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Corresponding Author: Ji-Young Cha

Authors: Ho-Jae Lee¹, Ji-Young Cha^{1,*}

Institution: ¹Department of Biochemistry, Lee Gil Ya Cancer and Diabetes Institute, Gachon University College of Medicine, Incheon 21999, Korea, ²Gachon Medical Institute, Gil Medical Center, Incheon 21565, Republic of Korea,

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Title: Recent insights into the role of ChREBP in intestinal fructose absorption and metabolism

Author's name: Ho-Jae Lee¹ and Ji-Young Cha^{1,2,*}

Affiliation: ¹Department of Biochemistry, Lee Gil Ya Cancer and Diabetes Institute, Gachon University College of Medicine, Incheon 21999, ²Gachon Medical Institute, Gil Medical Center, Incheon 21565, Republic of Korea

Running Title: Intestinal ChREBP is essential for fructose absorption and metabolism

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Corresponding Author:

Ji-Young Cha, Ph.D.

Department of Biochemistry

Lee Gil Ya Cancer and Diabetes Institute

Gachon University College of Medicine

155 Gaetbeol-ro, Yeonsu-gu, Incheon 406-840, Korea

Tel. No. +82-32-899-6070 and E-mail address jycha1014@gachon.ac.kr.

ABSTRACT

Fructose in the form of sucrose and high fructose corn syrup is absorbed by the intestinal transporter and mainly metabolized in the small intestine. However, excess intake of fructose overwhelms the absorptive capacity of the small intestine, leading to fructose malabsorption. Carbohydrate response element-binding protein (ChREBP) is a basic helix-loop-helix leucine zipper transcription factor that plays a key role in glycolytic and lipogenic gene expression in response to carbohydrate consumption. While ChREBP was initially identified as a glucose-responsive factor in the liver, recent evidence suggests that ChREBP is essential for fructose-induced lipogenesis and gluconeogenesis in the small intestine as well as in the liver. We recently identified that the loss of ChREBP leads to fructose intolerance via insufficient induction of genes involved in fructose transport and metabolism in the intestine. As fructose consumption is increasing and closely associated with metabolic and gastrointestinal diseases, a comprehensive understanding of cellular fructose sensing and metabolism via ChREBP may uncover new therapeutic opportunities. In this mini review, we briefly summarize recent progress in intestinal fructose metabolism, the regulation and function of ChREBP by fructose, and delineate the potential mechanisms by which excessive fructose consumption may lead to irritable bowel syndrome.

INTRODUCTION

Fructose is a monosaccharide that is derived mostly from fruits and a few vegetables. It is typically consumed as sucrose, a disaccharide composed of 50% fructose and 50% glucose, or as a component of high-fructose corn syrup (HFCS, mostly containing ~42-55% fructose and the remainder as glucose). HFCS and sucrose are commonly added as sweeteners to many processed foods and carbonated beverages, such as cakes, cookies, cereals, cured meats, flavored yogurts, and soft and fruit drinks. The use of HFCS has increased compared with that of sucrose because it is relatively inexpensive, enhanced flavor, and long shelf-life (1). In the early of 1970s, HFCS intake was 0.4% of the calories ingested through caloric sweeteners in the United States, reaching a rate of 42% in the 2000s (2). In recent years, although worldwide intake of added sugars has decreased or stabilized, but represents ~17% (300 kcal or 75 g/day) of total energy intake in the United States, still well above the recommended limit of 10% (3). The mean total consumption of sugar by Koreans from 2008 to 2011 was 61.4 g/day, corresponding to 12.8% of total daily energy intake (4). The sugar consumption of adolescents and young adults was substantially higher, and especially beverage-driven sugar accounted for almost 25% of the total sugar intake.

