an impaired insulin clearance [6]. |
| Fasting insulin level (FIL) | Insulin clearance is important as it regulates the cell response to insulin by reducing the availability of insulin [12]. Fasting insulin level (FIL) for insulin is the same as FBG for glucose. Insulin is drawn directly from a vein, and the level of insulin is an indicator of T2D. Insulin also binds to the insulin receptors on the erythrocytes. It is interesting to note that the red blood cells (RBC) in humans have 2,000 insulin-binding sites per erythrocyte [13]. Insulin, upon binding, increases the rate of glycolysis by activating 6-phosphofructo-1-kinase (PFK) [14]. This reduces the amount of glucose in the bloodstream, as the glucose is used up in glycolysis to produce ATP, NADH and pyruvate molecules. However, in a T2D patient, the insulin binding to erythrocytes was seen to decrease due to a decrease in the number of receptors rather than a reduction in affinity. This was inversely correlated with the concentration of insulin [15]. This means that at high insulin concentration, there is decreased binding of insulin to erythrocytes. This may be due to increased production of insulin due to insulin resistance, as seen in T2D patients. Therefore, if the amount of free insulin in higher than the amount of insulin bound to erythrocytes, this may indicate T2D. |

# Supplementary Table ST3. PRISMA Checklist [16] .

| **Section and Topic** | **Item #** | **Checklist item** | **Location where item is reported** |
| --- | --- | --- | --- |
| **TITLE** | | |  |
| Title | 1 | Identify the report as a systematic review. | Title |
| **ABSTRACT** | | |  |
| Abstract | 2 | See the PRISMA 2020 for Abstracts checklist. | Abstract |
| **INTRODUCTION** | | |  |
| Rationale | 3 | Describe the rationale for the review in the context of existing knowledge. | Introduction |
| Objectives | 4 | Provide an explicit statement of the objective(s) or question(s) the review addresses. | Introduction |
| **METHODS** | | |  |
| Eligibility criteria | 5 | Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses. | Methods |
| Information sources | 6 | Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted. | Methods |
| Search strategy | 7 | Present the full search strategies for all databases, registers and websites, including any filters and limits used. | Supplementary Table ST4 |
| Selection process | 8 | Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process. | Methods |
| Data collection process | 9 | Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process. | Methods |
| Data items | 10a | List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect. | Methods; Supplementary Table ST5 |
| 10b | List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information. | Methods; Supplementary Table ST5 |
| Study risk of bias assessment | 11 | Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process. | Methods |
| Effect measures | 12 | Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results. | Methods |
| Synthesis methods | 13a | Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)). | Methods |
| 13b | Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions. | Methods |
| 13c | Describe any methods used to tabulate or visually display results of individual studies and syntheses. | Methods |
| 13d | Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used. | Methods |
| 13e | Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression). | Methods |
| 13f | Describe any sensitivity analyses conducted to assess robustness of the synthesized results. | Methods |
| Reporting bias assessment | 14 | Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases). | Methods |
| Certainty assessment | 15 | Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome. | Methods |
| **RESULTS** | | |  |
| Study selection | 16a | Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram. | Results |
| 16b | Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded. | - |
| Study characteristics | 17 | Cite each included study and present its characteristics. | Results |
| Risk of bias in studies | 18 | Present assessments of risk of bias for each included study. | Results; Supplementary Figure SF1, SF2 |
| Results of individual studies | 19 | For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots. | Table 1 |
| Results of syntheses | 20a | For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies. | Results; Supplementary Figure S1, S2 |
| 20b | Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect. | Results, Table 2, Figure 2, Supplementary Table ST6, Supplementary Figure SF4 |
| 20c | Present results of all investigations of possible causes of heterogeneity among study results. | Results, Table 2, Supplementary Table ST4 |
| 20d | Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results. | Supplementary Table ST6, Supplementary Figure SF3, SF4. |
| Reporting biases | 21 | Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed. | Supplementary Figure SF1, SF2 |
| Certainty of evidence | 22 | Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed. | - |
| **DISCUSSION** | | |  |
| Discussion | 23a | Provide a general interpretation of the results in the context of other evidence. | Discussion |
| 23b | Discuss any limitations of the evidence included in the review. | Discussion |
| 23c | Discuss any limitations of the review processes used. | Discussion |
| 23d | Discuss implications of the results for practice, policy, and future research. | Discussion |
| **OTHER INFORMATION** | | |  |
| Registration and protocol | 24a | Provide registration information for the review, including register name and registration number, or state that the review was not registered. | Methods |
| 24b | Indicate where the review protocol can be accessed, or state that a protocol was not prepared. | Methods |
| 24c | Describe and explain any amendments to information provided at registration or in the protocol. | - |
| Support | 25 | Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review. | Acknowledgement |
| Competing interests | 26 | Declare any competing interests of review authors. | Acknowledgement |
| Availability of data, code and other materials | 27 | Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review. | Acknowledgement |

