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Impact of a low fructose, low glycemic index and low glycemic load dietary intervention on liver function, body composition and cardio metabolic risk factors in children and adolescents with nonalcoholic fatty liver disease

by

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a common liver disease in obese children. Diets high in added fructose (high fructose corn syrup; HFCS)/glycemic index (GI)/glycemic load (GL) in children with NAFLD are associated with increased inflammation and liver dysfunction. We hypothesized that a low GI/GL/fructose diet over six months would result in improvements in body composition, insulin resistance (IR), liver function and metabolic parameters in childhood NAFLD. Children with NAFLD and healthy children were studied at baseline (n=12 NAFLD, n=14 controls), 3-and-6 months (n=7 NAFLD; n=13 controls). Significant reductions in systolic blood pressure, body-fat percentage, plasma free fatty acids, Apo-B-100, HOMA-IR and ALT were observed in children with NAFLD at three-months ($p<0.05$). The strongest relationships between intake with HOMA-IR and plasma ALT were observed when children with NAFLD reduced their intake of GI ($p=0.009$), GL ($p<0.001$), total fructose ($p=0.001$), and HFCS ($p=0.004$). Treatment strategies aimed at reducing in fructose/GI/GL may contribute to therapy in childhood NAFLD.

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List of Abbreviations

ACAT Acyl-Cholesterol Acyl Transferase
ACC Acetyl Coenzyme-A Carboxilase
AHA American Heart Association
AHS Alberta Health Services
AI Adiposity Index
ALA α-Linolenic Acid
ALP Alkaline Phosphatase
ALT Alanine Transaminase
AMP Adenosine Monophosphate
ANOVA Analysis of Variance
Apo-B Apolipoprotein-B
Apo-B-100 Apolipoprotein B-100
Apo-B-48 Apolipoprotein B-48
Apo-C-III Apolipoprotein C-III
AST Aspartate Transaminase
ATP Adenosine Triphosphate
AUC Area Under the Curve
BMI Body Mass Index
BMR Basal Metabolic Rate
BP Blood Pressure
CAM Complementary and Alternative Medicine
CARDIA Coronary Artery Risk Development in young Adults
CCK Cholecystokinin
CDC Centre of Disease Control
CHREBP Carbohydrate Regulatory Element Binding Protein
CM Chylomicron
CRP C-reactive protein
CRU Clinical Research Unit
CT Computed Tomography
CVD Cardio Vascular Disease
DBP Diastolic Blood Pressures
DEXA Dual-Energy x-ray Absorptiometry
DHA Docosahexaenoic Acid
DM2 Diabetes Mellitus type 2
EI Energy Intake
ELISA Enzyme-Linked Inmunosorbent Assay
EPA Eicosapentaenoic Acid
FAS Fatty Acid Synthase
FFA Free Fatty Acids
GGT Gamma Glutamyl Transpeptidase
GHR Ghrelin
GI Glycemic Index
GL Glycemic Load
HAES Habitual Activity Estimation Scale
HbA-1c Glycated Hemoglobin 1 Ac
HDL High Density Lipoprotein
HFCS High Fructose Corn Syrup
HOMA Homeostatic Model Assessment
HOMA-IR Homeostatic Model Assessment-Insulin Resistance

IDF International Diabetes Federation
IGT Impaired Glucose Tolerance
IHL Intra Hepatic Lipid
IOTF International Obesity Task Force
IR Insulin Resistance
KHK Hexokinase
LBC Lifestyle Behavior Checklist
LCD Low Caloric Diet
LDL Low Density Lipoprotein
LPL Lipoprotein Lipase
MAPK Mitogen Activated Protein Kinase
MET Metabolic Equivalent of Task
MRI Magnetic Resonance Imaging
MRS Magnetic Resonance Spectroscopy
MS Metabolic Syndrome
MUFAs Monounsaturated Fatty Acids
NACTRC Northern Alberta Clinical Trials Centre
NAFLD Nonalcoholic Fatty Liver Disease
NASH Nonalcoholic Steatohepatitis
NEFA Non-Esterified Free Fatty Acids
NHANES National Health and Nutrition Examination Survey
NIDDK National Institute of Diabetes and Digestive and Kidney Diseases
PA Physical Activity
PAI Plasminogen Activator Inhibitor
PAQ Physical Activity Questionnaire
PPAR-α Peroxisome Proliferator-Activated Receptor-alpha
PUFAs Polyunsaturated Fatty Acids
ROS Reactive Oxidative Species
SBP Systolic Blood Pressure
SCD1 Stearoyl-CoA Desaturase 1
SFAs Saturated Fatty Acids
SREBP-1 Sterol Regulatory Element-Binding Protein-1
SS Simple Steatosis
TAGs Triacylglicerides
TER Trunk-to-Extremity Skinfold Ratio
TFAs Trans Fatty Acids
TG Triglycerides
TGF – β Transforming Growth Factor- β
TGF Transforming Growth Factor
TGV Thoracic Gas Volume
TLR4 Toll-Like Receptor 4
TNF- α Tumor Necrosis Factor α
US Ultrasound
USDA United States Department of Agriculture
VA Vaccenic Acid
VAT Visceral Adipose Tissue
VLDL Very Low Density Lipoprotein
VLDL-TG Very low Density Lipoprotein Triglycerides
WC Waist Circumference
WHO World Health Organization

Chapter 1- Review of literature

1.1 Introduction

Obesity has become one of the most alarming health public problems worldwide (Ogden, Yanovski, Carroll, & Flegal, 2007), affecting most global economies (Howe et al., 2011). This has led to an increase in chronic diseases and healthcare costs. For example, in the United States (USA) in 2000, about "$117 billion" was spent on obesity-related problems such as type 2 diabetes (T2D), hypertension and psychological disorders (Wellesley & Morgan, 2004).

The prevalence of overweight and obesity among industrialized countries ranges from 15% to 60% (Bluher et al., 2011). The prevalence of childhood obesity has also increased "in the last decade" (Biro & Wien, 2010). For example, 31% of children in the United States are considered overweight or obese (Y. C. Wang, Gortmaker, & Taveras, 2010). In Canada, the prevalence of childhood overweight (19.8%) and obesity (11.7%) accounts for 31.5% of the population (Roberts, 2012). Overall, the global prevalence among children and adolescents has been difficult to assess because classification cut-offs and measurement techniques are not standardized worldwide. This has influenced estimates (Cole, Bellizzi, Flegal, & Dietz, 2000), and possible prevalence could be underestimated. Moreover, children ands adolescents continually undergo physiological changes. Other factors such as age, sex, puberty and ethnicity make it even harder to classify obesity and overweight (Han, Lawlor, & Kimm, 2010).

The etiology of childhood overweight and obesity is associated with social, economic, educational, genetic and lifestyle factors (Han, Lawlor, & Kimm, 2010; Y. Wang & Lobstein, 2006). A longitudinal study from the U.K showed that the BMI of children decreases as maternal education increases (Howe et al., 2011). About 30%-70% of genetic predisposition seems to influence children BMI (Stunkard, Harris, Pedersen, & McClearn, 1990). Childhood obesity is associated with a high parental BMI. For example, 226 families were studied and children faced a higher risk of obesity when their same-sex parent was obese (Perez-Pastor et al., 2009a). Moreover an early adiposity rebound (point at which BMI increases after childhood nadir) is

associated with adult obesity (Dorosty, Emmett, Cowin, & Reilly, 2000). However, it is important to mention that BMI does not distinguish between muscle and adipose tissue. Therefore, the prevalence of obesity could be underestimated (van't Hof & Haschke, 2000).