The increased consumption of sugar and HFCS is associated with obesity, dyslipidemia, insulin resistance, non-alcoholic fatty liver disease, and diabetes (5-7). Moreover, excessive fructose consumption can overwhelm the absorptive capacity of the small intestine, resulting in incomplete absorption, *i.e.*, fructose malabsorption (8, 9). Fructose has the same chemical formula as glucose ($C_6H_{12}O_6$). However, its metabolic effects appear to be more harmful than those of glucose, with excessive fructose intake increasing *de novo* lipogenesis and serum triglyceride levels (10).

Recently, we reported that glucose sensing transcription factor ChREBP (carbohydrate response element-binding protein) plays a pivotal role in the expression of fructose-induced

transporter and the insufficient induction of Glucose transporter 5 (GLUT5) in small intestine leads to fructose malabsorption. In this review, we principally focus on fructose absorption and metabolism in the small intestine, the regulation of ChREBP by fructose, and extend the potential mechanisms by which excessive fructose consumption may contribute to irritable bowel syndrome (IBS).

Fructose absorption and concentrations in the blood

Fructose is primarily absorbed in the small intestine via GLUT5 (encoded by *Slc2A5*: K_m , 6-14 mM), the main fructose transporter in mammals (11, 12) in an energy-independent process, with carrier-limited absorptive capacity (13). GLUT5 is expressed in the apical lumen facing the border of intestinal mucosa and the basolateral enterocyte membranes (14, 15) (Figure 1). The liver is assumed as a primary site for fructose metabolism, but most ingested fructose is initially metabolized by the small intestine. More than 90% of fructose is actively metabolized into glucose and lactate in the small intestine and exported via portal blood (16). From the portal plasma, fructose is then efficiently taken up by the liver with limited amounts escaping the hepatic metabolism and entering the systemic circulation. The changes in fructose concentrations in the luminal, portal and peripheral blood reflect metabolic flux. Consumption of 65% fructose diets by young rats results in 26 mM fructose concentrations in the intestinal lumen (17). Portal and systemic fructose concentrations in rodents and other mammals are typically lower than 0.1 mM when fasting or consuming fructose-free, low-sucrose diets, and increase to ~ 1 mM following consumption of diets containing 40% to 60% fructose (18, 19). Meanwhile, glucose is transported by sodium glucose cotransporter 1 (SGLT1) on the apical membrane from the intestinal lumen into the enterocytes, and further into the portal blood by GLUT2 on the basolateral membrane (20) (Figure 1). The glucose diffuses from the small intestine into the body mostly passively.

Small intestine as the primary organ for fructose metabolism

Most previous studies have shown that the ingested fructose is mainly metabolized in the liver by fructokinase and aldolase B. However, recent evidence shows that the small intestine also expresses fructolytic and gluconeogenic enzymes, similar to liver, and thus plays an important role in fructose metabolism (Figure 2). Upon entering the intestinal epithelial cells via GLUT5, fructose is rapidly phosphorylated to fructose-1-phosphate (F-1-P) by fructokinase (KHK, ketohexokinase). KHK is a unique hexokinase characterized by the absence of feedback inhibition resulting in intracellular phosphate depletion and rapid generation of uric acid due to activation of AMP deaminase (21, 22). Although direct evidence suggesting uric acid synthesis upon fructose feeding in the intestine is unavailable, the small intestine is an important organ for uric acid production (23). KHK exists in two splicing isoforms, KHK-A and KHK-C, with KHK-C representing the major phosphorylating enzyme due to its lower K_m (24). KHK-C is primarily expressed in intestine, liver, kidney, and pancreas, whereas a splicing variant KHK-A is more ubiquitous (25). Fructose metabolism bypasses major regulatory steps in the glucose metabolic pathway: glucose-6-phosphate (G-6-P) generation by hexokinase and fructose-1,6-bisphosphate generation by phosphofructokinase (26). F-1-P is then directly cleaved into three-carbon units, dihydroxyacetone phosphate (DHAP) and glyceraldehyde by aldolase B (27). Glyceraldehyde, unlike the products of aldolase in glycolysis, must be phosphorylated into glyceraldehyde-3-phosphate by triokinase (TrioK). DHAP and glyceraldehyde-3-phosphate are identical to glycolytic intermediates and enter the gluconeogenic pathway for glucose or further catabolized via the lower glycolytic pathway to lactate (28, 29).

