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Alteration of the gut microbiome by scutellaria-coptis herb couple and metformin in type 2 diabetes

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Thesis

ALTERATION OF THE GUT MICROBIOME BY SCUTELLARIA-COPTIS

HERB COUPLE AND METFORMIN IN TYPE 2 DIABETES

by

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ABSTRACT

There is a rising global prevalence of type 2 diabetes (T2D), which poses both health and economic burdens. Effective research on T2D and clinical interventions can decrease the impact of these burdens and fill knowledge gaps in their efficacy, safety, and long-term outcomes. This comprehensive literature-based review examines the role of the gut microbiome in T2D and treatments, such as metformin and *scutellaria radix-coptidis rhizoma* (SC) herb couple, for gut microbiome (GM) modulation and improvement in T2D. For the purposes of this thesis, the GM is defined as a healthy nondiabetic GM, whereas dysbiosis, a deviation from a healthy GM maybe associated with the pathogenesis of T2D in terms of glucose metabolism, insulin sensitivity and inflammatory pathways and dysbiosis has been observed in individuals with a diagnosis of T2D. Microbial metabolites, such as short chain fatty acids and bile acids, and inflammatory processes have been associated with diabetes-related outcomes. Metformin, a traditional first line antidiabetic agent, as well as the traditional Chinese medicine herb couple, SC, modulate the composition of the GM in alleviating T2D. Based on the current body of knowledge, we propose to further investigate the effect of metformin and SC on the GM in T2D alleviation and measure the composition of the GM before and after an interventional clinical trial period. Investigating the effects of these study drugs

and their relation to the GM may provide new insights on the pathogenesis and

management of T2D.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

ADA	American Diabetes Association
AMPK	AMP-activated Protein Kinase
BA	Bile Acid
BMI	Body Mass Index
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
FBG	Fasting Blood Glucose
GLP-1	Glucagon-like Peptide 1
GM	Gut Microbiome
GPCR	G-protein Coupled Receptors
HbA1c	Hemoglobin A1c
ICD	Informed Consent Document
IR	Insulin Resistance
IRB	Institutional Review Board
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharide
MyD88	Myeloid Differentiation Primary Response 88
PBG	Postprandial Glucose
PYY	Peptide YY
SC	*Scutellaria radix-Coptidis rhizoma*

SCFA	Short Chain Fatty Acid
T2D	Type 2 Diabetes
TCM	Traditional Chinese Medicine
TG	Triglyceride Level
TGR5	Takeda G Protein Coupled Receptor 5
TLR4	Toll-like Receptor 4
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
WC	Waist Circumference

INTRODUCTION

Background

T2D is a chronic disease in which the body's ability to utilize and regulate glucose

metabolism is impaired. There is currently no cure for T2D, however, lifestyle

modifications, as well as pharmacologic treatments, can regulate blood sugar and

alleviate symptoms of T2D. Two key interrelated problems define the pathogenesis of

T2D: insulin resistance from muscle, fat and liver cells and beta-cell impairment, leading

to an inadequate insulin secretion from the pancreas and resulting in hyperglycemia.

Hyperglycemia is a gradual process in T2D and, long term, can lead to multiorgan

complications, including the gastrointestinal, cardiovascular, renal, and nervous

systems.[1–4]

According to guidelines of the American Diabetes Association, the diagnosis of

T2D can be made in a variety of ways. Patients can be classified as normal, prediabetic or

at risk for diabetes and diabetes mellitus. Methods use either fasting plasma glucose (in

mg/dl), oral glucose tolerance test or hemoglobin A1C, the percentage of glycosylated

hemoglobin (Table 1). In clinical practice, it is typical to require two abnormal results for

each test on two separate days for the diagnosis of diabetes. Classically, some of the

clinical symptoms include polyuria, polydipsia, polyphagia, blurry vision, fatigue,

malaise and infections. However, not all presentations of diabetes are uniform across

patients, as diabetes is a multifactorial disease and may manifest in a multitude of ways.

1

Table 1. Diagnostic Criteria for Prediabetes and Diabetes

Method of Diagnosis:	Normal Value:	Prediabetes or "at risk":	Diabetes Mellitus:
Hemoglobin A1C (%)	less than 5.7	5.7 to 6.4	6.5 or higher
Fasting Plasma Glucose (mg/dl)	less than 100	100-125	126 or higher
Oral Glucose Tolerance Test (mg/dl)	less than 140	140-199	200 or higher

Of the over 37 million Americans that have diabetes, it is estimated that 90-95% of these cases are affected with type 2 diabetes.[3] While most commonly diagnosed in adults over the age of 45, it is becoming increasingly common to diagnose younger Americans. Alongside the rise in obesity rates, T2D rates have also trended upward as the two have been found to be interconnected.[4,5] T2D represents both a healthcare and economic burden in the United States in the form of increased healthcare expenses, loss of productivity, reduced quality of life and premature mortality. In 2017, the cost of diabetes in the United States was estimated to be around $327 billion.[5,6]

The human microbiota is a dynamic ecosystem of various microbes including bacteria, archaea, viruses and fungi. The human microbiota is established at an early stage of life, however exogenous factors, such as mode of birth, age or antibiotic use, may affect its development and heterogeneity.[7,8] Variations between each individual's compositions may be attributed to these factors. These microbes have a profound effect on host physiology and immunology. Four major sites of these host-microbial

interactions include: oral, gut, vagina, and skin.[9] Of these, the human gastrointestinal

microbiota (GM) has proven to have clinical significance. Although the human GM is

vast, most of the available literature is focused on its bacterial component. Advancements

in sequencing technology and computational analysis have enabled scientists to

investigate the composition of the GM in greater detail, however, the relationships within

the human GM remain unclear.