The long-term metabolic and psychological consequences of childhood obesity are not clear, and they need to be followed closely. With regards to psychosocial consequences, obesity in childhood is associated with an increased likelihood for the child to have impaired peer relationships, poor self-esteem (Vander Wal & Mitchell, 2011) and to be involved in bullying (Kukaswadia, Craig, Janssen, & Pickett, 2011). Studies have shown that 30% and 25% of overweight girls and boys respectively are victimized either verbally or physically as a direct consequence of their body size and shape (Eisenberg, Neumark-Sztainer, & Story, 2003). Some other repercussions include increased concerns regarding body-image dissatisfaction, depression, eating disorders, and suicidal ideas (Robinson, 2006). Childhood obesity is also linked with problems such as pulmonary diseases, orthopedic and sleep disorders, and other chronic diseases such as nonalcoholic fatty liver disease (NAFLD), hypertension and type 2 diabetes (T2D) (Wang et al., 2011). Metabolic risk factors seem to affect more children with higher BMI than children who are lean (Beyerlein, Toschke, Schaffrath Rosario, & von Kries, 2011). For example, studies show that about 14.1% of obese children present a higher risk of cardiovascular diseases (Saha, Sarkar, & Chatterjee, 2011).

Obesity and metabolic syndrome are highly associated with the development of nonalcoholic fatty liver disease (NAFLD) (Vajro et al., 2012a). In fact, studies suggest that NAFLD may represent the liver component of the metabolic syndrome (Vanni et al., 2010). NAFLD involves a series of liver abnormalities. The first stage of the disease is known as simple steatosis (SS), which is an excessive accumulation of triglycerides in the liver. NAFLD can progress to nonalcoholic steatohepatitis (NASH), a liver disorder characterized by steatosis, hepatocellular damage, inflammation and, potentially, liver fibrosis (Alisi, Cianfarani, Manco, Agostoni, & Nobili, 2011; Ko et al., 2009). About 10%-29% of adult cases with NASH may evolve to cirrhosis and 4%-27% of the cases may develop hepatic carcinoma (Cohen, Horton, & Hobbs, 2011). Although rare in childhood, there are some reported

cases of liver cirrhosis developing in children with NASH (Takahashi et al., 2011).

The etiology of NAFLD is unclear but involves genetic, metabolic and lifestyle factors (Sinatra, 2012). For example NAFLD is strongly related to insulin resistance (IR), dyslipidemia, and excessive visceral adiposity (B. W. Smith & Adams, 2011). In fact, adults and children with obesity and metabolic syndrome tend to have excessive fat deposition in subcutaneous and visceral adipose tissue, contributing to IR (Biro & Wien, 2010). However, there are some children with NAFLD that have body weights within healthy ranges, but with the presence of visceral adiposity (J. Y. Yap, O'Connor, Mager, Taylor, & Roberts, 2011). Sedentary habits and a diet with an excessive intake of calories in the form of fat and simple sugars are thought to contribute to both obesity and NAFLD in children (Alisi, Cianfarani, Manco, Agostoni, & Nobili, 2011). Recent evidence indicates that dietary habits in children and adolescents with NAFLD are characterized by a high intake of saturated fatty acids, a low intake of polyunsaturated fatty acid, a high intake of simple sugar (e.g., commercially added fructose), and a low intake of fiber, vitamins (Vos et al., 2012), and minerals (Mager et al., 2010; Manco, Bottazzo et al., 2008; Mouzaki & Allard, 2012). High intakes of fructose have been associated with increased markers of inflammation, dyslipidemia and insulin resistance in children with NAFLD (Mager et al., 2010). This has also been reported in adults with NAFLD (Assy et al., 2008).

Children who consume high GI foods may experience higher postprandial rises in blood glucose and TG compared to those children whose diets are characterized by low GI foods (Wolever & Mehling, 2002). Chronic consumption of high GI foods is associated with markers of liver dysfunction in children (Mager et al., 2010). This may contribute to an increased risk for hepatic fat deposition (K. A. Le & Bortolotti, 2008). Foods with high glycemic load reflect the effect of the amount of carbohydrates. Therefore, large portion sizes of low-glycemic-index carbohydrate-containing foods will also affect glucose levels (Salmeron et al., 1997). Other dietary factors are related to hepatic fat accumulation. For example, it has been seen that among children, consuming sweetened beverages, processed foods and excess calories (e.g., GI of foods >60, fructose >9% of calories and fat >30% of

calories) is associated with fat accumulation in the liver; this is because such a diet decreases insulin sensitivity and increases postprandial TG levels (Berkey, Rockett, Field, Gillman, & Colditz, 2004; Jurgens et al., 2005). We have recently shown that acute intakes of meals characterized by high levels of saturated fat also contribute to insulin resistance, altered lipoprotein expression and dyslipidemia in obese children and adolescents with NAFLD. These are all factors associated with an increased risk for NAFLD (Mager et al., 2012). Hence, dietary intakes have all been shown to contribute to an increased risk for NAFLD.

Currently, the primary treatment of childhood NAFLD is to promote gradual weight loss through lifestyle modification (Alisi, Feldstein, Villani, Raponi, & Nobili, 2012; Janczyk & Socha, 2012). However, there are no specific evidence-based guidelines regarding the type of dietary or physical activity changes that are needed to treat children with NAFLD. For example, previous dietary modifications have focused on total calorie or fat restriction with special attention to saturated fats or carbohydrates to promote a weight loss of about 5% (Pacifico et al., 2012; C. L. Wang et al., 2008; Q. Wang et al., 2011). Typically, low carbohydrate diets recommend ingesting fiber and reducing simple sugars, but do not specify in specific methods to reduce simple sugars (e.g., GI or high corn fructose syrup added to food products). It is unknown to what extent the GI/GL/fructose needs to be restricted in children with NAFLD, especially in children who need to consume a diet high in vitamins and minerals from fruits and vegetables. The different composition in the diet may result in different and inconsistent results.

Lifestyle modifications in children with NAFLD seems to be the most appropriate strategy (Alisi, Feldstein, Villani, Raponi, & Nobili, 2012; Gossard & Lindor, 2011), because it has not been proven that drugs and surgery may alleviate the disease without secondary effects (Alisi & Nobili, 2011). However, authors suggest that carrying out lifestyle modifications can be quite challenging for both family and children. Parents and/or caregivers have an important role in their children's diets. It is not only important to find an appropriate treatment for this population. It is also important to explore whether or not these changes are sustainable. What do parents of children with NAFLD know have about nutrition? And what are their experiences with

lifestyle modification? The answers to these questions will make it possible to develop appropriate programs to help both families and children with NAFLD, providing them with strategies for a healthy lifestyle. The high incidence of NAFLD in the future will result in increased morbidity and mortality and will increase health-related costs globally (Saha et al., 2011). Therefore it is necessary to determine the most effective therapy for this population (Lomanco et al., 2013). The purpose of this literature review is to describe the risk factors associated with NAFLD in childhood and to describe the different treatment strategies (lifestyle modification) that have been used to treat childhood NAFLD. Different treatment approaches, with an emphasis on lifestyle modification on NAFLD will be compared. This chapter will also include information analyzing how the family influences lifestyle modifications in the child.

