SPECIAL REPORT

PHYTOSYNBIOTICS
Balance Your Gut & Achieve Better Health

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Date: 17th December 2013

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EXECUTIVE SUMMARY

Diabetes Mellitus ("diabetes") is a major, chronic disease that affects 382 million people worldwide and is set to grow to 592 million in 2035. The prevlance of diabetes affects about 8% of the world population and is so alarming that the World Health Organisation (WHO) maintains that diabetes has reached epidemic proportions worldwide.

Lifestyle changes are needed to combat Type-2 Diabetes Mellitus (T2DM), the most prevailing form of diabetes but in many cases, they are insufficient and need to be complemented with medication. However, medication can only slow down the progress of the condition and eventually become limited in their ability to control T2DM.

In recent years, studies from the Human Microbiome Project have shown that T2DM is associated with *disrupted microbiomes*. In every human being, and especially in the gut, dwells the microbiome: 100 trillion bacteria of several thousand species. This is 10 times more than the number of cells in the human body. This microbiome maintains health and performs beneficial functions such as food digestion, making vitamins and keeping bad organisms at bay. When this microbiome is disrupted, the body is unable to maintain health and the host falls ill. In addition to T2DM, disrupted microbiomes have also been associated with other problems such as obesity and malnutrition, atherosclerosis and heart disease, multiple sclerosis, asthma and eczema, liver disease, numerous diseases of the intestines including bowel cancer and autism.

A new class of food-grade dietary supplements called *phytosynbiotics* has been developed and shown tremendous promise to promote metabolic health by re-balancing disrupted microbiomes. *Phytosynbiotics* are formulations of plants with known healing properties which have undergone lactic acid fermentation, with production of natural occurring manno-oligosaccharide prebiotics as by products.

A clinical study on 166 diabetic patients involving a specific *phytosynbiotic* formulation developed from fermentation of bitter gourd and moringa has shown a new breakthrough in glucose and cholesterol management. The clinical study results are as follow:

- The **orally administered** formulation is **as effective as insulin in regulating blood glucose** in the body;
- The same formulation **reduces bad cholesterol** while **significantly increasing good cholesterol**; and
- The same formulation also **restores liver enzymes to normal levels**.

By adding beneficial bacteria and other active compounds into the gut via dietary supplements, the *phytosynbiotics* help to optimize gut health and maintain an ideal balance of beneficial to pathogenic bacteria in the gut. As illness is caused by a disrupted microbiome, re-balancing and stablising the upset microbiome resolves the problem, thereby helping the host to move away from a disease state into a healthy one.

CHAPTER 1. STATE OF TYPE 2 DIABETES MELLITUS IN THE WORLD

Diabetes Mellitus (diabetes) is a major chronic disease that affects 382 million people worldwide and is set to grow to 592 million in 2035 (see Figure 1). Table 1 shows the prevalence of diabetics in the regions of the world [1].

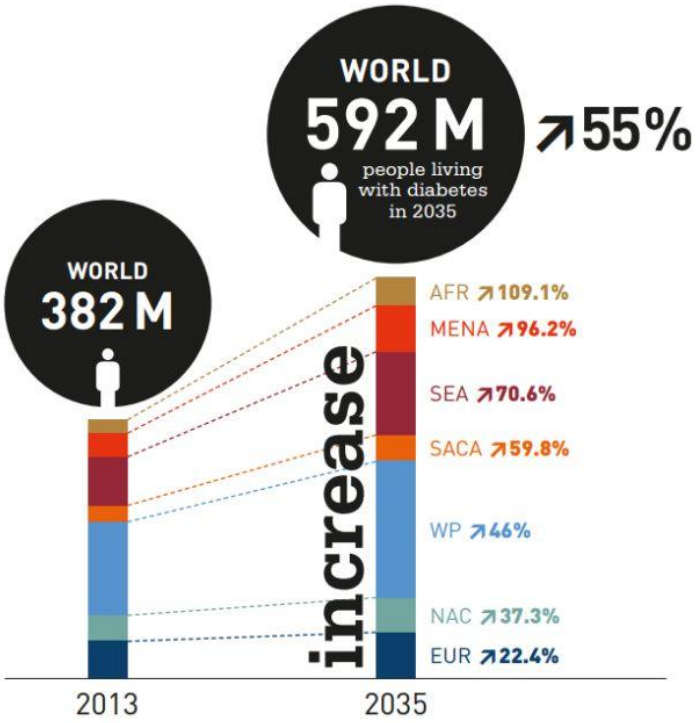


Figure 1 Global Prevalence Of Diabetes In The World

Source: IDF Diabetes Atlas Sixth Edition, International Diabetes Federation, 2013

Region	Prevalence	Percentage of Population
Western Pacific	138 million	8%
Southeast Asia	72 million	9%
Europe	56 million	7%
North America and Carribean	37 million	10%
Middle East and North Africa	35 million	11%
South and Central America	24 million	8%
Africa	20 million	6%
World	382 million	8%

Table 1: Prevalence of Diabetics in the World in 2013

Source: IDF Diabetes Atlas Sixth Edition, International Diabetes Federation, 2013

The World Health Organisation (WHO) maintains that diabetes has reached epidemic proportions worldwide. Although alarming, the above numbers mask the severity of the problem. It is estimated that for every one person diagnosed as having diabetes, there are 2.5 more people in the pre-diabetic stage who will develop diabetes in 3 – 6 years time [2].

Type 2 Diabetes Mellitus (T2DM) is the most common form of diabetes, accounting for between 85 and 95% of all diagnosed cases in adults. Untreated diabetes can lead to a range of serious medical problems, including heart disease, stroke and kidney failure [3].

Evidence points to the main causes of T2DM being lifestyle-related, including obesity and lack of exercise. According to the American Heart Association, many studies show that lifestyle changes such as losing weight, eating healthily and increasing physical activity can significantly reduce the progression of T2DM. But in many instances, lifestyle changes are insufficient and need to be complemented by a regimen of medications to control blood glucose levels, high blood pressure and cholesterol as well as to help prevent heart attack and stroke, which claim the lives of many diabetic patients.

The current modalities of treating T2DM consist of administering oral drugs as a first recourse. This is termed as the *non-insulin-dependent diabetes mellitus* (NIDDM) status. The oral treatment eventually fails when the diabetic patient progresses to an advanced stage, and treatment using insulin has then to be prescribed. This is termed as the *insulin-dependent diabetes mellitus* (IDDM) status. However, using insulin is cumbersome as it needs to be injected, and compliance of insulin is a real issue affecting its effectiveness.