Recent research indicates that dysbiosis contributes to the pathogenesis of T2D.[10–13] Given the importance of the GM in human wellbeing, there is mounting attention in

developing interventions to modulate its composition and function. In particular, there are

studies investigating oral therapeutics, such as antihyperglycemics and traditional

Chinese medications, and their effect on the GM.[13] However, while these studies hold

promise in elucidating the effects of the GM in T2D, much remains to be understood

about the mechanisms underlying the distinct interactions between the GM and human

physiology.

Statement of the Problem

Recent research has noted the involvement of an imbalanced GM in the pathogenesis of

T2D. Targeting the GM may be beneficial in the treatment of T2D. However, it is

complicated to translate the insights on the disrupted GM and T2D into remedial

measures. Current studies investigating the use of oral medications to modulate the GM

in humans are limited, owing to the small sample populations and variations in GM

compositions across different groups of people.[2,14] Further work is necessary to elucidate

the interactions of the GM, therapeutic measures and T2D and the safety and efficacy of targeting the GM in humans.

Hypothesis

The GM of T2D patients on treatment with metformin and traditional Chinese medications will result in ameliorated fasting blood glucose (FBG), 2-hour postprandial blood glucose (PBG), hemoglobin A1c (HbA1c) and insulin resistance (IR). There will be an associated shift in the GM toward a profile containing more beneficial bacteria (*Akkermansia muciniphila, Faecalibacterium prausnitzii, Eubacterium rectale, Roseburia inulinivorans, Roseburia intestinalis, Bacteroides acidifaciens, Bacteroides dorei, Bacteroides vulgatus, Lactobacillus intestinalis*) and less harmful bacteria (*Escherichia coli*, *Enterobacteriaceae*, *Enterococcus*, *Shigella* and *Enterobacter*).

Objectives and specific aims

Available literature suggests that oral medications, such as metformin and traditional Chinese medicine (TCM), may modulate the host's GM and, in turn, produce metabolites that ameliorate T2D.[15–18] In this study, we aim to elucidate the mechanisms of both metformin and a TCM herb couple, *scutellaria radix-coptidis rhizome*, in GM alteration that lead to decreased serum glucose and insulin resistance. Through a review of the current literature, this thesis seeks to contribute to our understanding of the GM, propose an experimental design and guide future research efforts aimed at improving human wellbeing. Specifically, this study aims to:

- Determine the primary (glycated hemoglobin) outcome at baseline and at 12 weeks in all patients.

- Determine the secondary (insulin resistance (IR), fasting blood glucose (FBG), 2-hour postprandial blood glucose (PBG), low density lipoprotein levels (LDL), triglyceride level (TG), body weight, body mass index (BMI), waist circumference (WC) and microbial composition of stool samples) outcomes at baseline and at 12 weeks in all patients.

REVIEW OF THE LITERATURE

Overview

As the seventh leading cause of death in the United States, diabetes continues to be a challenging metabolic disease.[5] Pathophysiologic factors, such as obesity, gastrointestinal microbiota (GM) dysregulation, and an impaired immune system, are important considerations in T2D, as they may exacerbate complications.[1,19] Recently, researchers have partially illustrated the role of an altered GM as a potential mechanism for the pathogenesis of T2D.[10–13] Dysbiosis and intestinal inflammation affect glucose regulation. While dysbiosis is referred to as a deviation from the normal microbiome, it is important to note there is no accepted defined standard of the microbiome. For the purposes of this paper, the human gut microbiota of healthy individuals without diabetes will be used for comparison. Of the trillions of microorganisms that have been identified in the GM, 6 phyla dominate: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia, the first and second representing 90% of the GM.[7] The exact compositions are controversial; for example, another study found that Firmicutes and Bacteroidetes represented roughly 82%.[4] Furthermore, studies tend to have discordant results in terms of abundance of bacteria in T2D but overall finds promising associations. For example, *Bacteroides* abundance may vary depending on studies but a review found an overall a negative association with T2D and attributes the results to a reported antibiotic effect of the widely used treatment metformin.[14] Another example is *Lactobacillus*, where discrepancies may be due to its effects being species or strain specific, leading to its controversy on the genus level.[14]

The microbiome's balance and complexity is considered a key factor in optimizing the health state of the host and is implicated in various metabolic diseases.[7,8,20] A study found that transferring synthetic GM from humans with T2D to mice fed with either normal chow diet or high fat diet leads to insulin resistance and glucose intolerance following dysbiosis in both groups.[21] Causal links between the GM and T2D indicate modulating the GM may be therapeutic.[22] Ongoing studies have outlined microbial metabolites, such as short chain fatty acid (SCFA), bile acid (BA) and trimethylamine N-oxide (TMAO), as vital modulators of host tissue and endocrine functioning of the host.[13,15,16]