1.2 Nonalcoholic liver disease

The liver is the largest organ in the body. Its major function is metabolic. For example, the liver metabolizes carbohydrates, lipids, and proteins. It processes all the food´s nutrients to produce energy. When this energy is not utilized, it is stored as fat and carbohydrates in the form of glycogen in the liver. The liver is also responsible for detoxifying waste products and medications and producing hormones, and other important proteins in the body. An imbalance of these liver functions may result in liver damage. In the case of NAFLD, a series of abnormalities affects the liver. Typically children with NAFLD are characterized by having Type 2 NAFLD. In contrast adults are often diagnosed with NASH **(See Table 1.1).**

Table 1.1 Types of NAFLD

Type 1	Simple steatosis: 5% fat accumulation (no inflammation or fibrosis)
Type 2	Steatosis with lobular inflammation but absent fibrosis or ballooning
Type 3	Steatosis, inflammation, and fibrosis of varying degrees (NASH)
Type 4	Steatosis, inflammation, ballooned cells, and fibrosis (NASH)
Reference: (Matteoni et al., 1999)	

In childhood, NAFLD is a diagnosis that must be determined by excluding other disorders related to excessive fatty deposition in the liver

(viral hepatitis, Wilson´s disease, excessive alcohol consumption **Table 1.2**). Once these diseases have been excluded, a diagnosis of NAFLD can be made **(see section 1.2.2 for Diagnosis).** The first reversible stage of NAFLD is simple steatosis, and is characterized by at least 5% of fat accumulation of liver volume weight (in the form of triglycerides) in the absence of excessive alcohol consumption (Angulo & Lindor, 2002; Kopec & Burns, 2011). The triglyceride composition in the liver of patients with NAFLD is characterized by increased diacylglycerol and free cholesterol, and decreased levels of phosphatidylcholine (Morita et al., 2012). A more severe stage called nonalcoholic steatohepatitis (NASH) includes the presence of fat infiltration, inflammation, and hepatic cellular damage with or without fibrosis. The pediatric population presents a different form of NASH, characterized by macro vesicular hepatocellular steatosis (rather than microvesicular fat deposition that occurs in adults) with portal inflammation, with or without portal fibrosis, in the absence of ballooning degeneration and perisinusoidal fibrosis (Vajro et al., 2012a). NASH can result in cirrhosis and hepatocellular carcinoma in adults (Gaemers & Groen, 2006). The presence of cirrhosis in children is not common, but several cases have been reported worldwide (Takahashi et al., 2011).

1.2.1 Prevalence of nonalcoholic fatty liver disease

NAFLD is considered the 12th leading cause of death in adults in the US (Vernon, Baranova, & Younossi, 2011) and the most frequent hepatic disease (J. Ludwig, McGill, & Lindor, 1997) in western countries (Niaz, Ali, Nayyar, & Fatima, 2011). Yet, like obesity, NAFLD is a global health problem. For example, during 2003 it was found that the prevalence in Japan was 26% in males and 12.7 % in females (Kojima, Watanabe, Numata, Ogawa, & Matsuzaki, 2003). The prevalence of NAFLD among adults in developed countries is estimated at 20%-35% and in developing countries almost 10% (Angulo, 2005). However, the exact prevalence of NAFLD may vary according the diagnostic methodology used **(See Section 1.3.1).** The prevalence of NAFLD can also increase in the presence of overweight and obesity, T2D, hyperlipidemia and IR. For example, in the presence of obesity,

the prevalence may increase up to 57.5%-74% (Clark & Diehl, 2003). NAFLD is also more prevalent in males than females. For example, one study found a prevalence ranging from 2.7% to 13.5% in male subjects regardless of their weight (Niaz, Ali, Nayyar, & Fatima, 2011).

In the pediatric population, the prevalence of NAFLD is even harder to determine. There are major difficulties diagnosing NAFLD due to physiological changes, and the method used for diagnosis **(See section 1.2.2).** For example, the prevalence within Hispanic and Asian populations is about 11.8% and 10.2% respectively (general population), while the rate of NAFLD occurrence among non-Hispanic Caucasian populations and individuals of Black ancestry has been reported to range between 1.5-9% (Schwimmer et al., 2006). Moreover, hepatic enzyme levels vary according to age, sex, and race (Devadason & Scheimann, 2012; Hou et al., 2011; Vajro et al., 2012a). The degree of fat infiltration in the liver and the progression of the disease are quite difficult to monitor among children (Patton et al., 2006). There is limited data regarding the progression of NAFLD in childhood, making it difficult to determine if there is a consistent pattern in the progression/expression of NAFLD.

According to epidemiologic studies from the US, 8% of adolescents present liver enzyme abnormalities. The prevalence assessed with altered liver enzymes was 17%-20% in adolescents (Aly & Kleiner, 2011). In Japan, NAFLD was diagnosed in 2.6% of children from 4-12 years, using ultrasound, and a higher proportion was found in boys than girls (3.4% vs. 1.8%) (Kojima, Watanabe, Numata, Ogawa, & Matsuzaki, 2003). Moreover, 13% of children and adolescents presented NAFLD after an autopsy was performed, and about 0.7% were 2-4 years and 17.3% were 15 -19 years (Schwimmer et al., 2006).

1.2.2 Diagnostic criteria in children

NAFLD diagnosis in the pediatric population is difficult to establish, because the majority of cases are asymptomatic. Multiple methods are used to diagnose NAFLD. These include a combination of hepatic laboratory markers, clinical parameters, demographic and anthropometric data, and imaging tests or liver biopsy. In order to diagnose children with NAFLD, it is necessary to

consider their dietary intake, perform an ultrasound (US), evaluate the presence of more than one metabolic syndrome feature, assess the liver enzyme levels and exclude the presence of other diseases associated with liver disease **(See Table 1.2).** In the clinical practice, are considered signs and symptoms such as fatigue, right upper abdominal pain, obesity (central and total body), acanthosis nigricans, hepatomegaly, insulin resistance and altered liver biochemistries (Ramesh & Sanyal, 2005; Schwimmer et al., 2006).

Table 1.2 Most common causes of fatty liver disease

General or systemic	Genetic-metabolic causes	Hereditary genetic disorders	Hepatotoxicity induced with drugs
Acute systemic disease with dehydration and/or severe infection Acute starvation Protein-energy malnutrition Anorexia nervosa Cachexia Total parenteral nutrition Obstructive sleep apnea Obesity/metabolic syndrome Polycystic ovarian syndrome Rapid weight loss Status post jejunoileal bypass or gastric reduction Celiac disease Nephrotic syndrome Chronic hepatitis C Type 1 diabetes mellitus and Mauriac syndrome Thyroid disorders Hypothalamo-pituitary disorders Blind loop (bacterial overgrowth)	Cystic fibrosis Shwachman syndrome Wilson's disease α1-Antitrypsin deficiency Galactosemia Fructosemia Cholesteryl ester storage disease Glycogen storage disease (type I and VI) Mitochondrial and peroxisomal defects of fatty acid oxidation Madelung lipomatosis Lipodystrophies Chanarin-Dorfman syndrome Porphyria cutanea tarda Homocystinuria Familial hyperlipoproteinemias Tyrosinemia type 1 Bile acids synthesis defects Congenital disorders of glycosylation Turner syndrome Organic acidosis Citrin deficiency HFE (hemochromatosis)	Alström syndrome Bardet-Biedl syndrome Cohen syndrome Cantu syndrome (1p36 deletion) Weber-Christian disease	Ethanol Ecstasy, cocaine Nifedipine Diltiazem Estrogens Corticosteroids Amiodarone Perhexiline Coralgil Tamoxifen Methothrexate Prednisolone Valproate Vitamin L-asparaginase Zidovudine and HIV treatments Solvents Pesticides Calcium channel blockers (case reports)
Reference: (Vajro et al., 2012a)			

Anthropometric parameters such as weight, BMI and waist circumference are also important to measures because these measurements are associated with IR, visceral adiposity and steatosis in children (Manco, Bedogni et al., 2008). Assessment subcutaneous fat with skin folds may also be helpful, because these measurements are associated with insulin and lipid concentrations in children (Freedman, Serdula, Srinivasan, & Berenson, 1999; Mager, Yap et al., 2012; D. P. Williams et al., 1992).

