Exploring the Relationship between Gut Microbiota and Type 1 & 2 Diabetes Mellitus

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INDEX

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Last but not the least, I would like to thank my parents because without their help this project would not have been successful.

Aim & Objectives

Through this project, I aim to find out and discuss the following :-

- Brief introduction to type 1 and type 2 Diabetes Mellitus
- Differences and SImilarities between type 1 and type 2 diabetes
- Introduction to the human gut microbiome
  - what is the microbiome?
  - what constitutes the microbiome?
  - why is it important for our body?
- The connections between the gut microbiome and type 1 & 2 diabetes
- What leads to diabtetes?
- Introduction to Eubiosis and Dysbiosis
  - How does dysbiosis affect the body?
- Can gut microbiome be another way of treating diabetes?
- How is the gut microbiome affected by diet as well as geographical features and surroundings?

Type 1 Diabetes Mellitus

INTRODUCTION:-

Type 1 diabetes (T1DM) is a chronic disease where the pancreas produces little to no insulin in the body. Due to low insulin levels, sugar accumulates the bloodstream rather than entering the cells. This causes high blood sugar in affected individuals.

In T1D, T cells attack the b-cell of islets, which in turn results in inadequate insulin production. High levels of glucose that remain in the blood cause excessive urination and dehydration, and damage tissues of the body.

Type 1 diabetes mellitus was previously known as insulin dependent diabetes or juvenile diabetes. It is diagnosed most commonly between ages 10 and 16. Type 1 diabetes equally affects males and females. It is an outcome of an interlinkage between varying degrees of genetic susceptibility and environmental factors.

Type 1 diabetes is an autoimmune disease. It begins when the body's immune system attacks cells in the body. In type 1 diabetes, the immune system destroys insulin-producing beta cells in the pancreas. It is not caused by the amount of sugar in a person's diet before the disease develops.

SYMPTOMS:-

Initial symptoms of the disease come on suddenly and strongly. The most prominent of these are:-

- Excessive urination and extreme thirst.
- Increased glucose in the blood causes the kidneys to create more urine than usual
- Weight loss
- Weakness, fatigue, confusion, nausea and vomiting
- Ketoacidosis occurs because cells can't use the glucose they need for energy.
- Ketoacidosis is the process of build up of ketones in the bloodstream.
    - It can cause heart problems and affect the nervous system. Within hours, it may put a person at risk of coma or death.

Chronic symptoms of T1DM Include the following:-

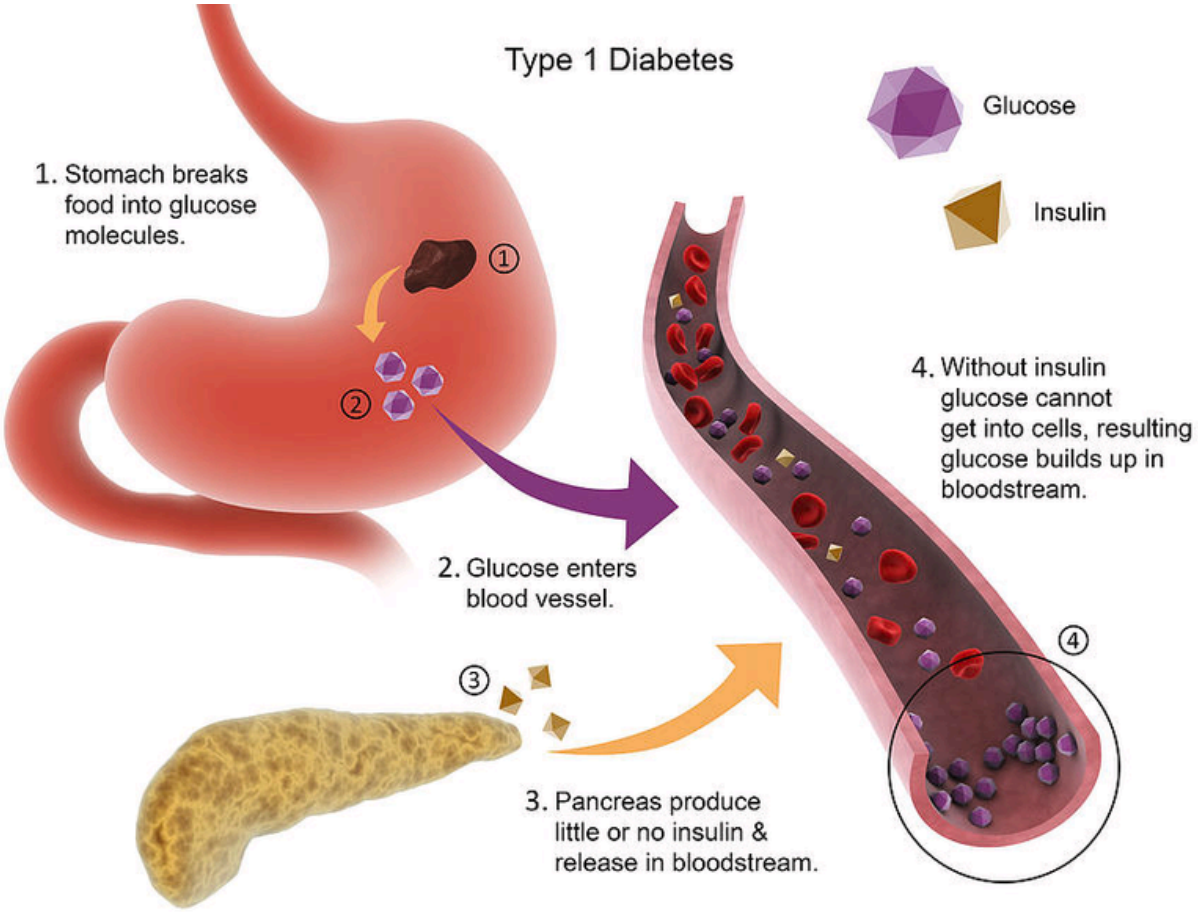
- Eye damage (retinopathy)
- Nervous damage (neuropathy)
- Foot problems
- Kidney disorders (nephropathy)
- Heart and Artery diseases
- Hypoglycemia (low blood sugar)

PREVENTION:-

There is no proven way to prevent type 1 diabetes. Vitamin D deficiency may increase risk of diabetes although it is not yet proven. Avoiding cow's milk during infancy may possibly prevent type 1 diabetes in genetically susceptible infants although this is not proven either.

TREATMENT:-

- Treatment of type 1 diabetes requires daily insulin injections. The injected insulin makes up for the insulin that is not produced by the body.
- Fast-acting insulin may be taken as needed, depending on the amount of carbohydrates ingested.
- Healthy diet and regular exercise.


Type 1 Diabetes
Glucose
Insulin
1. Stomach breaks food into glucose molecules.
①
②
2. Glucose enters blood vessel.
③
3. Pancreas produce little or no insulin & release in bloodstream.
④
4. Without insulin glucose cannot get into cells, resulting glucose builds up in bloodstream.

Type 2 Diabetes Mellitus

INTRODUCTION:-

Type 2 diabetes (T2D) is a chronic disease that affects the way sugar is processed by the body. It is characterised by high blood sugar levels. It is also known as type 2 diabetes mellitus or adult onset diabetes because it used to almost always start to develop in middle or late adulthood although now it's becoming common even in younger ages.

Islets of beta cells fail in T2D where the body becomes immune to the insulin it creates which in turn causes obesity as well.

This condition is milder and much more common than type 1 diabetes. It accounts for about 90% of total diabetes cases worldwide.

Type 2 diabetes occurs when your body's cells resist the normal effect of insulin, which is to drive glucose in the blood into the inside of the cells. This condition is called insulin resistance. As a result there is a build up of glucose in the blood.

Type 2 diabetes runs in families. Obesity greatly increases the risk of diabetes.

SYMPTOMS:-

- Excessive thirst hunger and urination
- Weight loss
- Increased susceptibility to infections
- Very high amounts of blood sugar can lead to a condition known as hyperosmolar syndrome, which is a life threatening form of dehydration. It causes weakness, confused thinking, nausea and even seizure or coma.