The most prevalent SCFA-producing bacteria in the GM (*Roseburia spp., Eubacterium rectale, Faecalibacterium prausnitzii, Clostridium* (groups IV and XIVa)) ferment undigested dietary fibers from its host to produce most commonly: acetate, propionate and butyrate.[9] These SCFAs act as ligands to G-protein coupled receptors (GPCR) promoting the secretion of glucagon-like peptide 1 (GLP1), improving satiety and insulin sensitivity, and have been directly linked to decreasing low-grade inflammation.[9,20] Butyrate has also been found to enhance gut membrane integrity and promotes peptide YY (PYY) release, increasing satiety and delays gastric emptying.[14,23]

GM are also responsible for the biotransformation of primary BAs into secondary BAs, mainly by *Bacteroides, Eubacterium* and *Clostridium* (groups XIVa and XI).[9] Secondary BAs are potent agonists of the Takeda G protein coupled receptor 5 (TGR5), which has a role in mitigating intestinal inflammation and GLP1 secretion, leading to

increased beta-pancreatic cell release of insulin. This increases hepatic and pancreatic functioning in regards to maintaining glucose homeostasis.[4,20]

The conversion of choline to the absorbable trimethylamine (TMA) by intestinal microbes leads to the product, TMAO, after conversion in the liver.[9,11,15] Increases in TMAO decrease insulin sensitivity and glucose tolerance, increase inflammation and have been linked to T2D.[9,11,24]

Lipopolysaccharide (LPS) is another microbial derived product (from the phylum Proteobacteria, which includes *Escherichia coli*) that is implicated in T2D. A rise in LPS is linked as a source of potentiated low-grade metabolic inflammation and increased intestinal permeability, leading to glucose intolerance and insulin resistance in T2D.[11,13,24–26] The use of antibiotics corroborates this, as administration leads to a decrease in LPS intestinally and within the serum with a concomitant increase in glucose tolerance.[11] It has been found that the LPS inflammatory response is mediated by toll-like receptor 4 (TLR4) and increases circulating cytokines found in obesity and T2D.[13,27,28] LPS recognition by TLR4 activates the TLR4/MyD88 pathway, producing proinflammatory cytokines, and leads to insulin resistance.[18]

Current treatment options for those who present with a prediabetic condition involve introduction of lifestyle modifications, which can either slow or stop the progression to T2D. These changes in lifestyle may include dietary changes, physical activity and weight loss. In terms of diet, enriching meals with fruits, vegetables, whole grains and fiber may be beneficial.[29,30] Physical activities may include moderate to vigorous aerobic exercise. As for weight loss, a modest reduction and maintenance of at

least 7% can decrease the risk of developing T2D.[30] Moreover, antihyperglycemic agents, such as metformin, the first line agent in T2D treatment in combination with lifestyle modifications, is often the initial treatment standard.[31] Recent research provides novel insight on several therapeutic methods which target the human GM and, therefore, its metabolites in disease control.

Metformin is a biguanide medication that works to inhibit hepatic gluconeogenesis via stimulation of the AMP-activated protein kinase (AMPK) signaling pathway. Side effects of metformin include nausea, diarrhea and bloating.[23] Although the benefits of metformin on glucose regulation are clear, the exact mechanism in which this widely used drug operates has not been fully elucidated. However, evidence suggests the GM plays a key role in the mechanism of metformin in glucose metabolism. [22,32–34]

Various interventions targeting the GM in diabetic management are being studied. Among these interventions, metformin and traditional Chinese medicines (TCM) show promising results in diabetic management via modulation of the GM. TCM has been used in the treatment of diabetes for thousands of years, although the causal relationship between herbal remedies and amelioration of diabetes is not yet defined. A popular herb couple, *scutellaria radix-coptidis rhizome* (SC), has been used in many TCM compound formulations for the treatment of T2D.[18,35–37] Cui et al. found that SC worked synergistically to attenuate inflammation, insulin resistance, hyperglycemia and hyperlipidemia in T2D rats.[36] Huang et al. found similar results, as well as damaged islet cell amelioration and GM dysbiosis improvement.[38]

Overall, evidence suggests the GM and its metabolites may influence glucose

levels and insulin resistance in T2D. Therefore, the role of oral medications in restoring a

healthy GM and its metabolites may contribute to its healing effects in T2D.[15,16,18,22,35–37]

Existing research

As an emerging field in research, literature focused on the GM has grown

remarkably in the last few years. Even more, the research on involvement of the GM in

T2D has provided insights into innovative approaches to treatment. Still, there are

discrepancies in the literature due in part to variances in sample sizes, population models,

analysis methods, novelty of the field and the complexity of roles, depending on the

environment of the microbes. Nonetheless, current research has provided a tremendous

number of clues and recurring themes in the interconnectedness of the GM, oral

medications and T2D.