Serum biomarkers that are used to diagnose of NAFLD include alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT). ALT alone is associated with liver, heart and muscle damage and is highly correlated with visceral fat (Patton et al., 2006). In a Japanese study with 310 obese children, plasma levels of ALT were highly sensitive in detecting fatty liver correlated well with previous results using US (Tazawa, Noguchi, Nishinomiya, & Takada, 1997). By contrast, elevated ALT levels are not always found in the earliest stages. For example, normal weight child may have normal liver enzymes but the presence of NAFLD is confirmed with US or liver biopsy (J. Y. Yap, O'Connor, Mager, Taylor, & Roberts, 2011). Furthermore, the permeability of the liver is considered lost when mild to moderate abnormalities of the liver enzymes are found. The ratio between AST:ALT is often used to assess for the presence of hepatic fibrosis. However, it seems to be a weak marker, because some participants with steatosis will not always present elevated enzyme levels. In addition, the reference values of the liver enzymes have not been well defined in children and adolescents (Rodriguez, Gallego, Breidenassel, Moreno, & Gottrand, 2010). Many clinicians and scientists propose that normal cut-offs for serum ALT should be in the range of 20 UI/L rather than the standard cut-offs of 40 UI/L (Mager, Yap et al., 2012; Patton et al., 2006; Poustchi, Esmaili, Esna-Ashari, & Ardalan, 2011). A clinical criteria diagnosis in children has been recently proposed, which requires the presence of central obesity (waist-to-height ratio >0.5 or waist circumference >90th percentile for age), IR (HOMA-IR>3.15), dyslipidemia (fasting hypertriglyceridemia or hypercholesterolemia) and imaging hepatic steatosis (J. Y. Yap, O'Connor, Mager, Taylor, & Roberts, 2011).

The gold standard to diagnose NAFLD is liver biopsy; this histological test is the only one that can accurately assess the degree of fatty infiltration in the liver, the degree of inflammation, and the presence of hepatocellular injury and fibrosis. Therefore, the use of liver biopsy can be used to determine the stages of the disease (Pacifico, Nobili, Anania, Verdecchia, & Chiesa, 2011). Liver biopsy is based on a scoring histologic system proposed by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) Pathology Committee (**See Table 1.3**). However, the use of liver biopsy to diagnose NAFLD is controversial in childhood, because this test is considered highly invasive and expensive (Chalasani et al., 2012; Vajro et al., 2012a). Many factors should be considered in order to perform a liver biopsy in children. These factors include the presence of severe NAFLD history in the family; clinical presence of hepatosplenomegaly; persistent/abnormal laboratory parameters such as hypertransaminasemia, insulin resistance, and non organ-specific autoantibodies; and biochemical tests for other related liver diseases (E. A. Roberts, 2007).

NAFLD is often diagnosed with non-invasive methods, but their accuracy is still in doubt. For example, these methods include computed tomography (CT), ultrasonography (US), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS) (Pacifico, Nobili, Anania, Verdecchia, & Chiesa, 2011). US is one of the preferred methods due to its low cost and non-invasive character. It estimates steatosis by classifying the echogenicity of the liver (Roldan-Valadez, Favila, Martinez-Lopez, Uribe, & Mendez-Sanchez, 2008). US classified as the minimal degree of steatosis when hyper echogenicity is found to be <25%, moderate when hyper echogenicity is 20%-50%, important when it is 50-75%, and massive when it is >75%. US can detect steatosis with a sensitivity and specificity that goes from 80% to 100%. However, its sensitivity is diminished with obese patients; in these cases it cannot detect the degree of steatosis (Wieckowska, McCullough, & Feldstein, 2007).

Table 1.3 Clinical Research Network Scoring System Definitions

Item	Score	Degree	Definition
Steatosis grade (S)	0 1 2 3	<5% 5%-33% >33%-66% >66%	Low to medium power evaluation of parenchymal involvement by steatosis
Steatosis location	0 1 2 3	Zone 3 Zone 1 Azonal Panacinar	
Microvesicular steatosis	0 1	Not present Present	
Inflammation Lobular (L) Inflammation	0 1 2 3	None <2 foci per 200x field 2-4 foci per 200x field >4 foci per 200x field	Overall assessment of all inflammatory foci
Microgranulomas	0 1	Absent Present	
Large lipogranulomas	0 1	Absent Present	
Portal inflammation	0 1	Absent Present	
Liver cell injury Ballooning (B)	0 1 2	None Few balloon cells Many cells/prominent ballooning	
Acidophil bodies	0 1	None to rare Many	
Pigmented macrophages	0 1	None to rare Many	
Megamitochondria	0 1		
Fibrosis	0 1 1a 1b 1c 2 3 4	None Perisinusoidal or periportal Mild, zone 3, perisinusoidal Moderate, zone 3, perisinusoidal Portal/periportal Perisinusoidal and portal/periportal Bridging fibrosis Cirrhosis	
NASH Activity grade = S + L+ B (Range 0-8) Adapted from (Lerret et al., 2011).			

One disadvantage of US is that this technique depends on the ability of the technician. The use of US in children was assessed in a large prospective study and the US scores were well correlated with the NAFLD activity score (NAS) and with the degree of steatosis, but not with inflammation or fibrosis found in a biopsy test (Shannon et al., 2011).

CT is a fast, non-invasive method highly used to quantify mineral density. This test is able to identify the presence of fatty liver (Mendler et al., 1998). An intravenous contrast material is administered and the diagnosis is made when the image of the liver is attenuated 10 or more Hounsfield units lower than the attenuation of the spleen (Ramesh & Sanyal, 2005). This procedure is able to distinguish hepatic steatosis with a 76% positive value (J. E. Jacobs et al., 1998). This procedure has some disadvantages. For example, CT does not provide information regarding the severity of steatosis and cannot detect early stages of the disease. CT is more expensive than other imaging procedures, and is not often used in children due to the increased exposure to radiation (Duman et al., 2006; Saadeh et al., 2002).

MRI is one of the preferred methods used to diagnose NAFLD in pediatric patients. It measures the degree of fat infiltration in the liver using a double-gradient echo chemical imaging technique that can detect water from fat in different tissues. This technique is the most sensitive of all non-invasive techniques (Vajro et al., 2012a). For example, in adults, it correlates well with the degree of steatosis shown in the biopsy (M. Fishbein et al., 2005). A recent study showed that MRI had 80% sensitivity and 87% specificity to diagnose NAFLD among obese children (Pacifico, Nobili, Anania, Verdecchia, & Chiesa, 2011). Some of the limitations of this method are that it is not always available in hospitals, is expensive, and requires well-trained professionals who can interpret and perform the tests accurately.

