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Effects of mitochondrial NADP+-dependent isocitrate dehydrogenase deficiency on fructose-induced obesity in mice

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II. Abstract

Obesity prevalence in the United States continues to increase and is associated with health consequences such as type 2 diabetes, hypertension, atherosclerosis, and hyperlipidemia. Among many contributing factors to obesity, fructose may be one of the major reasons as it disrupts the antioxidant system thereby resulting in an accumulation of reactive oxidative species and leading to obese conditions. The enzyme, isocitrate dehydrogenase 2 (IDH2), reduces nicotinamide adenine dinucleotide phosphate from the TCA Cycle, hence might be implicated with not only energy metabolism but also cellular redox homeostasis. Therefore, the hypothesis was that IDH2 deficiency in mice would exacerbate hepatic lipid metabolism in response to fructose intervention. The study consisted of a total of 24 IDH2 knockout female mice and their background strain (C57BL/6N) mice; the animals were assigned to either fructose or control diet group. After intervention of fructose over six weeks (34%; v/v), tissue weight, liver expression of lipogenesis gene (*SREBP-1*, *SCD1*, *FAS*, and *DGAT2*), and liver expressions of lipolysis genes (*AMPK*, *SIRT1*, and *PPARa*) were measured. There was a significant increase in visceral fat, while the body mass remained the same. Further, there was a trend of increase in expression of lipogenesis genes, whereas no change was shown in lipolysis gene expression in IDH2 mice fed fructose. In conclusion, even though changes in visceral fat mass were statistically significant in IDH2 deficient mice, hepatic lipid metabolism did not support the phenotypes in this study. Additional studies with larger sample size are warranted to find a potential link between IDH2 and increase in visceral fat accumulation.

III. LITERATURE REVIEW

Obesity Prevalence and Chronic Illness Association

Obesity is a state of excess accumulation of fat tissue, which occurs through an increase of size in adipocytes or increase of fat cells. It can be caused by an imbalance between energy intake and expenditure (1). Overweight and obesity have become a major problem in multiple Western countries since the 1950s, and their prevalence has been increasing steadily (2). Indeed, in the past 35 years, obesity prevalence has doubled worldwide and, in the United States, there is an obesity prevalence of 38.3% for women and 34.3% for men (3). Interest in the prevention of obesity has been on the rise since it is one of the most important risk factors for other diseases such as hypertension, type 2 diabetes, hyperlipidemia, and nonalcoholic fatty liver disease (NAFLD) (4). For example, the prevalence rate of NAFLD increases with the elevation of body mass index, a marker of obesity (5). Additionally, the medical cost of overweight-related diseases such as obesity or type 2 diabetes has tripled since the 1980s (6). Knowing the medical implications of obesity, it is critical to understand what factors play a role in causation in the development of obesity and abnormal increase of adipocytes.

Obesity Risk Factors

Factors implicated in the development of obesity include educational level, obesity trends within families, the promotion of a sedentary, high-fat, and high-sugar intake. According to the National Health and Nutrition Examination Survey (NHANES), as women's educational level decreased, the trend for obesity increased. Additional risk factors for obesity are social determinants. Socioeconomic conditions (e.g., concentrated poverty) also play a part in obesity

trends. Examples are residential segregation, access to health care services, transportation options, availability of resources to meet daily needs, and social support (7). Not surprisingly, genetics and ethnicity play a major role in obesity prevalence as well. For instance, according to the NHANES study, the prevalence of obesity was lowest among non-Hispanic Asian adults (11.7%), followed by non-Hispanic white (34.5%), and Hispanic (42.5%). Non-Hispanic black (48.1%) adults had the highest level of obesity rates (7).

As expected, diet also plays a huge role in an individual developing obesity. At the surface level, a high ratio of caloric input verse caloric output will cause an increase in weight. Consuming more calories than the individual expends will cause an increase in adipocyte size, leading to obesity. Additionally, the type of diet one consumes plays a factor into obesity. A high-fat and high-sugar based diet, along with excess calories and poor physical activity, will lead to a gain in weight and eventually obesity. Common food tendencies that fall into this category include meals eaten out, processed or fast foods, and sugary drinks (8).

Beverages containing high-fructose corn-syrup are the most common sugary drinks in United States today. In 2011-2014, 63% of youth and 49% of adults drank sugary drinks on any given day (9). On average, U.S. youth consume 143 calories from sugary drink and U.S. adults consume 145 calories from sugary drinks on a given day (9). High fructose corn-syrup is a commonly used substitute for sucrose, and is found in beverages, baked goods, canned fruits, and dairy products. High fructose corn-syrup is the major source of calories in these products, and therefore high fructose corn-syrup is the nation's major source of artificial sweetener and dietary fructose (10). Knowing the close correlation between obesity and chronic illnesses, it is beneficial to look into factors that contribute to increasing obesity rates, particularly fructose given its predominant use in processed foods.