When comparing the GM in healthy adults to. T2D patients, shifts in the

microbiota composition were observed, although some trends were more consistent than

others (Table 2). In a study that aimed to characterize the GM in T2D, Ge et al. found

that *Roseburia inulinivorans, Bacteroides* and *Eubacterium rectale* had a significantly

higher abundance in the control group of randomized non-T2D participants compared to

a recently diagnosed T2D group and *Roseburia inulinivorans* most consistently showed a

negative association with T2D.[12] A study conducted by Ejtahed et al. that also analyzed

the composition of gut bacteria between patients with and without diabetes similarly

supports higher levels of *Roseburia* in nondiabetic adults, while *Escherichia* load was

significantly lower. In the same study, *Bacteroides* load was found to be decreased in

T2D compared to nondiabetics.[28] The relative abundance of *Lactobacillus* appears most controversial across studies but Ge et al. found no significant differences in the composition of *Lactobacillus* in T2D and control groups. There were also no significant findings on the changes of abundance of *Faecalibacterium prausnitzii.* However, other literature supports a decrease in *Faecalibacterium prausnitzii* in T2D.[26] Pai et al., found *Faecalibacterium prausnitzii* as the highest abundance in healthy individuals.[39] Additionally, Bakir-Gungor et al. found *Faecalibacterium prausnitzii* to be a promising biomarker for T2D as it is negatively associated with the disease[40]. In patients with untreated T2D, Zhang et al., found that both *Faecalibacterium* and *Ruminococcus* levels were low.[41] *Enterococcus* was found to have a higher abundance in T2D compared to control. *Bifidobacterium* abundance also showed no significant differences but this is a discrepant finding considering *Bifidobacterium* is most frequently reported as a beneficial genera glycemic control with decreased in abundance in T2D.[26,28,39] Research from Pai et al. supports a decreased abundance of *Akkermansia muciniphila* in T2D.[39] They also found that *Escherichia coli* had a higher abundance in T2D. In a study in which inoculation of GM from T2D population to mice caused dysbiosis, inflammation and T2D, Liaqat et al. found that *Bacteroides vulgatus* and *Akkermansia muciniphila* was present in mice treated with normal chow diet without GM treatment from humans with T2D.[21]

Table 2. Compositional Changes in T2D Compared to Non-T2D [12,26,28,39–41]

Bacteria:	Overall Compositional Changes of GM:	Reference:
Roseburia inulinivorans	Decreased in T2D compared to non-T2D	(Ge et al., 2022)
Roseburia	Decreased in T2D compared to non-T2D	(Ejtahed et al., 2020)
Eubacterium rectale	Decreased in T2D compared to non-T2D	(Ge et al., 2022)
Lactobacillus	No significant changes in abundance Controversial in various studies	(Ge et al., 2022)
Enterococcus	Increased in T2D compared to non-T2D	(Ge et al., 2022)
Bacteroides	Decreased in T2D compared to non-T2D	(Ge et al., 2022)
Bacteroides	Decreased in T2D compared to nondiabetics	(Ejtahed et al., 2020)
Faecalibacterium prausnitzii	Not significant	(Ge et al., 2022)
Faecalibacterium prausnitzii	Decreased in T2D	(Hung et al., 2021)
Faecalibacterium prausnitzii	Highest abundance in healthy individuals	(Pai et al., 2022)
Faecalibacterium prausnitzii	Decreased in T2D	(Bakir-Gungor et al., 2021)
Faecalibacterium	Low in untreated T2D	(Zhang et al., 2019)
Ruminococcus	Low in untreated T2D	(Zhang et al., 2019)
Bifidobacterium	Not significant	(Ge et al., 2022)

Bifidobacterium	Decreased in T2D	(Hung et at., 2021)
Bifidobacterium	Decreased in T2D	(Pai et al., 2022)
Bifidobacterium	Higher in nondiabetics	(Ejtahed et al., 2020)
Akkermansia muciniphila	Decreased in T2D	(Pai et al., 2022)
Escherichia coli	Increased in T2D	(Pai et al., 2020)
Escherichia	Decreased in nondiabetics	(Ejtahed et al., 2020)
B. vulgatus	Found in normal chow diet mice	(Liaqat et al., 2021)
Akkermansia muciniphila	Found in normal chow diet mice	(Liaqat et al., 2021)

To illustrate the role of bacterial taxa in disease, we will discuss common findings reported on the following genera: *Bifidobacterium, Bacteroides, Akkermansia, Faecalibacterium, Roseburia, Blautia and Escherichia* (Table 3). In a murine study conducted by Kikuchi et al., administration of sterilized *Bifidobacterium* decreased LPS levels and lowered intestinal inflammation. Additionally, glucose tolerance and insulin resistance improved after modulation of anti-inflammatory cytokines.[42] They also produced a SCFA, butyrate.[39,42] Yang et al. found that at the species level, *Bacteroides acidifaciens* was found to ameliorate insulin resistance and glucose tolerance.[43] In a study conducted by Yoshida et al., treatment with live Bacteroides in mice strengthened the gut barrier. Specifically, the dominant species *Bacteroides vulgatus* and *Bacteroides dorei* directly decreased colon LPS leading to a decrease in TLR4 response.[44]

Akkermansia muciniphila has been found to improve glucose tolerance, increase production of SCFA, and reduce serum LPS.[26] The low abundance of *Akkermansia*

muciniphila in T2D found in a study conducted by Pai et al. indicated a negative

relationship with insulin resistance. It is known mucin degrading bacteria, which in turn

helps enhance the thickness of the mucus layer and stabilize the membrane barrier,

protecting against intestinal permeability and inflammation.[39] *Faecalibacterium* produces

SCFA.[28,39] *Roseburia* produces butyrate, an SCFA.[28] *Escherichia coli* of the phylum

Proteobacteria produces LPS, leading to increased membrane permeability and decreases

in insulin sensitivity.[26] *Blautia* spp. have been found to produce the SCFAs butyrate and

acetate with improvements in glucose and lipids.[22] In summary, the mechanisms of the

aforementioned bacteria associated with T2D are fairly consistent throughout the

literature.