Supplementary Table ST4. Detailed Search Strategy in Each Database, last updated 10 April 2022**.**

|  |  |  |
| --- | --- | --- |
| Database | Search Strategy | |
| PubMed | ("Probiotics"[MeSH Terms] OR "probiotics"[Title/Abstract] OR "probiotic"[Title/Abstract] OR "Prebiotics"[MeSH Terms] OR "prebiotic"[Title/Abstract] OR "prebiotics"[Title/Abstract] OR "Synbiotics"[MeSH Terms] OR "synbiotics"[Title/Abstract] OR "synbiotic"[Title/Abstract] OR "symbiotic"[Title/Abstract] OR "symbiotics"[Title/Abstract] OR "gastrointestinal microbiome"[MeSH Terms] OR "gut microbiome"[Title/Abstract] OR "gut flora"[Title/Abstract]) AND ("diabetes mellitus, type 2"[MeSH Terms] OR "T2D"[Title/Abstract] OR "type 2 diabetes"[Title/Abstract]). Limit to clinical and animal studies. | |
| Scopus | (INDEXTERMS ("clinical trials" OR "clinical trials as a topic" OR "randomized controlled trial" OR "Randomized Controlled Trials as Topic" OR "controlled clinical trial" OR "Controlled Clinical Trials" OR "random allocation" OR "Double-Blind Method" OR "Single-Blind Method" OR "Cross-Over Studies" OR "Placebos" OR "multicenter study" OR "double blind procedure" OR "single blind procedure" OR "crossover procedure" OR "clinical trial" OR "controlled study" OR "randomization" OR "placebo")) OR (TITLE-ABS-KEY (("clinical trials" OR "clinical trials as a topic" OR "randomized controlled trial" OR "Randomized Controlled Trials as Topic" OR "controlled clinical trial" OR "Controlled Clinical Trials as Topic"  OR  "random allocation"  OR  "randomly allocated"  OR  "allocated randomly"  OR  "Double-Blind Method"  OR  "Single-Blind Method"  OR  "Cross-Over Studies"  OR  "Placebos"  OR  "cross-over trial"  OR  "single blind"  OR  "double blind"  OR  "factorial design"  OR  "factorial trial" ) ) )  OR  ( TITLE-ABS ( clinical  AND trial\* OR  rct\*  OR  random\*  OR  blind\* ) ) AND  ( ( ( TITLE-ABS-KEY ( probiotics  OR  probiotic  OR  prebiotic  OR  prebiotics ) )  OR  ( TITLE-ABS-KEY ( synbiotics  OR  synbiotic  OR  symbiotic  OR  symbiotics ) )  OR  TITLE-ABS-KEY ( "gastrointestinal microbiome" )  OR  TITLE-ABS-KEY ( "gut microbiome" )  OR  TITLE-ABS-KEY ( "gut flora" ) AND  ( TITLE-ABS-KEY ( diabetes  AND mellitus  AND type  2 )  OR  TITLE-ABS-KEY ( t2d )  OR  TITLE-ABS-KEY ( type  2  diabetes ) ) ) ) | |
| Web of Science | TOPIC ((probiotic\* OR prebiotic\* OR sy\*biotic OR symbiotic\* OR synbiotic\*) OR ("gastrointestinal microbiome" OR "gut microbiome" OR "gut flora")) AND TOPIC ("diabetes mellitus type 2" OR t2d OR "type 2 diabetes"). Limit to: Clinical Trials. | |
| Embase | (probiotic\*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR prebiotic\*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR sy\*biotics.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR sy\*biotic.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR gut flora.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR gastrointestinal microbiome.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR gut microbiome.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]) AND (diabetes type 2.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR t2d.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR type 2 diabetes.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] OR diabetes mellitus.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]. Limit to (clinical trial or randomized controlled trial or controlled clinical trial or multicenter study or phase 1 clinical trial or phase 2 clinical trial or phase 3 clinical trial or phase 4 clinical trial) | |
| Clinical Trials | (“Diabetes Mellitus, Type 2” OR “Type 2 diabetes” OR Diabetes) AND (prebiotic OR probiotic OR symbiotic OR synbiotic OR "gastrointestinal microbiome" OR “Gut Microbiota”). Filtered by Completed Studies | |
| ProQuest Dissertations and Theses | (Probiotic\* OR Prebiotic\* OR Synbiotic\* OR Symbiotic\* OR "gastrointestinal microbiome" OR "Gut Flora" OR "gut microbiome") AND ab(diabetes AND ("type 2" OR t2d OR "Type II")) | |
| Cochrane | 1      MeSH descriptor: [Probiotics] | 12   "gut flora" |
| 2      probiotic | 13   "gastrointestinal microbiome" |
| 3      prebiotic | 14   "gut microbiome" |
| 4      MeSH descriptor: [Prebiotics] | 15   "diabetes mellitus" |
| 5      symbiotic | 16   MeSH descriptor: [Diabetes Mellitus] |
| 6      synbiotic | 17   t2d |
| 7      MeSH descriptor: [Synbiotics] | 18   "type 2" AND diabetes |
| 8      probiotics | 19   1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 |
| 9      prebiotics | 20   15 or 16 or 17 or 18 |
| 10   sy\*biotics | 21   19 and 20 |
| 11   MeSH descriptor: [Gastrointestinal Microbiome] | 22   Limit to: Controlled trials |