Health Implications of Fructose

Diets high in sugar may promote the development of metabolic diseases. Recent epidemiological studies have strengthened pre-clinical and clinical observations; for instance, Mattei et al., showed that consumption of sugar sweetened beverages (e.g., instant sugary powder mix beverages and carbonated non-diet beverages) increased plasma triglycerides, waist circumference, and metabolic syndrome (11). Similarly, increased frequency of sugar sweetened beverage intake was positively associated with increased proportion of visceral to subcutaneous abdominal adipose tissues (12). Particularly, fructose is often added in processed foods such as soft drinks, canned fruits, and dairy products (1), and its intake over the years has increased thus it may be a major contributor for the increase in the overweight and obese population (12). Not limited to obesity per se, chronic diseases (e.g., NAFLD, type 2 diabetes, and hyperglycemia) are also associated with a high-fructose, high-fat diet (13). In particular, fructose is known to induce hypertension (14) and, potentially, lipogenesis (15) hence increasing the potential for the development of hyperlipidemia and cardiovascular diseases.

There are several mechanisms proposed with regard to fructose and its implications to chronic diseases. To be specific, fructose directly affects lipid and carbohydrate metabolism and indirectly promote positive energy balance, thereby resulting in dysregulation of energy homeostasis (16). Fructose is transported into cells via GLUT5, which is highly expressed in the liver, small intestine, and pancreas (15); this transporter is specific to fructose and the aid of digestion (20). Hepatocytes have high fructose extraction rates thus, virtually an entire ingested fructose molecule can be metabolized in the liver (21). In the hepatocytes, fructose is phosphorylated to fructose-1-phosphate, initiating the breakdown of fructose (also known as fructolysis), eventually forming pyruvate and subsequently acetyl-CoA, a primary substrate for

lipogenesis (22). While glucose and fructose metabolism both yield acetyl-CoA, glucose-derived acetyl-CoA is tightly regulated by insulin (21) and by feedback inhibition of glycolytic flux by adenosine triphosphate (ATP) generated from glycolysis (22). In contrast, acetyl-CoA generation via fructolysis continues uninterrupted, and is not ATP-sensitive (22). Thus, unabated fructolysis leads to unabated lipogenesis (15, 22).

An additional mechanism of fructose-mediated obesity is closely related to a disruption of the antioxidant defense system (17). In this, antioxidant enzymes are susceptible to post-translational modification (e.g., glycation) in which kinetic activities can be inhibited [e.g., nicotinamide adenine dinucleotide phosphate ($NADP^+$)-dependent isocitrate dehydrogenase, (IDH2); (18)]. Therefore, it is possible that fructose-induced glycation of enzymes may lead to the perturbation of the cellular antioxidant defense system and accumulation of reactive oxygen species (ROS), subsequently resulting in obesity conditions. In that regard, nicotinamide adenine dinucleotide phosphate, reduced (NADPH) is critical because oxidized glutathione (i.e., endogenous cellular antioxidant) is regenerated via NADPH-consuming glutathione reductase and peroxidase systems (19).

Summary and Study Hypothesis

Fructose may be one of the major factors for high prevalence of obesity in the United States due to disruption of hepatic lipid metabolism via multiple mechanisms. Particularly, it causes post-translational modification of key metabolic enzymes including IDH2; this mitochondrial enzyme catalyzes the oxidative decarboxylation of isocitrate into α-ketoglutarate with concurrent reduction of $NADP^+$ to NADPH. Since NADPH is used as a cofactor of glutathione reductase to maintain the redox systems, impaired IDH2 activity, either by

environmental (fructose) or genetic mutation (e.g., single nucleotide polymorphism), may disrupt hepatic lipid metabolism. Therefore, it is hypothesized that lack of IDH2 may exacerbate dysregulation of hepatic lipid metabolism that is induced by fructose exposure.

IV. MATERIALS AND METHODS

Animals and study diets

A total of 24 female IDH2 knockout (KO) mice and their background strain (C57BL/6N) mice [wild type (WT)] were assigned into either a fructose group or a control group: WT control group (WT CON, n=6), WT fructose group (WT FRU, n=6), IDH2 KO control group (IDH2 CON, n=6), and IDH2 KO fructose group (IDH2 FRU, n=6). The IDH2 CON and IDH2 FRU mice study was carried out at Gyeongnam National University of Science and Technology (Jinju, South Korea) and all harvested tissues were shipped to the United States. The WT CON and WT FRU mice study was carried out at The University of Arkansas (Fayetteville, Arkansas). Both FRU groups were subjected to 34% fructose solution for six weeks to induce obesity phenotypes as previously described and validated (16). Control group animals (WT CON and IDH2 CON groups) were maintained with deionized water. AIN-76A purified diet was used as a control diet and Table 1 shows the detailed dietary composition of the AIN-76A purified diet. All animal handling and experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Arkansas (Protocol Approval Number: 17044).