Table 3. Bacteria and Associations with T2D Mechanisms[26,28,39,42–44]

Bacteria:	Association in T2D:	Reference:
Bifidobacterium	Improved glucose tolerance Decrease LPS Improve insulin resistance	(Kikuchi et al., 2018)
Bifidobacterium	Butyrate producer	(Pai et al., 2022)
Bacteroides acidifaciens	Improved insulin resistance	(Yang et al., 2017)
B. vulgatus	Decrease gut permeability Decrease LPS	(Yoshida et al., 2018)
B. dorei	Decrease gut permeability Decrease LPS	(Yoshida et al., 2018)
Akkermansia muciniphila	Improve glucose and adipose tissue inflammation	(Hung et al., 2021)
Akkermansia muciniphila	Butyrate producer Enhance mucus layer	(Pai et al., 2022)

	Reduce serum LPS	
Faecalibacterium	Butyrate producer	(Pai et al., 2022)
Faecalibacterium	Butyrate producer	(Ejtahed et al., 2020)
Escherichia coli	Produces LPS	(Hung et al., 2021)
Roseburia	Butyrate producer	(Ejtahed et al., 2020)
Blautia spp	Produces SCFA, improves glucose and lipids	(Tong et al., 2018)

Changes in GM due to oral medications, such as metformin, have been found in various studies (Table 4). A study conducted by Zheng et al. found that metformin increased the abundance of *Akkermansia*.[45] This is supported by Ryan et al., who found metformin also increased *Akkermansia* of phylum Verrucomicrobia in a murine model. Metformin-mediated increases in the number of mucin secreting goblet cells enables proliferation of *Akkermansia,* which utilizes mucin as an energy source. This proliferation enables further strengthening of the barrier and attenuation of inflammation in the gut. Even more, this study found an increased level of *Parabacteroides. Parabacteroides distasonis* has been found to generate BA. Increased levels of *Bacteroides,* and *Clostridiales* and decreased levels of *Muribaculum, Clostridiaceae, Lachnoclostridium, Coprococcus, Dorea, Papillibacter, Oscillospira, Ruminococcus, Desulfovibrio* and *Desulfovibrionaceae* were found in mice treated with metformin. Additionally, a decrease in diversity was seen in metformin treatment likely due to population inflation of *Akkermansia*.[23] Zhang et al. found an increase in *Lactobacillus intestinalis* in metformin treated fatty diabetic rats.[46] Ahmadi et al. found increases in

Ruminococcaceae, Lactococcus and decreases in *Coriobacteriaceae, Lactobacillus,*

Dorea, Roseburia, Veilonellaceae, and *Dehalobacterium* when looking at fed a high fat

diet and treated with metformin compared to control mice only fed a high fat diet. They

found a causal relationship between metformin-modulated GM promoting mucin

secretion via intestinal epithelial goblet cells, which improved inflammation and barrier

function.[47] The same study also found significantly increased butyrate production in mice

treated with metformin. Tong et al., found that SCFA producing *Blautia* spp. were

increased after metformin treatment, however *Akkermansia* decreased, contrary to

previous studies, which the authors attribute to variations between strain functioning.[22]

Zhang et al., found that metformin treated mice increased the number of

Prevotellaceae.[48]

Table 4. Changes of GM with Metformin Treatment of T2D[22,23,45–48]

Reference:	Change in GM:
(Zheng et al. 2018)	Increased abundance *Akkermansia.*
(Ryan et al. 2020)	Increased abundance *Akkermansia, Parabacteroides, Bacteroides, Clostridiales.* Decreased abundance of *Muribaculum, Clostridiaceae, Lachnoclostridium, Coprococcus, Dorea, Papillibacter, Oscillospira, Ruminococcus, Desulfovibrio* and *Desulfovibrionaceae.*
(Zhang et al. 2019)	Increase in *Lactobacillus intestinalis.*

(Ahmadi et al. 2020)	Increases in *Ruminococcaceae, Lactococcus.* Decreases in *Coriobacteriaceae, Lactobacillus, Dorea, Roseburia* and *Veilonellaceae,* and *Dehalobacterium.*
(Tong et al., 2018)	Increased *Blautia* and decreased *Akkermansia* in metformin treatment
(Zhang et al., 2020)	Increased *Prevotellaceae*

SC herb couple is a widely used combination in TCM formulations for the treatment of diabetes. To elucidate the mechanisms of this herb couple, a study conducted by Zhang et al. found SC extracts to ameliorate glucose tolerance, insulin resistance, inflammation, triglyceride levels and modulate the GM in diabetic mice. Mainly, SC inhibited the TLR4/MyD88 pathway, decreasing downstream effects of inflammation and insulin resistance. Active ingredients included baicalin, which decreases insulin resistance, and berberine, which lowers blood glucose.[18] In a study by Tong et al., berberine, a major active component of *Coptis chinensis,* has been found to ameliorate diabetes, likely through upregulating SCFA producing bacteria.[22] Xu et al., found that berberine improved hyperglycemia, glucose clearance capacity and insulin resistance in rats.[17] In another study by Zhang et al., SC was found to protect the intestinal mucosal barrier in mice through upregulating tight junction proteins and reducing LPS, leading to

inhibition of inflammatory pathways. LPS is known to induce islet cell apoptosis and reduce the efficacy of insulin.[48]