1.2.3 Risk factors and pathophysiology

The etiology of NAFLD is currently unknown because multiple factors are associated with its development. For example, NAFLD is associated with metabolic dysregulation (lipid and carbohydrate metabolism) (Manco, 2011)

genetic disorders, and an unhealthy lifestyle. It is also associated with sex, age and ethnicity especially in children. **See Table 1.4.**

Table 1.4 Associated risk factors with nonalcoholic fatty liver disease

Metabolic Factors	Genetic Factors	Lifestyle Factors	Pediatric
• Insulin resistance • Overweight /Obesity • Visceral adiposity • Other Metabolic syndrome features: Hypertension Hypertriglyceridemia	• Adiponutrin polymorphism (rs738409) • Interleukin-6 polymorphism (174G/C) • TNF-α polymorphism • Tumor suppressor gene Kruppel-like factor 6 mutation	• Sedentary behaviors • Excess intake of calories: **Simple sugars:** (added sugar beverages and food products, HFCS products, high GI/GL foods) **Dietary fat:** (saturated fat, low intake of polyunsaturated fatty acids • Low dietary intake of fiber and vitamins	• Gender (More case series in boys) • Puberty • More cases during pre puberty
(Gaemers & Groen, 2006; Kohli et al., 2010; Mager, Mazurak et al., 2012; Patton et al., 2006; A. J. Sanyal et al., 2001; Vajro et al., 2012b; Valtuena et al., 2006)			

Insulin resistance (IR) is thought to play a key role in the pathogenesis of NAFLD. However, the exact mechanisms are still unclear. The no-longer supported two-hit theory proposed that the first hit (peripheral IR) promoted an increased efflux of FFA to the liver by enhancing lipolysis from the adipose tissue. The second hit was related with oxidative injury and inflammation that leads to steatohepatitis. More recent data has shown that there are multiple hits that affect the liver, but the order of such hits is unknown. IR is thought to induce lipogenesis by activating the gene transcription factor involved in lipogenesis and hyperglycemia (Lemoine & Serfaty, 2012). In addition, hyperinsulinemia decreases the synthesis of VLDL by enhancing Apo-B degradation (Charlton, Sreekumar, Rasmussen, Lindor, & Nair, 2002). Donnelly proposed that 26% of the efflux of fat comes from de novo lipogenesis (adipose tissue and liver), 59% from lipolysis and 15% from the diet (K. L. Donnelly et al., 2005).

Overweight and obesity are associated with NAFLD. For example, overweight and obese children tend to have higher levels of ALT (M. H.

Fishbein, Miner, Mogren, & Chalekson, 2003; Schwimmer et al., 2003). Specifically, the presence of central adiposity seems to contribute to hyperinsulinemia, hyperglycemia, hyperlipidemia, which all of them are linked with IR (Kopec & Burns, 2011). However, there is evidence of normal weight patients with excessive visceral adiposity and NAFLD (J. Y. Yap, O'Connor, Mager, Taylor, & Roberts, 2011). Moreover, studies suggest that the presence of obesity is independently correlated with fibrosis (Malavolti et al., 2012; Petta et al., 2012). Certain individuals of specific ethnicities such as Hispanic and non-Hispanic white populations are at higher risk for NAFLD, because they present with higher levels of ALT than the black population and are at higher risk to develop fibrosis (Bambha et al., 2012; Patton et al., 2006). Other populations, such as South Asian, which are at high risk of MS, T2D and CVD, suggest that they may also be at risk of NAFLD (Fan et al., 2007). Certainly there is an increasing rate of diagnosis of NAFLD in south Asian populations (V. W. Wong, 2013).

In children it is important to consider the age, sex and pubertal stage of development. NAFLD is more common in boys than girls (Schwimmer, McGreal, Deutsch, Finegold, & Lavine, 2005). Studies have revealed that boys tend to have higher levels of transaminases (Schwimmer et al., 2006; Suzuki & Abdelmalek, 2009). There is evidence that child older than 10 years are more prone to develop NAFLD than younger children (Schwimmer, McGreal, Deutsch, Finegold, & Lavine, 2005). However, NAFLD has been reported in children as young as 18 months of age (Mager et al., 2006, Fishbein et al., 2003. Recent data showed that puberty and post-puberty stages were associated with a lower risk of developing steatosis and portal inflammation, and pre-puberty stages were associated with severe steatosis (Suzuki et al., 2012). However, more data is necessary to clarify the influence of puberty in the development of NAFLD. The risk for IR increases with the rapid onset of pubertal development. This may predispose an obese adolescent with an increased risk for developing NAFLD (Pilia et al., 2009). The exact mechanism by which diet relates to the development of NAFLD has not been fully elucidated. Studies have shown that excessive calorie intake is stored in the form of fat in the human liver.

When fat (triglycerides) exceeds the liver's capacity, it leads to liver damage by impairing its clearance (Cohen, Horton, & Hobbs, 2011). Sources of free fatty acids come from dietary TAGs, adipose tissue and the intestine (K. L. Donnelly et al., 2005). Dietary TAGs are transported via chylomicrons from the intestine to adipose tissue and liver, where they are secreted via lipoproteins. Other hepatic TAGs are synthesized from fatty acids and glycerol in the liver (Tamura & Shimomura, 2005). In other words, greater rates of hepatic de novo lipogenesis or/and an imbalance of fat uptake in the liver associated with unhealthy diets (e.g., excessive saturated fat and commercially added fructose) could increase the risk of NAFLD (Mager, Mazurak et al., 2012).

1.3 Contribution of fructose to metabolic syndrome features and NAFLD

The worldwide intake of fructose in food products such as sweetened beverages has increased in the same extent as overweight and obesity (K. L. Stanhope & Havel, 2010; Vos, Kimmons, Gillespie, Welsh, & Blanck, 2008a). Particularly, high intakes of HFCS have been associated with high prevalence of chronic diseases such as diabetes, cardiovascular disease, metabolic syndrome and obesity (Angelopoulos et al., 2009; Ostos et al., 2002; Perez-Pozo et al., 2010). The implicated mechanisms are unclear. However, there is evidence that consume excessive HFCS can lead to dyslipidemia levels and insulin resistance, which are all linked to the development of NAFLD (Cornejo E. & Raimann B., 2004; D. Huang, Dhawan, Young, Yong, Boros, & Heaney, 2011a; Jurgens et al., 2005; H. Koo, 2008; Ouyang et al., 2008). The next section of this chapter will focus on the concept of fructose, its use as a sweetener in the food industry, and its implication in contributing to metabolic abnormalities.

1.3.1 Structure of fructose

Fructose is a monosaccharide that shares its formula with glucose but which has a different structure (**See Figure 1).** Fructose contains 6 atoms of carbon with a ketone group in the second carbon. By contrast, glucose is also composed of 6 carbon-atoms, but contains an aldehyde group in the first carbon. The major source of fructose is sucrose, known as table sugar, which contains

glucose and fructose (R. J. Johnson et al., 2009). Fructose can also be found in natural sources such as fruits, some vegetables, and honey.

