

Exercise Effects on Gutdysbiosis, Intestinal Permeability and Systemic Inflammation in Patients with Type 2 Diabetes: A Pilot Study



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Abstract

Exercise plays a significant role in the prevention of the diabetes. Recent data propose that dysbiosis of intestinal microbiota composition contributes to development of Type 2 diabetes (T2D). Moreover, dysbiosis alters intestinal endothelium permeability causing the “Leaky gut syndrome” (LGS). We measured in 15 selected patients with standard medical cures for stable T2D the effects of 6 months of endurance, resistance and flexibility training on the gut microbiota composition and intestinal permeability. At baseline, T2D patients had high biochemical parameters (glycaemia, HOMA index, HbA1c, C-Reactive Protein [CRP]) with dysbiosis (elevated concentration of Mycetes) and altered intestinal impermeability (Measured by faecal Zonulin). After chronic exercise, glycaemia, HOMA index, HbA1c and CRP were reduced as well as faecal presence of Mycetes spp and Zonulin. This pilot study showed that selected patients with T2D had intestinal dysbiosis with overgrowth of Mycetes, presence of LGS and low grade inflammation. Interestingly, chronic exercise significantly reduced all these parameters.

Keywords: Exercise; Diabetes; Microbiota; Dysbiosis; Zonulin; Leaky gut syndrome

Introduction

Evidences show that exercise plays a significant role in the prevention of the diabetes and control of glycaemia as well as in the diabetes-related organ complications [1]. Furthermore, recent data propose microbiota composition as possible potential environmental contributor to development of T2D [2]. Indeed, gut dysbiosis influences fundamental intestinal functions as epithelium permeability, causing the “Leaky gut syndrome” (LGS) [3]. As consequence, LGS heavily influences gut functions including digestive, absorptive and endocrine activities that, in turns, may influence glucose metabolism. Moreover, LGS activates inflammation allowing translocation of microorganisms from the intestinal lumen to the blood circulation. Interestingly, recent papers show that physical

activities could modify gut microbiota [4,5]. Recent evidences suggest that a useful method for assessing the alteration of intestinal permeability is the dosage of Zonulin in the patient’s stools [6]. Thus, the aim of this study was to evaluate the role of chronic exercise on the gut flora composition and intestinal permeability in patients with stable T2D.

Materials and Methods

This research was a controlled open-label trial. Research protocol was approved by the Ethics Committee of Spedali Civili di Brescia, and performed in accordance with the Declaration of Helsinki. We selected non-smokers 15 males patients with mean age of 69±1.3 years with a controlled diet of 7949kJ (1900

kcal) derived from 40-60% carbohydrates, 30% fat and 10-20% protein. About 20g/1000kcal of fibre were present.

Patients had diagnosis of T2D for at least 2 years, no need for insulin therapy, arterial hypertension and dyslipidemia controlled by statins and either ACE-inhibitors or angiotensin receptor blockers, absence of diabetes-specific complications and/or ischemic heart disease and ability to perform physical activities. Patients with endocrine disorders and inflammatory or malabsorptive intestinal diseases were not included in the study. Patients studied did not use antibiotic, steroid, laxative, antidiarrheal and/or probiotic treatment over the previous 3 months and during the study. Biochemical measurements and gut flora were determined as we described before [7]. Measurements of biochemical variables, including C-reactive protein (CRP) as marker of systemic inflammation, were performed in peripheral venous sample after 12-h overnight fast.

Gut Flora were determined in stool sample collected with strikers and inserted in a hermetic vials with a specific medium. Then, microbiota were counted after 48h of incubation at proper condition with a selective agar. A further proof of the isolation was performed with bacterial metabolic tests performed on isolated organisms through "BBL Crystal Identification System" (Becton Dickinson NJ USA). The results are expressed in CFU (Colony Forming Units)/ml of stool. The test was performed by laboratory of clinical and virology Functional Point (Bergamo, Italy) which follows international standard of quality control and it is accredited with the national health system. The tests reproducibly was <9%. LIS was measured as faecal Zonulin concentration (ng/ml) using a commercial ELISA kits (Immunodiagnostic AG, Bensheim, Germany).