# Supplementary Table ST5. Data Extraction Variables

|  |  |  |
| --- | --- | --- |
| **1.** | **Study characteristics** | First author’s last name, year of publication, country of study, primary outcomes, study design, trial duration and investigated biomarker(s). |
| **2.** | **Participant characteristics** | Mean and standard deviation (SD) of age and baseline body mass index (BMI), ratio and number of participants sexes, total number of participants, presence of inclusionary comorbidities for both intervention and placebo/control groups. |
| **3.** | **Intervention characteristics** | Type, composition and daily dosage of nutraceutical and control/placebo substance. |

# Supplementary Table ST6. Meta-Regression Analyses of Continuous Variables.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Biomarker | Covariate | Number of Trials | Participants | Intercept Estimate (95% CI) | β effect (95% CI) | p-value for β effect | Heterogeneity measures | | | |
| **Accounted** | | **Residual** | |
| **I² (%)** | **I² (%)** | | **p-value** |
| FBG (mg/dl) | Age | 66 | 3,735 | -48.92 (-79.46; -18.37) | **0.66 (0.11; 1.20)** | **0.0177** | 11.67 | 93.46 | | <0.0001 |
| Baseline BMI | 66 | 3,735 | -24.85 (-69.23; 19.52) | 0.42 (-1.08; 1.93) | 0.5796 | 3.10 | 93.59 | | <0.0001 |
| Baseline FPG | 64 | 3,645 | 45.20 (25.25; 65.15) | **-0.38 (-0.52; -0.25)** | **<0.0001** | 24.68 | 91.92 | | <0.0001 |
| Intervention Duration | 66 | 3,735 | -14.28 (-23.24; -5.32) | 0.15 (-0.70; 1.00) | 0.7311 | 0.0 | 94.50 | | <0.0001 |
| Year | 66 | 3,735 | -330.97 (-2440.1; 1778.2) | 0.16 (-0.89; 1.20) | 0.7673 | 0.0 | 94.56 | | <0.0001 |
| HbA1c (%) | Age | 48 | 2,669 | -1.52 (-2.31; -0.74) | **0.02 (0.01; 0.03)** | **0.0044** | 2.36 | 95.21 | | <0.0001 |
| Baseline BMI | 48 | 2,669 | 0.25 (-0.85; 1.34) | -0.02 (-0.06; 0.02) | 0.2523 | 0.0 | 95.44 | | <0.0001 |
| Baseline HbA1c | 46 | 2,579 | 0.24 (-0.62; 1.09) | -0.083 (-0.196; 0.029) | 0.1474 | 0.0 | 95.67 | | <0.0001 |
| Intervention Duration | 48 | 2,669 | -0.56 (-0.75; -0.37) | 0.018 (-0.000; 0.035) | 0.0515 | 0.0 | 95.20 | | <0.0001 |
| Year | 48 | 2,669 | 9.40 (-39.75; 58.55) | -0.01 (-0.03; 0.02) | 0.6963 | 0.0 | 95.44 | | <0.0001 |