Furthermore, there has been no new class of diabetic drugs launched in the market in the past 20 years.

CHAPTER 2. ABOUT PHYTOSYNBIOTICS

2.1. Introduction

Figure 2 Work Stress

Modern day diets and living conditions place tremendous stress on our bodies. Metabolic syndrome ailments such as diabetes mellitus (high glucose levels), cholesterolemia (high cholesterol levels), hypertension, obesity and gout are growing at an alarming rate. People are both physically and mentally overworked, and pollutants in our environment are subjecting our bodies to even more stress.

Much research has been dedicated to find natural solutions to overcome the above problems. A new type of formulations called *phytosynbiotics* is showing tremendous promise to meet these challenges headon.

2.2. History Of Phytosynbiotics

Figure 3 Traditional Fermented Foods in a Market in Asia

The *phytosynbiotics* has been researched over the course of the past 20 years. The development came about from the discovery that some villagers in rural parts of Southeast Asia and North Asia were living longer and healthier lives than their counterparts in other parts of their countries. Observations showed that the secrets of their longevity and health were in the consumption of traditional fermented concoctions handed down from generations. These findings were painstakingly recorded and the data used to re-invent traditional fermentation in a modern-day setting.

Research and development of the *phytosynbiotics* technology was carried out in Singapore, one of the most vibrant hub for bio-medical research and development in the world. The research was supported by Spring Singapore, a government agency which promotes research in small and medium size enterprises in Singapore.

This outcome of this research and development effort is a series of condition-specific *phytosynbiotics* formulations.

2.3. What Are Phytosynbiotics ?

Phytosynbiotics are a new class of food-grade dietary supplements which have shown promise to promote metabolic health and help our bodies cope with the rigorous demands of modern day living.

Figure 4 Ingredients For Making *Phytosynbiotics*

Phytosynbiotics are formulations of plants with known healing properties which have undergone lactic acid fermentation, with production of natural occurring manno-oligosaccharide prebiotics as by products.

Phytosynbiotics = Plants + Synbiotics

Synbiotics = Prebiotics + Probiotics

Lactic acid fermentation significantly increases the efficacy of the healing properties of the plants by making their nutrients and compounds more readily available and more easily absorbed by the body. The fermentation process allows small quantities of the plants to be used, thus significantly reducing the risks of toxic effects resulting from over dosage and use.

2.4. How Do Phytosynbiotics Work ?

In every human being, and especially in the gut, dwells the microbiome: 100 trillion bacteria of several thousand species. This is 10 times more than the number of cells in the human body. This microbiome maintains health and performs beneficial functions such as food digestion, making vitamins and keeping bad organisms at bay. More information about the microbiome can be found in Chapter 4.

Disrupted microbiomes have been associated with problems such as obesity and malnutrition, diabetes, atherosclerosis and heart disease, multiple sclerosis, asthma and eczema, liver disease, numerous diseases of the intestines including bowel cancer and autism.

The active ingredients of the *phytosynbiotics* help to stablise the upset microbiomes. If the upset microbiome causes illness, stablising it might resolve it.

Phytosynbiotics are more than probiotics or prebiotics or plant extracts. They are produced when selected plants with healing properities are fermented in a tightly controlled process with specific Lactobacillus probiotics. For example, a *phytosynbiotics* formulation for glucose management can be produced by co-fermenting lactic acid bacteria with bitter gourd and horse radish tree, plants which are known for their ability to control blood glucose in the body. In Chapter 3, results from a clinical study show that this particular orally administered *phytosynbiotics* formulation is as effective as subcutaneously-administered insulin in lowering blood glucose level to an ideal non-diabetic state in people with Type-2 Diabetes Mellitus. The *phytosynbiotics* thus has shown glucose management capabilities beyond the characteristics of the original raw plants.

During the fermentation process, prebiotic manno-oligosaccharides are produced as a by product. In additional to the beneficial effects on gut health, the prebiotics also play an important role in protecting the probiotics and plant nutrients from acidic digestive juices as they make their way through the digestive system. They arrive intact and are released in the small intestine, the environment where they are designed to work.

The controlled fermentation brings about beneficial results through a series of biochemical transformations:

- Unlocking of nutrients and vitamins from raw plants, eg hydrolisation of starch into shorter chains of glucose and dextrose, increasing levels of vitamins particularly thiamine, nicotinic acid, biotin and riboflavin;

- Production of enzymes that aid in increasing the bio-availability of the nutrients so that our bodies can absorb and assimilate them more easily;

- Reduction of naturally occurring toxins in the plants thus rendering them safe for consumption;

- Presence of probiotics and prebiotics to support a healthy gut microbiome and maintain proper digestion function. When digestion is functioning properly and the body is absorbing and assimilating all the nutrients needed, the immune system tends to be better equipped to wage war against disease and illness.

2.5. Key Differences Between Phytosynbiotics And Probiotics

Phytosynbiotics have a number of key features which differentiate them from conventional probiotics.

Supplement Facts

Serving Size 1 Capsule Servings Per Container 60

	Amount Per Serving	% DV
Probiotic Blend	10 Billion Organisms	*
L. acidophilus LA-02	*L. rhamnosus* LR-04	
L. paracasei LPC-00	*B. lactis* BS-01	
L. plantarum LP-01	*B. breve* BR-03	

* Daily Value not established.

Figure 5 Typical conventional probiotics label

Conventional probiotics contain a single pure strain or a blend of pure strains of microbes. These microbial strains are individually grown in bio-reactors, harvested and then blended together to form the final product. Since these microbes are cultivated in artificial bio-reactors, they are not adapted to the environment of our

digestive tract. Despite the large numbers of microbes claimed in many conventional probiotics often in excess of billions, in reality, almost all the microbes cannot survive the passage through the stomach and the digestive juices present inside. Even if they do arrive at their target destination, the small intestine, the pure strains of probiotics do not proliferate well together as a team. They have not been conditioned to grow together with each other and more often than not, because of their different growth dynamics, one of the blended strains will typically outgrow and suppress the other microbes.

Phytosynbiotics overcome the problems faced by conventional probiotics by using the dynamics of microbial communities and consortia (see Figure 6). Using complex liquid and solid fermentation processes, the microbial community will undergo its own natural selection process and at the end of the process, the remaining microbes have all gone through the growth process together and are able to work together in a synergistic manner. When introduced into the intestines, the different microbes in the community will support each other and carry out their work in a synergistic manner.