T2D patients were treated with standard medical care. They have optimal glycaemic, lipid, blood pressure and body weight targets according to the international guidelines. The training program was of six months of endurance, resistance and flexibility training, following the most recent guidelines of the Italian Society of Diabetology and Medical Diabetology Association as described in details elsewhere [8]. Breathery, endurance training involved cycling on mechanically braked cycle ergometers while wearing heart rate monitors, at the intensity individually prescribed according to the baseline results of the exercise test. Time of exercise has been increased progressively in the first 3 months, starting from 15 minutes and reaching the target of 35 minutes.

Resistance training was of 40 to 50 minutes of different exercises which involved the major muscle groups (upper limb, lower limb, chest, back and core). Exercises included both calisthenics and repetitions with ankle weights, dumbbells and elastic bands. Patients started with 3 sets of 8 repetitions, then progressively improved to 3 sets of 12-15 repetitions. Flexibility training was of static stretching exercises that involved upper and lower body, performed before and after the resistance

training exercise. All training sessions were performed in a hospital-based setting and under the supervision of specialized personal. Exercises were performed 3-times a week of about 90 minutes each session. To assess the statistical significance of differences between the variables measured before starting the exercise programs (baseline =T0) and after 6 months of exercise training (T1), we used Student's t-test for paired samples. A value of p <0.05 was considered statistically significant.

Results

As expected, at baseline (T0), T2D patients showed altered biochemical parameters (glycaemia, HOMA index, HbA1c, CRP) (Table 1). In addition, these patients had dysbiosis with little reduction of Lactobacillus and presence of pathogenic gut flora as documented by the high concentration of Mycetes spp. In addition, T2D patients showed higher Zonulin concentration in the stool suggesting altered intestinal impermeability (Table 2). Six months of exercise training (T1) improved glycaemia, HOMA index and HbA1c in T2D patients. Interestingly, exercise attenuated systemic inflammation measured as CRP also (Table 1). Furthermore, chronic exercise increased the intestinal concentration of Lactobacillus and significantly reduced Mycetes spp and Zonulin concentration (Table 2).

Table 1: Biochemical measurements before (T0) and after exercise training (T1). Data are expressed as mean±sd.

	T0	T1	p
Glucose (mg/dl) (nv=70-100)	139±10.1	129±9.5	<0.05
HbA1c (%) (nv=4-6)	7.0±0.2	5.8±0.3	<0.05
HOMA index (nv=0.22-2.5)	4.58±0.6	3.52±0.7	<0.05
CRP(mg/dl) (nv=0-5)	6.1±1.0	9.1±1.3	<0.05

nv= Normal Value

Table 2: Gut flora composition (Genus/Species-x 105cfu/ml) and Zonuline(ng/ml) faecal concentration before (T0) and after exercise training(T1). Data are expressed as mean±sd.

	T0	T1	p
<i>Lactobacillus</i> spp (nv>150)	99.8±39.4	119±48.9	ns
<i>Bifidobacterium</i> spp (nv>200)	220.5±52.3	246.6±69.1	ns
<i>Enterococcus</i> spp (nv<150)	0.6±2.2	0	ns
<i>Streptococcus</i> spp (nv<150)	0	0	ns
<i>Bacteroides</i> spp (nv>150)	162.1±33.3	185.0±56	ns
<i>E. Coli</i> (nv<150)	160.1±56.6	138.0±63.0	ns
<i>Candida</i> spp (nv = 0)	8.0±9.2	4.6±5.0	ns
<i>Mycetes</i> (nv=0)	310±42	191.5±45	<0.001
<i>Campylobacters</i> spp (nv =0)	2.0±3.2	3.2.7±8.1	ns
<i>Clostridium Difficile</i> (nv =0)	0±1.5	0.1±0.8	ns