| Insulin (µU/ml) | Age | 40 | 2,480 | -4.96 (-10.47; 0.55) | 0.06 (-0.03; 0.16) | 0.2143 | 4.12 | 83.69 | | <0.0001 |
| Baseline BMI | 40 | 2,480 | 12.75 (3.60; 21.91) | **-0.48 (0.79; -0.17)** | **0.0022** | 15.13 | 79.88 | | <0.0001 |
| Baseline Insulin | 38 | 2,392 | -1.19 (-2.46; 0.07) | -0.02 (-0.12; 0.07) | 0.6017 | 0.0 | 85.44 | | <0.0001 |
| Intervention Duration | 40 | 2,480 | -0.50 (-2.00; 1.01) | -0.11 (-0.27; 0.04) | 0.1500 | 0.0 | 84.91 | | <0.0001 |
| Year | 40 | 2,480 | 52.13 (-334.50; 438.76) | -0.03 (-0.22; 0.17) | 0.7857 | 0.0 | 82.65 | | <0.0001 |
| HOMA-IR | Age | 36 | 2,255 | -3.21 (-7.26; 0.85) | 0.05 (-0.03; 0.12) | 0.2217 | 0.0 | 97.64 | | <0.0001 |
| Baseline BMI | 36 | 2,255 | -5.72 (-13.94; 2.49) | 0.17 (-0.11; 0.44) | 0.2312 | 0.0 | 97.65 | | <0.0001 |
| Baseline HOMA-IR | 32 | 2,091 | 0.14 (-1.23; 1.50) | -0.23 (-0.55; 0.09) | 0.1567 | 0.0 | 97.76 | | <0.0001 |
| Intervention Duration | 36 | 2,255 | -0.03 (-1.23; 1.16) | -0.07 (-0.18; 0.04) | 0.2342 | 0.0 | 97.67 | | <0.0001 |
| Year | 36 | 2,255 | -100.22 (-525.95; 325.52) | 0.05 (-0.16; 0.26) | 0.6469 | 0.0 | 97.05 | | <0.0001 |
| QUICKI | Age | 9 | 479 | -0.09 (-0.15; -0.02) | **0.0017 (0.0006; 0.0027)** | **0.0026** | 65.32 | 58.26 | | 0.0189 |
| Baseline BMI | 9 | 479 | 0.07 (-0.13; 0.28) | -0.0020 (-0.0087; 0.0047) | 0.5568 | 0.0 | 82.59 | | <0.0001 |
| Baseline QUICKI | 9 | 479 | 0.03 (-0.04; 0.09) | -0.0456 (-0.2609; 0.1696) | 0.6779 | 0.51 | 81.60 | | <0.0001 |
| Intervention Duration | 9 | 479 | -0.03 (-0.08; 0.013) | **0.0043 (0.0003; 0.0083)** | **0.0373** | 42.36 | 70.28 | | 0.0014 |
| Year | 9 | 479 | -8.01 (-13.79; -2.24) | **0.0040 (0.0011; 0.0068)** | **0.0064** | 64.37 | 58.58 | | 0.0181 |
| C-peptide (ng/ml) | Age | 12 | 867 | -2.79 (-5.42; -0.17) | **0.049 (0.003; 0.096)** | **0.0372** | 0.0 | 96.67 | | <0.0001 |
| Baseline BMI | 12 | 867 | -0.80 (-3.52; 1.93) | 0.027 (-0.067; 0.121) | 0.5723 | 0.0 | 96.77 | | <0.0001 |
| Baseline C-peptide | 10 | 777 | -0.24 (-0.78; 0.29) | 0.141 (-0.107; 0.388) | 0.2647 | 0.0 | 97.43 | | <0.0001 |
| Intervention Duration | 12 | 867 | 0.43 (-0.050; 0.92) | **-0.030 (-0.059; -0.002)** | **0.0358** | 1.13 | 96.38 | | <0.0001 |
| Year | 12 | 867 | 113.39 (-101.31; 328.10) | -0.056 (-0.163; 0.050) | 0.3005 | 0.0 | 96.61 | | <0.0001 |