The disposition of fructose-derived carbon among the major metabolic pathways depends on the overall nutritional and endocrine status of the animal as well as the status of key regulatory

checkpoints in intermediary metabolism. Fructose is absorbed from the small intestine, where it is phosphorylated and cleaved into three-carbon units, and converted into glucose and organic acids. A recent study using isotope tracing and mass spectrometry has shown that in mice, the small intestine converts ~42%, ~20% and ~10% of the fructose to glucose, lactate, and alanine, respectively (16). Only ~14% of fructose is released into the portal blood by the small intestine. Additionally, ~3% of fructose is converted into other organic acids such as glycerate, TCA intermediates, and amino acids. The passage of unmetabolized fructose through the small intestine to the liver depends on dose rate. Low doses of fructose are nearly entirely cleared by the small intestine, but high doses of fructose overwhelm intestinal fructose absorption and clearance capacity. The extra fructose is digested by the liver and colonic microbiota. Notably, intestinal fructose absorption and metabolism are augmented both by prior exposure to fructose and by feeding (16).

Regulation of ChREBP activity by glucose and fructose

ChREBP is a basic helix-loop-helix leucine zipper (bHLH-ZIP) transcription factor initially discovered as a deleted gene product in Williams-Beuren syndrome, WBSCR14 and later found to recognize the carbohydrate response element (ChoRE) within the promoter of the liver-type pyruvate kinase (LPK) (30, 31). ChREBP forms a heterodimeric complex with Max-like protein X (Mlx) and activates the transcription of ChoRE-containing target genes in response to glucose and fructose (32-34).

ChREBP regulation by glucose

The transcriptional activity of ChREBP is regulated by its phosphorylation status, subcellular localization, glucose metabolites, post-translational modification, and protein stability. ChREBP contains two major cAMP-activated protein kinase (PKA) phosphorylation sites, Ser¹⁹⁶ (P1) and Thr⁶⁶⁶ (P3), which are regulated by cAMP and glucose. When glucose levels

are low, the phosphorylation of ChREBP by PKA inactivates DNA-binding activity, whereas protein phosphatase 2A (PP2A) dephosphorylates P1 site of ChREBP, which results in stimulation of ChREBP import into the nucleus. Following nuclear translocation of ChREBP, glucose signaling activates the transcriptional activity of ChREBP by dephosphorylation of the P3 site by PP2A (31, 35). Under conditions of low energy, AMP-activated protein kinase (AMPK) phosphorylates P1 sites of ChREBP, resulting in its transcriptional inactivation by reducing DNA binding (36, 37).

ChREBP is activated by glucose metabolites, such as G-6-P, xylulose-5-phosphate (Xu-5-P), and fructose-2,6-bisphosphate (F-2,6-BP). The phosphorylation of glucose to G-6-P catalyzed by glucokinase, is the first step of glycolysis and leads to the production of essential metabolites for ChREBP activation. Dentin R *et al.* showed that G-6-P synthesis via over-expression of glucose-6-phosphate dehydrogenase, is required for glucose-induced activation of ChREBP (38). Xu-5-P, a metabolite of the pentose phosphate pathway, activates PP2A, which in turn dephosphorylates P1 and P3 sites of ChREBP and hence, facilitates ChREBP nuclear translocation and activation (39, 40). F-2,6-BP, a key regulator of glycolysis and gluconeogenesis, has been implicated in ChREBP activation. Arden C *et al.* demonstrated that the enzymatic depletion of F-2,6-BP inhibits glucose-induced recruitment of ChREBP to the promoter of target genes (41).