MANAGEMENT OF TYPE 2 DIABETES:-

- Healthy Eating
- Regular exercise
- Weight loss
- Diabetes medication/ insulin therapy
- Blood sugar monitoring

PREVENTION:-

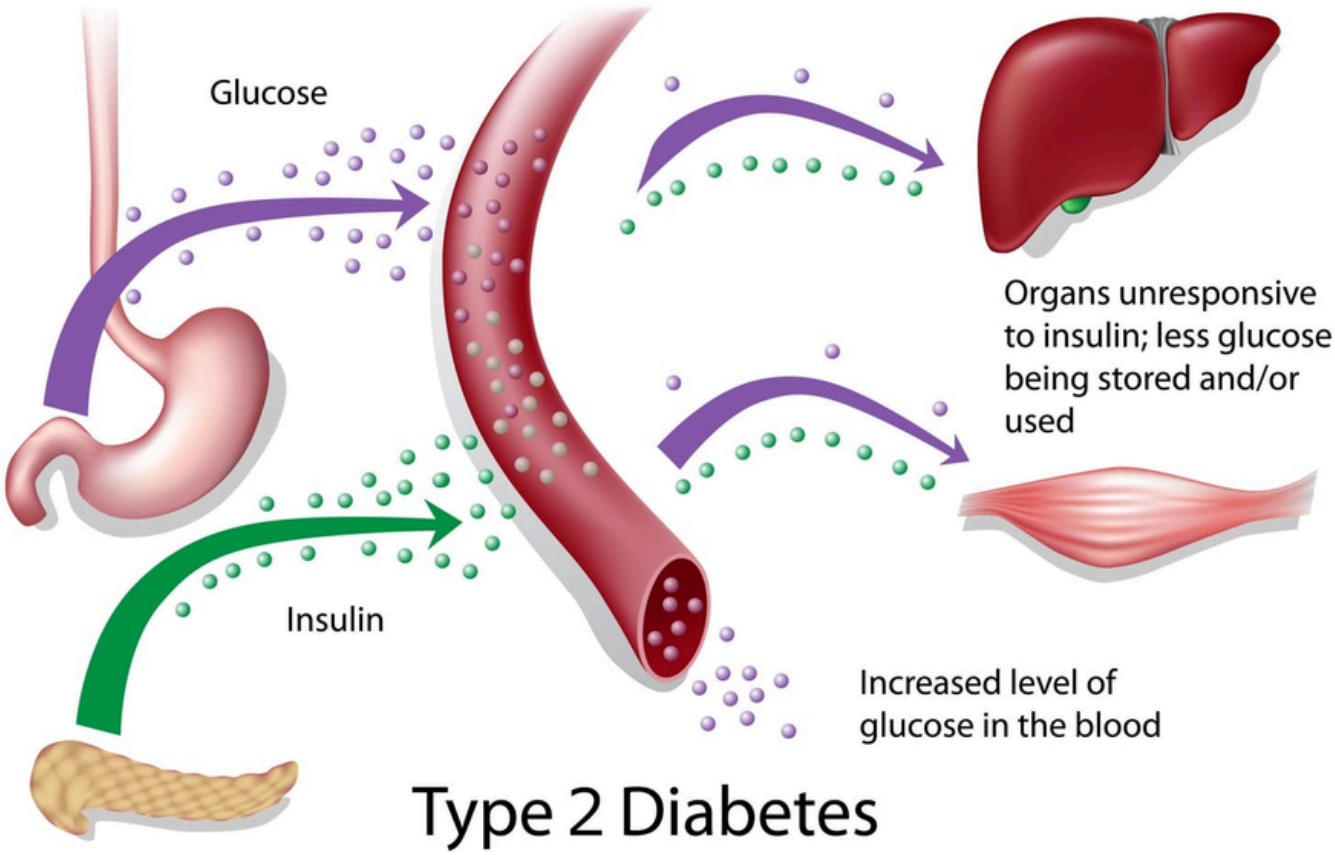
- Maintaining ideal body weight
- Regular exercise
- Healthy diet

If a close relative has the condition the diagnosis of a person shows a condition known as pre diabetes defined as blood glucose levels between 100 and 125 mg/dL. In such a case the person is at risk of developing type 2 diabetes.

In this case medication can offer some protection or prevent further complications.

TREATMENT:-

- Weight loss
  - Weight loss is often a primary goal for people with diabetes as weight loss can help improve insulin resistance.
- Physical activity
  - Physical activity can be particularly effective in controlling blood glucose levels.
  - After the exercise, the body will start replenishing its stores of glucose by steadily taking in available glucose from the blood.
- Medication
  - Tablets
    - The main role of diabetes medication is to help lower blood glucose levels, although more recently developed medications can also aid weight loss.
    - Metformin is often the first tablet prescribed to people with type 2 diabetes and stronger-acting medication may be prescribed in addition to metformin, or in place of it, if blood glucose levels remain too high.
    - Diabetes medication needs to be supported with diet changes and regular physical activity to keep you healthy.
  - Insulin
    - Insulin is the most well-known type of injectable medication for diabetes.
    - Insulin is the hormone which helps to move sugar out of the blood and into cells to be used as energy or to be stored as fat.
  - Incretin mimetics.
    - Incretin mimetics are medications which mimic the action of a hormone called glucagon-like-peptide-1.
    - This hormone plays a number of roles in response to digestion such as increasing the amount of insulin released and decreasing the amount of glucagon released.



Type 2 Diabetes

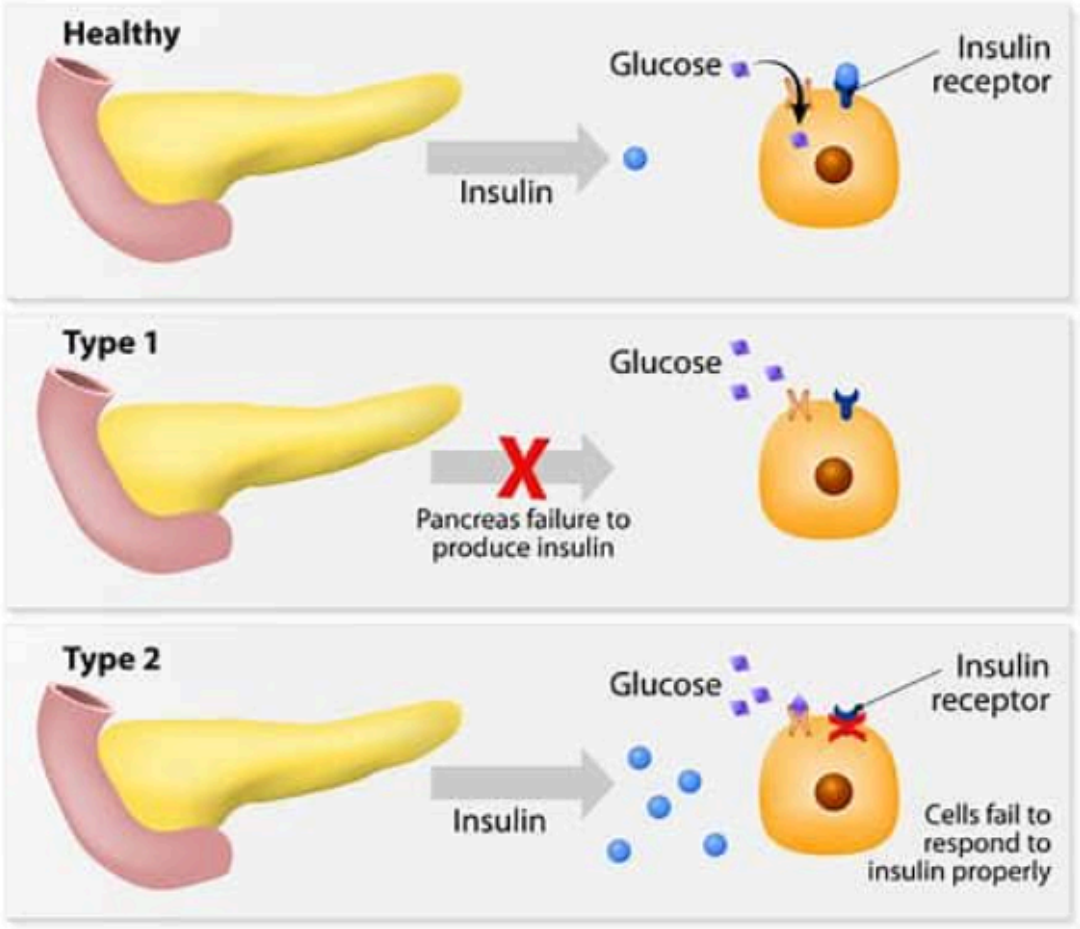
Differences between Type 1 & Type 2 Diabetes Mellitus

