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Correlations of changes in inflammatory factors, glucose and lipid metabolism indicators and adiponectin with alterations in intestinal flora in rats with coronary heart disease

Y. PENG[1], N. ZHANG[2], W.-J. LI[1], K. TAN[3], Y. ZHOU[1], C. SHE[1], H.-N. CHEN[1]

[1]Department of Geriatric, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha, China
[2]Hunan Institute of Geriatrics, Changsha, China
[3]Clinical Lab, Hunan Provincial People's Hospital, The First Affiliated Hospital of Hunan Normal University, Changsha, China

Abstract. – OBJECTIVE: The aim of this study was to explore the correlations of changes in inflammatory factors, glucose and lipid metabolism indicators and adiponectin with alterations in intestinal flora in rats with coronary heart disease.

MATERIALS AND METHODS: A total of 30 male specific pathogen-free rats were randomly assigned into two groups, including: blank group (n=15) and coronary heart disease group (n=15). The rats in the coronary heart disease group were given high-fat diets and pituitrin to establish the model of coronary heart disease. Meanwhile, rats in the blank group were administered with an equal volume of double-distilled water. The alterations in the intestinal flora of rats were detected in the two groups, respectively. In addition, the changes in the levels of inflammatory factors, glucose and lipid metabolism indicators, adiponectin, creatine kinase (CK) and its isoenzyme, as well as, troponin were also examined.

RESULTS: Statistically, significant differences in the levels of glucose and lipid metabolism indicators low-density lipoprotein (LDL) (p=0.040), total cholesterol (TC) (p=0.039), high-density lipoprotein (HDL) (p=0.044), triglyceride (TG) (p=0.000) and blood glucose (p=0.046) were observed between the rats in the coronary heart disease group and blank group. The content of all the glucose and lipid metabolism indicators (except HDL) in coronary heart disease group was significantly higher than the blank group (p<0.05). The rats in the coronary heart disease group had evidently higher levels of CK (p=0.000) and its isoenzyme (p=0.019), as well as troponin (p=0.021), than those in the blank group. The level of serum adiponectin in rats in coronary heart disease group was distinctly lower than that in the blank group, showing statistically significant differences (p<0.05). Besides, the levels of the inflammatory factors interleukin (IL)-2 (p=0.011), transforming growth factor (TGF)-β (p=0.048), tumor necrosis factor-α (TNF-α) (p=0.025) and IL-6 (p=0.038) in rats in the coronary heart disease group were dramatically higher than those in blank group. Rats in coronary heart disease group had remarkably more *Actinobacteria, Desulfovibrio*, *Aristipus* and *Escherichia coli* in the intestine. Meanwhile, the abundance of *Flavobacterium*, *Burkhofer* and some probiotics increased significantly in the intestine of rats in blank group (p<0.05). The changes in the abundance of *Actinobacteria, Desulfovibrio*, *Aristipus* and *Escherichia coli* in the intestine of rats were probably correlated with increased levels of glucose and lipid metabolism indicators, inflammatory factors and adiponectin in coronary heart disease group. Moreover, the abundance of intestinal probiotics such as *Bifidobacterium* and *Lactobacillus* in rats in coronary heart disease group was notably lower than that in blank group (p<0.05). The decline in the abundance of such intestinal probiotics as *Bifidobacterium* and *Lactobacillus* was correlated with the changes in the levels of glucose and lipid metabolism indicators, inflammatory factors and adiponectin. In addition, decreased levels of probiotics weakened normal physiological functions of the intestine and promoted disease progression.

CONCLUSIONS: Inflammatory factors, glucose and lipid metabolism indicators and adiponectin have evident changes in rats with coronary heart disease, which may be correlated with the alterations in the intestinal flora.

Key Words:
Coronary heart disease, Intestinal flora, Adiponectin.