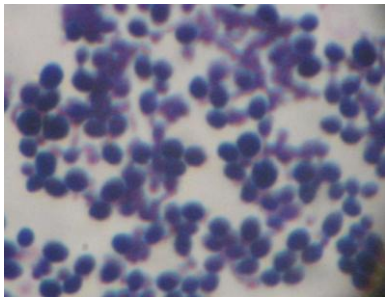


Figure 6 Microbial Community

During the fermentation process, the probiotics produce prebiotic substances such as manno-oligosaccharides. These prebiotic substances envelope the probiotic microbes to form a protective matrix which protects the *phytosynbiotics* during their passage through the acidic environment in the stomach, thus ensuring their integrity (see Figure 7). Upon arrival in the small intestine, the prebiotic matrix is dissolved in the acid neutral environment of the small intestine, thus releasing the probiotics intact into the intestinal flora.

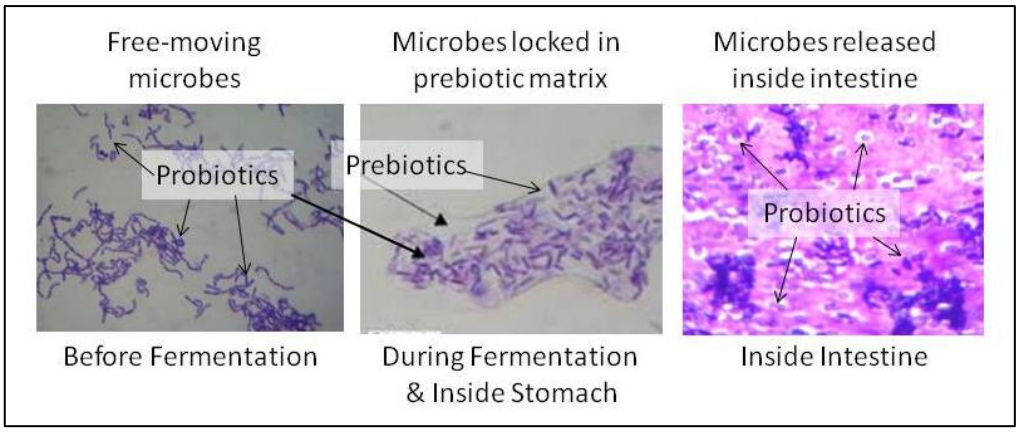


Figure 7 Protection of Probiotic Microbes By Prebiotic Manno-oligosaccharides

2.6. Phytosynbiotics And Our Health

The socio-economic impact of non-communicable diseases – or NCDs for short – like heart attacks and strokes, cancers and diabetes, is very high. Every year, NCDs account for over 63% of deaths in the world. An estimated 17 million people die of cardiovascular diseases, particularly heart attacks and strokes [4]. Globally, it is estimated that 382 million people suffer from diabetes [1].

Physical inactivity and unhealthy diets are some of the main risk factors which increase incidences of NCDs. Today's diet comprises many industrially processed foods which are laden with sugar, fat and salt. New discoveries in science prove that such food is biologically addictive, leading to craving and

binging. This leads to profound physiological consequences that drive up calorie consumption and weight gain. This in turn results in profound health consequences such as high blood pressure, diabetes, heart disease, arthritis and even cancer [5].

When we eat, we're not only feeding yourself, but also the 100 trillion of microbugs that thrive in our intestines (called the gut flora or **microbiome**). Recent research shows that these bugs not only help us digest foodstuff, ferment carbohydrates and proteins but also affect numerous processes in the body, including the immune system, metabolism, energy production, nutrition, genetic expression and general health. The choice of foods can alter the microbiome in a matter of days, for better or worse.

70% of our immune system is located in the gut as a vast network of lymph tissue referred to as GALT (gut associated lymphatic tissue). Disruption of the microbiome, through the use of antibiotics or unhealthy food, would negatively impact our ability to fend off diseases and has been associated with problems such as obesity and malnutrition, diabetes, atherosclerosis and heart disease, multiple sclerosis, asthma and eczema, autism, liver disease, and numerous diseases of the intestines, including bowel cancer [6], [7]. There's even tantalizing evidence that the disruption could extend to the brain, influencing mood, anxiety and cognition.

To stay healthy and disease-free, it is important to maintain good gut health or re-balance a disrupted microbiome. Optimizing gut health is about maintaining an ideal balance of beneficial to pathogenic bacteria in your gut which is about 85 percent good bacteria and 15 percent bad. One convenient way of doing this is to selectively foster beneficial bacteria by adding bacteria via food or supplements directly into the gut.

CHAPTER 3. DETERMINING THE EFFICACY OF PHYTOSYNBIOTICS

3.1. Introduction

The technology underlying *phytosynbiotics* has been researched over the course of the past 20 years. The development came about from the discovery that some villagers in rural parts of Southeast Asia and North Asia were living longer and healthier lives than their counterparts in other parts of their countries. Observations showed that the secrets of their healthy and longevity were in the consumption of traditional fermented concoctions handed down from generations. These findings were painstakingly recorded and the data used to re-invent traditional fermentation in a modern-day setting.

This outcome of this research and development effort is a series of condition-specific *phytosynbiotics* formulations.

3.2. Formulation For Glucose And Cholesterol Management

In Asian tradition, some plants are known for their glucose lowering effect and in particular, the fruit of the bitter gourd or bitter melon (*Momordica charantia*) and the leaf of the white radish tree (*Moringa oleifera*) [8] , [9], [10], [11]. When used in their raw forms, these plants have not proven to be as effective as insulin.

However, when these two above-mentioned plants are co-fermented with proprietary probiotics (food-grade *Lactobacillus* species), prebiotic manno-oligosaccharides are created as by products, and the resulting *phytosynbiotics* formulation (termed PSB) exhibited excellent glucose and cholesterol balancing properties.

3.3. Clinical Study

In the mid 1990s, a clinical study [12] was commissioned in a hospital setting to determine whether the PSB formulation is effective in lowering the blood glucose level of T2DM diabetic patients to an ideal non-diabetic state.

An equal number of male and female volunteers were recruited for the study. Selection criteria were:

a) Age ≥ 20 years old.

b) Diagnosed to have T2DM for more than 3 years and Hb_{A1C} result at the time of test of 7.0% ± 0.2 %.

c) They were currently on oral medication, *e.g*. metformin, glimepiride, but not on insulinotherapy.

A total of 166 samples were eventually assayed.