In terms of GM changes, *Lactobacillus intestinalis* was found to significantly increase in SC treated mice. More *Bacteroides vulgatus* was present in the GM of mice treated with high dose SC compared to non-SC treated model group.[18] It was also found that SC favorably changed abundances of potential enteropathogenic bacteria, particularly by decreasing levels of Proteobacteria, *Enterobacteriaceae*, *Enterococcus*, *Escherichia-Shigella* and *Enterobacter*, while increasing the abundance of *Lachnospiraceae* and *Prevotellaceae*, which contain butyrate producers. However, Zhang et al., noted that SC treatment decreased the overall amount and diversity of the GM, including some beneficial *Lactobacillus,* and may warrant pre/probiotic use alongside SC treatment.[48] Effects of SC treatment and its changes to the GM and T2D are outlined in Table 5.

Table 5. Effects on GM and T2D Associated with SC Treatment[17,22,46,48]

Reference:	Change to GM and T2D:
(Zhang et al., 2019)	Improve glucose tolerance, insulin resistance, inflammation, triglyceride levels. SC increased *Lactobacillus intestinalis* and *Bacteroides vulgatus*
(Tong et al., 2018)	Berberine increases SCFA producing bacteria
(Zhang et al., 2020)	SC protects intestinal membrane barrier and increases insulin efficacy. SC decreases Proteobacteria, *Enterobacteriaceae*, *Enterococcus*, *Escherichia-Shigella* and *Enterobacter* while increasing *Lachnospiraceae* and *Prevotellaceae*

(Xu et al., 2020)	Berberine improved hyperglycemia and insulin resistance

In recent years, the GM, consisting of trillions of microorganisms, has gained significant attention due to is role in various health conditions, including T2D.[7] Metformin, a commonly prescribed antidiabetic medication and a traditional Chinese herb couple, SC, have been studied for their properties on the GM and their possible implications in managing T2D. They have been associated with alterations in the composition and diversity of the GM, with increased beneficial bacteria and decreased pathogenic bacteria. It is speculated that the shift seen in the GM produce metabolites that likely contribute to decreased insulin resistance and hyperglycemia, alleviating effects.[18,22,33,45,48] While there is promising research on the potential effects of metformin and SC on the GM in T2D, further studies are warranted to fully uncover their mechanisms and establish clinical safety and efficacy.

METHODS

Study design

The proposed project is a multicenter, randomized, double-blind controlled clinical trial. One of the two following treatments—metformin or scutellaria-coptis herb couple extract—will be examined in eligible patients diagnosed with type 2 diabetes based on the American Diabetes Association (ADA) criteria over the duration of 12 weeks. Metformin, a traditional well-established treatment of T2D will be used as the control. Participants will be evaluated at weeks 0, 4, 8 and 12. Stool samples will be collected at weeks 0 and 12. Additionally, there will be an extension trial at 6 weeks post study to obtain measurements (blood draw and stool sample) after the study drug has been removed and participants are on their original regimen to determine whether effects have been sustained.

Study population and sampling

Participants will be recruited over a 12-week period from outpatient clinics throughout the Boston area including the 5 major academic institutions: Boston Medical Center, Brigham and Women's Hospital, Tufts Medical Center, Massachusetts General Hospital, and Beth Israel Deaconess Medical Center. Participants receiving treatment must have a new diagnosis of T2D based on the ADA criteria with no prior treatment. Inclusion and exclusion criteria can be found in Table 5 below.

Table 6. Inclusion and Exclusion Criteria for Participants

Inclusion	Exclusion
1. All patients must be 18 years old or older to provide informed consent. 2. All patients with a new diagnosis (within 4 weeks) of T2D based on current ADA criteria who have not been treated with an antidiabetic medication in the past three months.	1. Patients having constipation, blood in stool, diarrhea, or any other gastrointestinal disease. 2. Patients who have taken antibiotics (oral or injectable), corticosteroids, antidiabetic medications or probiotic supplementation in the past three months. 3. Any patient who is pregnant or currently lactating. 4. Any patient who has had major abdominal surgery. 5. Patients who had any recent GI infection in the past three months. 6. Any patient who has contraindications to Metformin or SC herb couple treatment. 7. Patients on any other antidiabetic medications. 8. Inability to understand the consent or illiterate persons. 9. Patients with eGFR <45 mL/min/1.73 m^2. 10. Significant laboratory abnormalities on CBC and metabolic panel.

The estimated sample size based on the primary outcome, HbA1c. Using a sample size calculator (sample-size.net) for Paired T-test, at least 68 participants will be required, 34 people in each group, based on alpha level 5%, beta at 20% and effect size of 0.500 with an assumption of a decrease in HbA1c in both groups receiving treatment. Calculation was made using the T statistic and non-centrality parameter. Each of the

study arms will be increased by 10% (rounded up to the nearest integer) to adjust for potential attrition.