Corresponding Author: Hanna Chen, BM; e-mail: friendpeng123@163.com

Introduction

With the improvement of people's living standard, cardiovascular diseases have gradually become major diseases endangering human health. Statistics[1] have shown that the morbidity rate of cardiovascular diseases is higher than that in malignancies. Of them, coronary heart disease is a kind of disease caused by the shortening of coronary atherosclerotic heart disease. Coronary artery stenosis and occlusion resulted from various reasons may induce myocardial ischemia, hypoxia and necrosis, thereby leading to severe clinical symptoms[2,3]. Current studies have shown that the development of coronary heart disease is correlated with the increase in age. The decline in the ability to regulate metabolism in the middle-aged and elder may give rise to a persistently high level of blood lipid, which further induces the disease[4,5]. Meanwhile, excessive inflammatory responses in the body are likely to damage the coronary artery to drive the development of coronary heart diseases[6]. Therefore, it is helpful to search for indicators related to the development and progression of coronary heart disease for elucidating its mechanism and identifying a new treatment direction.

Intestinal flora refers to microorganisms residing in the intestine for a long time in the body, the composition and abundance of which can affect multiple physiological processes[7]. In recent years, the changes in intestinal microorganism composition and content have been considered as important causes affecting the progression of many diseases, such as necrotizing enterocolitis[8] and systemic sclerosis[9]. Coronary heart disease, a disease affected by the metabolic state in the body, is much likely to influence the composition of the intestinal flora. It can further affect the changes in other vital indicators in the body, thereby promoting the progression of the disease. In the present study, therefore, the model of coronary heart disease was first successfully established in rats using high-fat diets and pituitrin. The levels of inflammatory factors, glucose and lipid metabolism indicators, adiponectin, creatine kinase (CK) and its isoenzyme, as well as troponin were determined. Furthermore, the alterations in the intestinal flora of rats with coronary heart disease were analyzed to verify the correlations of such alterations with the changes in inflammatory factors, glucose, and lipid metabolism indicators and adiponectin.

Materials and Methods

Laboratory Animals and Grouping

A total of 30 specific pathogen-free male Sprague-Dawley rats weighing (250±30) g were randomly assigned into two groups, including: blank group (n=15) and coronary heart disease group (n=15). During experiments, all the rats were fed based on the standard, with free access to food and water. Specifically-assigned researchers regularly changed the food and padding and examined the status of rats. This study was approved by the Animal Ethics Committee of Hunan Normal University Animal Center.

Establishment of Coronary Heart Disease Model in Rats

The rats in the coronary heart disease group were administered with high-fat diets and pituitrin to establish the model of coronary heart disease according to a previous study[10]. Briefly, the rats were fed with 35 g of high-fat diets daily for 2 months to induce blood lipid abnormality. Subsequently, they were intraperitoneally injected with pituitrin at 30 U/kg for 3 d to prepare the model of coronary heart disease. Similarly, the rats in the blank group were given an equal volume of double distilled water daily. Other feeding conditions were the same in the two groups.

Collection of Blood Specimens and Detection of Glucose and Lipid Metabolism Indicators

After the model of coronary heart disease was successfully established in rats, abdominal aortic blood was collected from each group of rats into pro-coagulation tubes. After centrifugation at 3,000 rpm for 10 min, the upper-layer serum was harvested and cryopreserved in a refrigerator at -20°C for use. Glucose and lipid metabolism indicators low-density lipoprotein (LDL), total cholesterol (TC), high-density lipoprotein (HDL), triglyceride (TG) and blood glucose were determined in the Biochemical Room of the Laboratory Department in our hospital.

Adiponectin Assay and Detection of CK and Its Isoenzyme As Well As Troponin

Serum adiponectin of rats in each group was quantitatively detected according to the instructions of the adiponectin assay kit. The buffer-diluted serum of rats was added into wells, shaken using an oscillator, incubated, washed and added

Table I. Levels of glucose and lipid metabolism indicators in blank group and coronary heart disease group (mmol/L, $\bar{x} \pm s$).