The T2DM volunteers were divided into 3 groups:

a) In the first group, the CONTROL group, volunteers were advised to continue their oral medication as prescribed by their doctors. They did not administer PSB nor insulin.

b) In the second group, the Insulin-treated ISL-GP group, each volunteer was prescribed a pre-standardized dosage of insulin without any other oral medication nor PSB.

c) In the third group, the *phytosynbiotic*-treated PSB-GP group, each volunteer was prescribed a pre-standardized dosage of PSB and was advised to administer only PSB daily without any other oral medication.

Volunteers in all groups were advised to maintain a proper diet. The volunteers visited the attending clinician on a monthly basis during the 5-month study period, during which blood samples were collected and used to determine the serum levels of Hb_{A1C} (glycated haemoglobin), HDL-C (mg/dl), LDL-C (mg/dl), ALT (IU/l) and AST (IU/l) using standard laboratory techniques.

3.4. Results And Analysis From The Clinical Study

3.4.1. Serum Glucose

3.4.1.1. About Glycated Haemoglobin (Hb_{A1C}) [13]

The Hb_{A1C} assay is widely used by clinicians to assess the efficacy of anti-diabetic drugs over a period of time. As described in the literature [14], [15], the clinical study of novel treatments and drugs using Hb_{A1C} assay precedes the fasting plasma glucose test, because the former gives a reliable assessment of the efficacy of the drug over a period of time without inconveniencing the volunteer for the need to fast and possibly leading to false results. Hb_{A1C} assay is hence an important marker for diagnosing and treating T2DM and has been chosen for this study. Glycated haemoglobin is present in non-diabetics at a level of around 5.0% of total haemoglobin, while T2DM patients have levels that are > 7.0% of total haemoglobin. Hb_{A1C} levels are relatively unaffected by short-term fluctuations in blood glucose level. Thus, Hb_{A1C} levels are indicative of the average blood glucose concentration over the past 1-3 months and are used to assess the severity of glucose intolerance in a T2DM patient and in management of the disease.

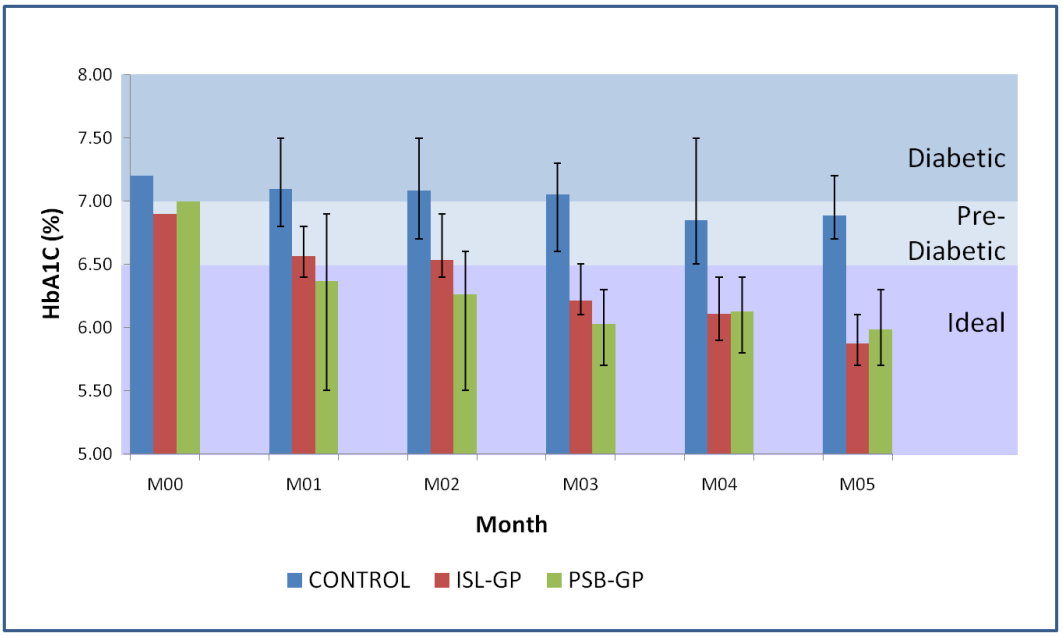
Table 6 summarizes the different Hb_{A1C} levels and their risk of complication to the T2DM patient [9]:

Assay	**Assessment of glucose control**			
	Ideal	Optimal	Sub-optimal	Unacceptable
Hb_{A1C} levels (%)	4.5 – 6.4	6.5 – 7.0	7.1 – 8.0	>8.0
Risk of complication	None	Low	Moderate	High
Stage	Normal	Pre-diabetic	NIDDM	IDDM

Table 6: Risk of Complication in Relation to Hb_{A1C} Levels

3.4.1.2. Results And Analysis

To better appreciate the relative efficacy of PSB, the kinetics of the Hb_{A1C} average of the 3 groups over the 5-month study period are succinctly represented in Graph 1:



Graph 1. Comparison of Hb_{A1C} Levels over 5 Months

- *Phytosynbiotic*-treated PSB-GP. T2DM volunteers who were on the PSB-treatment regimen managed to reduce their Hb_{A1C}, on the average, from 7.0% to well below 6.5% from the 1st month onwards. This rapid decrease in the average Hb_{A1C} levels had allowed them to move directly from a *non-insulin-dependent diabetes mellitus* (NIDDM) status to an ideal non-diabetic status.

- Insulin-treated ISP-GP. T2DM volunteers who were on the insulin-treatment regimen managed to also reduce their Hb_{A1C}, on the average, from 6.9% to around 6.5% on the 1st and 2nd month of the study period, and finally well below 6.5% from the 3rd month onwards.

- Oral medication CONTROL. The above results were in contrast to T2DM volunteers who were on oral medication prescribed by their doctors. In this CONTROL group, Hb_{A1C} average fell to below 7.0% only after the 4th month of the study period. This meant that the oral medication prescribed by their doctors took a longer time to see improvement from an NIDDM status.

The results for the insulin-treated ISL-GP group and for the CONTROL group on oral medication were within the expected efficacy of these drugs. In effect, the American Diabetes Association and the World Health Organization acknowledge the difficulty in achieving an ideal state of normal blood glucose level over the long-term. For patients who are diagnosed with NIDDM and are on oral medication, the long-term goal is to achieve and maintain a pre-diabetic status where the Hb_{A1C} level is between 6.5% and 7.0%. However, this pre-diabetic status can revert to NIDDM status over time and this reversion is commonly observed by doctors treating T2DM patients with oral medication.

