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The Effect of Butyric Acid on Gene Expression of GLUT2 and IRS1 on Human Hepatocytes *in vitro*

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Abstract Diabetes mellitus type 2 (DMT2) affects hundreds of millions of people globally and costs billions of dollars each year. The importance of research toward prevention and treatment of this disease cannot be overestimated. Butyric acid is a short chain fatty acid that has been shown in mice and in intestinal cell culture studies to increase insulin sensitivity at the level of gene expression. However, little or no work has been reported on its effects on human liver cells. The present study determined the changes in gene expression of glucose transporter 2 (GLUT2) and insulin receptor substrate 1 (IRS1) on insulin shocked THLE-2 human liver cells exposed *in vitro* to the following concentrations of butyric acid in mg/ml: 0.05, 0.1, and 1.0. GLUT2 and IRS1 had increases in expression at doses of butyric acid previously found to be nontoxic in human serum. This work indicates that more studies involving the effects of butyric acid on gene expression of insulin resistant human hepatocytes are warranted.

Keywords GLUT2, IRS1, Diabetes Mellitus Type 2, Butyric Acid, THLE-2 Cells

1. Introduction

The World Health Organization projects that diabetes mellitus will be the seventh leading global cause of death by 2030 [1]. This disease already contributes to billions of dollars spent, with 80% of deaths from diabetes mellitus occurring in low and middle income countries where this money is not readily available. 90% of diabetes mellitus cases are type 2, which is most commonly caused by extended periods of physical inactivity and being overweight. This form of the disease is best treatable by physical activity, maintaining body weight, and eating a healthy diet.

One component of a healthy diet that shows great promise in preventing and treating diabetes mellitus type 2 (DMT2) is butyric acid. This short chain fatty acid is found naturally in milk and butter, but can also be fermented in the intestines by genera of bacteria such as Roseburia, Faecalibacterium, and Eubacterium in people who consume diets high in fiber [2-5]. Increased levels of butyric acid in the intestine have been correlated with increased insulin sensitivity in research involving mice, human tissue culture, and in human studies directly.

Gao *et al.* [6] fed C57BL/6J mice sodium butyric acid, along with a high fat diet, and determined that dietary butyric acid improved insulin sensitivity and reduced adiposity. These mice showed an increase in mitochondrial function with elevated adenosine monophosphate (AMP) kinase and p38, as well as stable levels of blood glucose and insulin levels. This study indicated that butyric acid can have affects at the level of gene expression.

In a study involving tissue culture, butyrate stimulated intestinal gluconeogenesis in human colorectal adenocarcinoma Caco-2 cells with coinciding increases in glucose-6-phosphatase, catalytic subunit (G6PC) and phosphoenolpyruvate carboxykinase 1 (PCK1) gene activities [7]. Intracellular cyclic AMP also increased and it was hypothesized that its activity was likely responsible for the increases in activities of PCK1 and G6PC since the activity of one gene can affect others. As this barely touches the surface of the many genes involved in insulin pathways and glucose metabolism, this study indicates that there is much more to be understood as to the effects of butyric acid on genes of various cell types involved in the metabolism of glucose.

In humans with decreased insulin response, bacteria such as *Roseburia intestinalis* and *Eubacterium hallii* correlated with increased butyrate levels and a coinciding improvement of insulin sensitivity when they received donations of intestinal bacteria from lean, insulin-sensitive donors [8,9]. This study shows a direct correlation between intestinal bacteria, butyrate levels, and insulin response. Although some work has been done to determine the effects of butyric acid on insulin sensitivity in mice, human tissue culture, and in humans directly, more comprehensive work is necessary to assess the effects of butyric acid on pathways of gene expression that impact insulin sensitivity and energy metabolism in humans and to determine what positive effects there may be from this

dietary fatty acid on DMT2.

The aim of this research was to determine changes in gene expression of GLUT2 and IRS1 on insulin shocked THLE-2 human liver cells exposed *in vitro* to various concentrations of butyric acid. GLUT2 transports glucose into and out of hepatocytes through facilitated diffusion, with various sugars and hormones affecting its activity in addition to glucose and insulin [10,11]. IRS1 has been shown to play a role in the development of insulin resistance in liver cells along with mitochondrial dysfunction [12]. It follows that substances that affect the expressions of GLUT2 and IRS1 in liver cells may have an impact on increasing insulin sensitivity. Determining the effects of dietary components, such as butyric acid, on these and other genes involved in molecular pathways of insulin sensitivity and glucose metabolism can provide insight into the mechanisms involved in preventing and controlling DMT2.

2. Materials and Methods

2.1. Cell Culture

THLE-2 human liver cells (CRL-2706) were purchased from American Type Culture Collection (ATCC) and cultured in precoated flasks in bronchial epithelial cell growth medium (BEGM) with additives (Lonza), as specifically recommended by ATCC in 37°C, 5% CO_2 incubator [13]. Medium was renewed every 2 to 3 days and subcultured as needed.

2.2. Insulin Shocking and Exposure of Cells to Butyric Acid

1.25×10^5 cells per well were seeded in a 96 well plate and insulin shocked with 5.6×10^{-4} mg/ml insulin (Sigma) and 4.5 mg/ml glucose (Fisher Scientific) in fortified BEGM for 24 hours [14]. Insulin shock suspensions were removed and replaced with 2.5×10^{-8} mg/ml insulin and 2.1 mg/ml glucose along with the following concentrations of butyric acid (Sigma), each group in triplicate for 24 hours: 0 mg/ml (control group), 0.05 mg/ml, 0.1 mg/ml, 1.0 mg/ml.