Group	No.	LDL	TC	HDL	TG	Blood glucose
Blank group	15	0.43 ± 0.05	0.32 ± 0.04	0.89 ± 0.06	1.64 ± 0.14	6.87 ± 0.12
Coronary heart disease group	15	0.97 ± 0.12	0.78 ± 0.08	0.54 ± 0.05	3.76 ± 0.41	9.47 ± 0.76
t		8.01	8.23	7.44	14.87	6.53
p		0.040	0.039	0.044	0.000	0.046

with enzyme conjugate solution, substrates and stop buffer. Optical density (OD) at 450 nm was detected by a micro-plate reader. Besides, CK and its isoenzyme, as well as troponin, were determined using the kits for plasma CK, CK-MB and troponin, respectively. OD value was finally determined by a micro-plate reader as well.

Measurement of Serum Immune and Inflammatory Factor Level

The levels of inflammatory indicators interleukin (IL)-2, transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α) and IL-6 in the two groups of rats were measured by the enzyme-linked immunosorbent assay (ELISA, BD Biosciences, Franklin Lakes, NJ, USA). During the detection, 3 replicates were set in each group, and OD value at 550 nm was measured by a micro-plate reader. Finally, the actual concentration of each indicator detected was converted based on the standard curves.

Detection of Intestinal Flora

Rats in both blank and coronary heart disease groups were first sacrificed. 3-4 g of cecal feces were then taken from rats in each group and cryopreserved in a refrigerator at -80°C for flora detection. Subsequently, deoxyribonucleic acids (DNAs) were extracted from the feces of rats using the QIAGEN QlAamp DNA Stool Mini Kit (QIAGEN, Duesseldorf, Germany). After the sequencing was completed, the flora species with low abundance were eliminated, and the main intestinal flora of rats in the blank group and the coronary heart disease group were analyzed for microbial diversity.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Measurement data were presented as mean ± standard deviation. Intergroup comparisons were made using unpaired *t*-test. $p<0.05$ was considered statistically significant.

Results

Differences in the Glucose and Lipid Metabolism Indicators Between Blank Group and Coronary Heart Disease Group

Statistically significantly differences were observed in the levels of glucose and lipid metabolism indicators LDL ($p=0.040$), TC ($p=0.039$), HDL ($p=0.044$), TG ($p=0.000$) and blood glucose ($p=0.046$) in rats between coronary heart disease group and blank group. The content of all the glucose and lipid metabolism indicators except for HDL in coronary heart disease group was remarkably higher than the lank group ($p<0.05$) (Table I).

Differences in the Levels of CK and its Isoenzyme As Well As Troponin Between Blank Group and Coronary Heart Disease Group

Rats in coronary heart disease group had evidently higher levels of CK ($p=0.000$) and its isoenzyme ($p=0.019$) as well as troponin ($p=0.021$) than those in blank group (Table II).

Table II. Levels of CK and its isoenzyme as well as troponin ($\bar{x} \pm s$).

Group	No.	CK (IU/L)	CK-MB (IU/L)	Troponin (ng/mL)
Blank group	15	7.88 ± 0.58	5.32 ± 0.74	8.21 ± 0.68
Coronary heart disease group	15	17.45 ± 1.23	12.64 ± 0.84	12.41 ± 2.41
t		14.21	10.23	9.87
p		0.000	0.019	0.021

Changes in Adiponectin Level in Blank Group and Coronary Heart Disease Group

The level of serum adiponectin in rats in coronary heart disease group was distinctly lower than that in blank group, and the difference was statistically significant (p<0.05) (Figure 1).

Differences in Inflammatory Factor Levels Between Blank Group and Coronary Heart Disease Group

The levels of inflammatory factors IL-2 (p=0.011), TGF-β (p=0.048) and IL-6 (p=0.038) in rats in coronary heart disease group were dramatically higher than those in blank group (Figure 2).