ChREBP has two isoforms, ChREBP- α and ChREBP- β , and each ChREBP isoform complexed with Mlx regulates ChREBP target gene expression (42). ChREBP- α contains a low-glucose inhibitory domain (LID) and a glucose-response activation conserved element (GRACE) (42, 43). The glucose responsiveness of ChREBP is associated with the intramolecular inhibition between LID and GRACE. Under low glucose conditions, the transcriptional activity of GRACE is inhibited by the LID, whereas high glucose conditions release this inhibition (43). In addition, upon glucose stimulation, ChREBP- α is translocated from the cytosol to the

nucleus, whereas ChREBP- β , which lacks LID and is constitutively active, is mainly localized in the nucleus. Glucose-activated ChREBP- α also induces the transcription of ChREBP- β suggesting a feed-forward mechanism in which ChREBP- α is first activated by glucose metabolism and, in turn, stimulates the expression of ChREBP- β , a more potent activator of ChREBP transcriptional targets (42). In addition, ChREBP-interacting proteins, such as 14-3-3 proteins, CRM1, and importins play an essential role in subcellular trafficking of ChREBP in response to variations in nutritional status (44-46).

The transcriptional activity of ChREBP is also modulated via post-translational modification. Bricambert *et al.* showed that glucose-activated histone acetyltransferase (HAT) coactivator p300 acetylates ChREBP and increases its transcriptional activity by enhancing its binding to target gene promoters (47). In addition, high glucose concentrations activate O-linked N-acetylglucosamine transferase (OGT)-induced O-glycosylation of ChREBP and increase DNA binding of ChREBP. The O-glycation of ChREBP is suggested to decrease the ubiquitin-mediated degradation of ChREBP (48, 49).

ChREBP regulation by fructose

Fructose also activates ChREBP transcriptional activity by increased DNA binding, phosphorylation via Xu-5-P, O-glycosylation and acetylation (41, 50, 51). Recently, Kim *et al.* showed that high fructose diet (HFrD) markedly induces hepatic and intestinal ChREBP- β expression, followed by increased glycolytic, fructolytic, and lipogenic gene expression (52, 53). We also showed that the intestinal protein but not mRNA levels of ChREBP were increased by HFrD, accompanied by increased fructolytic and gluconeogenic gene expression (54).

ChREBP target genes

ChREBP is highly expressed in key metabolic tissues, including liver, adipose tissue, small intestine, kidney, and pancreatic β -cells, where it regulates carbohydrate metabolism in an

insulin-independent manner (55, 56). Most of ChREBP targets have been identified in hepatocytes, adipocytes, and pancreatic β cells, where it contributes to the regulation of glucose sensing and/or *de novo* fatty acid synthesis. Since its discovery, ChREBP has proven to be a key regulator of genes involved in glycolysis and pentose phosphate pathway (*Glut2*, *Pklr*, *G6pdh*), lipogenesis (*Acaca*, *Fasn*, *Scd1*, *Elovl6*), and triglyceride formation (*Gpdh*, *Dgat2*). ChREBP also regulates some genes involved in fructolysis (*Khk*, *Aldolase B*, *Triokinase*), gluconeogenesis (*G6pase*, *Fbp1*), and hormone and hormone receptors (fibroblast growth factor 21, glucagon receptor, and adiponectin receptor 2) (34, 55, 57-63). ChREBP was first identified as a transcriptional activator of their target genes, although ChREBP has been shown to repress target genes directly (55, 57, 64, 65).

Although ChREBP is primarily a glucose-responsive factor, recent observations in different animal models suggest a crucial role in fructose metabolism and metabolic disease. In rats, high-fructose feeding, when compared with glucose, was associated with increased ChREBP activity and the expression of its target genes (50). Accordingly, mice fed with HFrD showed an increase in hepatocellular carbohydrate metabolites, expression of ChREBP target genes, and hepatic steatosis, due to hepatic ChREBP activation (52). Moreover, ChREBP is essential for fructose-induced increases in circulating FGF21, which is required for a normal hepatic metabolic response to fructose consumption, and the absence of FGF21 leads to liver disease in mice exposed to HFrD (66).