2.3. RNA Extraction and Production of cDNA

Suspensions were removed and RNA was extracted from cells using the RNeasy Mini Kit (Qiagen) and converted to complementary DNA (cDNA) using the RT^2 First Strand Kit (Qiagen).

2.4. Quantitative PCR and Analysis

Quantitative polymerase chain reaction (PCR) was performed using the Applied Biosystems 7300 real-time PCR system with RT^2 SYBR Green Mastermix (Qiagen) and GLUT2 (RefSeq accession number NM_000340.1) and IRS1 (RefSeq accession number NM_005544.2) primers (Qiagen). PCR settings were as follows: 1 cycle at 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Fold changes in gene expression for each group compared to the control group were determined using the $\Delta\Delta C_t$ method (Qiagen) [15].

3. Results

After cells were insulin shocked and exposed to the various concentrations of butyric acid, the GLUT2 and IRS1 genes were upregulated for all concentrations of butyric acid when compared to cells not exposed (Table 1). The activity of the GLUT2 gene was upregulated 3.0-fold at 0.05 mg/ml, 4.1-fold at 0.1 mg/ml, and 9.1-fold at 1.0 mg/ml ($p < 0.01$). The activity of the IRS1 gene was increased 15.8-fold at 0.05 mg/ml, 9.3-fold at 0.1 mg/ml, and 1.8-fold at 1.0 mg/ml ($p < 0.01$).

Table 1. Fold-changes in gene expression for glucose transporter 2 (GLUT2) and insulin receptor substrate 1 (IRS1) for each concentration of butyric acid (mg/ml) compared to the control

	Butyric acid concentrations (mg/ml)		
Genes:	**0.05**	**0.1**	**1.0**
GLUT2	3.0	4.1	9.1
IRS1	15.8	9.3	1.8

4. Discussion

Results of this study suggest that butyric acid plays a role in increasing sensitivity in insulin resistant human liver cells by affecting the expression of key genes in the insulin pathway. Insulin shocked human liver cells had increases in expression of GLUT2 and IRS1 genes for all concentrations of butyric acid when compared to the control (Table 1). GLUT2 had increasing expressions with increasing concentrations of butyric acid. IRS1 had its greatest increase in expression at the lowest concentration of butyric acid tested, with a smaller increase in expression at the highest concentration of butyric acid tested. There are numerous genes in the insulin pathway, which interrelate in a complex manner. Butyric acid likely effects genes downstream from IRS1, which may explain why this fatty acid had greater effects on the expression of IRS1 at lower concentrations than at high concentrations. A key point, however, is that both genes were upregulated when cells were exposed to a level of butyric acid similar to that previously found in serum to be tolerable to humans [16]. Conley *et al.* (1998) showed that plasma butyrate levels of 0.04 mg/ml were nontoxic to human subjects.

Results of this study of the effects of butyric acid on human liver cells *in vitro* are consistent with previous studies in mice and in human tissue culture that butyric acid improves insulin sensitivity at the level of gene expression [6,7]. How butyric acid affects each gene in the insulin pathway in liver cells has

not yet been determined. However, it has been suggested that butyric acid works to increase insulin sensitivity by promoting energy expenditure in mice by inducing mitochondrial function in skeletal muscle and brown fat [6]. It has also been shown to regulate fatty acid metabolism as well as electron transport and oxidative stress pathways in human colonic mucosa [17]. Another study found that the greatest overall effect of butyrate on bovine kidney cells was on cell cycle control while a study on human colonic epithelial cells indicated that effects of butyric acid were opposite of those reported on colon cancer tissue [18,19].

While some work has been done on the effects of butyric acid on various cell types, more extensive molecular research will provide additional insight into how butyric acid improves insulin response in patients with DMT2. It has been established that there are many genes involved in the intracellular pathway affected by insulin, including influencing glycolysis once glucose has entered cells through facilitated transport, increasing glycogen and fatty acid synthesis, and decreasing gluconeogenesis [20-22]. Each part of the insulin pathway involves numerous genes, with GLUT2 and IRS1 being two of the primary genes involved. GLUT2 facilitates transport of glucose across the membrane in hepatocytes, and in mice this has been found to be an insulin receptor-mediated form of glucose regulation [23]. Other genes are dependent upon GLUT2 activity as it has been shown by live imaging in kidney epithelial cysts that GLUT2 inhibition correlates with protein kinase C inhibition. It has been shown in primary mouse hepatocytes that GLUT2 is upregulated by sterol response element-binding protein 1c binding to the GLUT2 promoter [24,10]. Further, IRS1 has been shown in obese mice to be phosphatidylinositol 3-kinase dependent while in SK Hep1 hepatocytes IRS1 was found to be part of the mechanism of insulin resistance induced by mitochondrial dysfunction [25,12]. Further work involving the effects of this fatty acid on each of these and other genes in the insulin pathway would provide more insight into the overall impacts butyric has on insulin sensitivity and glucose utilization within cells.

5. Conclusions

This study showed that butyric acid increases the gene expression of both IRS1 and GLUT2 in insulin resistant human liver cells *in vitro.* This further supports previous research that this fatty acid increases insulin sensitivity in insulin resistant cells. Additional understanding of the effects of butyric acid at the gene level of a variety of cell types could aid in the prevention and treatment of DMT2.

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Conflicts of Interest

There are no conflicts of interest.

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