In this clinical study, both insulin and PSB allowed the NIDDM patients to achieve, and probably maintain, an ideal state of normal blood glucose level over the long-term. Such a goal opened up the

reality of markedly reducing the risk of complication due to T2DM, thereby improving the patient's quality of life. What was more remarkable was that insulin had been injected subcutaneously whereas the PSB had been given *per os* (administered orally). This provided PSB with a clear advantage over insulin, as *per os* was a vastly more convenient and safer route of administration with respect to a subcutaneous injection. This tremendously improved compliance by the T2DM patients.

3.4.2. Serum Cholesterol

3.4.2.1. About Low-Density Lipoprotein (LDL) And High-Density Lipoprotein (HDL)

Beneficial effect on serum cholesterol is based on decreasing LDL-C and increasing HDL-C levels respectively. The key objective in managing both serum LDL-C and HDL-C levels is to reduce the risk of heart attack and stroke.

People with diabetes have the same risk for heart attack and stroke as people who already have cardiovascular disease [16]. According to the American Heart Association, T2DM patients should target their LDL-C level to be well below 100 mg/dl and HDL-C level well above 40 mg/dl for males and 50 mg/dl for females. Ideally, to be considered protective against cardiovascular disease, the American Heart Association recommends LDL-C and HDL-C targets of 70 mg/dl and 60 mg/dl respectively [16]:

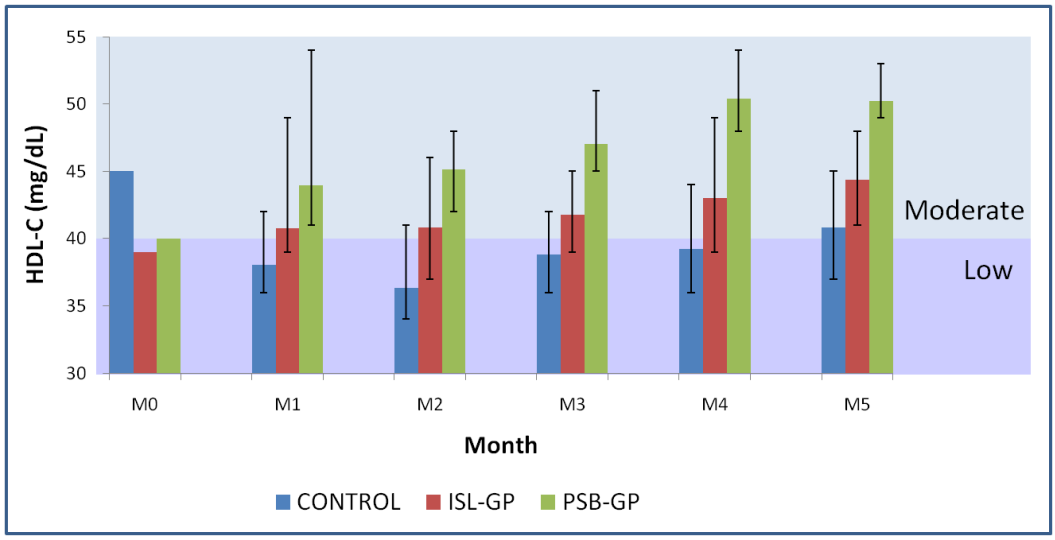
	Optimal	Ideal
Risk of cardiovascular disease	Significantly lowered	Negligible
HDL-C	> 40 mg/dl	60 mg/dl
LDL-C	< 100 mg/dl	70 mg/dl

Table 7: Optimal and Ideal Levels of Cholesterol Markers for T2DM Patients

In a U.S. study on T2DM patients (study population = 7,692), it is interesting to note that 49.5% of them have low HDL-C levels (< 40 mg/dl for males and <50 mg/dl for females) and that by increasing the HDL-C levels alone in these T2DM patients can actually bring about a lowering of risk of cardiovascular disease [17]. In our clinical study, the HDL-C levels in T2DM patients were not considered as high risk of acquiring cardiovascular disease. However it is reported that T2DM tends to lower HDL-C and raise LDL-C levels over time, which subsequently increases the risk for heart attack and stroke [16]. This common condition is called *diabetic dyslipidemia*.

3.4.2.2. Results And Analysis

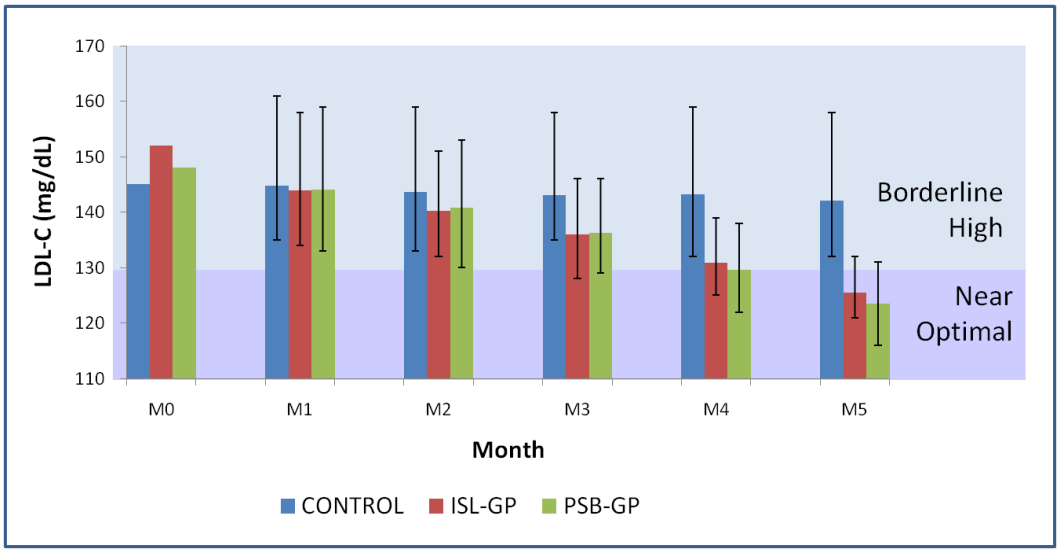
In our study, we noted that the average HDL-C levels in both the PSB-GP and ISL-GP groups showed a rise over a 5-month period. The PSB-GP group's average HDL-C level showed an even more rapid and marked rise in levels – *up to 50.8 mg/dl at the 4th month onwards* – than that of the insulin-treated ISL-GP group. The average HDL-C level in the CONTROL group did not improve beyond 41 mg/dl. This can be better appreciated in Graph 2:



Graph 2. Comparison of HDL-C ("good" cholesterol) Levels over 5 Months

It is reported that for every 1 mg/dl increase in HDL-C levels above the 40 mg/dl mark, the risk of mortality from cardiovascular disease is reduced by 3.7% in male T2DM patients and 4.7% in female T2DM patients [18]. Although the ideal level of 60 mg/dl was not achieved in this study for both the PSB-GP and ISL-GP groups, we could postulate that T2DM patients who were on PSB-treatment would have a 41% reduction in risk of succumbing to cardiovascular disease *vs.* T2DM patients on insulinotherapy who would have 15% reduction in risk of succumbing to cardiovascular disease.

