https://doi.org/10.52973/rcfcv-e34320 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34320

The effect of fructose–induced metabolic syndrome on the histological structure and enteroendocrine cells in duodenum of rats

Efecto del síndrome metabólico inducido por fructosa sobre la estructura histológica y las células enteroendocrinas en el duodeno de ratas

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ABSTRACT

Metabolic syndrome is a worldwide common disorder that coexists with abdominal obesity, high blood pressure, dyslipidemia, and high blood sugar. The purpose of this investigation was to examine the effects of metabolic syndrome on enteroendocrine cell count and histological structure in duodenum. The rats were separated into control and metabolic syndrome groups. Duodenum tissue sections were stained with hematoxylin–eosin for histomorphological examinations. Additionally, immunohistochemical analysis of duodenum showed the presence of somatostatin and gastrin immunoreactive cells. In duodenum, it was found that villus height, villus width, villus area and thickness of tunica muscularis significantly increased in metabolic syndrome group compared to control group. Similarly, it was observed that the numbers of somatostatin and gastrin immunoreactive cells in duodenum were significantly higher in metabolic syndrome group compared to control group. As a result, it was revealed that metabolic syndrome caused structural disorders in duodenum and changed enteroendocrine cell population. Thus, it can be assumed that intestinal functions may be negatively affected due to metabolic syndrome and digestive physiology may be disrupted.

Key words: Duodenum; gastrin; metabolic syndrome; rat; somatostatin

RESUMEN

El síndrome metabólico es un trastorno común a nivel mundial en el que coexisten obesidad abdominal, presión arterial alta, dislipidemia y niveles altos de azúcar en sangre. En el estudio presentado, el objetivo fue evaluar los efectos del síndrome metabólico en la estructura histológica y las células enteroendocrinas del duodeno. Las ratas se dividieron aleatoriamente en dos grupos: control y síndrome metabólico. Mientras que las ratas del grupo de control recibieron agua del grifo durante 16 semanas, las ratas del grupo con síndrome metabólico recibieron agua del grifo que contenía un 20% de D–fructosa. Se tomaron muestras de duodeno del intestino delgado al final del experimento. Se aplicó el método de tinción con hematoxilina y eosina a secciones de tejido para exámenes histomorfológicos. Además, se detectaron inmunohistoquímicamente células inmunorreactivas de somatostatina y gastrina en el duodeno. Se determinó que la altura de las vellosidades, el ancho de las vellosidades, el área de las vellosidades y el grosor de la túnica muscular en el duodeno aumentaron significativamente en el grupo con síndrome metabólico en comparación con el grupo de control. De manera similar, se observó que el número de células inmunorreactivas de somatostatina y gastrina en el duodeno aumentó significativamente en el grupo con síndrome metabólico en comparación con el grupo de control. Como resultado, se reveló que el síndrome metabólico causa trastornos estructurales en el duodeno y cambia la población de células enteroendocrinas. Por tanto, se puede pensar que las funciones intestinales pueden verse afectadas negativamente y la fisiología digestiva puede deteriorarse debido al síndrome metabólico.

Palabras clave: Síndrome metabólico; duodeno; somatostatina; gastrina; rata

Received: 11/09/2023 Accepted: 28/11/2023 Published: 26/02/2024

INTRODUCTION

Metabolic syndrome (MS) is a disease characterized by insulin resistance, hypertension, dyslipidemia and an increase in body mass index [1]. An essential factor in development of this disease is the rise in the consumption of high-calorie, low-fiber fast food as well as the decline in physical activity [2]. Additionally, people with metabolic syndrome have a higher chance of developing cardiovascular disease, which is the main cause of mortality. Nowadays, frequency of metabolic syndrome has significantly increased and it is now recognized as a serious health issue on a global scale [3]. In addition, loss in economy due to care of metabolic syndrome patients has reached high costs [2].