Measurement of body weights, tissue weights, and fat mass

Body weight was measured once a week, during the six weeks of intervention. After six weeks of intervention, the mice were killed by exsanguination via cardiac puncture under anesthesia using 2, 2, 2-tribromoethanon (Sigma-Aldrich, St. Louis, MO). Liver, white adipose tissue (visceral fat depot) and subscapular brown adipose tissue were harvested and stored in *RNALater* solution (Invitrogen, Carlsbad, CA) at -80°C until analyzed.

RNA extraction and cDNA synthesis

Total RNA was isolated using the *RNeasy Plus Mini* kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. Briefly, total RNA was extracted from 20-30 mg of tissues in 0.6 mL lysis buffer containing 1% β-mercaptoethenol. Genomic DNA residues were eliminated using the genomic DNA eliminator spin column in the kit. Extracted RNA was precipitated using one volume of 70% ethanol, and then collected onto an *RNeasy* spin column. Collected total RNA was washed with a series of washing buffers. Finally, purified RNA collected on the spin column membrane was eluted using 30 µL nuclease-free water. The quality of extracted RNA was assessed using the conventional A260/280 ratio and A260/230 ratio measurement for spectrophotometric (SpectraMax i3x; Molecular Devices, Sunnyvale, CA). Isolated RNA (2 µg) was then reversely transcribed using the *High Capacity cDNA Reverse Transcription* kit (Applied Biosystems, Foster City, CA) for the cDNA synthesis per the manufacturer's instruction. The cDNA synthesis was performed under the following conditions: one cycle at 25°C for 10 min, cycle two at 37°C for 120 min, followed by cycle three at 85°C for 5 min, and cycle four at 4° C for 1 hr. The cDNA samples were then stored at -80°C until analyzed.

Quantitative Real Time PCR Analysis for Lipogenic mRNA Expression

The cDNA was used as a template for the relative quantitation of the following lipogenic target genes with predesigned *TaqMan* gene expression assay kits: sterol regulatory element-binding protein-1 (*SREBP-1*), fatty acid synthase (*FAS*), stearoyl-CoA desaturase 1 (*SCD1*), and diglyceride acyltransferase 2 (*DGAT2*). All reactions were performed in triplicate with the StepOnePlus system (Applied Biosystems) in a reaction mixture containing *TaqMan Gene Expression Mastermix*, primers with *TaqMan* probe (TaqMan Gene Expression Assay; Applied Biosystems), and cDNA. Each 20 μL reaction contained 100 ng of cDNA, 2× *TaqMan Gene Expression Master Mix*, and forward and reverse primers with *TaqMan* probes. Amplification was conducted under the following conditions: one cycle at 50°C for 2 min, and 95°C for 10 min, followed by 40 cycles of denaturation (95°C for 15 s) and annealing (60°C for 1 min). Genes of interest were normalized to that of reference genes. Data was analyzed with Step One Software (Ver. 2.1; Applied Biosystems) using the ΔΔCT method.

Quantitative Real Time PCR Analysis for Lipolytic mRNA Expression

Expression of mRNAs was measured by quantitative real time RT-PCR analysis using the StepOnePlus system (Applied Biosystems) in a reaction mixture containing *TaqMan Gene Expression Mastermix*, primers with *TaqMan* probe (TaqMan Gene Expression Assay; Applied Biosystems) and cDNA. Briefly, the cDNA was amplified using specific primers for the following lipolytic target genes: AMP-activated protein kinase alpha (*AMPKα*), sirtuin 1 (*SIRT1*), and peroxisome proliferator-activated receptor-alpha (*PPAR-α*). Each 20 μL reaction contained 100 ng of cDNA, 2× *TaqMan* Gene Expression Master Mix, forward and reverse primers with *TaqMan* probe. Amplification was conducted under the following conditions: one

cycle at 50°C for 2 min, and 95°C for 10 min, followed by 40 cycles of denaturation (95°C for 15 s) and annealing (60°C for 1 min). Genes of interest were normalized to that of reference genes. All samples were analyzed in triplicate.

Statistical analysis

All results are expressed as least squares mean ± standard error of means. Gene expression data were expressed as relative quantity to the value of WT CON group. Differences between the groups were tested using one-way ANOVA followed by multiple comparisons using the difference matrix of least squares mean (SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically different.

V. RESULTS

Effects of IDH2 Deficiency on Visceral Fat Depot

Body weight, weight gain, and a ratio of tissue weight to body weight were used as phenotypic markers of fructose-induced obesity. There was no difference in weight gain throughout the study period ($p > 0.05$; Figure 1). In spite of no differences in body weight and weight gain, a ratio of visceral fat mass to body weight was significantly larger in the IDH2 KO groups than those of the WT groups (WT CON vs IDH2 CON, $p = 0.04$; WT FRU vs IDH2 FRU, $p = 0.0008$; Figure 2). No changes were observed in the ratio of brown fat mass to body weight ($p > 0.05$ for all). There was a significant decrease in the ratio of liver weight to body weight in IDH2 KO groups (WT CON vs IDH2 CON, $p = 0.001$; WT FRU vs IDH2 FRU, $p = 0.018$; Figure 2). In addition, there was no significant influence of fructose in all parameters

between IDH2 CON and IDH2 FRU (i.e., body weight, weight gain, and tissue weight; Figure 2).