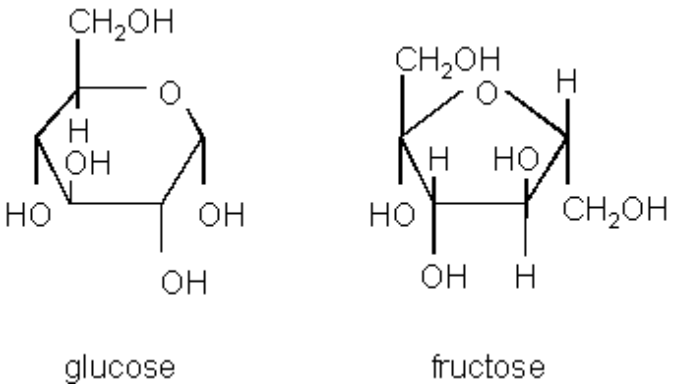

Figure 1 . 1 Biochemical structure of fructose (Gregory M.J, 2012)

1.3.2 Fructose as new sweetener

The food industry started to use fructose as an alternative sweetener almost 50 years ago (Jones, 2009; White, 2008). Fructose became popular in the food industry because it is cheaper than sugar and has the highest sweetening power of all carbohydrates (twice the sweetener capacity of glucose). Fructose also confers organoleptic properties to food products, such as long shelf life and adding softening and texture to food products (Hanover & White, 1993). This popularity of this new sweetener was not limited to the food industry. For example, the health-care professional considred fructose in a more positive light when they found that fructose unlike other sugars, does not induce hyperglycemia and hyperinsulinemia in healthy populations or in people with diabetes (Bantle et al., 1983; Mehnert, 1976). For these reasons, fructose was considered an appropriate option as a sweetener of foods for people with diabetes, and a good choice by the food industry.

The United States introduced another source of fructose in the American diet in the 70s. This new form is high fructose corn syrup (HFCS), which has since become the major sweetener in the food industry (K. L. Stanhope & Havel, 2010). In order to produce HFCS, it is first necessary to extract starch from corn and hydrolyze it to produce glucose molecules (Hanover & White, 1993; Kaneko, Takahashi, & Saito, 2000; White, 2008). After this process, glucose is transformed to fructose through a process called enzymatic isomerization. Then,

the molecules of fructose and glucose are mixed, resulting in HFCS.

HFCS is utilized in the food industry in multiple variations that range from 42 to 90% of fructose content. The most common form of HFCS is HFCS-55; this form contains 42% glucose, 55% fructose and about 3% of other sugars such as maltose (Hanover & White, 1993). HFCS-55 is added to carbonated beverages, and HFCS-42 is added to food products such as breakfast cereals, ice cream, fast food products and juice (Hanover & White, 1993). The United States is the largest consumer and producer of HFCS in the world, followed by Canada, Argentina, Japan, China and South Korea (Bray, Nielsen, & Popkin, 2004; Vos, Kimmons, Gillespie, Welsh, & Blanck, 2008b; Vuilleumier, 1993). According to NHANES 1999-2004, in the United States HFCS consumption increased 26% from 1978 to 1998 among all genders and ages, while sugar consumption decreased (Tappy & Le, 2010; Welsh, Sharma, Grellinger, & Vos, 2011).

1.3.3 Fructose intake and consequences

The use of fructose brought advantages to the food industry and to people with DM2 (Cox, 1995). However, new evidence with animals and humans indicates that commercially added fructose sources might have negative effects associated with metabolic syndrome features (Bantle, 2009; Cornejo E. & Raimann B., 2004; Morris et al., 2012; Sheludiakova, Rooney, & Boakes, 2012). For example, rats fed high-fructose drinks had increased triglyceride levels, became overweight and obese, and developed induced insulin resistance and increased inflammatory markers (Alzamendi, Castrogiovanni, Gaillard, Spinedi, & Giovambattista, 2010; Cannizzo et al., 2012; D. Huang, Dhawan, Young, Yong, Boros, & Heaney, 2011b; Ishimoto et al., 2012).

1.3.4 Metabolism of fructose

After being ingested, fructose as a free form is transported by the portal circulation and absorbed at the duodenum and jejunum, where it is transported through GLUT5, independent of sodium or ATP. High-saturated fat can enhance fructose absorption (Tappy & Le, 2010). At this level, fructose in converted into lactate (Tappy et al., 1986). After this process, about 12% of fructose is

transported to the liver through GLUT2 transporter. It is then metabolized (Tappy & Le, 2010). The rest of the fructose is metabolized in the kidney and adipocytes (Van den Berghe, 1986).

In the liver, fructose is phosphorylated by fructokinase to fructose-1-phosphate. In this process, ATP and phosphate molecules are lost, and AMP is generated. The generated AMP follows a degradation pathway to produce uric acid (Van Den Bergh et al., 1996). Then, aldolase-B transforms fructose-1-phosphate into 3-phosphate glyceraldehyde and phosphate dihydroxyacetone. The enzymes, fructokinase and aldolase-B, have a high affinity with fructose and are not regulated by insulin. Therefore, excessive intake of fructose is directly metabolized in the hepatocyte (Tran & Tappy, 2012). These final products can generate pyruvate and lactate via glycolysis and produce Acetyl-coA (Spruss & Bergheim, 2009). Finally, acetyl-coA is used to synthesize free fatty acids, triglycerides, VLDL and cholesterol (Szendroedi & Roden, 2009) **See Figure 1.2.**

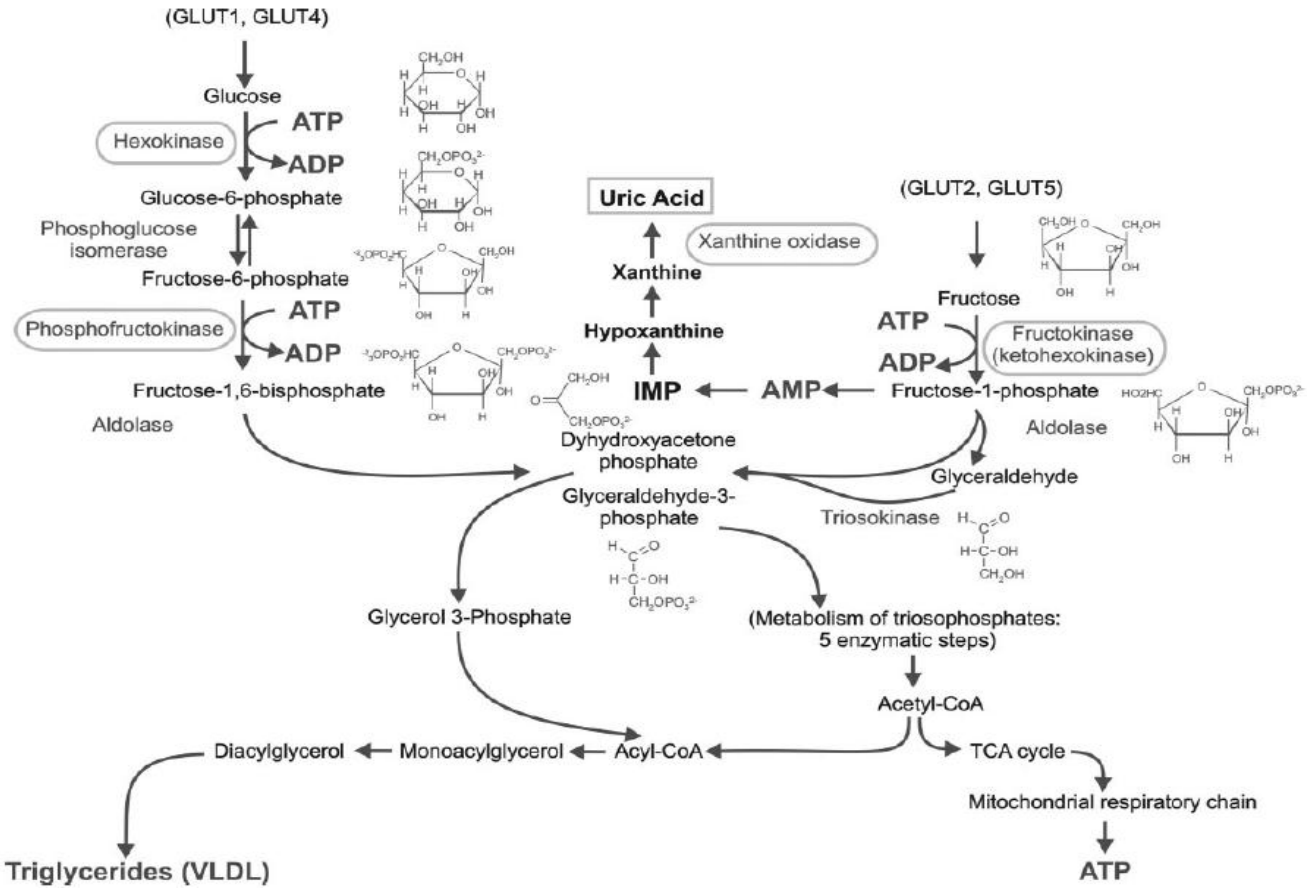

Figure 1.2 Fructose Metabolism (R. J. Johnson et al., 2009)