Fructose is a six-carbon monosaccharide. It is consumed as a component of fruit, vegetables, honey, artificial sweeteners and corn syrup with high fructose [4]. Fructose mediates weight gain by stimulating lipogenesis. Besides, excessive fructose consumption has become an important factor in development of metabolic syndrome, as it can cause an increase in blood pressure and triglycerides as well as insulin resistance [5]. In addition, it has been reported that an experimental metabolic syndrome model can be created in rodents with high fructose intake [1].

The metabolic syndrome induced by feeding with a high fructose or fatty diet causes various degrees of damage such as irregularity in microvilli [5], epithelial swelling, epithelial degeneration, villus collapse and chronic inflammatory cell infiltration in small intestine [6]. Also, it increases intestinal permeability, decreases intestinal barrier integrity and stimulates the formation of apoptosis [7]. Furthermore, metabolic syndrome has been linked to an increase in the number of goblet cells [8], villus length [9], crypt depth and mucosal thickness in small intestine [5]. In addition, metabolic syndrome increases small intestine weight, mitotic activity, and absorption of short-chain fatty acids. On the other hand, metabolic syndrome induces oxidative stress in intestinal tissue by increasing lipid peroxidation and increases the levels of cytokines interleukin β1 (IL-β1) and interleukin 6 (IL-6) [10].

Enteroendocrine cells are found in digestive tract and produce peptide hormones. It is reported that they consist of at least 15 cell types. Somatostatin and gastrin hormones are secreted by D and G cells, respectively [11]. The somatostatin hormone inhibits the secretions of insulin and glucagon [12]. It reduces blood flow by contracting smooth muscle in intestine [13]. The gastrin hormone stimulates gastric acid secretion [14] and angiogenesis [15]. Besides, it suppresses apoptosis [16] while increasing mucosal growth [14].

The purpose of this study was to look into the effects of fructose-induced metabolic syndrome on histological structure and enteroendocrine cells in duodenum that secrete somatostatin and gastrin hormones.

MATERIALS AND METHODS

Ethical approval

Local ethics committee for animal experiments at Aydin Adnan Menderes University granted permission for study (Decision number: 64583101/2020/042).

Animals

All methods in experiment were conducted in accordance with guidelines of Declaration of Helsinki. In study, 20 two-month-old male Sprague Dawley rats (*Rattus norvegicus*), with average body weight 200-250 g were used. Rats were obtained from Aydin Adnan Menderes University, Experimental Animals Unit. The rats were kept under conventional conditions at 22 ± 1°C with a 12-hour light/dark cycle.

Experimental design

The rats were randomly divided into two groups as control (n=10) and metabolic syndrome (n=10). Tap water and food *ad libitum* were given to the rats in control group for 16 weeks. The rats of metabolic syndrome group were fed with tap water containing 20% D-fructose (Merck D(-)-fructose for biochemistry 104007.0250) [17] and food *ad libitum* for 16 weeks [18, 19].

Histomorphological analysis

At the end of experiment, rats were killed by cervical dislocation under ether anesthesia. Then, duodenum samples were taken from small intestine. Tissue samples were fixed in 10% buffered formalin for 24 hours. After routine histological procedures, tissue samples were embedded in paraffin and serial sections of 5 μm thickness were taken. Tissue sections were stained with hematoxylin-eosin staining method and two sections from each animal were examined. The number of villi in the duodenum was determined by counting in five different microscopic areas at 20× magnification in both sections of animals. Also, five measurements of villus height, villus width, villus area, crypt depth and thickness of tunica muscularis were made in two sections from each animal. The measurements were carried out with a light microscope (SOIF BK5000-TR/L, Denmark) equipped with an image analysis system (MShot Digital Imaging System, China). In addition, photographs were taken with a camera (MShot MD 50, China) from the necessary parts of the sections.