Effects of IDH2 Deficiency on Lipogenesis Genes in Liver

Expression levels of key lipogenic genes in liver were measured. Figure 3 shows the effects of IDH2 KO on *SREBP-1*, *SCD1*, *FAS*, and *DGAT2* mRNA in the liver tissue of mice fed with fructose-supplemented water. Overall, the expressions of all four lipogenic genes in IDH2 CON groups showed no statistical significance when compared to the WT groups, due to large variation ($p > 0.05$ for all; Figure 3). The expression of *SREBP-1*, however, was higher in IDH2 FRU group compared to the IDH2 CON group ($p = 0.0148$). The expression of *SCD1* in fructose groups was also remarkably higher compared to those of respective control groups (WT CON vs WT FRU, $p = 0.003$; IDH2 CON vs IDH2 FRU, $p = 0.02$). Moreover, there was a trend of increased *FAS* expression in WT FRU group when compared to WT CON group ($p = 0.0627$; Figure 3). The *DGAT2* expression was not found to be statistically significant when the fructose groups were compared to control groups ($p = 0.27$). However, IDH2 KO groups did not show change in expression levels of the genes compared to those of WT groups ($p > 0.05$).

Effects of IDH2 Deficiency on β-oxidation Genes in Liver

In order for further investigation of potential mechanisms underlying the phenotypes, expression of genes involved in the fatty acid β-oxidative pathway were examined by quantitative PCR analysis. There was no difference in the *AMPKα* expression among the groups ($p > 0.05$; Figure 4). In addition, the expression levels of *SIRT1* and *PPAR-α* were the same for

both WT and IDH2 mice. Via measurement of *AMPKα*, *SIRT1*, and *PPAR-α*, there was no significant changes in any of the three mRNAs involved in fatty acid β-oxidation (p >0.05).

VI. DISCUSSION

Maintaining balanced regulation of lipogenesis and fatty acid β-oxidation pathways are crucial as lipid metabolism is highly implicated with multiple metabolic diseases [e.g., obesity, type 2 diabetes, and fatty liver disease; (23)]. Not surprisingly, lipid metabolism can be disrupted by excessive intake of dietary sugars such as fructose. Thus, fructose is known as one of the major causes of obesity-related metabolic syndromes (23). Further, fructose induces dysregulation of lipid metabolism via excessive production of ROS in obesity (23). Therefore, the study aimed to investigate effects of IDH2 deficiency, a redox regulatory enzyme in mitochondria, on fructose-induced dysregulation of hepatic lipid metabolism (i.e., lipogenesis and fatty acid β-oxidation).

Accumulation of fatty acids is controlled by a lipogenic pathway, in which fatty acids are synthesized from acetyl-CoA within the cytosolic fractions of cells. When there is an excess consumption of fructose this creates a burden on the metabolic pathway and causes an overproduction of acetyl-CoA in the mitochondria (24). The tricarboxylate transport system then transports acetyl-CoA into the cytoplasm for fatty acid synthesis from the liver X receptor (LXR), SREBP-1, acetyl-CoA carboxylase (ACC), FAS, and SCD1 (24). If there is an increase in lipogenesis then malonyl-CoA accumulates; malonyl-CoA is synthesized from acetyl-CoA by ACC and used by FAS for fatty acid synthesis (24). In the present study, three representative genes were selected of the fatty acid synthesis pathway (i.e., both upstream and downstream genes): *SREBP-1*, *FAS*, and *SCD1*. In this, fructose intervention either induced *SCD1* gene

expression in both WT and IDH2 mice (p = 0.0003 and 0.0223 for WT and IDH2 mice, respectively; further fructose intervention resulted a trend of increase in *FAS* gene expression in WT mice, which was somewhat expected (p = 0.0627; Figure 3).

In addition, the *DGAT* family (i.e., *DGAT1*, and *DGAT2*) has a role in fat deposition by be a major gene involved in triglycerides (TG) synthesis (25). Among the *DGAT* family, *DGAT2* has a more extensive role in TG synthesis than *DGAT1* (25, 26). Further, previous findings demonstrated that a reduction of *DGAT2* expression was positively correlated with a decrease in hepatic steatosis (26). In this study, fructose intervention did not result in an increased *DGAT2* gene expression for both WT FRU and IDH2 FRU groups compared to respective control groups, possibly due to small sample size and experimental variation (1.25 ± 0.109, p = 0.145; 1.00 ± 0.109, p = 0.138 for WT and IDH2 respectively). Of note, liver tissue mass was decreased in all IDH2 KO groups compared to respective counterpart groups which indicates impaired liver regeneration (p = 0.001 and 0.018 for IDH2 CON vs WT CON and IDH2 FRU vs WT FRU, respectively). Since hepatic TGs are used for liver tissue regeneration (27) this is somewhat in agreement with the *DGAT2* expression data; for instance *DGAT2* gene expression decreased, although it was not statistically significant, in IDH2 CON compared to the WT CON (approximately 25% reduction; 0.75 ± 0.109). Overall, gene expression patterns of *SCD1*, *FAS*, and *DGAT2* were relatively consistent with liver mass phenotype as the expression levels of the genes tended to be lower in IDH2 KO mice compared to the WT mice, although *SCD1* was the only gene which showed statistical significance (p < 0.05 for both WT and IDH2 groups; Figure 3).