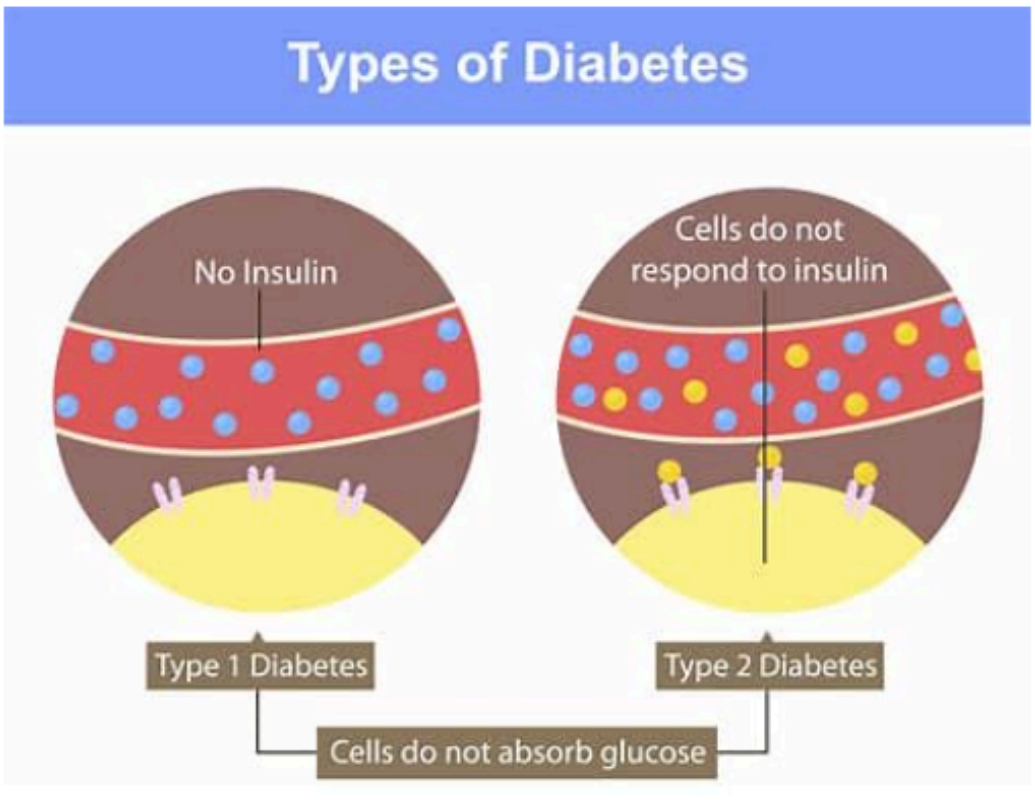
TYPE 1 DIABETES MELLITUS	TYPE 2 DIABETES MELLITUS
Often diagnosed in children and young adults	Usually diagnosed in adults
Caused by an autoimmune response against insulin-producing beta cells	Cause is unknown, but related to weight, age, inactivity and genetics
Treatment must include insulin, as the body no longer produces it	Treatment usually includes some combination of medications, diet, exercise and insulin

Similarities between Type 1 & Type 2 Diabetes Mellitus

- Elevated blood glucose levels.
- Well balanced diet and regular exercise is a must for both type 1 and 2 diabetes mellitus as it helps improve insulin sensitivity and manage blood sugar levels.
- Long-term complications arising from increased blood sugar levels are common to both forms of disease and include neuropathy, retinopathy and nephropathy.

DIABETES MELLITUS





Gut Microbiome

The human intestine is inhabitant to trillions of micro-organisms not only bacteria but also viruses, fungi and archaea, that make up a complex ecological community with which an individual lives in a symbiotic manner all his life.

Numerous micro-organisms reside on and within our bodies. These microorganisms constitute our microbiota and their complete genome is called the microbiome. It has been estimated that human gut microflora contains over 35,000 species and over 10 million non-redundant genes. The human gut microbiota has been found to influence a large number of diseases including type 1 and 2 diabetes mellitus, hypertension, obesity, cardiovascular diseases as well as cancer.

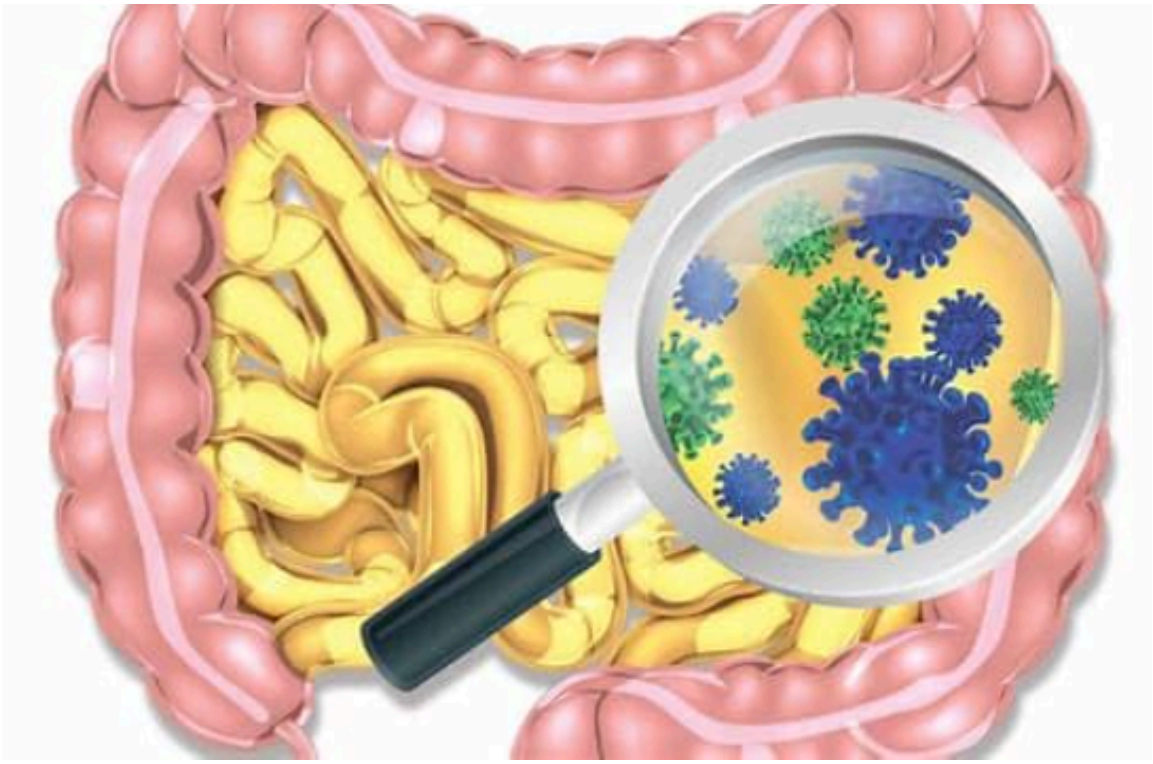
The gastrointestinal system represents one of the biggest connections between the microbial world inside a human and the exterior world. The gut microbiota also functions as a supplementary endocrine system. It has a significant role in the systemic and intestinal immune and metabolic homeostasis.