Immunohistochemical analysis

Firstly, sections were deparaffinized. For antigen retrieval, the sections were boiled three times for 5 min each time in a microwave oven at 98°C in 0.01 M pH 6 sodium citrate. Then, they were kept in 3% H_2O_2 that was prepared with distilled water for 10 min to remove endogenous peroxidase activity. After that, sections were kept in blocking solution for 5 min. After this process, sections were incubated (NUVE, FN 055, NUVE Factory, Turkey) in primary antibody (anti-gastrin, bs-1189R; anti-somatostatin, bs-1132R) diluted 1/100 for 2 hours at 37°C. Then, sections were incubated in Primary Antibody Amplifier Quanto and HRP Polymer Quanto for 10 min each, respectively. Subsequently, sections were kept in 3,3'-diaminobenzidine (DAB) for 3-5 min. Finally, sections were stained with Harris Hematoxylin for 20 s and closed with entellan. The numbers of enteroendocrine cells were determined by manually counting on the lamina epithelialis and crypts in randomly selected different microscopic areas at 20× magnification in two sections of each animal. Then, the microscopic area was calculated and the results were normalized to a unit area of 1 mm^2.

Statistical analysis

The data were statistically analyzed with package program SPSS 20.00. *t*-test and Mann-Whitney U test were used to determine whether or not there were differences between groups. The data were presented using mean ± standard deviation format. The values with differences of $P<0.05$ (*) and $P<0.001$ (***) were deemed statistically significant.

RESULTS AND DISCUSSIONS

Studies have stated that metabolic syndrome increased the mucosal thickness [5], villus height [9] and mitotic activity in small intestine [10]. In the present study, it was determined that the villus height in duodenum significantly increased in metabolic syndrome group compared to control group (TABLE I; FIG. 1). This finding in this study shows parallelism with the literature. In addition, it has been detected that the increase in villi height is associated with an increase in intestinal surface area and absorption of nutrients [20]. It is also known that metabolic syndrome causes obesity [1]. Therefore, in the present study; villi height increasing in duodenum as a result of metabolic syndrome may increase the absorptive capacity of small intestine and may play a role in pathogenesis of weight gain in metabolic syndrome by leading to excessive nutrient absorption.

TABLE I
Histomorphological values in duodenums of control and metabolic syndrome groups

Parameters	Control (n=10)	Metabolic syndrome (n=10)	*P*–value
Number of villus	5.81 ± 1.12	5.80 ± 1.04	NS
Villus height (µm)	570.73 ± 122.98[b]	685.10 ± 81.37[a]	***
Villus width (µm)	120.11 ± 26.24[b]	137.63 ± 28.38[a]	***
Villus area (µm²)	65703.69 ± 54084.47[b]	81226.94 ± 19579.99[a]	***
Crypt depth (µm)	252.07 ± 55.32	252.33 ± 54.86	NS
Thickness of tunica muscularis (µm)	92.56 ± 23.03[b]	99.28 ± 24.52[a]	*

[a,b]: Different superscripts in the same row indicate the significant difference. NS: Non–significant, *P<0.05, ***P<0.001