Comparison of Differences in Intestinal Flora Between Blank Group and Coronary Heart Disease Group

According to the linear discriminant analysis effect size (LEfSe) analysis results (Figure 3), rats in coronary heart disease group had remarkably more *Actinobacteria*, *Desulfovibrio*, *Aristipus* and *Escherichia coli* in the intestine (p<0.05). However, those in blank group exhibited distinctly increased abundance of *Flavobacterium*, *Burkhofer* and some probiotics in the intestine (p<0.05). Moreover, the changes in the abundance of *Actinobacteria*, *Desulfovibrio*, *Aristipus* and *Escherichia coli* in the intestine of rats were correlated with increased levels of glucose and lipid metabolism indicators, inflammatory factors and adiponectin in coronary heart disease group.

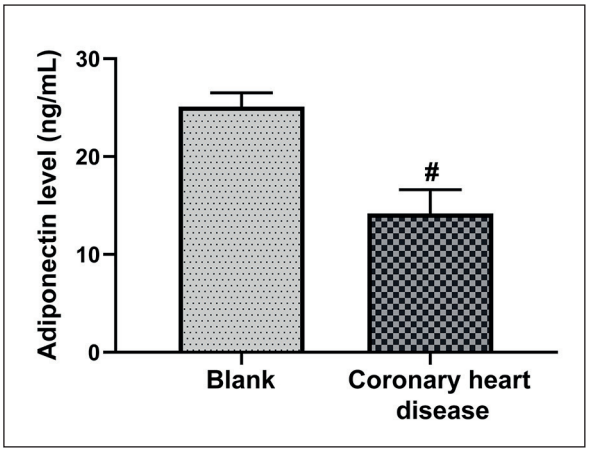

Figure 1. Adiponectin level in blank group and coronary heart disease group ($^{\#}p$<0.05 *vs.* blank group).

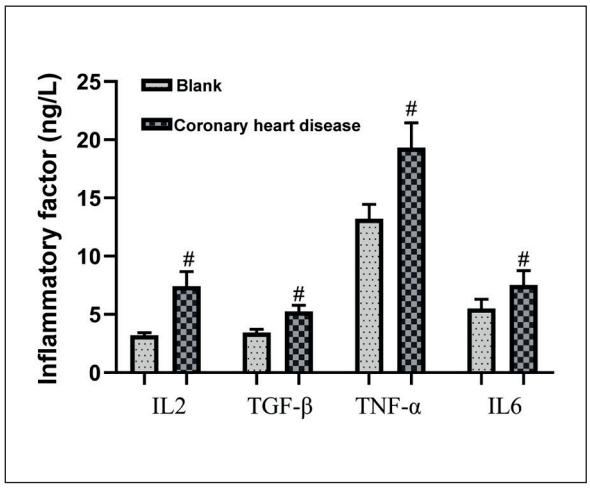

Figure 2. Inflammatory factor levels in blank group and coronary heart disease group ($^{\#}p$<0.05 *vs.* blank group).

Comparison of Probiotic Abundance Between Blank Group and Coronary Heart Disease Group

The abundance of intestinal probiotics, such as *Bifidobacterium* and *Lactobacillus* in rats in coronary heart disease group was notably lower than that in blank group (p<0.05) (Figure 4 and 5). The decline in the abundance of such intestinal probiotics as *Bifidobacterium* and *Lactobacillus* might be correlated with the changes in the levels of glucose and lipid metabolism indicators, in-