Various factors can affect the composition of the microflora. Some of these include host genetics, food habits, age, exposure to drugs, stresses or toxins. The composition of microflora changes with age. Microbial composition increases with age and becomes highly complicated in adults and reaches peak productivity. Other factors that affect the composition of the gut microbiome are geographical location, use of antibiotics, probiotics and prebiotics.

The microflora of the gut can be classified into 6 major phyla i.e. Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Out of these, Bacteroidetes and Firmicutes constitute about 90% of total microflora. Most of the major genera found under these categories are gram negative (do not retain the crystal violet stain used in the Gram staining method of bacterial differentiation).

Certain compounds formed in the body are indigestible by humans in the sense that humans do not produce the enzymes required to digest them. Gut Bacteria can ferment these compounds for their own benefit and produce short chain fatty acids (SFCAs). These SCFAs are also beneficial for humans as they function as a source of energy.

The bacteroidetes phyla have a role in fermenting these otherwise indigestible carbohydrates. Major role of firmicutes is believed to be metabolic degradation of an energy source. They can therefore be associated with calorie bioavailability and utilisation.



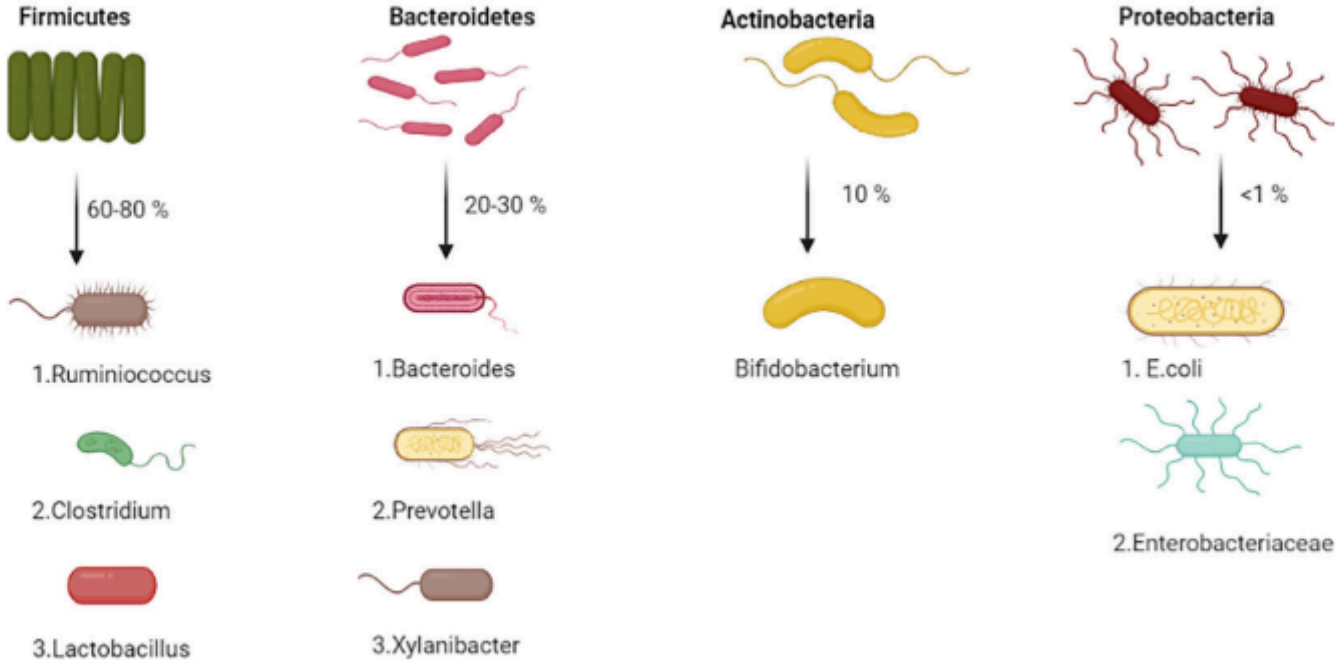


Fig. 1. The four major phyla of human gut bacteria.

Connection of T1DM with the Gut Microbiome

Diabetes is suggested to have a direct connection with gut microbiota that determine our metabolic outcomes. Healthy people have a different microbiome that leads to achieving more short chain fatty acids and more branched amino acids while diabetic individuals are more likely to produce compounds that affect glucose metabolism.

In a healthy individual, the composition of the gut microbiota is such that the ratio of amount of bacteroidetes and firmicutes favours Bacteroidetes.The low number of Firmicutes present results in adequate energy uptake of the host that does not lead to high calorie availability, thus disfavoring positive energy balance.

In obese and/or affected individuals, firmicutes concentration is greater.

Recent studies show that gut microbiota can contribute to development of gut associated lymphoid tissues and also enhance immunity to pathogens. The microbiome is thus related with the development of T1D.

T1D usually originates in the early stages of life. At this stage the intestinal microbiota is still developing. Microbial composition increases with age and becomes highly complicated in adults and reaches peak productivity. Children advancing to T1D have a reduced abundance of bacteria that manufacture butyrate or lactate following the emergence of the first disease predictive autoantibodies.

According to a study in finland in the year 2011:-

- Normal healthy individual
  - High ratio of *Bacteroidetes:Firmicutes* as compared to affected individuals
  - A high proportion of mucin degrading and butyrate producing bacteria in controls as compared to the cases.
- Affected individual
  - The ratio of other short chain fatty acid producing bacteria were higher.

Another study done in year 2013 showed raise in levels of Clostridium, Bacteroides and Veillonella and a decrease in Lactobacillus, Bifidobacterium, the Blautia coccoides–Eubacterium rectale group and Prevotella in the children with T1D.

<u>Altered gut bacteria</u>:-

- correlation of altered gut microbiota with b-cell autoimmunity has been seen in paediatric groups who are at potential chance of developing T1D.
- Alteration of microbial occurrence in childhood would lead to abnormal microbial prevalence in adulthood that would affect a particular species of bacteria.
- altered gut microbiota are strongly linked to b-cell autoimmunity and T1D in either composition or/and function.

Connection of T2DM with the Gut Microbiome

Gut microbiota has a close interaction with various systems like inflammatory, renal, cardiovascular and endocrine. T2D patients show altered intestinal microbiota where decreased Bacteroidetes:Firmicutes ratio is observed along with some other reduced bacterias. In T2D patients, ratio prefers Firmicutes over Bacteroidetes while in healthy individuals it is the other way around.

Problems in the body that may lead to T2D:-

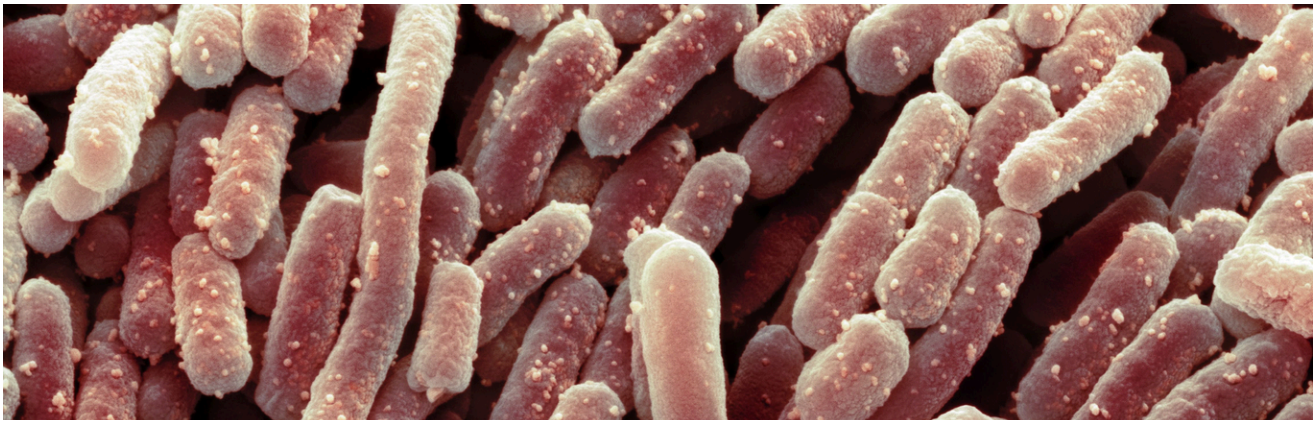
- Insulin resistance in fat muscle and liver cells.
- Insufficient insulin production.