At the same time both the PSB-GP and ISL-GP groups had their average serum LDL-C levels decreased noticeably when compared to that of the CONTROL group. This trend is succinctly represented in Graph3:



Graph 3. Comparison of HDL-C ("bad" cholesterol) Levels over 5 Months

Unlike its effect on HDL-C levels which stabilized from the 4th month onwards, the graph above showed that the PSB's lowering effect on LDL-C had not slowed down over the 5-month study period. Using linear regression (with an R^2 value of 0.987), we could postulate, by extrapolation, that it would take 10 months of daily intake of PSB to bring the serum LDL-C level to below 100 mg/dl,

considered as the optimal level for T2DM patients. Further studies would be required to confirm our postulate.

3.4.3. Serum Liver Enzymes

3.4.3.1. About Alanine Aminotransferase (ALT) And Aspartate Aminotransferase (AST)

It has been long established that elevated activities of serum alanine aminotransferase or ALT (> 45 IU/l) and aspartate aminotransferase or AST (> 35 IU/l) are common signs of liver injury, and are observed to be 4 times more likely among diabetics than in the general population [19]. This is because the liver plays a critical role in the maintenance of carbohydrate homeostasis, glucose regulation, and insulin degradation. A survey conducted on 2,999 men and women, aged 20-74 years in southwestern United States, found that diabetes appeared to be associated with liver injury, as indicated by elevated activity of serum AST, independent of alcohol consumption and obesity [19]. In a more recent study, higher ALT and γ-glutamyltransferase values were significantly ($p < 0.01$) associated with both diabetes and pre-diabetic stage, whereas AST was only significantly associated with pre-diabetic stage ($p = 0.0001$) [20].

Unlike HDL-C and LDL-C where there are clear and stringent targets to achieve for T2DM patients, clear targets to achieve for liver enzyme markers like ALT and AST by T2DM patients have yet to be established. In absence of such targets, we take the commonly acceptable threshold levels of 45 IU/l and 35 IU/l for ALT and AST respectively.

3.4.3.2. Results And Analysis

From our study, we observed the following:

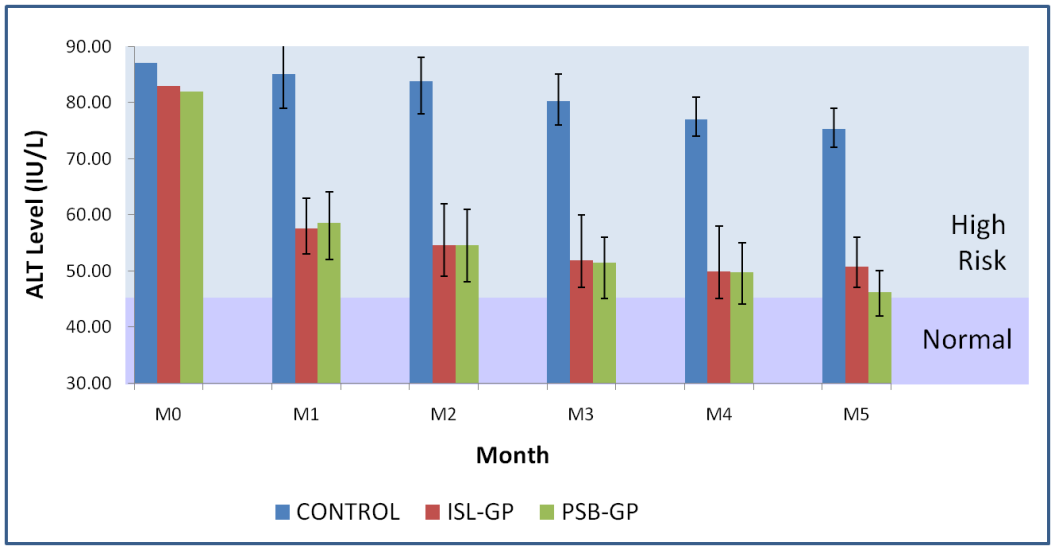
- In the 1st month of the study, serum ALT showed a sharp 30% decrease in activity for both the PSB-GP and ISL-GP groups, whereas the serum ALT activity decreased only by a marginal 2% in the CONTROL group;

- Over the next 4 months, the average rate of decrease in serum ALT activity for the CONTROL and ISL-GP groups was the same, *i.e.* 3%, whereas the average rate of decrease in serum ALT activity for the PSB-GP group was double that at 6%.

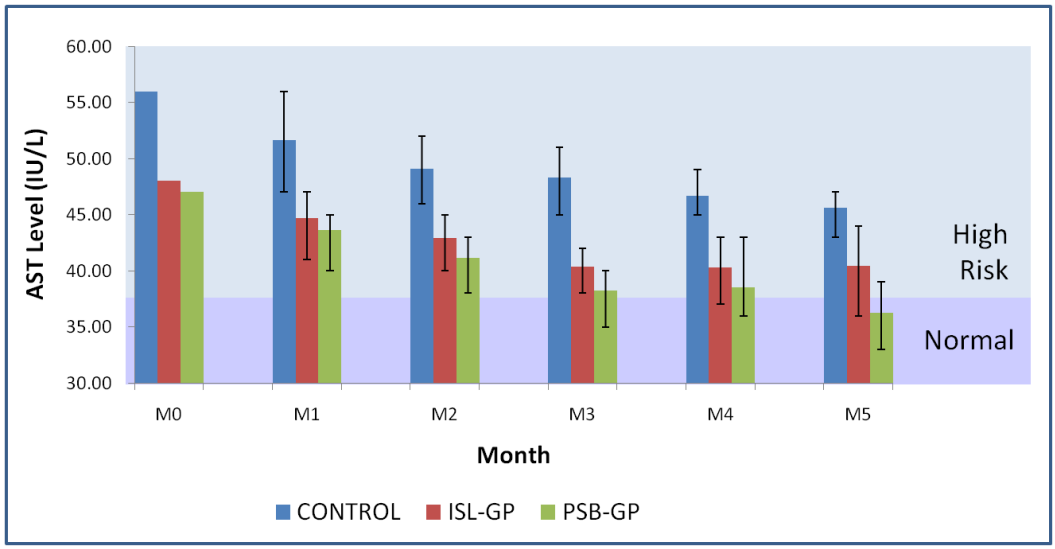
- This same phenomenon was repeated for serum AST activity.