Intestinal ChREBP as an essential mediator for fructose tolerance

In the intestine, fructose rapidly induces genes involved in its uptake and catabolism. GLUT5 plays a major role in regulating the fructose entry in our body. Deletion of GLUT5 reduces intestinal fructose absorption as well as serum fructose concentration by >80% (67). GLUT5 mRNA and protein abundance are markedly increased within a few hours of fructose

consumption, resulting in enhanced fructose absorption (14, 67). Patel *et al.* showed that fructose absorption via GLUT5 and KHK-mediated fructose metabolism are required for the regulation of intestinal fructolytic and gluconeogenic enzymes (68). The feedforward regulation of fructolytic and gluconeogenic enzymes may proactively enhance the intestinal ability to process the anticipated increases in dietary fructose concentrations. It has been reported that ChREBP directly upregulates hepatic *Khk* following high fructose intake (51). Recently, we have reported that ChREBP is a key transcription factor in fructose-mediated upregulation of fructose transporter (*Glut5*), fructolytic enzymes (*Khk*, *AldoB*, *TrioK*, *Ldh*), and gluconeogenic enzymes (*G6pc* and *Fbp1*) in the intestine (Figure 3) (54). We demonstrated that ChREBP directly regulates *Glut5* gene expression through functional ChREBP-ChoRE (-2165 - 2149bp) interaction(54).

The significant role of ChREBP in fructose metabolism is supported by the observations of rapid weight loss, hypothermia, and moribund state observed in global ChREBP knockout mice exposed to diets containing sucrose or fructose (54, 55). The diminished expression of enzymes required for fructose entry into glycolysis, such as aldolase B and KHK in the liver of ChREBP KO mouse was proposed as the reason for the fructose intolerance (52, 56). However, recent reports suggest that intestinal, but not hepatic ChREBP is essential for fructose tolerance (53, 54, 69). ChREBP knockout mice are fructose intolerant mainly due to a decreased capacity for fructose absorption following insufficient induction of GLUT5 upon high fructose ingestion (Figure 3). As a result, a high concentration of fructose in the gut leads to water influx into the lumen due to osmotic pressure, and rapid propulsion of bowel contents into the colon. Colonic bacterial fermentation of unabsorbed fructose may generate gas, bloating, or diarrhea and results in decreased food intake.

ChREBP deficiency leads to fructose malabsorption and irritable bowel disease

IBS, one of the most frequent functional gastrointestinal disorders (FGIDs), is characterized by symptoms of abdominal pain, bloating, and altered bowel habits, which include diarrhea, constipation, or both. The pathophysiology of IBS remains incompletely understood, but factors such as altered gastrointestinal (GI) motility, abnormal sensory function of the intestine, malabsorption of food products, bacterial overgrowth in the small intestine and psychosocial factors may play a role (70). In normal physiology, fructose is absorbed across the intestinal mucosa by facilitated diffusion via GLUT5, and its absorption capacity is limited (71). Therefore excess dietary intake of fructose can quickly overwhelm the absorptive capacity of the small intestine, leading to incomplete absorption and resulting in fructose malabsorption (72). Fructose malabsorption, previously described as dietary fructose intolerance, was first described in 1978 based on a breath test report of fructose malabsorption (73) and later described in IBS patients (74, 75). The unabsorbed fructose represents an osmotic load propelling the sugar into the colon, where contact with anaerobic microbiota causes fermentation and production of gas, abdominal bloating, and diarrhea (76). However, the precise pathophysiology leading to fructose malabsorption is not clear. Barone S *et al.* have previously shown that HFrD-fed GLUT5 knockout mouse model exhibits a decreased absorption of dietary fructose, with consequent distension of the large intestine with fluid and gas. The model presented the hallmarks of fructose malabsorption (67). Recently, we and others demonstrated that HFrD-fed ChREBP knockout mice exhibit a severely distended cecum, with proximal colon containing both gas and fluid content, and severe diarrhea, suggesting incomplete fructose absorption (53, 54, 69).