1.3.5 Fructose and overweight

High fructose intake has been related to increases in energy intake and consequently to increases in weight and changes in body composition, as shown by Stanhope and colleagues. In their study, participants who consumed sweetened beverages (25% of energy from either fructose or glucose beverages) showed significantly increased weight, fat mass and visceral adipose tissue as compared to participants who consumed glucose beverages (Stanhope & Hanover, 2009). In addition, humans cannot adjust their energy intake after consuming high quantities of fructose-sweetened beverages and consequently increase their intake of solid foods (Teff et al., 2004). Moreover, as previously described, fructose does not stimulate insulin production and therefore satiety potentially increases as leptin-insulin mediated secretions are low (Basciano, Federico, & Adeli, 2005). In addition, chronic high fructose consumption increases ghrelin levels, which is considered an orexigenic hormone (Bowen, Noakes, & Clifton, 2007; Lindqvist, Baelemans, & Erlanson-Albertsson, 2008; Melanson et al., 2007; Teff et al., 2004).

Epidemiological studies have associated high fructose consumption with childhood obesity. These studies highlight that children are important consumers of fructose-sweetened beverages (F. B. Hu & Malik, 2010; Vos, Kimmons, Gillespie, Welsh, & Blanck, 2008a). For example, high consumption of fructose-sweetened beverages (492 g) was associated with obesity among children (Mrdjenovic & Levitsky, 2003). In the Bogalusa heart study, children (10 years old) were followed over 21 years. The study showed that children reduced the frequency with which they consumed sweetened beverages, but not the amount or total energy intake, and consequently they significantly increased their BMI (Rajeshwari, Yang, Nicklas, & Berenson, 2005).

1.3.6 Fructose and the risk of cardiovascular disease

High intake of fructose-sweetened beverages has been associated with the presence of cardiovascular risk factors such as dyslipidemia, hypertension, and endothelial dysfunction (Havel, 2005). Acute and chronic excessive consumption of fructose increased fasting triglyceride levels by stimulating de novo lipogenesis in animal studies (Chong, Fielding, & Frayn, 2007b; Crescenzo et al., 2012; H. Y.

Koo et al., 2008; Reaven, Ho, & Hoffman, 1990). One suggested theory of how fructose raises triglyceride levels is that fructose increases Apo-B synthesis and hepatic VLDL-TG secretion. Another theory is that increased triglyceride levels may result from low triglyceride clearance, probably due to low activity of the enzyme lipoprotein lipase (LPL), which is activated by insulin (Havel, 2005). However, fructose does not stimulate insulin secretion. This lack of stimulation of insulin secretion leads to low LPL activity and impaired triglyceride clearance (Chong, Fielding, & Frayn, 2007a).

Evidence from human studies is inconclusive. Some short-term studies have shown an increase in fasting triglycerides after high intakes of fructose (Couchepin et al., 2008; Sunehag, Toffolo, Campioni, Bier, & Haymond, 2008). For example, fructose drinks (15% solution) could increase triglyceride levels more in obese insulin-resistant subjects than in obese non-insulin-resistant subjects (Teff et al., 2009). These differences suggest that subjects with more cardiovascular risk factors may be more susceptible to high fructose diets than those without. In children and adolescents, HFCS and sweetened beverages have been associated with smaller LDL particles, high blood pressure, high triglyceride levels, and high glucose levels (Pollock et al., 2012).

In terms of hypertension, some studies were conducted on rats that were fed high fructose diets. Those rates showed high blood pressure levels (Reed, Ho, Donnelly, & Reaven, 1994). In the CARDIA study, young adults who consumed sweetened beverages showed high blood lipid levels and high blood pressure (Duffey, Gordon-Larsen, Steffen, Jacobs, & Popkin, 2010). Studies suggest that fructose might increase uric acid levels (Hollander, 1974; M. T. Le et al., 2012; Perez-Pozo et al., 2010; Vos et al., 2012), which is highly related to the risk for hypertension. High fructose diets that range from 50 g/d to 200 g/d in healthy males induced high levels of uric acid (Livesey & Taylor, 2008). However, when this effect was tested with women who received different ratios of HFCS and sucrose drinks, HFCS did not affect uric acid levels (Angelopoulos et al., 2009). This suggests that the effect of fructose on uric acid levels could be more deleterious in males, a factor that may make the male with NAFLD more susceptible to adverse effects of dietary fructose. The mechanisms that relate hyperuricemia high intakes of fructose are not clear. However, it is known that the metabolism of fructose induces AMP accumulation, which can stimulate AMP

deaminase and generate purines such as uric acid (Van Den Bergh et al., 1996). High fructose diets seem to make humans more susceptible to increased uric acid levels, because humans do not synthesize vitamin C (R. J. Johnson, Andrews, Benner, & Oliver, 2010), which is involved in reducing uric acid levels (X. Gao, Curhan, Forman, Ascherio, & Choi, 2008).

1.3.7 Fructose and insulin resistance

In terms of insulin resistance, animal studies have shown that overconsumption of fructose reduces insulin sensitivity (K. A. Le & Tappy, 2006). Human studies have shown different results. For example, short-term studies (7 days to 4 weeks) with healthy subjects have demonstrated hepatic and adipose IR with an average of 25% kcal of fructose (Beck-Nielsen, Pedersen, & Lindskov, 1980; Faeh et al., 2005). In contrast, lower doses of fructose (100g/d during 4 weeks) did not induce IR (K. A. Le et al., 2006). It seems that the presence of MS risk factors (such as obesity and insulin resistance) may enhance the negative effect of fructose diets and enhanced IR. For example, for 10 weeks Stanhope and colleagues gave fructose-sweetened beverages to insulin-resistant men and women and found that 15 to 25% of energy from fructose induced IR and dyslipidemia, and increased visceral adiposity (K. Stanhope, 2008).

The mechanisms by which fructose may lead to insulin resistance are not clear. However, there are some possible explanations. For example, epidemiological studies have associated high fructose intake with high levels of the plasma peptide C (T. Wu et al., 2004). High levels of plasma peptide C were shown to exacerbate insulin resistance in overweight children (Aeberli et al., 2007). One study, on rats infused with fructose, associated an increase in the synthesis of glucose-6-phosphate, which seems to be associated with impaired glucose uptake and insulin resistance (Dirlewanger, Schneiter, Jequier, & Tappy, 2000). Fructose intake is related to the up regulation of inflammatory processes, one of which may occur via mitogen-activated protein kinase (MAPK), a kinase that is involved in cell proliferation, T-helper cells differentiation, migration, and apoptosis (Wissing et al., 2007), and has been linked to insulin resistance (Lim, Mietus-Snyder, Valente, Schwarz, & Lustig, 2010).