Treatment

The study population will be randomized into two groups to receive either SC herb couple or metformin. All subjects that will undergo treatment will be allocated to each group randomly in a proportion of 1:1 using a blinded pharmacist who will not interact with the participant. The blinded pharmacist will use R software to assign randomized treatment groups and the results of the group assignments will remain sealed until the end of the study. Both treatments will be made into capsules that look the same to reduce potential unblinding. This will be outsourced to a third-party company.

The TCM formula in our study will consist of the SC herb couple obtained from Sichuan Neo-Green Pharmaceutical Technology Development Co., Ltd. (Chengdu, China) and quality controlled. The dosing will be a total of 9 g of *scutellaria radix* and 9 g of *coptidis rhizome* in a ratio of 1:1 for the strongest synergistic effect. The herbal combination will be extracted for its main active ingredients for administration. For patients initiating metformin, a total of 1500 mg of immediate-release metformin will be prescribed. The total dose of medication will be divided equally by three and will be taken with meals three times daily by mouth.

Preparation of SC Extract

Extraction methods have been adapted from Shin et al. and Zhang et al.[18,33] Baikal skullcap root and Chinese goldthread in dried form will be used. The ratio of baikal

skullcap root and Chinese goldthread will be 1:1. These herbs will be placed in distilled water for 30 minutes and boiled for 60 mins at 100 degrees C in a 1:8 mixture of herb to water. After extraction, the fluid will be centrifuged and the supernatant will be condensed to 1g/mL and stored at -20 degrees C.

High-performance liquid chromatography with ultraviolet detector analysis will be performed to assess the quality of SC. This will be used to obtain information on the main contents of SC.

Study variables and measures

Primary outcome will include glycated hemoglobin. Secondary outcomes will include insulin resistance (IR), fasting blood glucose (FBG), 2-hour postprandial blood glucose (PBG), low density lipoprotein levels (LDL), triglyceride level (TG), body weight, body mass index (BMI), waist circumference (WC) and microbial composition of stool samples. Upon initiation of their study period, participants will have a baseline evaluation. At the end of the 12-week period, the study participants will have a post treatment evaluation. During the extension period 6 weeks post trial, participants will have a final evaluation to determine if effects have been sustained.

Recruitment

The clinical trial design will be subjected to Institutional Review Board (IRB) approval at all participating institutions. Subjects will be provided with an informed consent document (ICD) with guidelines, policies, and safeguards alongside a verbal explanation of the project design. There will be ample opportunity in which subjects and clinical investigators may exchange information concerning the study. Subjects will be required

to sign the ICD to enroll. Participants of at least 18 years of age will be recruited by a clinical research coordinator in outpatient endocrine and primary care provider settings of the major academic teaching hospitals in the Boston area over a 12-week period. They will be approached during their office visit and offered to participate. Only subjects with an official diagnosis of T2D based on ADA criteria made by a licensed health care provider who meet the inclusion and exclusion criteria will be enrolled to receive treatment with either SC or metformin.

At the time of recruitment, a healthcare provider will perform an initial evaluation to establish the subject's baseline. This will entail demographic information, a complete history and physical examination along with laboratory tests, including a complete blood count (CBC), a comprehensive metabolic panel (CMP), and urine biochemistry.

Data collection

The initial evaluation will include a complete history and physical exam with lab work (CBC, CMP, urine biochemistry, HbA1c, fasting plasma glucose test) to assess the participant's baseline. Tests will be performed after 12-14 hours of overnight fasting by Quest Diagnostics. Participants will also follow up at weeks 4, 8 and 12 of treatment to receive lab work. Participants are to record all adverse events and report to the provider of such events during follow up visits. At weeks 0 and 12 of treatment, fresh stool samples will be collected by the participant at home and immediately stored in the home freezer at 0 degrees F until brought to the laboratory no later than 12 hours. The stool samples will be stored at -80 degrees C in a sterile collection tube with a screw cap for up to 3 days prior to processing. QIAamp PowerFecal Pro DNA Kit manufactured by

Qiagen will be used to obtain bacterial DNA from each participant's stool samples and processed in accordance with the manufacturer's guidelines and stored at -20 degrees C until use.

Data analysis

Blood tests will be performed by Quest Diagnostics. For stool sample analysis, shotgun metagenomic sequencing (SMS) will be used to reveal the profile of the gut microbiome. Although historically amplicon sequencing has been more popular due to cost constraints, SMS provides greater resolution and functional information and has been recently become more financially feasible.[49] Additionally, as an untargeted method, there is less risk of bias. Novogene platforms will be used to sequence the genes according to the manufacturer's protocol.

The primary outcome will be expressed as a percent change between baseline and final follow up at 12 weeks. Intermediate follow up at weeks 4 and 8 will be used as exploratory data and will not be included as primary endpoints. Similarly, data for secondary endpoints will be calculated and compared to baseline. Statistical analysis will be performed by the team biostatistician. A P value of 0.05 was considered as the level of significance. A reduction by 0.5% in HbA1c will be considered a positive response in each intervention. The comparison of primary data from baseline values will be conveyed as a percent change and lab results can be analyzed using a paired t-test. This method will be employed to analyze secondary endpoints of IR, FBG, PBG, LDL, TG, body weight, BMI and WC as well. For comparison of stool sample composition pre and post treatment, the study will look at Beta diversity using Principal Coordinate Analysis and

permutational multivariate analysis of variance to determine a statistically significant difference in beta diversity. Adverse events will be recorded throughout the clinical trial as reported by patients in both SC and metformin cohorts.