CONCLUSIONS AND PERSPECTIVES

In this review, we summarized the role of ChREBP in fructose absorption and metabolism, particularly in the small intestine, discussed recent findings involving ChREBP knockout mice,

manifesting diarrhea-predominant IBS symptoms following exposure to HFrD. The underlying pathophysiology of IBS is complex and has yet to be fully understood. The animal model facilitates the investigation of the pathogenesis of IBS without the risks associated with a human study. Until now, the exact mechanism responsible for IBS was limited by the lack of animal models with high face validity. The generation of IBS animal model is mostly based on induction of psychological stress. Thus, our recent study of ChREBP knockout mice fed with HFrD may represent an appropriate model for the understanding of the mechanisms of IBS, particularly diarrhea-predominant IBS caused by fructose malabsorption. Furthermore, investigations into the changes associated with the intestinal microbiota may elucidate the pathophysiology of IBS and provide a new therapeutic strategy.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1: Models for glucose and fructose transport across the intestinal epithelium.

Glucose is transported into the enterocyte across the brush border membrane by the sodium glucose cotransporter 1 (SGLT1) and released across the basolateral membrane down the

concentration gradients by glucose transporter 2 (GLUT2). The Na⁺/K⁺ pump in the basolateral membrane maintains the functional gradient of SGLT1. Fructose is transported through the brush border membrane and extruded basolaterally by facilitated diffusion via GLUT5.

Figure 2: Fructose metabolism in the small intestine. Fructose is efficiently metabolized by ketohexokinase (KHK) into fructose-1-phosphate (F1P). F1P is then cleaved by aldolase B (AldoB) into dihydroxyacetone phosphate (DHAP) and glyceraldehyde. Glyceraldehyde is phosphorylated by triokinase (TrioK) to provide glyceraldehyde 3-phosphate (GAP). The triose phosphates derived from fructolysis are resynthesized into glucose via gluconeogenesis or further metabolized into lactate or acetyl-CoA, which are oxidized or used for lipogenesis.

Figure 3: Model showing the effects of ChREBP deletion on fructose transport and metabolism upon high-fructose diet consumption Upon high fructose ingestion, genes involved in fructose transport (*Glut5*), fructolysis (*Khk*, *AldoB*, *Triok*, and *Ldh*), and gluconeogenesis (*G6pc* and *Fbp1*) are significantly increased in the intestinal cells. However, ChREBP knockout mice show impaired induction of GLUT5 as well as fructolytic and glucogenic enzymes in the intestine. As a result, HFrD-fed ChREBP knockout mice develop diarrhea-dominant irritable bowel symptoms, such as gas, bloating, abdominal pain, or diarrhea. Genes significantly increased by high fructose in WT mice but not in ChREBP knockout mice are indicated in red color. ALDOB, aldolase B; ChREBP, carbohydrate response element-binding protein; DHAP, dihydroxyacetone phosphate; F1P, fructose-1-phosphate; F-1,6-BP, fructose-1,6-bisphosphate; FBP1, fructose-1,6-bisphosphatase; GA, glyceraldehyde; GAP, glyceraldehyde-3-phosphate; GLUT2, glucose transporter 2; GLUT5, glucose transporter 5; G-6-P, glucose-6-phosphate; G6PC, glucose-6-phosphatase; KHK, fructokinase; LDH, lactate dehydrogenase; TrioK, triokinase.