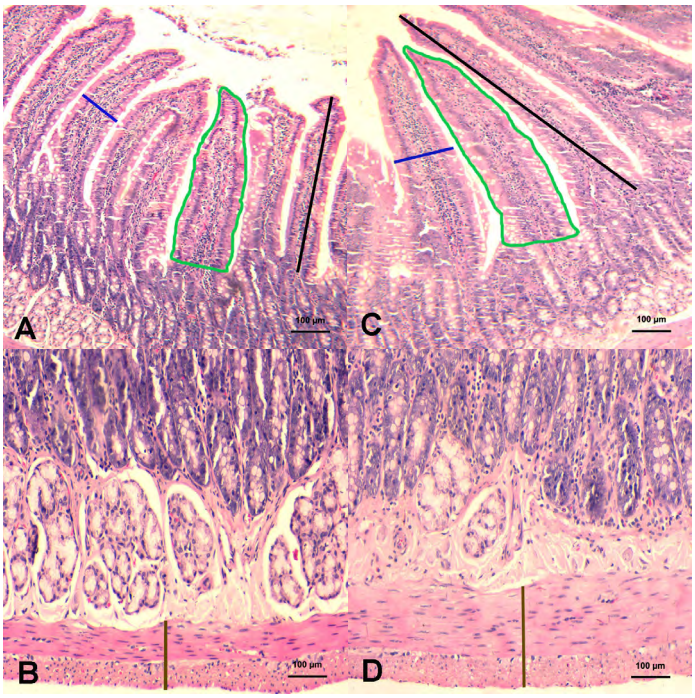

FIGURE 1. Microscopic views of the duodenum in the control (A, B) and metabolic syndrome groups (C, D). It is seen that villus height (black lines), villus width (blue lines), villus area (inner regions of green line) and thickness of the tunica muscularis (brown lines) increased in the metabolic syndrome group compared to the control group. Hematoxylin–eosin staining method. Bars: 100 µm

It has been reported that feeding a high sucrose and fatty diet increased blood tumor necrosis factor–alpha (TNFα) and IL–6 cytokines, lipopolysaccharide (LPS) endotoxin levels, as well as caused swelling of villus epithelium in small intestine [6]. In addition, it has been determined that high–fat diet together with sedentary behavior triggered plasmacytoid and lymphocyte infiltration in intestine, causing an increase in villus width [21]. Similarly, it has been observed that the toxic effect of fluoride led to severe mononuclear cell infiltration in small intestine, resulting in villus thickening [22]. In the present study, it was noticed that villus width and villus area in duodenum significantly increased in metabolic syndrome group compared to control group (TABLE I; FIG. 1). In light of this information, it can be thought that feeding with a high fructose diet may have triggered inflammation process in intestine and increased size of villi in the present study.

In the studies, it has been stated that the increase in thickness of intestinal muscle layer mostly resulted from hypertrophy. The reason of this, the factors released in intestinal inflammation act as hypertrophic stimuli for smooth muscle cells [23]. Also, it has been found that increased muscle thickness in intestine may be due to the increase in connective tissue between muscle fibers [24]. In the present study, it was determined that the thickness of tunica muscularis significantly increased in metabolic syndrome group compared to control group (TABLE I; FIG. 1). Based on this data, it is considered that a high fructose diet may have induced the inflammation process in duodenum, leading to hypertrophy in muscle cells or connective tissue accumulation between muscle cells. On the other hand, it has been emphasized that there may be an increase in the thickness of tunica muscularis in order to increase the digestibility of nutrients due to an increase in food intake during pregnancy [25]. Also, it has been detected that the increase in the thickness of tunica muscularis increased the contact between the intestinal contents and mucosa [26]. According to these findings, it can be deduced that the muscle layer thickness may have increased in order to increase digestive and absorption capacity of duodenum as a result of feeding with a high fructose diet in our study.

Somatostatin regulates gastric acid secretion. It decreases smooth muscle contraction and blood flow in small intestine [13]. It has been found that somatostatin immunoreactive cells were localized in villus epithelium and crypts of small intestine [11]. In addition, it has been determined that the number of somatostatin–producing D cells increased in cardia and antrum parts of stomach but decreased in corpus part of stomach in streptozotocin and fructose diet–administered rats compared to control group [27]. Also, it has been detected that 3–aminoisobutyric acid (BAIBA) metabolite was higher in plasmas of subjects with high L and D cell density in duodenal biopsies of individuals with metabolic syndrome. Thus, it has been emphasized that there may be a link between increased L and D cell density, plasma metabolites and clinical features of metabolic syndrome [28]. In the current study, somatostatin immunoreactive cells were found in epithelial layer and crypts of duodenum. It was noticed that they were more intensely localized in crypts. In addition,

it was detected that the number of somatostatin immunoreactive cells in duodenum significantly increased in metabolic syndrome group compared to control group (TABLE II ; FIG. 2). The obtained findings are in parallel with the literature. On the basis of this information, it is possible to conclude that the increase in the number of somatostatin immunoreactive cells with effect of metabolic syndrome may cause problems in functions such as regulation of gastric acid secretion in stomach, blood circulation and motility in duodenum.