Timeline and resources

Table 7. Proposed Study Timeline

Fall 2023	IRB Submission and Approval
Winter 2023-Spring 2024	Patient Recruitment (3 months)
	Treatment Intervention (3 months)
Summer 2024	Data Analysis

The proposed study will require a principal investigator, a study coordinator, a volunteer to recruit participants, a manufacturer for the herb couple, a pharmacy for treatment, a blinded pharmacist, and a biostatistician who will be primarily responsible for the data analysis.

Institutional Review Board

The protocol of the proposed study will be submitted to the IRB at Boston Medical Center and Boston University Medical Campus and to the IRBs of the corresponding participating institutions for full review as participants are receiving medical treatment and genetic material will be obtained. Research team members participating in this trial will require current training credentials for human clinical trials.

CONCLUSION

Discussion

It has been demonstrated that both TCMs and metformin can modulate GM in alleviating T2D.[18,22] Still, this field of research requires additional investigations and has great developmental potential. This proposal seeks to make a meaningful contribution to the understanding of T2D, the GM and TCMs, further reveal the complexities of their interrelatedness and inform potential interventions.

Despite the valuable insights obtained from the current body of knowledge, there are limitations that should be addressed when interpreting this review and proposal. Limitations stem from the paucity of information of the GM and lack of a defined baseline GM composition for different groups of people, which may affect results and interpretation, as there may be difficulty comparing data. Also, many of previous trials either lack human subjects or have too few human subjects. This study aims to fill these gaps and provide formative information for future studies by using human subjects and shotgun metagenomic sequencing. Furthermore, one must consider that the administration of antidiabetic drugs may modulate GM from an untreated diabetic baseline, since it has been found that antidiabetics modify the GM.[46] To diminish the impact of this effect, only newly diagnosed T2D patients (within 4 weeks) who have not initiated any antidiabetics will be included in the trial. Additionally, differences between individual diets may greatly affect results; the GM is influenced by dietary intake. Genetic sequencing technology itself is limited, as it cannot differentiate between living and nonliving material.[9] Additionally, when investigating GM changes, sequencing

strategies are difficult to reproduce between studies giving varying results even in the same disease.[50]

Moreover, considering the generalizability of findings is essential in this proposed study. TCMs vary widely between available remedies and may affect the generalizability. In this proposed study, a specific herb couple has been chosen and the main bioactive compounds will be extracted for administration. It is important to note that, since only SC and metformin are being investigated, this study may not be as applicable to patients receiving an alternative treatment for T2D. However, looking at these study drugs in a way that has rarely been studied before using a standardized procedure will lay groundwork for future research. Moreover, the GM varies significantly between individuals and is influenced by several factors. Utilizing a diverse sample population within the Boston hospital community will add to the generalizability of findings as a diverse sample will represent a larger population. Using standardized protocols for data collection and analyzation are also important steps to take for comparison to other studies. By taking these precautions, the results will be applicable to greater contexts.

Anticipated obstacles in this proposal include the paucity of scientific literature on both TCMs and the GM. As mentioned, there is no accepted defined standard of the GM, making it more difficult to compare results. Furthermore, as individuals are unique in her habits, we must consider variables such as dietary and physical activity habits, medication adherence or even differing dosage of medications especially if adjustments are needed.

Strengths of this study include using a human model instead of murine model since there is a scarcity of trials involving humans. Furthermore, the extended trial period will allow researchers to investigate whether the effects of the treatments on the GM are sustained after discontinuation of the study drug and resuming the original regimen. Additionally, this proposal includes metagenomic sequencing which provides more information on strain-specific functioning. This is beneficial as contradictory results in previous studies may be attributed to differences in strains, which their sequencing was unable to capture.[22]

Summary

T2D is a highly prevalent, multifactorial disease affecting several organ systems. Out of the many contributing factors in the pathogenesis of T2D, GM research is relatively new and expanding. Microbial metabolites influence serum glucose, insulin resistance, satiety and ultimately the progression of T2D. TCMs have been used for centuries to treat T2D in Asia. Although there is a baseline understanding of current interventions and how they modulate the GM, studies are limited with many things to be uncovered. Current clinical trials are limited in human based data, reproducibility in GM analysis, and lack overall understanding of TCMs in western practice. The proposed study will allow for a deeper understanding of the efficacy of TCMs and reveal data about the GM and how it is modulated during the treatment of T2D.

Clinical and/or public health significance

Worldwide, T2D continues to be a problematic disease that is an economic burden, reduces the quality of life and negatively affects several systems in the human body.[1,6,51]

29

Additionally, T2D is diagnosed more frequently, even in younger ages.[5,51] Moreover, the GM is a complex ecosystem of microbes that is implicated in the pathophysiology of T2D and is of recent interest in medical research.[1,2,11,40] On top of that, medications and how they alter the GM are relatively novel ideas in the scientific world.[18,22] Exploring a combination of these will provide better insights and different angles in understanding T2D and give way for innovative therapeutic strategies for combating this disease.

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