In addition to the lipogenesis pathway, expressions of genes involved in fatty acid β-oxidation were examined. Fatty acids break down via fatty acid β-oxidation to acetyl-CoA and

function as cellular signaling molecules (28). In the present study, the mRNA expressions were measured of *SIRT1*, *AMPKα*, and *PPAR-α*, key genes in fatty acid β-oxidation. In recent studies, *SIRT1* has been suggested in having a critical role in regulating lipid metabolism, as *SIRT1* is an upstream regulator of *SREBP-1* (key gene for lipogenesis and cholesterol synthesis) (29). Moreover, *SIRT1* inhibits the expression of fat storage genes, thereby reducing TG accumulation. Additionally, AMPK is a sensor of cellular energy status, and is activated by a level of AMP/ATP or upstream kinases (30). Recent studies indicate *SIRT1* positively regulates AMPK signaling through cell types such as hepatocytes (30). *SIRT1* -dependent AMPK inhibits downstream regulators of AMPK, such as ACC and FAS, and reduces lipid accumulation through fatty acid β-oxidation (30). Therefore, *SIRT1* and AMPK both have roles in regulating lipid metabolism, especially fatty acid oxidation. In addition to the connection with AMPK, *SIRT1* positively regulates *PPAR-α* (31), which controls fatty acid β-oxidation (32) in which PPAR-α ligand binding leads to activation of fatty acid catabolism and oxidative degradation of fatty acid derivatives (32). In the study, no difference was shown in these three genes throughout the experimental groups ($p > 0.05$). However, *SIRT1* and *PPAR-α* gene expression were not changed throughout the groups, which is puzzling to interpret (Figure 4). Further, the expression of *AMPKα* was unchanged by fructose intervention and IDH2 KO, which warrants validation by examining phosphorylation of AMPKα at threonine 172 ($p > 0.05$).

VII. Future Directions

Limitations of the study expressed below can be address in future studies. First, as discussed above, obesity is closely related to production of ROS, which causes cellular damage and can initiate inflammatory signaling (33). In this study, however, ROS-related genes or

inflammatory markers (such as inflammatory cytokines or neutrophils, to examine if inflammation reactions were triggered within the liver in response to fructose intervention or IDH2 deficiency) were not measured. Studies show that inflammatory cytokines may lead to an accumulation of TGs and increase cellular oxidative stress (34). In turn, an increase of hepatic TGs results in more production of the cytokines such as tumor necrosis factor alpha and interleukin-6. These cytokines can generate more ROS and lead to lipid peroxidation (35, 36). Therefore, it would be interesting to see if inflammatory signaling was exacerbated by fructose intervention in IDH2 KO mice, possibly due to a dysregulated redox system.

In addition to inflammation, the glutathione redox system is one of the most prominent antioxidant defense systems in protecting against oxidative damage in cells. Glutathione (GSH) is a key in repairing and preventing oxidative damage to lipids, proteins, and nucleic acids. If there is a reduction in GSH level and an increase in GSSG level (an oxidized form of glutathione), there could also be an increase in ROS and oxidative damage. Hence, NADPH supply is important as it produces reducing power to convert GSSG to GSH. Therefore, in future studies, there can be an examination if IDH2 deficient mice are more susceptible to oxidative damage due to their inability to provide sufficient NADPH in the TCA cycle in which a ratio of GSH/GSSH will be directly assessed.

Even though key genes that are related to lipid metabolism were measured, four genes in lipogenesis and three genes in lipolysis, there are other genes involved in obesity and fat accumulation. For instance, in lipogenesis, *LXR* plays a significant role in metabolism of cholesterol and lipogenesis (37). LXR is an important transcription factor that is the main regulator of *SREBP-1* in the synthesis of fatty acids (38). Additionally, measuring more genes involved in lipolysis, such as Peroxisome proliferator-activated receptor (*PPAR-γ2*), Fatty Acid

Binding Protein 4 (*FABP4*), and Lipoprotein lipase (*LPL*), could strengthen results in support of the phenotypes. *PPAR-ɣ2*, *FABP4*, and *LPL* are normally expressed in adipose tissue, and are associated with fatty acid oxidation (39). FABPs are a superfamily of lipid-binding proteins, and *FABP5* is specifically associated with liver fat content. LPL functions as a rate limiting protein for TG particles rich in lipoprotein. LPL activity is high in the adipose tissue of obese humans (39). In future studies these genes could be examined to better explain phenotypes found in mice used in this experiment.