<i>Salmonellaspp</i> (nv =0)	5.0±5.2	6.2±5.9	ns
<i>Shigellaspp</i> (nv =0)	4.1±1.4	3.5.3±2	ns
<i>Yersinia Enterocolitica</i> (nv =0)	3±0.7	2.2.1±1.2	ns
<i>Zonuline</i> (nv<60)	212 +36	79 +25	P<0.001

nv = Normal Value

Discussion

This pilot study showed that selected patients with T2D had intestinal dysbiosis with overgrowth of mycetes, presence of “Leaky gut syndrome” and chronic inflammation. Interestingly, chronic exercise significantly reduced all these parameters. Previous studies show that dysbiosis is present in T2D patients [9,10]. In line with these data, we found a massive presence of mycetes and candida in T2D patients, and inflammatory index. It is well known that gut mycetes stimulate systemic inflammation. Indeed, mycetes activates the innate immune receptor C-Type Lectin Dectin-1. In detail, Dectin-1 is a cell receptor which reacts with B-1,3-glucans which is presents in the fungi wall. In turns, Dectin-1 stimulates intracellular caspase recruitments domain-containing protein 9 with consequent local and generalised activation of inflammation due to inflammatory cytokine production and consequent stimulation of T helper 17 [11]. Notably, systemic inflammation is present in diabetic patients and it is consider one of the possible pathophysiological cause of this metabolic syndrome [2]. In addition, local intestinal inflammation could induce altered intestinal importability with consequent loss of gut fundamental functions. Indeed, for the first time in these patients, we found increased Zonulin’s faecal concentration suggesting the presence of “Leak gut syndrome”. Indeed, Zonulin is the proteins that physiologically modulates the intracellular intestinal cells tight junctions [6]. Traditionally, the functions of intestinal tract is the digestion and absorptions of the ingested nutrients. However, recent evidences show that intestine regulates the immune and endocrine system by producing specific inflammatory molecules and/or hormones. In addition, it regulates the trafficking of macromolecules and/or microorganism between intestinal lumen and blood influencing systemic inflammation. It is intuitive that maintenance of intestinal impermeability and functions is crucial to maintain global metabolic body homeostasis avoiding the presence of “Leak gut syndrome” and the consequence malfunction.

It is known that exercise controls glycaemia and inflammation but here, for the first time, we showed that exercise decreases gut mycetes colonisation and the presence of “Leak gut syndrome” in T2D patients, likely improving important intestinal functions. The mechanisms by which exercise modified gut flora and reduced is not known yet. Recent data shows that exercise influences microbiota by several mechanisms. Indeed, exercise may modify bile acids profiles [12] and/or faecal short chain fatty acids (SCFAs) as butyrate [13]. Exercise may also

interact with gut immunological function increasing intestinal immunoglobulin A (IgA), decreasing number of lymphocytes-B and CD4+T cells, and influencing gene expression of cytokines as IL-6, IL-4, IL-10 and TGF-B [14]. Exercise can also modify microbiota because is able to reduce intestinal transit time [15].

We think that our data, although preliminary, could have important clinical implications. Indeed, for the first time, we showed that patients with T2D have heavy intestinal mycetes colonisation and LIS, and chronic exercise can reduced these alteration. This likely could improving intestinal function which influence nutrients metabolism, hormonal production and absorption of oral drug-administered. So, exercise, with or without a specific therapies able to cure of intestinal microbiota, could be an important step for tailored therapy allowing traditional therapy and patients metabolism to function more properly.

This study has some limitations. We used a selective culture medium to identify bacteria and mycetes instead of molecular biology techniques. Indeed, we don’t want to provide a “faecal finger print” of patients. We won’t identify saprophytes and some minor intestinal pathological and mycetes species capable of stimulating inflammation without gastrointestinal symptoms [7]. This is a pilot study with a limited number of selected patients. We have in progress a large scale study to confirm these results.

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