In cases where the quantitative measurements for the mean age or baseline BMI were not reported, the values were imputed from the pooled median of the remaining trials. **Bold text** has been used to highlight significant effect of a covariate on the effect sizes for that respective biomarker.

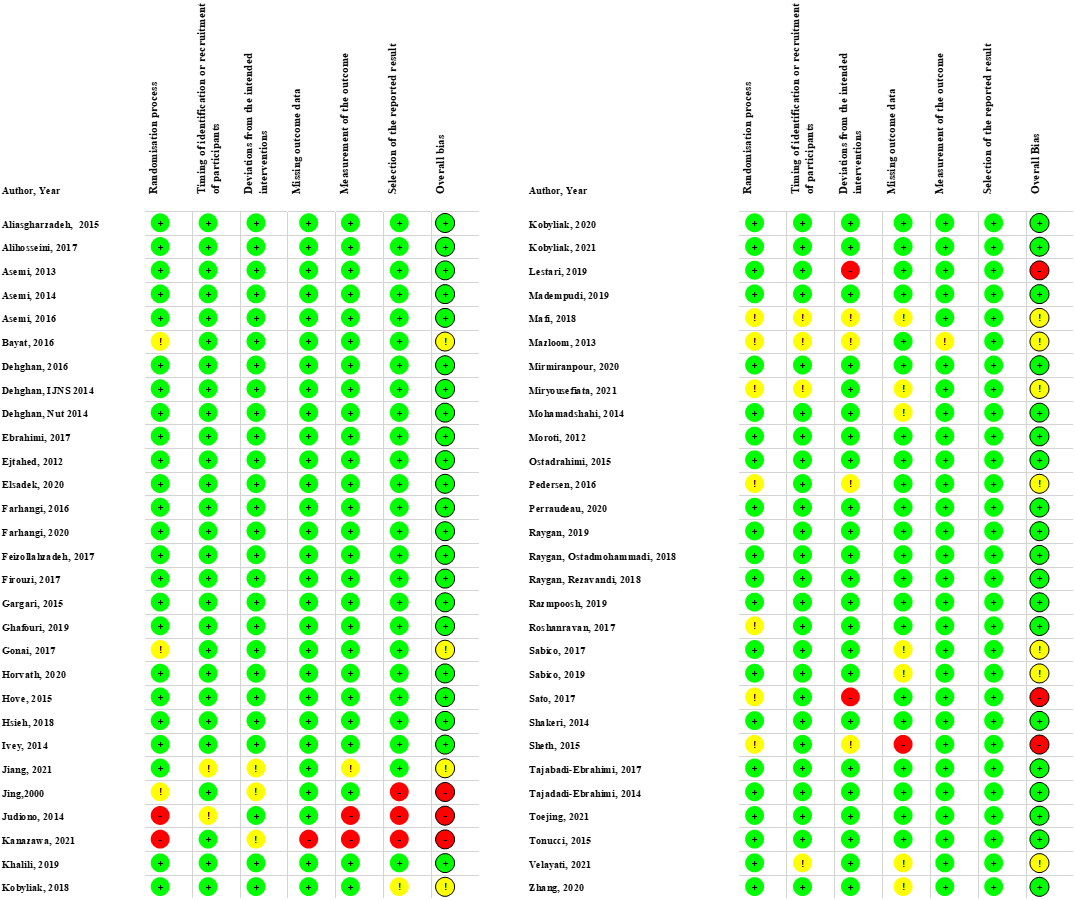
# Supplementary Table ST7. Summary of the Order of Efficacies of the various Nutraceutical Types classified by Biomarker.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Biomarker | Most Efficacy |  |  |  | Least Efficacy |
|  |  |  |  |  |  |
| FPG | PREBIOTIC [12] | SYNBIOTIC(M) [12] | PROBIOTIC(S) [12] | PROBIOTIC(M) [24] | SYNBIOTIC(S) [6] |
| HbA1c | SYNBIOTIC(M) [8] | PREBIOTIC [12] | PROBIOTIC(M) [16] | SYNBIOTIC(S) [2] | PROBIOTIC(S) [10] |
| Insulin | SYNBIOTIC(S) [4] | SYNBIOTIC(M) [6] | PROBIOTIC(M) [19] | PROBIOTIC(S) [5] | PREBIOTIC [6] |
| HOMA-IR | SYNBIOTIC(M) [7] | SYNBIOTIC(S) [4] | PROBIOTIC(S) [5] | PREBIOTIC [4] | PROBIOTIC(M) [16] |
| QUICKI | PROBIOTIC(M) [5] | SYNBIOTIC(M) [2] | PROBIOTIC(S) [1] | SYNBIOTIC(S) [1] | PREBIOTIC-NR [0] |

(S)=single strain; (M)=multistrain; [n]=number of studies reporting results for that nutraceutical type-biomarker combination; blue background represents statistically significant pooled results for that nutraceutical