Another limitation was the small sample size of this project. The tissue harvested was from a total of six mice per group, with intervention for six weeks. The small sample size may have played a factor in the inconclusive results. With a larger quantity of mice a larger pool of liver tissue could be utilized to test for further gene expression analyses. In addition, in the present study, there was no measurement of protein expression and enzymatic activity. In the future in addition to measuring mRNA, future studies could measure protein expression and enzymatic activity to determine any agreement with these mRNA expression data patterns.

VIII. Conclusion

In this project, it was found that IDH2 deficiency aggravated fructose-induced obesity conditions hence, related hepatic gene expressions were examined. Specifically, visceral fat mass was significantly higher in IDH2 KO groups compared to the WT groups. In contrast, liver mass was decreased in IDH2 KO groups compared to WT groups. Although liver mass phenotype was generally in agreement with the lipogenesis gene expression results, due to the small sample size, it is difficult to draw a solid conclusion with limited statistical power. Thus further analyses will be required.

Abbreviations:

Acetyl-Coenzyme A Carboxylase (ACC); AMP-activated Protein Kinase alpha (AMPKα); Adenosine Triphosphate (ATP); Diglyceride Acyltransferase 2 (DGAT2); Fatty Acid Binding Protein 4 (FABP4); Fatty Acid Synthase (FAS); Glutathione (GSH); Oxidized GSH (GSSG); Knockout (KO); Lipoprotein Lipase (LPL); Mitochondrial $NADP^+$-dependent Isocitrate Dehydrogenase (IDH2); IDH2 KO Control (IDH2 CON); IDH2 KO Fructose (IDH2 FRU); National Health and Nutrition Examination Survey (NHANES); Nicotinamide Adenine Dinucleotide Phosphate ($NADP^+$); Nicotinamide Adenine Dinucleotide Phosphate, reduced (NADPH); Nonalcoholic Fatty Liver Disease (NAFLD); Peroxisome Proliferator-Activated Receptor-alpha (PPAR-α); PPAR-gamma2 (PPAR-ɣ2); Reactive Oxidative Species (ROS); Sirtuin 1 (SIRT1); Stearoyl-CoA Desaturase 1 (SCD1); Sterol Regulatory Element-Binding Protein-1 (SREBP-1); Triglycerides (TG); Wild Type CON (WT CON).

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XI. Tables and Figures

Table 1. AIN-76A Purified Diet Formulation

Ingredient	g/kg
Casein	200.00
DL-Methionine	3.00
Dyetrose	--
Cornstarch	150.00
Sucrose	500.00
Cellulose	50.00
Soybean Oil	--
Corn Oil	50.00
t-Butylhydroquinone	--
Salt Mix #200000	35.00
Salt Mix #210025	--
Salt Mix #210050	--
Vitamin Mix #300030	10.00
Vitamin Mix #310025	--
L-Cystine	--
Choline Bitartrate	2.00

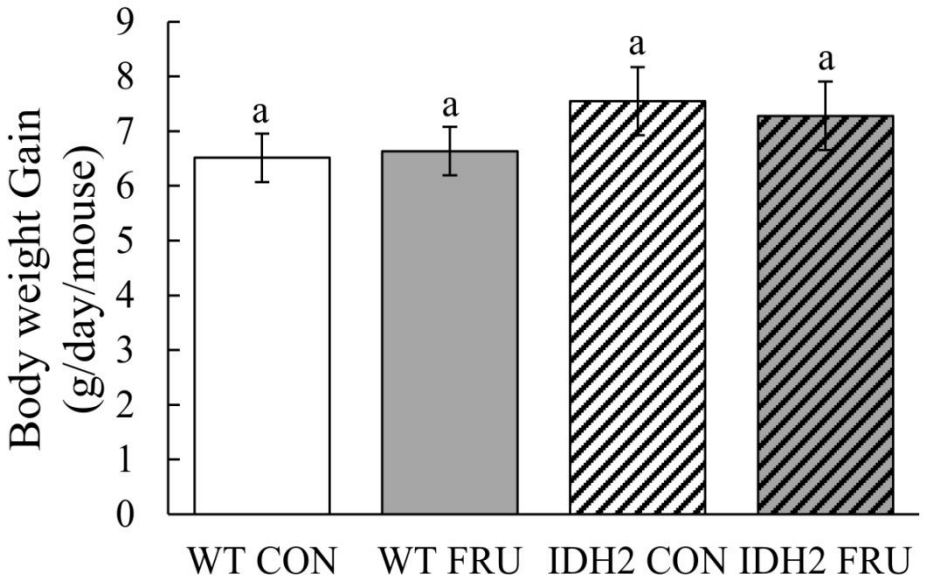

Figure 1. Body weight gain was not changed by fructose intervention in mice.