T2D patients also show an increase in some endotoxins-producing gram-negative bacteria that modify the metabolic activity of the host.
Among the class of probiotic bacteria, lactobacillus is the most predominant.

- Among T2D patients, various species of lactobacillus were found to be reduced.
- Several species of this genus also work as probiotics.

Relation of T2D with Obesity:-

- High fat diets typically result in poor microbiome health which leads to dysbiosis in the gut.
- Obesity is a global epidemic interestingly seen both in developed and developing countries.
- Factors contributing to obesity:-
    - hypercaloric diets
    - Sedentary lifestyles
- Can lead to health complications like heart diseases, high blood pressure and diabetes.



Features of Gut Microbiota that may lead to T2D Development

- Endotoxins
  - moderate increase in endotoxins like cytokines which impair insulin interaction with receptor is produced by gram negative bacteria
  - If there is any change in gram negative gut microbiota, it may lead to a change in intestinal permeability which is directly linked to resistance to insulin.
- Brown adipose tissue
  - encourages immunity to insulin.
  - Brown fat cells arise in white adipose tissue by a mechanism called browning in cold environments or during exercise.
  - Upon antibiotic treatment, the beige fat formation in adipose tissue eradicates microbiota due to which there is increase in glucose tolerance and insulin sensitivity.
- Drugs
  - Many researchers have suggested metformin as a therapy for patients with T2D. Not only this, but metformin has also been proposed as a treatment of gestational diabetes and T2D prevention in prediabetic individuals.
- Changes in incretin secretion
  - increase in Bifidobacterium spp. is related to increased secretion of glucagon-like peptide-1 (GLP-1) by the bowel that has favourable effects in reducing insulin resistance.
- Secondary bile acids
  - Secondary bile acids have been reported to have an insulin sensitising role.
- Vitamins
  - Few vitamins like choline and niacin can be broken down by some bacteria; Firmicutes, Actinobacteria, Proteobacteria due to oxidative stress and their end products have been linked with diabetes development.

Eubiosis Vs. Dysbiosis

EUBIOSIS

- Eubiosis refers to the normal/healthy profile of gut microflora
  - In a state of eubiosis, the microbiome plays several roles in producing SCFAs, branched chain amino acids, affecting lipid metabolism, and generating other key metabolites.
  - Eubiosis in the gut typically is a condition in which there is a vibrant gut bacterial population composed of 95% Bacteroidetes, and 5% Firmicutes, forming an ideal B/F ratio.
  - When the B/F ratio is such, the microbiome extends optimized gut health, regulates and controls opportunistic pathogens, and contributes to the entire body.

DYSBIOSIS

- Dysbiosis refers to imbalance between the types of organism present in a person's natural microflora, especially that of the gut, thought to contribute to a range of conditions of ill health.
  - Dysbiosis can lead to obesity and several other health complications chronic in nature including type 2 diabetes mellitus and CVD.

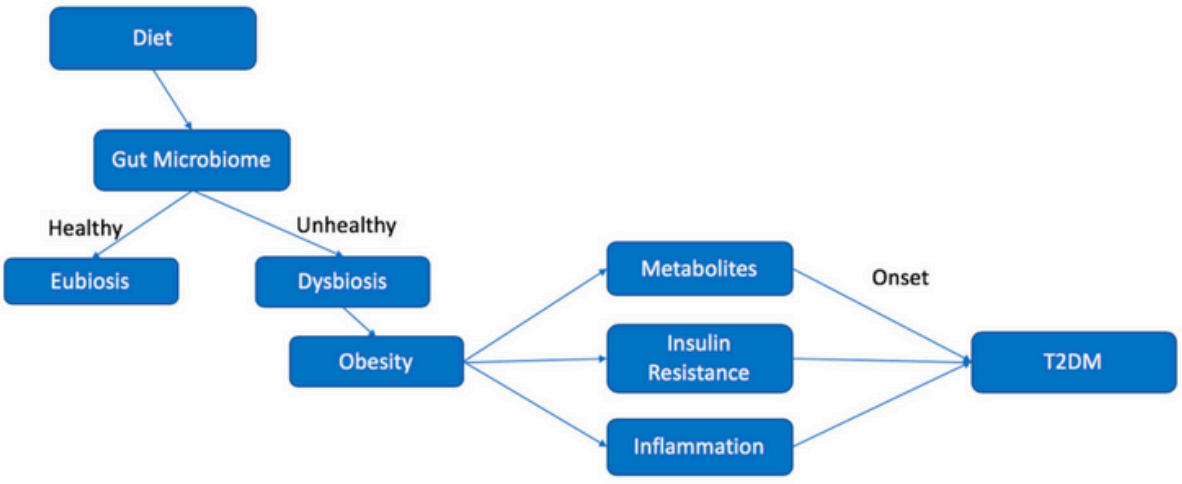
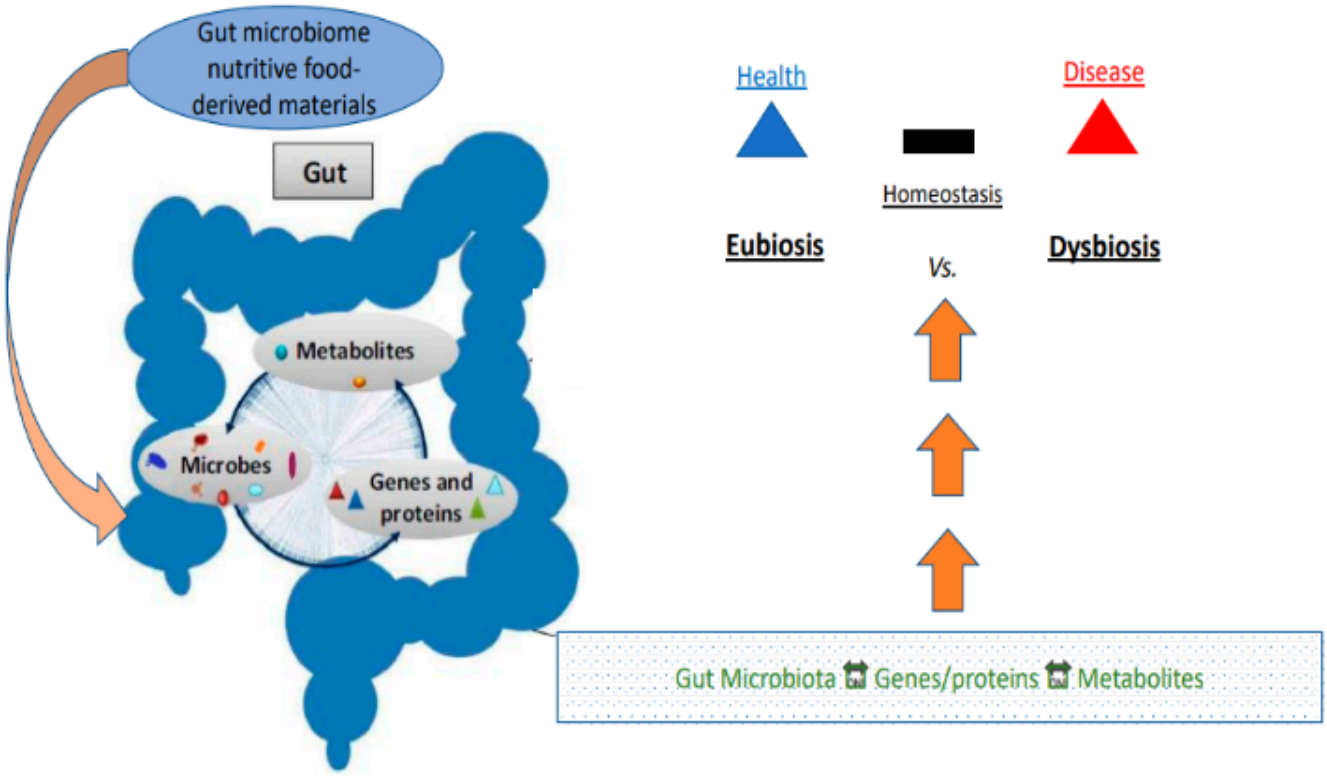


Figure 2. Chart illustrating the effect of diet on the microbiome and the relationship leading to increased risk for the development of type 2 diabetes mellitus (T2DM).