- With PSB, both serum ALT and AST activities were very close to optimal levels by the 5th month of the study period.

These observations are succinctly represented in Graphs 4 and 5:



Graph 4. Comparison of ALT Levels over 5 Months



Graph 5. Comparison of AST Levels over 5 Months

This would imply that the PSB formulation had a superior beneficial effect vs. insulin in lowering the activities of serum liver enzymes over the 5-month study period. The findings are in agreement with reports on the "hepato-protective" effect of probiotics, and possibly also prebiotics, albeit in rat models [21], [22], [23], [24]. More specifically, several species of *Lactobacillus* rather than *Bifidobacterium* spp. are shown to be capable of significantly lowering the activity of serum ALT in liver-injured subjects [21], [24]. The PSB is an association of several proprietary food-grade *Lactobacillus* species.

3.5. Implications

The clinical study, over a 5-month period and on 166 volunteers diagnosed with T2DM, was the first of its kind in comparing insulin (considered as the gold standard) with a novel, proprietary *phytosynbiotics* formulation comprising of plants known to have anti-diabetic properties and potentiated by a proprietary manufacturing process with selected probiotics and prebiotics. The apparent potentiation of the anti-diabetic properties of the formulation is evidence by the resultant *phytosynbiotics* formulation to be equally as effective as, or even superior to, insulin in the

parameters that are key medical indicators of the diabetes metabolic syndrome. We believe that the efficacy of the *phytosynbiotics* formulation in controlling T2DM is not a coincidence. This is evidence by the same improvement in all key metabolic parameters related to T2DM – and not only the Hb_{A1C} parameter – in both the PSB-treated and insulin-treated groups, whereas only marginal improvement is found in the same key metabolic parameters in the CONTROL group that are on the normal, oral medication for T2DM.

In all cases of T2DM, the pancreas is impaired at varying severity and peripheral organs have either insufficient or impaired receptors to bind with insulin so as to allow sufficient glucose into the cells for normal function. This causes the pancreas to compensate by pumping more insulin into the system. Current medical opinions therefore warrant that insulinotherapy should be instituted in all cases of patients diagnosed with T2DM, so as not to impose undue stress on the pancreas. The reality lies in the patient's compliance. Any novel insulin-mimetic compound or insulin analogues that are given *per os* will indeed improve the therapeutic outcome for T2DM patients.

A point of interest that is noteworthy: the average HDL-C level in PSB-treated group rises more noticeably than that of the insulin-treated group. It may be an additive effect due to the presence of probiotics in the *phytosynbiotics* formulation. It has been recently discovered that a key component in the formation of HDL, called apo A-1, is generated by the intestinal epithelial cells [25]. In the *phytosynbiotics* formulation, we can postulate that the proprietary probiotic species have somehow incited the intestinal epithelial cells to increase production of apo A-1, as shown by a higher rise in serum HDL-C level *vs*. that of the insulin-treated group. If so, such a novel product will indeed compliment the current use of statins, which have only LDL-C lowering effect. Medical experts opine in agreement that a HDL-C raising drug will be a more useful research pathway for the biomedical industry than trying to develop yet another statin drug.

In addition, the superior hepato-protective effect of the *phytosynbiotics* formulation to that of insulin would be an added advantage to T2DM patients, as liver injury is a common condition of the chronic nature of diabetes mellitus.

The results thus obtained confirm the efficacy of the *phytosynbiotics* formulation to manage blood glucose and cholesterol in T2DM patients.

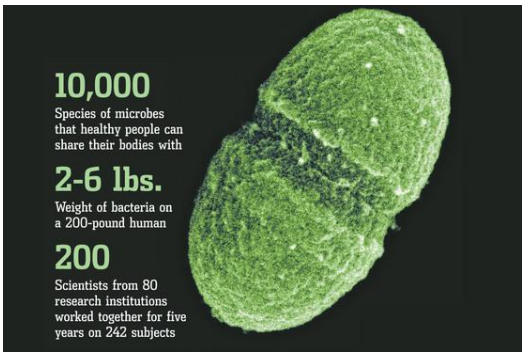
CHAPTER 4. THE HUMAN MICROBIOME

4.1. Introduction

The domain of the human microbe is one of the most exciting areas of research in understanding ailments and metabolic disorders which until now remain poorly understood. Results from the research of the human microbiome are already turning heads, and are set to completely revolutionise the way we treat diseases and ailments in the years to come.

There is an explosion in the amount of research and new knowledge in this area. Every week, new research data and findings are released at a frenzied pace, providing astounding new insights into the relation between the microbiome and our health. As such, this chapter will only provide a basic introduction to the concept of the human microbiome and provide key references for the reader to further his or her exploration in this fascinating subject.

4.2. The Human Microbiome



In every human being, and especially in the gut, dwells 100 trillion microbes of several thousand species. This is 10 times more than the number of cells in the human body. This aggregate of microbes that reside on the surface and in deep layers of skin, in the saliva and oral mucosa, in the conjunctiva, and in the gastrointestinal tracts is called the human microbiome.

Studies in 2009 questioned whether the decline in the microbiome as a result of human intervention might impede human health. Most of the microbes associated with humans appear to be not harmful at all, but rather assist in maintaining processes necessary for a healthy body. A surprising finding was that at specific sites on the body, a different set of microbes may perform the same function for different people. For example, on the tongues of two people two entirely different sets of organisms will break down sugars in the same way. This suggests that medical science may be forced to abandon the one-microbe model of disease, and rather pay attention to the function of a group of microbes that has somehow gone awry. However, this topic has been too poorly researched to properly understand the role that microbes play.

In response to this, the United States National Institutes of Health launched the Human Microbiome Project (HMP) in 2008 with the goal of identifying and characterizing the microbes which are found in association with both healthy and diseased humans. Armed with a budget of $115 million, the first five-year phase was focused on development of analytic tools, data analysis and access.