Data was expressed as least squares mean ± standard error of means. Statistical analysis was carried by one-way ANOVA followed by multiple comparisons using the difference matrix of least squares mean (SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically different. Sharing same superscript indicates no statistical difference. WT CON, Wild type mice fed control diet; WT FRU, Wild type mice fed fructose supplemented water (34% v/v); IDH2 CON, IDH2 KO mice fed control diet; IDH2 FRU, IDH2 mice fed fructose supplemented water (34% v/v). See the method section for detail experimental conditions.

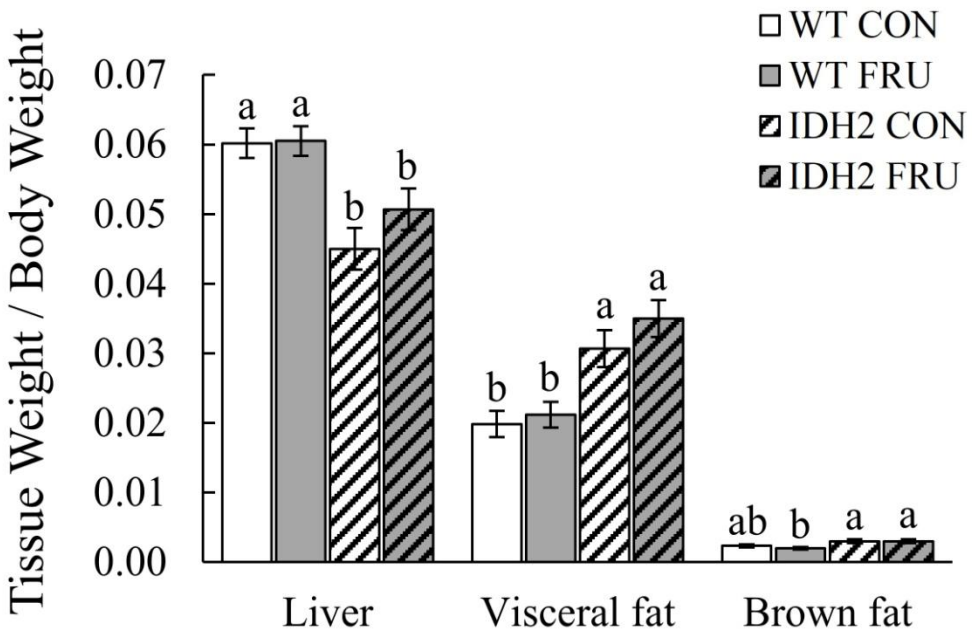

Figure 2. Effects of fructose intervention and IDH2 deficiency on ratios of tissue weight to body weight.

Data was expressed as least squares mean ± standard error of means. Statistical analysis was carried by one-way ANOVA followed by multiple comparisons using the difference matrix of least squares mean (SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically different. Sharing same superscript indicates no statistical difference. WT CON, Wild type mice fed control diet; WT FRU, Wild type mice fed fructose supplemented water (34% v/v); IDH2 CON, IDH2 KO mice fed control diet; IDH2 FRU, IDH2 mice fed fructose supplemented water (34% v/v). See the method section for detail experimental conditions.

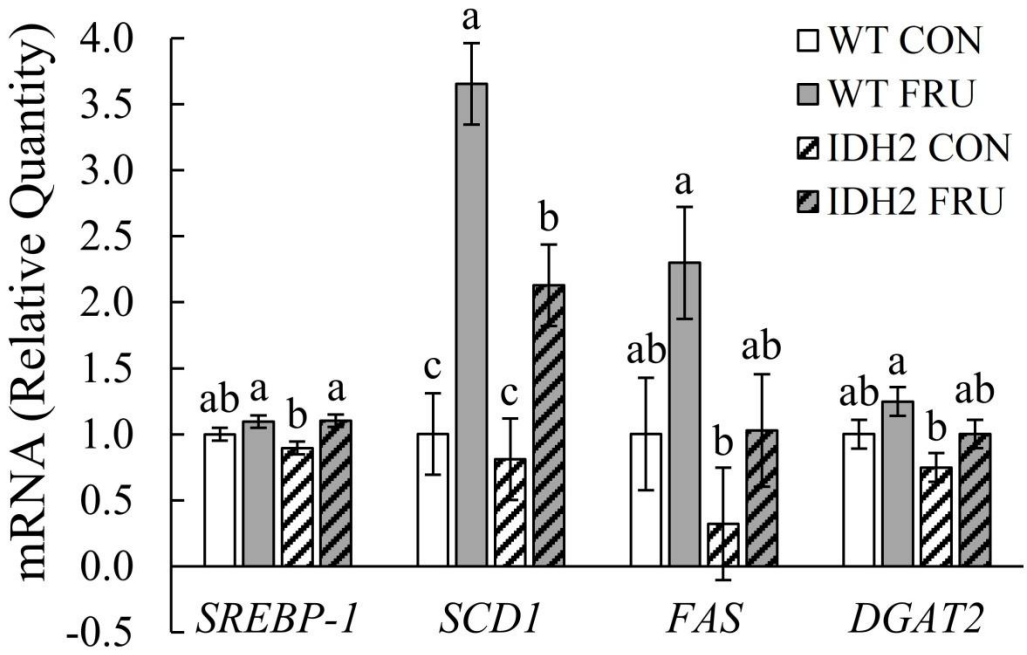

Figure 3. Effects of fructose intervention and IDH2 deficiency on expression of hepatic lypogenesis genes.