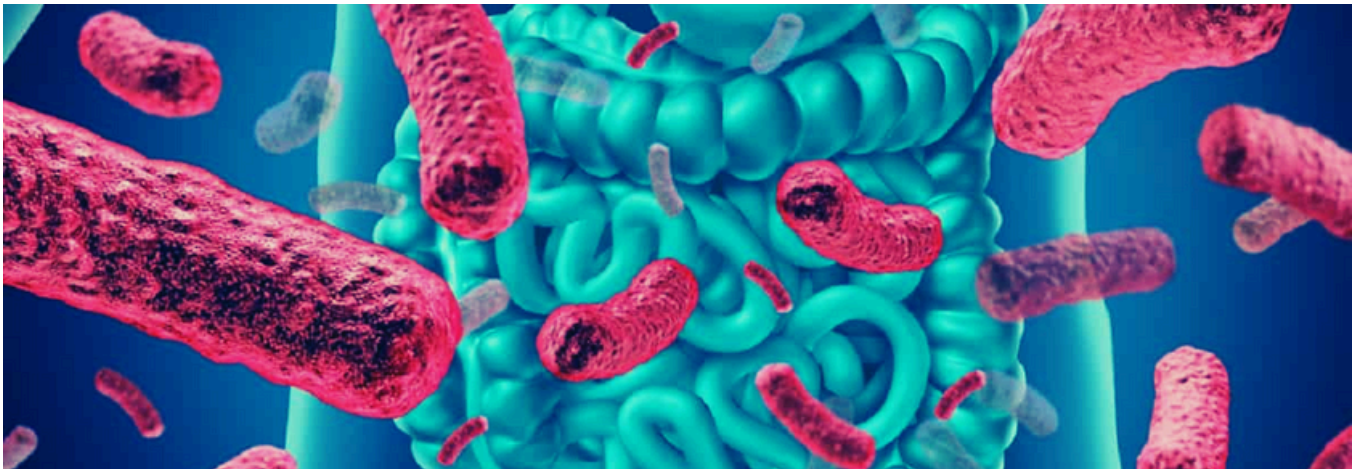


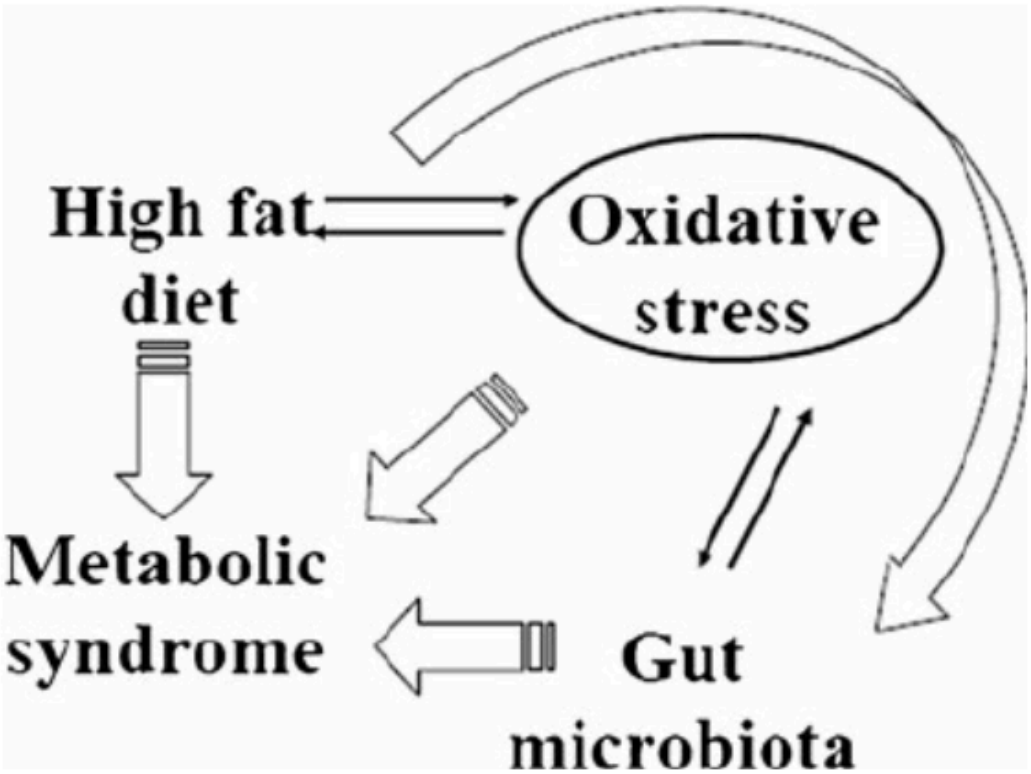
Eubiosis vs Dysbiosis

Dysbiosis and development of T2D

Many factors affect the development of T2D such as obesity, a sedentary lifestyle, genetics, diet, environmental factors and the microbiome.

- Inflammation
  - There is increasing evidence supporting a definite relationship between induced inflammation and increased risk for insulin resistance, which in turn leads to T2DM.
  - Individuals in a pre-diabetic state compensate for insulin resistance by β-cells insulin hypersecretion
  - As the disease progresses, β-cells progressively grow less able to supply the needed amount of insulin, gradually become exhausted, and eventually die.
  - Anti-inflammatory diets are considered to help reduce risk of diabetes.
  - Inflammatory response activation can be mediated by the microbiome.
  - Accumulation of inflammatory molecules like flagellin and peptidoglycans that are derived from gut- bacteria in the intestine hasten the inflammation in T2D.
- Insulin resistance
  - While the direct connection between the microbiome and insulin resistance is not clear, it is evident that the microbiome plays an important role in regulating insulin resistance.
- Oxidative stress
  - Reactive oxygen species (ROS) form as a result of metabolism, and transfer unpaired electrons causing oxidation of cellular machinery
  - In a healthy individual, antioxidants, to a large extent, counter this process, neutralising ROS and hence defending body homeostasis.
  - Imbalance, due to ineffective antioxidant defence, results in oxidative stress, which is closely related to glycation phenomena and diabetes onset.