At the end of phase 1 In June 2012, the HMP published the most extensive investigation on the human microbiome to date. And those publications have revealed many surprises and have given us the clearest picture ever of the microorganisms that call our body home — and this knowledge may result in actionable discoveries that are likely to affect our lives in ways we would never expect.

These could someday lead to new approaches to treat malnutrition, asthma, obesity, diabetes and other conditions but the research is still in its early stages.

In the same year, in a meeting where scientists met to review progress made under phase 1 of HMP and consider future directions, Dr Francis S. Collins, NIH director, called HMP a "very exciting area of science, which is just bursting with opportunity and potential for understanding aspects of human health that had been outside our reach until recently".

The second phase of HMP has just begun and will be probing the microbiome's impact on health. The ultimate goal of HMP is to test how changes in the human microbiome are associated with human health or disease.

4.3. Type 2 Diabetes Mellitus And The Human Microbiome

Studying gut bacteria can reveal a range of human illnesses. A joint European and Chinese research study showed that the composition of a person's intestinal bacteria played an important role in the development of T2DM [26]. By studying the intestinal bacteria of 345 people from China, the research team discovered that the 171 subjects who had T2DM were found to have a "more hostile bacterial environment in their intestines" than those not suffering from the disease. The study suggests that this out-of-balance gut flora could increase resistance to different medicines as well as likely be the trigger for T2DM. Similar studies carried out on T2DM patients in Denmark also showed a significant imbalance in the function of their intestinal bacteria and composition.

Together with obesity, insulin resistance is the harbinger of metabolic syndrome. Insulin resistance is when the body cannot use insulin effectively. Insulin is needed to help control the amount of sugar in the body. As a result, blood sugar and fat levels rise which in turn leads to morbid obesity, T2DM, stroke and heart problems. So, what is the connection of metabolic disease to bacteria? A group of researchers in Brazil answered the question in a study on insulin resistance and gut bacteria. The study, albeit on mice, showed that the bacteria living in our gut are responsible for our metabolism. And it is the interaction between the bacteria and our immune system that not only protects us from pathogens, but also protects us (or not) from metabolic disease [27]. This outcome has been repeated again and again in many other studies that followed.

Another landmark study on the link between metabolic syndrome and gut microbes was carried out by Professor Zhao Liping of Shanghai Jiao Tong University. In a paper published in December 2012, Prof Zhao's team isolated a bacterium from a 385-pound man's intestines, and used it to plump up mice that are specially bred to resist obesity. They found that the bacteria, a toxin-producing microbe called *enterobacter cloacae*, made up 35 percent of all the microorganisms in the human volunteer's digestive tract. But a diet formulated to specifically to kill off those bacteria succeeded in reducing his levels to below what could be detected in a laboratory. He lost 113 pounds in 23 weeks. His high blood pressure disappeared. So did his T2DM and his fatty liver disease [28]. In an interview with China Daily, Prof Zhao explained how it works. He said, "The endotoxin released by the bacterium can activate a gene that helps generate fat. And it also deactivates a gene that consumes fat."

4.4. Suggested Readings

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Accessed at: http://www.economist.com/node/21560523

Microbes Maketh Man, The Economist, 16th August 2012
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A Study in Balance: How Microbiomes Are Changing the Shape of Environmental Health, Kellyn S. Betts, Environmental Health Perspectives, Journal of the National Institute of Environmental Health Sciences, 7th August 2011.
Accessed at: http://www.eoearth.org/view/article/168674/

The Human Microbiome Project
Accessed at: http://www.hmpdacc.org/

Human Microbiome Science: Vision for the Future (videos)
Accessed at:
http://www.youtube.com/watch?v=eFGhKzLSwGA&list=PL1ay9ko4A8smvKyR0HIjyNSpf9CRhqzjJ

CHAPTER 5. CONCLUSION

T2DM is a progressive disease. Although there is a wide range of medication available to treat T2DM, evidence is showing that T2DM in the majority of patients is still poorly controlled. This failure to achieve optimal glycemic control partly results from the limitations of current therapies, which in most cases target the symptoms of the disease but not its underlying causes. Although control of T2DM can be achieved with the timely use of insulin, the intrusive nature of insulin therapy often leads to non-compliance by diabetic patients.

New research on the human microbiome is finally providing insight into the underlying causes of T2DM. It is now known that diabetic patients exhibit an intestinal microbiome which is significantly different from healthy microbiomes. Studies have also concluded that stabilising the disrupted microbiomes of obese and diabetic patients can shift the hosts into a healthy state.

Although still in its infancy, research and development on the human microbiome is showing the potential of managing T2DM through the stabilisation of the intestinal microbiome. It is anticipated that in the years to come, new treatments will emerge which offer the promise of even better glycemic control through mechanisms of action that tackle the disease pathophysiology, ie targeting to re-balance the microbiome, not just its symptions.

In this report, we have presented one such solution – *phytosynbiotics*. *Phytosynbiotics* exhibit characteristics which offer great potential to become a breakthrough solution in the fight against T2DM and its features can be summarised as follow:

- *Phytosynbiotics* produce **exceptional results**. Efficacy of the *phytosynbiotics* formulation to manage glucose, cholesterol and liver enzymes is demonstrated in the clinical study which concluded that the *phytosynbiotics* formulation tested is as effective as insulin in controlling blood glucose. In addition to the clinical study, there is much anecdotal evidence showing that *phytosynbiotics* really do work and produce exceptional results.

- *Phytosynbiotics* are **safe**. They are made from food ingredients and their GRAS (Generally Recognized As Safe) status make them perfectly safe for consumption. This is further borne out in the clinical study where the liver enzymes of diabetic patients are restored to normal levels as against those taking chemically synthesized medication.

- *Phytosynbiotics* are **effective**. They can survive the stomach environment which would destroy most commercial probiotics. The prebiotics secreted by the probiotics during fermentation constitute a protective matrix which ensure the safe passage of the probiotics through the stomach into the small intestines where they are released to do their work.

- *Phytosynbiotics* are **condition specific**. This is not a single formulation to cure all ailments. Because of the possibility of combining different plants with their specific healing properties, *phytosynbiotics* can be formulated to address specific metabolic conditions. Different conditions can be addressed using different combinations of plants and probiotics to achieve maximal optimisation of the microbiome.

- *Phytosynbiotics* are backed by **real science**. The *phytosynbiotics* technology is backed by over 20 years of research in one of the most exciting scientific discoveries of our time–microbial communities and the human microbiome. *Phytosynbiotics* stand at the cutting edge of the new technology which is poised to revolutionize medicine in the years to come.

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