type-biomarker combination, whereas gray background denotes non-significant results; effect of prebiotics for change in QUICKI was NR=not reported; C-peptide was not summarized due to non-significant overall pooled results (see main analysis in table 2); nutraceutical types are ordered based on decreasing order of mean difference among significant results first, followed by decreasing order of mean difference among the non-significant results (table 2).

# Supplementary Figure SF1. Summary of risk of bias assessment for the included studies.

# Supplementary Figure SF2. Individual study risk of bias assessments.



# Supplementary Figure SF3. Usual and contour-enhanced funnel plots assessing publication bias for (A) FPG, (B) HbA1c, (C) Insulin, (D) HOMA-IR, (E) QUICKI and (F) C-peptide.

|  |  |
| --- | --- |
| 1. **FPG; Egger test p-value = 0.047** | |
|  |  |
| **(B) HbA1c; Egger Test p-value = 0.124** | |
|  |  |
| 1. **Insulin; Egger Test p-value = 0.328** | |
|  |  |
| 1. **HOMA-IR; Egger Test p-value = 0.103** | |
|  |  |
| 1. **QUICKI; Egger Test p-value = 0.453** | |
|  |  |
| 1. **C-peptide; Egger Test p-value = 0.356** | |
| Chart  Description automatically generated | A picture containing chart  Description automatically generated |

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