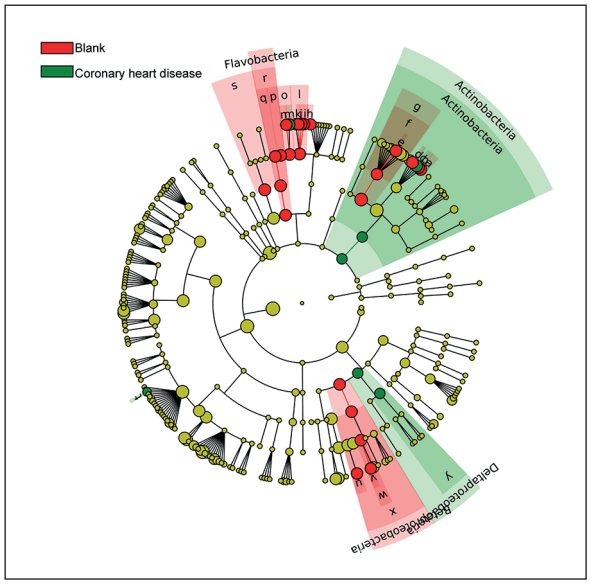

Figure 3. LEfSe analysis results of intestinal flora in blank group and coronary heart disease group.

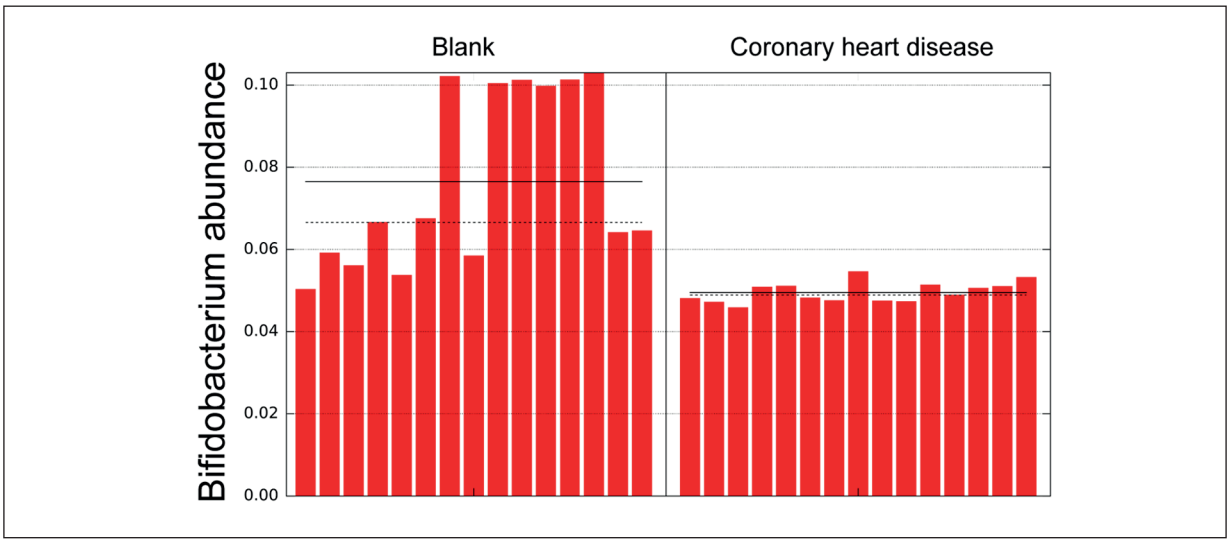

Figure 4. Comparison of Bifidobacterium abundance in the intestine of rats between blank group and coronary heart disease group.

flammatory factors and adiponectin. In addition, decreased levels of probiotics weakened normal physiological functions of the intestine and promoted disease progression.