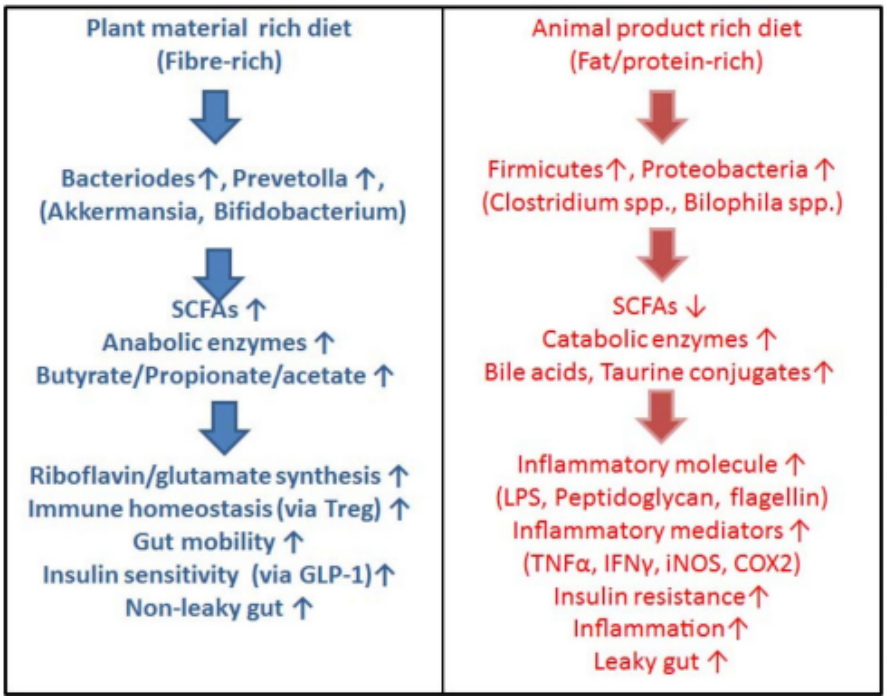




Effect of oxidative stress

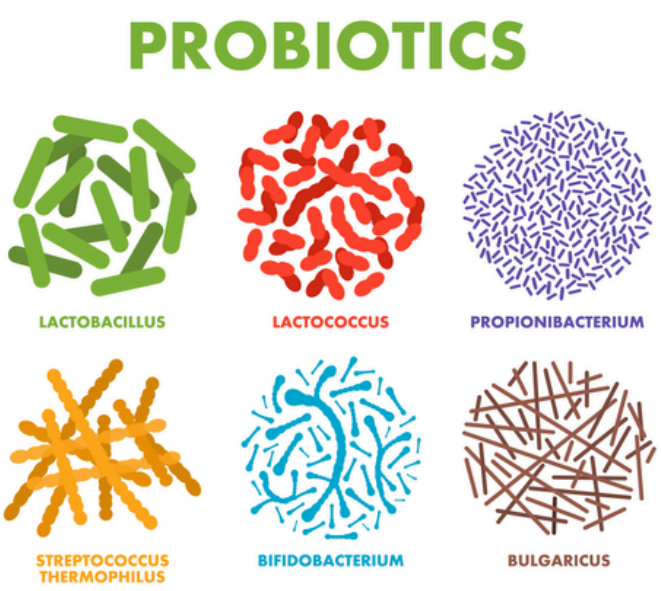
Effect of Diet on Gut Microbiota

- The microbial composition of T2DM patients is quite different compared to non-T2DM individuals.
- Diet is shown to extend a significant effect on gut health, and a healthy gut is responsible for more optimally regulating many pathways in the body. In the case of dysbiosis, many of these pathways are negatively impacted, contributing to the eventual onset of chronic disease.
- Major components of diet such as fats, proteins etc affect the constitution of gut microbiota.
- Degradation of proteins and carbohydrates results in end products like short chain fatty acids, acetate and butyrate in the intestinal gut tract.
- Proteins are degraded by proteolytic bacteria present in the gut.
- Ammonia, amino acids and amines are the end products of protein degradation.
- Prebiotics are non-digestible food components that benefit the host by promoting microbiota development.
- The Microbial Diet as a dietary approach is claimed to restore gut health (promoting eubiosis), increase metabolism, and decrease inflammation. It runs in three parts, with the overarching idea of eating less processed foods and more foods rich in prebiotics.
- Commonly, T2DM patients must follow a strict diet, low in simple carbohydrates and refined sugars, rich in complex carbohydrates, and low glycemic index foods/meals.



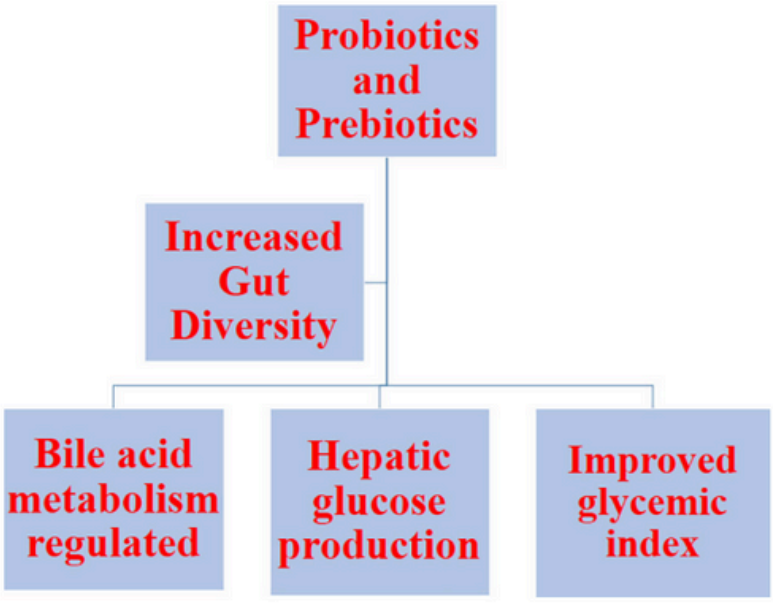
PROBIOTICS vs PREBIOTICS

Beneficial live bacteria to promote healthy digestive health	Food to promote growth of health bacteria
Where are they found?	**Where are they found?**
1. Yogurt	1. Banana
2. Sauerkraut	2. Onion
3. Kefir	3. Artichoke
4. Pickles	4. Garlic
5. Kimchi	5. Oatmeal
6. Tempeh	6. Honey
7. Supplements	7. Asparagus



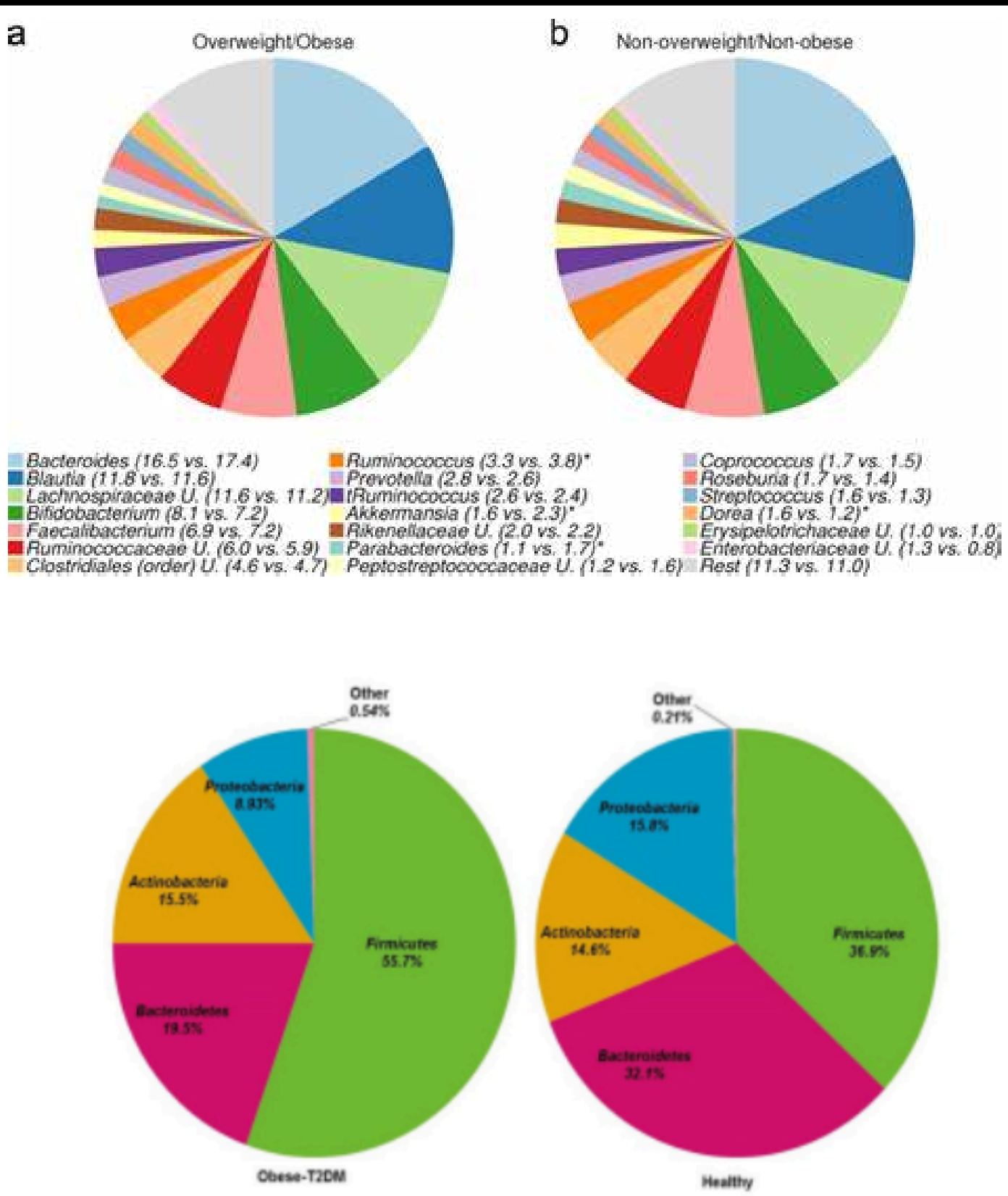
Gut Microbiota as an Alternate Therapy for Diabetes

