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HYPOTHESIS

Long-Term Substitute of Intestinal Micro-flora with Health Bacteria May Play a Role in Preventing Certain Diabetic Complications

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Abstract. Diabetes nowadays is the most common cause of progressive kidney failure, leading to dialysis or transplantation. Intestinal microflora play an important role in health and disease, but this effect remains incompletely characterized. Cytokines and acute phase reactants such as C-reactive proteins, interleukins, tumor necrosis factor α and lipopolysaccharides, may have a major role in diabetes, because inflammatory cytokines induce peripheral insulin resistance by impairing the insulin receptor-dependent signaling. A key action of gut microflora is the induction of inflammatory factors and cytokines production. The gut may also be capable of producing cytokines in response to an inflammatory stimulus, even in the absence of portal or systemic spread of bacteria. We hypothesize that gut microflora may increase the risk of diabetic nephropathy through production of inflammatory factors and cytokines. In addition, long-term regulation of gut microflora by prebiotics and/or probiotics may have preventive effects on certain diabetic complications such as nephropathy.

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Abbreviations used: ILs, interleukins; TNF, tumor necrosis factor; LPS, lipopolysaccharides; IR, insulin receptor; CKD, chronic kidney disease; AGE product, advanced glycation end products.

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1. Introduction

Diabetes currently affects more than 170 million people world-wide and projections for the future are alarming [1]. According to the World Health Organization, it is expected that the number of patients with diabetes will double within the next 20 years due to an epidemic rise in the prevalence of type 2 [2]. Without specific intervention, 20 to 40% of all diabetic patients will develop diabetic nephropathy characterized clinically by hypertension, a progressive increase in albuminuria, a high cardiovascular risk, and a relentless decline in GFR leading towards ESRD. Despite improved prognosis over the last decades, diabetic nephropathy remains a major health problem, and many patients still progress to ESRD. Today nephropathy due to type 2 diabetes has become the single most common cause of ESRD in the Western world accounting for 45% of all patients on dialysis in the US and 22% in Denmark [3]. It is therefore of uttermost importance to identify early modifiable risk factors for progression of diabetic nephropathy for prompt treatment of high risk individuals and identifying new targets for intervention. Furthermore, new and more effective strategies for prevention and treatment of diabetic nephropathy are urgently needed [4, 5].

The two main risk factors for DN are hyperglycemia and arterial hypertension. However, DN develops in only about 40% of patients, even in the presence of hyperglycemia and elevated BP for long periods of time [4, 5].

During the nearly 80 years since its discovery, insulin therapy led to only partial success in the treatment of hyperglycemia and even less in preventing the chronic complications that often decrease life expectancy and adversely affect quality of life. This observation raised the concept that DN will develop only in a susceptible subset of patients. Furthermore, family studies have confirmed a genetic contribution for the development of DN in both type 1 and type 2 DM. Other conventional risk factors for DN are smoking, dyslipidemia, proteinuria, dietary factors [6, 7] Different inflammatory molecules, including adipokines, Toll-like receptors, chemokines, adhesion molecules and pro-inflammatory cytokines, may be critical factors in the development of microvascular diabetic complications, including nephropathy [8]. Findings from various studies support an association between increased secretion of inflammatory molecules, such as cytokines, growth factors, Tumor necrosis factor α (TNF-α), interleukin (IL)-1 and IL-6 and development of diabetic nephropathy [9, 10]. TNF-α was the first inflammatory cytokine postulated as a critical mediator of insulin resistance. Another cytokine, IL-6, has the strongest correlation with insulin resistance and type 2 diabetes [11]. Oxidative stress also seems to play a central role. Studies that have used inhibitors of the pathways involved in genesis of diabetic nephropathy have shed light on the pathogenesis of this condition but have not led to expansion of the therapeutic armamentarium to halt the disease process [9]. The gastrointestinal tract functions as a barrier against antigens from microorganisms and food. The generation of immunophysiologic regulation in the gut depends on the establishment of indigenous microflora [12]. Gut flora might also be an essential factor in certain pathological disorders, including multisystem organ failure, colon cancer, and inflammatory bowel diseases. Nevertheless, bacteria are also useful in promotion of human health. Probiotics and prebiotics are known to have a role in prevention or treatment of some diseases [13].

2. Recent Evidence

Recent studies have highlighted the importance of relation between gut microflora and inflammatory status in diabetes [14, 15]. One third of diabetic dialysis patients with a presumptive diagnosis of diabetic nephropathy, glycemic control improves spontaneously the progression of chronic kidney disease (CKD). Diminished food intake and diabetic gastroparesis or other changing gastrointestinal factors can improve glycemic

states in patients with diabetic nephropathy treated with hemodyalysis [16]. This new hypothesis can improve the prognosis of disease in the future. Gut microflora composition is involved in the regulation of energy homeostasis. Creely *et al.* [17] showed that lipopolysaccharide may act as a gut microbiota-related factor involved in the development of type 2 diabetes and obesity in humans. In another study, small intestinal bacterial overgrowth, increased intestinal permeability, elevated endotoxin, and tumor necrosis factor α (TNF-α) levels contribute to the development of non-alcoholic steatohepatitis [18]. Endotoxin lipopolysaccharide (LPS) or possibly TNF derived from the overgrowth of intestinal gram-negative bacteria is responsible for TPN-associated hepatic steatosis [20]. Bacterial lipopolysaccharide derived from the intestinal microbiota may act as a triggering factor linking inflammation to high fat diet–induced metabolic syndrome [21]. Bifidobacteria was found to reduce the intestinal endotoxin levels and improve mucosal barrier function [22- 24].