Discussion

Coronary heart disease, one of the most common cardiovascular diseases, can cause chronic myocardial ischemia in patients. Therefore, angina pectoris and myocardial infarction may occur, seriously threatening human health[11]. Due to large population base and many basic metabolic diseases, coronary heart disease has become an important public health problem in China. The development of coronary heart disease is a very complex process, which is mainly caused by persistent metabolic disorders, such as hyperlipidemia and hyperglycemia in organisms[12]. Additionally, bad dietary styles, smoking, intemperance, the increase in age, sex difference

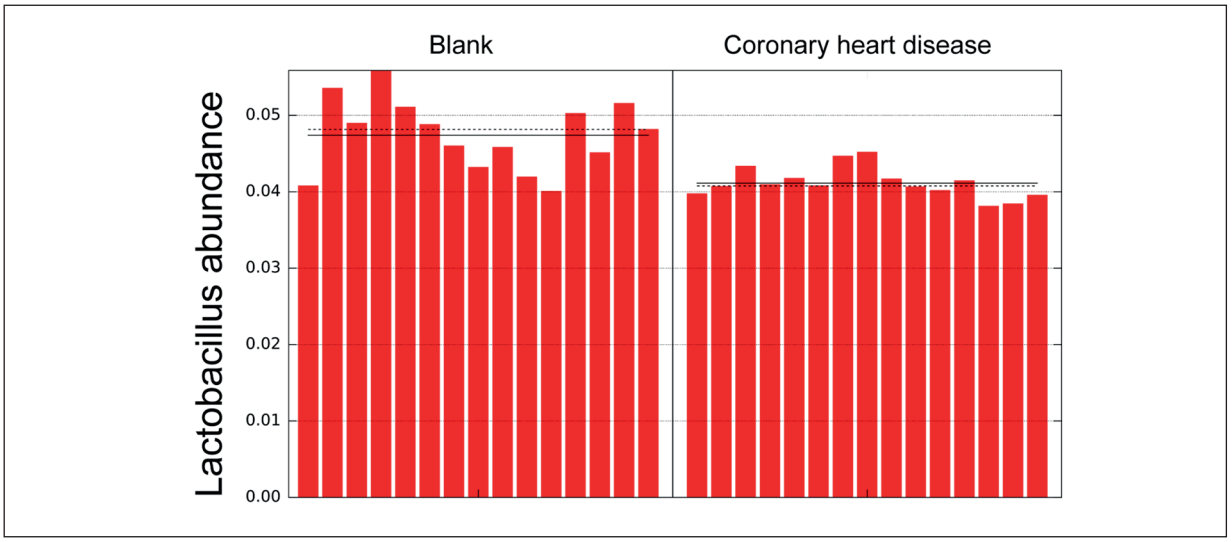

Figure 5. Comparison of Lactobacillus abundance in the intestine of rats between blank group and coronary heart disease group.

and genetic factors can drive the development of coronary heart disease[13]. Previous studies[14,15] have demonstrated that this disease occurs with changes in multiple substances, including metabolism-related molecules, immune molecules, inflammatory molecules and various cells in the body. It can also change the environment in the body to facilitate disease progression. There are many risk factors for coronary heart disease, with a complex pathogenesis. Therefore, searching for the molecules or substances with characteristic changes in the body will greatly help to elucidate its pathogenesis and formulate novel treatment strategies.