Data was expressed as least squares mean ± standard error of means. Statistical analysis was carried by one-way ANOVA followed by multiple comparisons using the difference matrix of least squares mean (SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically different. Sharing same superscript indicates no statistical difference. WT CON, Wild type mice fed control diet; WT FRU, Wild type mice fed fructose supplemented water (34% v/v); IDH2 CON, IDH2 KO mice fed control diet; IDH2 FRU, IDH2 mice fed fructose supplemented water (34% v/v). *SREBP-1*, Sterol Regulatory Element-Binding Protein-1; *SCD1*, Stearoyl-CoA Desaturase 1; *FSA*, Fatty Acid Synthase; *DGAT2*, Diglyceride Acyltransferase 2; See the method section for detail experimental conditions.

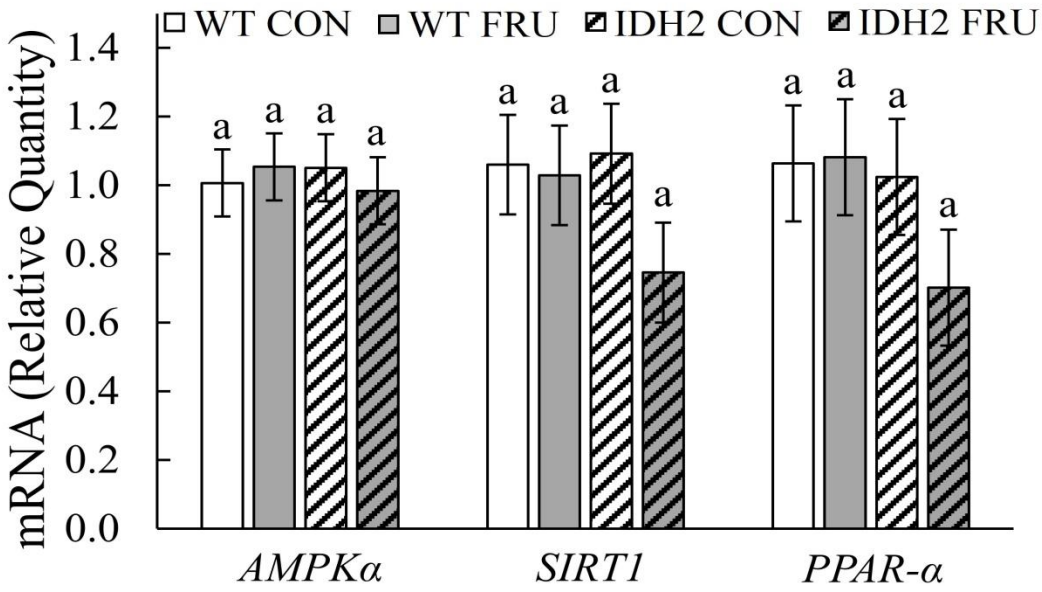

Figure 4. Effects of fructose intervention and IDH2 deficiency on hepatic expression of fatty acid oxidation genes.

Data was expressed as least squares mean ± standard error of means. Statistical analysis was carried by one-way ANOVA followed by multiple comparisons using the difference matrix of least squares mean (SAS Institute, Cary, NC, USA). P-values less than 0.05 were considered statistically different. Sharing same superscript indicates no statistical difference. WT CON, Wild type mice fed control diet; WT FRU, Wild type mice fed fructose supplemented water (34% v/v); IDH2 CON, IDH2 KO mice fed control diet; IDH2 FRU, IDH2 mice fed fructose supplemented water (34% v/v). *AMPKa,* AMP-activated Protein Kinase alpha; *SIRT1,* Sirtuin 1; *PPAR-a*, Peroxisome Proliferator-Activated Receptor-alpha; See the method section for detail experimental conditions.

Contributions of authors

Abstract	Allison Montalbano & Kaleigh Beane
Literature Review	Kaleigh Beane: Obesity Prevalence and Chronic Illness Association & Obesity Risk Factors Allison Montalbano: Health Implications of Fructose & Summary and Study Hypothesis
Materials and Methods	Allison Montalbano & Kaleigh Beane
Animals and Study Diets	Allison Montalbano & Kaleigh Beane
Results	Allison Montalbano: Lipogenesis Genes in Liver Kaleigh Beane: β-oxidation Genes in Liver
Discussion	Allison Montalbano: Lipogenesis Kaleigh Beane: Lipolysis
Future Direction	Allison Montalbano & Kaleigh Beane
Conclusion	Kaleigh Beane
Acknowledgments	Allison Montalbano
Tables and Figures	Allison Montalbano & Kaleigh Beane