Gastrin immunoreactive cells are observed in villus epithelium and crypts in all segments of small intestine [11]. Gastrin is a regulator of gastric acid secretion. It stimulates cell proliferation and angiogenesis [15]. It has been reported that it reduced apoptosis in crypt cells in intestinal damage induced by ischemia reperfusion [16]. It has been determined that the number of gastrin-positive endocrine cells decreased in corpus and antrum parts of stomach in streptozotocin and fructose diet administered rats compared to control group [27]. In this study, gastrin immunoreactive cells were localized in epithelial layer and crypts. Also, they were found to be more common in crypts. On the other hand, the number of gastrin immunoreactive cells in duodenum significantly increased in metabolic syndrome group compared to control group (TABLE II) (FIG. 2). This result differs from the study findings of Gulubova *et al* [27]. The reason for obtaining different results in the studies may be due to the differences in the tissue, experimental model and duration. In addition, it can be considered that the increase in the number of gastrin immunoreactive cells in duodenum with effect of metabolic syndrome may induce disturbances in regulation of gastric acid secretion as well as intestinal apoptosis and proliferation processes.

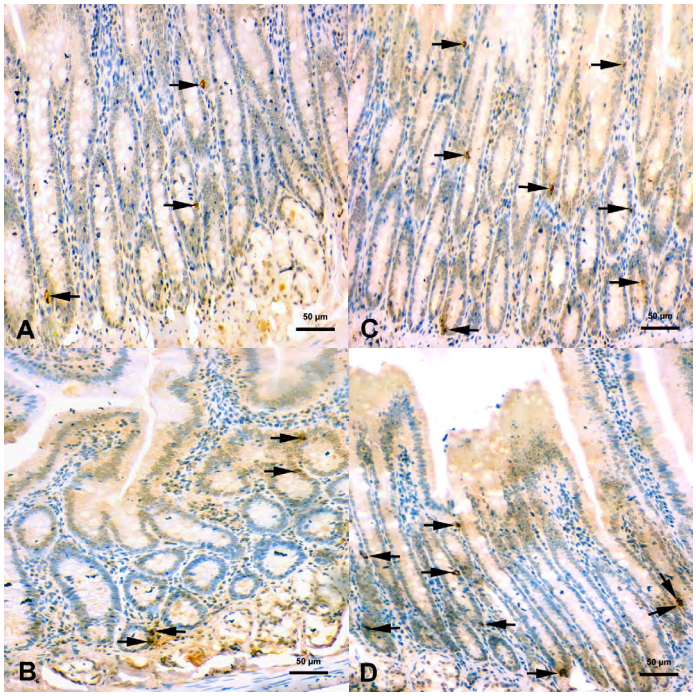

FIGURE 2. Microscopic views of somatostatin (A, C) and gastrin immunoreactive cells (B, D) in the duodenum (arrows). It is observed that the number of enteroendocrine cells increased in the metabolic syndrome group (C, D) compared to the control group (A, B). Polymer-based immunohistochemical method. Bars: 50 µm

TABLE II
The numbers of enteroendocrine cells in duodenums of control and metabolic syndrome groups (cell number per mm²)

Enteroendocrine cells	Control (n=10)	Metabolic syndrome (n=10)	*P*-value
Somatostatin IR cells	7.17±4.34[b]	12.96±3.06[a]	***
Gastrin IR cells	10.02±4.91[b]	13.43±4.00[a]	***

[a.b]: Different superscripts in the same row indicate the significant difference. *** *P*<0,001, IR: Immunoreactive

CONCLUSIONS

In conclusion, it was revealed that metabolic syndrome caused changes in enteroendocrine cell population and histomorphological structure in duodenum. Furthermore, it was drawn attention that these alterations may lead to digestive physiology issues by adversely influencing intestine functions. On the other hand, the data in the present study will be a reference for future studies that affect digestive system.

Conflict of interests

The authors of this study declare that there is no conflict of interest with the publication of this manuscript.

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