Intestinal flora physiologically plays a pivotal role in substance decomposition, absorption and nutrient conversion in the intestine of organisms[16]. Moreover, intestinal flora can directly or indirectly affect homeostasis in the body, and increase the expressions of some important molecules, thereby promoting or inhibiting immune and inflammatory responses[17]. The composition and abundance of intestinal flora may be changed in diseases. Meanwhile, they can further affect substance absorption and promote inflammation, thereby regulating the progression of the disease[18,19]. Currently, the development and progression of coronary heart disease have been believed to be correlated with intestinal microorganisms[20]. In fact, chitooligosaccharide can increase intestinal probiotics to enhance anti-oxidative capacity, thereby preventing coronary heart disease to a certain extent[21]. In this study, the rat model of coronary heart disease was first successfully established using high-fat diets and pituitrin. It was found that the levels of glucose and lipid metabolism indicators LDL (p=0.040), TC (p=0.039), HDL (p=0.044), TG (p=0.000) and blood glucose (p=0.046) were significantly different between coronary heart disease group and blank group. The content of all the glucose and lipid metabolism indicators except HDL in coronary heart disease group was significantly higher than blank group (p<0.05). Rats in coronary heart disease group had evidently higher levels of CK (p=0.000) and its isoenzyme (p=0.019) as well as troponin (p=0.021) than those in blank group. These results imply that the rat model of coronary heart disease was successfully established and that coronary heart disease has a certain effect on blood glucose. Meanwhile, it can also be inferred that cardiac cells are damaged in the progression of coronary heart disease. According to the results of intestinal flora detection, the rats in coronary heart disease group had markedly more *Actinobacteria*, *Desulfovibrio*, *Aristipus* and *Escherichia coli* in the intestine (p<0.05). The abundance of *Flavobacterium*, *Burkhofer* and some probiotics in the intestine of rats increased significantly in the blank group (p<0.05). Besides, the abundance of intestinal probiotics, such as *Bifidobacterium* and *Lactobacillus* in rats in coronary heart disease group was notably lower than that in blank group (p<0.05). These results suggest that intestinal flora is indeed affected in the progression of coronary heart disease, with decreased probiotics and affected intestinal microenvironment. This may be an important reason for promoting the further progression of coronary heart disease.

Adiponectin, a cytokine secreted by adipose tissues, can potently attenuate vascular damage and inflammation. The expression of adiponectin decreased significantly in obese and diabetic patients, which suggested that adiponectin plays an important regulatory role in the process of internal metabolism and has a crucial inhibiting effect on the development of endocrine system diseases[22,23]. In patients with coronary heart disease, adiponectin, secreted by adipose tissue and released into the serum, plays a protective role *via* binding to the receptors on coronary vascular endothelial cells. According to the findings of this study, the level of serum adiponectin in rats in coronary heart disease group was distinctly lower than that in blank group, and the difference was statistically significant (p<0.05). It can be inferred that the anti-atherosclerosis ability in the body is greatly weakened in the progression of coronary heart disease, further promoting disease progression. The effects of coronary heart disease on inflammatory factors were detected as well. It was discovered that the levels of inflammatory factors IL-2 (p=0.011), TGF-β (p=0.048), TNF-α (p=0.025) and IL-6 (p=0.038) in rats in coronary heart disease group were dramatically higher than those in blank group. Furthermore, the alterations in the intestinal flora of rats with coronary heart disease were analyzed. The results indicated that changes in the abundance of *Actinobacteria*, *Desulfovibrio*, *Aristipus* and *Escherichia coli* were probably correlated with increased levels of glucose and lipid metabolism indicators, inflammatory factors and adiponectin. Moreover, the decline in the abundance of such intestinal probiotics as *Bifidobacterium* and *Lactobacillus* might be correlated with the changes in the levels of glucose and lipid metabolism

indicators, inflammatory factors and adiponectin. In addition, decreased levels of probiotics weakened normal physiological functions of the intestine and promoted disease progression. All these findings indicate that alterations in the intestinal flora in the development of coronary heart disease may have important correlations with the levels of glucose and lipid metabolism indicators, inflammatory factors and adiponectin in the body. They probably act as the risk factors for coronary heart disease, jointly facilitating the progression of this disease.

Our findings showed that reduced adiponectin levels may be one of the key causes of dysregulation of blood glucose and lipid metabolism, as well as an important factor in promoting the development of coronary heart disease. In addition, intestinal flora disorders, such as reduced probiotics abundance, may further lead to the deterioration of coronary heart disease. All these results suggested that changes of intestinal flora may be significantly related to glucose and lipid metabolism, inflammatory factors and adiponectin levels in the development of coronary heart disease. Intestinal flora disorders, as a risk factor of coronary heart disease, may play a role in the progress of coronary heart disease.

Conclusions

Briefly, the novelty of this study was that inflammatory factors, glucose, and lipid metabolism indicators and adiponectin have evident changes in rats with coronary heart disease, which may be correlated with the alterations in the intestinal flora.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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