3. Hypothesis

Based on the effects of health intestinal bacteria on inflammatory markers and systemic inflammation and due to the major role of systemic inflammation and inflammatory cytokines in the pathogenesis of diabetic complications, we hypothesize that, long-term substitution of harmful bacteria in the gastrointestinal tract may play an important role in the progression of diabetic complications such as diabetic nephropathy, and changes the intestinal microflora to health bacteria with perbiotics and/or probiotics may have a protective role in the progression of diabetic complications such as diabetic nephropathy.

4. Evaluation and Discussion of the Hypothesis

Diabetes and its complications have become a public health problem. One of the most important complications is diabetic nephropathy, which is nowadays the main cause of chronic renal failure. In spite of our greater understanding of this complication, the exact mechanisms leading to the development and progression of renal injury are not well understood. New perspectives in activated innate immunity and inflammation appear to be relevant factors in the pathogenesis of diabetes. Moreover, different inflammatory molecules, including adipokines, Toll-like receptors, chemokines, adhesion molecules and pro-inflammatory cytokines, may be critical factors in the development of microvascular diabetic complications, including nephropathy [8].

The low degree of inflammation in type 2 diabetes mellitus results from increased expression and production of cytokines and acute phase reactants such as C-reactive proteins, interleukins (ILs), TNFα, or lipopolysaccharides (LPS). Inflammatory cytokines induce peripheral insulin resistance by impairing the IR-dependent signaling. Administration of anti-inflammatory agents (*e.g.*, high doses of aspirin) or experimental deletion of receptors for inflammatory cytokines results in increased insulin sensitivity and improved pancreatic beta-cell function, which is paralleled by a reduction of hyperglycemia and loss of body weight. Despite numerous studies describing the activation of pro-inflammatory cytokines in both major metabolic disorders little is known about the underlying mechanisms. In addition studies in recent years have shown that inflammation and more specifically inflammatory cytokines, are determinant in the development of microvascular diabetic complications, including neuropathy, retinopathy and nephropathy. Presently, it is known that among inflammatory cytokines, IL1, IL6, IL18 and TNF-α are relevant to diabetic nephropathy, retinopathy, atherosclerosis, and other complications [25-27]. IL 18 and TNF-a are increased at a very early stage in the serum of patients with diabetic nephropathy and correlate with the degree of albuminuria [25]. Emerging evidence also suggested that atherosclerosis itself is also mediated in large part by inflammatory activation by in-

crease expression of vascular cell adhesion molecule-1 (VCAM-1 and ICAM-1) [26]. Recently, digested food-derived advanced glycation end products (AGE) are also found to play an important role in the pathogenesis AGE-related disorders such as diabetic complications [28].

On the other hand, the human gut is the natural habitat for a large and dynamic bacterial community; Major functions of the gut microflora include metabolic activities that result in salvage of energy and absorbable nutrients, important trophic effects on intestinal epithelia and on immune structure and function, and protection of the colonized host against invasion by alien microbes. Gut flora might also be an essential factor in certain pathological disorders, including multisystem organ failure, colon cancer, and inflammatory bowel diseases. Nevertheless, bacteria are also useful in promotion of human health [13]. Clinical observations suggest that certain intestinal and extraintestinal bacterial infections sometimes precede or reactivate chronic intestinal inflammation [29]. Specific strains of the healthy gut microbiota have been shown to induce the production of IL-10 and transforming growth factor-β, which possess an important regulative role in the development of allergic type immune response [12, 30]. The gut may also be capable of producing cytokines in response to an inflammatory stimulus, even in the absence of portal or systemic spread of bacteria. The magnitude of the cytokine response does not correlate with the magnitude of bacterial translocation [31]. It appears, in addition, that the translocation of bacteria and endotoxin may lead to local activation of the immune inflammatory system and the local production of cytokines and other immune inflammatory mediators. These intestinally derived mediators may then exacerbate the systemic inflammatory response and potentially lead to a further increase in gut permeability. A vicious cycle of increased intestinal permeability, leading to toxic mediator release, resulting in a further increase in gut permeability is generated [32]. Prebiotics and probiotics are known to have a role in prevention or treatment of some disease. Among the possible mechanisms of probiotic therapy is promotion of a nonimmunologic gut defense barrier, which includes the normalization of increased intestinal permeability and altered gut microecology. Probiotics also strengthen gut defense barrier mechanisms, reduce antigen load in the gut and improvement of the intestine's immunologic barrier, particularly through intestinal immunoglobulin A responses and alleviation of intestinal inflammatory responses, which produce a gut-stabilizing effect. Moreover, distinct regulatory effects have been detected in healthy subjects and in patients with inflammatory diseases [12, 30]. Many probiotic effects are mediated through immune regulation, particularly through balance control of pro-inflammatory and anti-inflammatory cytokines. These data show that probiotics can be used as innovative tools to alleviate intestinal inflammation, normalize gut mucosal dysfunction, and down-regulate hypersensitivity reactions. More recent data show that differences exist in the immunomodulatory effects of candidate probiotic bacteria. Moreover, distinct regulatory effects have been detected in healthy subjects and in patients with inflammatory diseases. These results suggest that specific immunomodulatory properties of probiotic bacteria should be characterized when developing clinical applications for extended target populations [12].

This new pathogenic perspective leads to important therapeutic considerations, with new pathogenic pathways becoming important therapeutic targets that can be translated into clinical treatments for diabetic nephropathy and others diabetic complications.

In conclusion, long-term change in intestinal microflora during years of diabetes may produce dysregulation and also elevation of inflammatory cytokines. This continuing imbalance of pro inflammatory and anti-inflammatory cytokines may have a critical role in producing and progression of diabetic complications such as diabetic nephropathy. Based on this evidences we hypothesize that substitution of health bacteria in gastrointestinal tract by using prebiotics

and/or probiotics may have a role in prevention of diabetic complications such as diabetic nephropathy.

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