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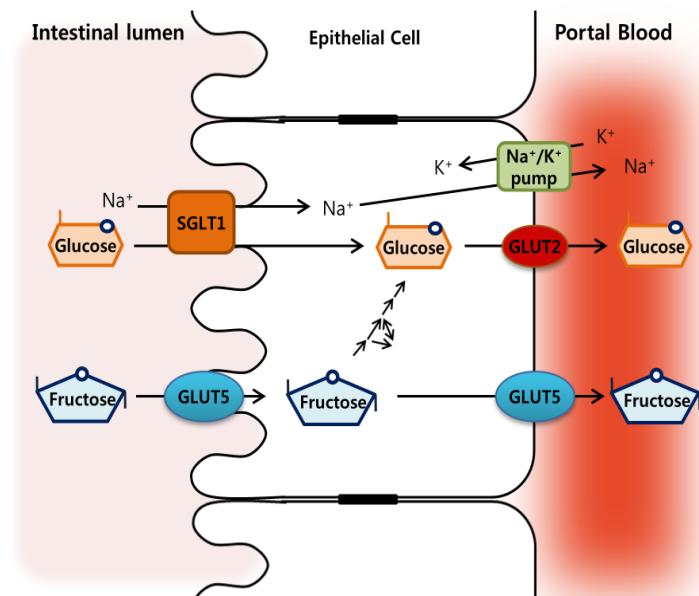
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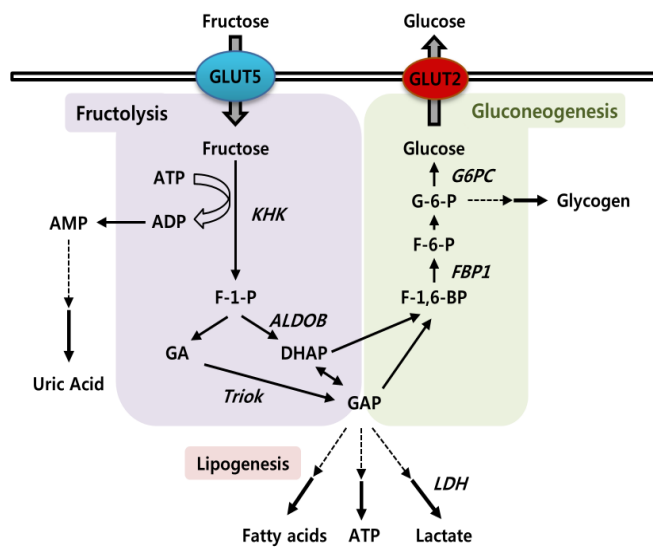
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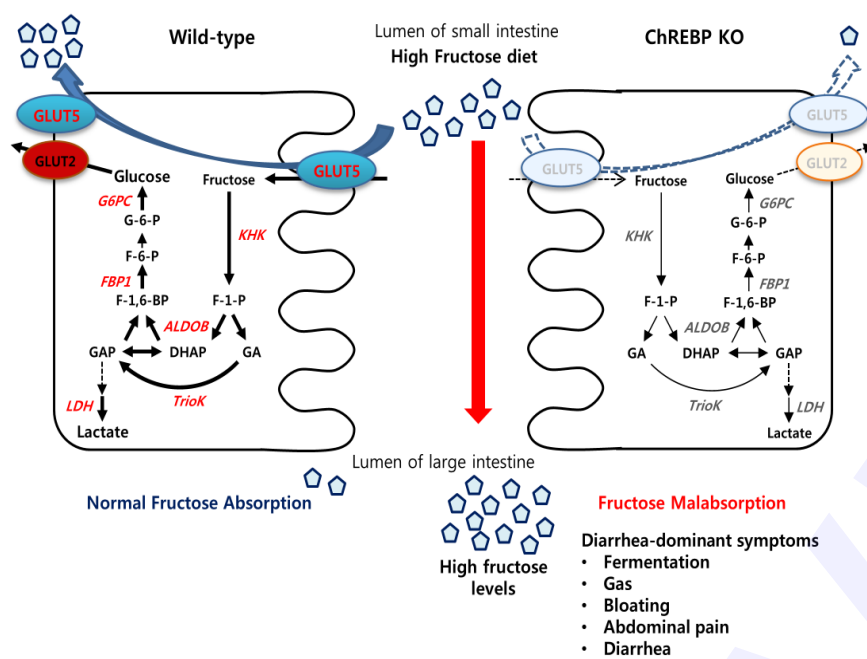
HJ Lee & JY Cha. BMB Reports, Figure 1

Fig. 1.



HJ Lee & JY Cha. BMB Reports, Figure 2

Fig. 2.



HJ Lee & JY Cha. BMB Reports, Figure 3

Fig. 3.