- Dysbiosis refers to an imbalance in a body's natural microflora (as previously explained.)
- A change in microbial dysbiosis can be achieved by supplementation of probiotics.
- Various researchers have suggested strategies for the prevention of dysbiosis of the gut microbiota through supplementation of beneficial live bacteria.
- A study also showed most of the patients after bariatric surgery had improved T2D manifestations.
    - Bariatric surgery is a surgery that helps reduce food consumption and help weight loss by removing a part of the stomach thereby reducing its size.
- Probiotics when administered may provide health benefits to the host.
    - By decreasing insulin resistance, probiotics regulate the glycemic condition and may decrease the development of inflammatory markers in people with T2D.
- Studies also indicate that administration of Bifidobacterium animalis, B. breve, and B. longum can improve glucose intolerance
- New therapies like neuropathy and retinopathy that are associated with diabetes have too gained importance nowadays.



Trans-Ethenic Gut Microbiota Signatures of T2DM in Denmark & India

- Type 2 diabetes (T2D), a multifactorial disease influenced by host genetics and environmental factors. It is the most common endocrine disease.
- Gut microbiota is an environmental mediator which influences host physiology including metabolism.
- How was this study conducted?
    - 16S ribosomal RNA gene amplicon sequencing was performed on stool samples from 279 Danish and 294 Indian study participants and differences in microbial signatures were observed.
- Overall, the gut microbial communities of Danes and Indians are compositionally very different.
- T2D patients have an increased relative abundance of two operational taxonomic units (OTUs) from the Lachnospiraceae family, and a decreased abundance of Subdoligranulum and Butyricicoccus.
- T2D-related microbial changes at the taxonomic level were observed within the Danish population only.
- No significant difference between normoglycemic individuals and metformin-naive T2D patients, whereas microbial richness is significantly decreased in metformin-treated T2D patients compared to metformin-naive T2D patients and normoglycemic individuals
- Major compositional differences of the gut microbiota between Danish and South Indian individuals, some of which may relate to differences in ethnicity, lifestyle, and demography.


a
Overweight/Obese
b
Non-overweight/Non-obese
Bacteroides (16.5 vs. 17.4)
Blautia (11.8 vs. 11.6)
Lachnospiraceae U. (11.6 vs. 11.2)
Bifidobacterium (8.1 vs. 7.2)
Faecalibacterium (6.9 vs. 7.2)
Ruminococcaceae U. (6.0 vs. 5.9)
Clostridiales (order) U. (4.6 vs. 4.7)
Ruminococcus (3.3 vs. 3.8)*
Prevotella (2.8 vs. 2.6)
tRuminococcus (2.6 vs. 2.4)
Akkermansia (1.6 vs. 2.3)*
Rikenellaceae U. (2.0 vs. 2.2)
Parabacteroides (1.1 vs. 1.7)*
Peptostreptococcaceae U. (1.2 vs. 1.6)
Coprococcus (1.7 vs. 1.5)
Roseburia (1.7 vs. 1.4)
Streptococcus (1.6 vs. 1.3)
Dorea (1.6 vs. 1.2)*
Erysipelotrichaceae U. (1.0 vs. 1.0)
Enterobacteriaceae U. (1.3 vs. 0.8)
Rest (11.3 vs. 11.0)
Other 0.54%
Proteobacteria 8.93%
Actinobacteria 15.5%
Firmicutes 55.7%
Bacteroidetes 19.5%
Obese-T2DM
Other 0.21%
Proteobacteria 15.8%
Actinobacteria 14.6%
Firmicutes 36.9%
Bacteroidetes 32.1%
Healthy

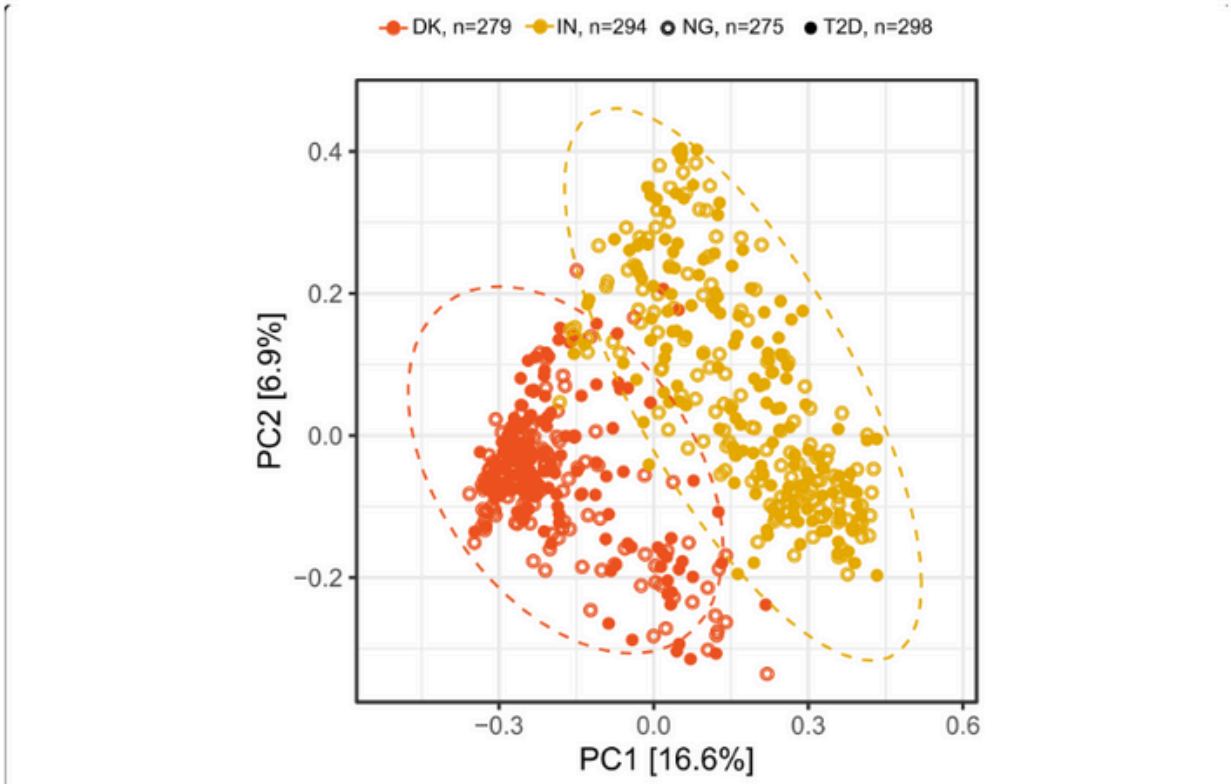


Fig. 1 Country of origin is the main driver of gut microbial community structure. Danish and Indian microbiota profiles are clearly separated at OTU level in the principal coordinate analysis using Bray–Curtis dissimilarity measure. Country of origin also explains 12% of the beta-diversity variation ($p < 0.001$, PERMANOVA test). Ellipses represent 95% confidence intervals for the Danish and Indian gut microbiota profiles. Gut microbiota compositions do not separate based on T2D status. DK, Denmark; IN, India; NG, normoglycemic controls; T2D, type 2 diabetes

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