1.3.8 Fructose and NAFLD

Many studies with animal models assessed the effect of fructose (25-60% of energy) on cardiovascular and metabolic syndrome features (O. J. Park et al., 1992; Tetri, Basaranoglu, Brunt, Yerian, & Neuschwander-Tetri, 2008). These studies have suggested a link between dietary fructose intakes and the development of NAFLD. In fact, in animal studies, fructose seems to increase intrahepatic lipid accumulation (IHL), and induce hepatic IR and oxidative stress (Faeh et al., 2005). Short clinical humans studies (<10 weeks) that used doses from 100g/d to 250g/d of fructose found that fructose increased not only MS features, it also increased ALT and AST levels and IHL, and reduced insulin sensitivity (K. A. Le et al., 2006; Perez-Pozo et al., 2010; Sheludiakova, Rooney, & Boakes, 2012). Cross-sectional studies have also suggested that chronic consumption of high fructose diets could contribute to lipid accumulation in the liver (Assy et al., 2008).

The mechanisms by which fructose may contribute to NAFLD are not clear. However, one implicated mechanism is the depletion of ATP during fructose metabolism. Fructose is phosphorylated to fructose-1-phosphate by the enzyme fructokinase, which is not rate-limited and requires ATP. Therefore, fructose can induce ATP depletion. ATP depletion may induce hepatic inflammation as shown in animal models and human subjects (Bode, Zelder, Rumpelt, & Wittkamp, 1973; Cortez-Pinto et al., 1999). It has been found that fructose pathways may contribute to the lipid accumulation in the liver by inducing VLDL-TG production. Glyceraldehyde-3-phosphate is degraded to glycerol-3-phosphate, which is then transformed to monoacylglycerol (in excess of acyl-coA), and then to diacylglycerol, and finally triglycerides are produced and packaged into VLDL-TG particles (R. J. Johnson et al., 2009). Glyceraldehyde-3-phosphate will also contribute to *de novo* lipogenesis by producing acetyl-coA. Acetyl-coA enters the mitochondrial respiratory chain in the liver to participate in β-oxidation. Excessive acetyl-coA is exported to the cytosol in the form of citrate, which contributes to *de novo* lipogenesis (Nomura & Yamanouchi, 2012). Fructose is associated with increased activity of *de novo* lipogenesis mediators such as the carbohydrate response element binding protein (CHREBP), sterol regulatory element binding protein 1c (SREBP-1c), lipogenic

genes fatty acid synthase (FAS), stearoyl coenzyme –A desaturase-1, and acetyl co-A carbocxilase (ACC) (Lim, Mietus-Snyder, Valente, Schwarz, & Lustig, 2010; Nomura & Yamanouchi, 2012).

Another theory is that fructose induces the production of reactive oxidative species (ROS), which are associated with pro-inflammatory markers. This theory was demonstrated after rats were fed with high-fat diet or a western diet rich in fructose (55% of calories) and sucrose (45% of calories) drinks. The combination of the western diet and sweetened drinks increased hepatic triglycerides, cholesterol, AST levels and liver weight more than the high-fat diet. This combined diet also produced liver fibrosis, which was positively correlated with increased oxidative stress assessed with oxidized coenzyme Q9 (Kohli et al., 2010).

Finally, fructose overconsumption is related to intestinal bacteria overgrowth and increased intestinal permeability. These characteristics have been found in some patients with NAFLD and NASH (Farhadi et al., 2008; Thuy et al., 2008). However, fructose's key role in intestinal bacteria overgrowth is unknown. Intestinal bacteria overgrowth can generate pyruvic components (Baraona, Julkunen, Tannenbaum, & Lieber, 1986) and induce endotoxemia that may contribute to inflammation and the synthesis of lipids in the hepatic cells (Busserolles et al., 2003) by increasing the concentration of plasminogen activator inhibitor (PAI)-1 and hepatic toll-like receptor 4 (TLR4) (Thuy et al., 2008). First, bacteria overgrowth may induce increased production of reactive oxidative species and Kupffer cells, which mediate pro-inflammatory markers involved in the progression of IR (Spruss & Bergheim, 2009), an important component in the development of NAFLD. More long-term studies are needed to distinguish the effect between naturally occurring fructose, fructose added to fast food products, total dietary fructose, and HFCS. Recent studies have suggested that HFCS intake may be more detrimental to the overall health than naturally occurring sucrose and fructose from fruits, vegetables and honey (M. T. Le et al., 2012; Madero et al., 2011).

1.4 Contribution of glycemic index and glycemic load to metabolic syndrome features and NAFLD

1.4.1 Glycemic index and glycemic load concept

Jenkins and colleagues developed the term glycemic index (GI) as the incremental area under the curve (AUC) of blood glucose response after the intake of a food containing carbohydrate and compared it with the same amount of glucose, within a 2-hour period (D. J. Jenkins et al., 1981). Carbohydrate-rich foods are categorized according to their impact on glucose plasma levels. Foods with GI above 70 are classified as high GI, and foods below 55 are classified as low GI foods (D. J. Jenkins et al., 1981). Glycemic load (GL) is defined as the GI of a food, times its available carbohydrate in grams, divided by 100 (Salmeron et al., 1997).

Table 1.5 Examples of foods with low-high glycemic index

Low GI	Medium GI	High GI
Vegetables Alfalfa sprouts Asparagus Cabbage Brocoli Celery Cucumber Green peas Lettuce	Vegetables Carrots Sweet potato Sweet corn Squash Potato Beet root	Vegetables Boiled potato Mashed potato French fries
Fruit Cranberries Blackberries Blueberries, Cherries Grapefruit, Orange Limes Peach	Fruit Banana Dates Kiwi Mango Grape Apple	Fruit Dried fruits Pineapple Strawberry jam Watermelon Roll up fruit bars Fruit juices
Grain products Whole grain cereal Whole grain bread Rolled oats Whole grain pasta Brown rice Whole grain muffin Whole grain cracker	Grain products Pita bread Couscous White bread Chapatti Noodles White flour muffin Rice cakes White rice White pasta	Grain products White bread/ refined Cornflakes cereal Sugar added cereal Coated cookies Croissant Cupcake and cakes Instant oatmeal Waffles
Milk and alternatives Milk Yogurt (no added sugar)	Milk and alternatives Sugar added milk and yogurt	Milk and alternatives Rice milk
Reference: (Foster-Powell, 2002)		

1.4.2 High glycemic index/glycemic load intake and metabolic consequences

Epidemiological studies suggest that a diet characterized as having high GI (Hodge, English, O'Dea, & Giles, 2004), high GL (Salmeron et al., 1997; C. Zhang, Liu, Solomon, & Hu, 2006), and low in fiber (Salmeron et al., 1997) is associated with increased risk of T2DM (C. J. Chiu et al., 2011). Other cohort studies found a strong relationship between risk of T2DM and high GI/GL (Sluijs et al., 2010; Villegas et al., 2007). In contrast to this argument, other epidemiological studies found no associations between GI/GL diets and risk of DM2 (Mosdol, Witte, Frost, Marmot, & Brunner, 2007; A. V. Patel et al., 2007). More recently, a cross sectional study found that a breakfast that had a high GI and low fiber was associated with metabolic syndrome in adults with T2DM (Silva et al., 2011). These studies suggest that these foods, specifically, increase plasma glucose and insulin levels and consequently contribute to the development of diabetes (Sahyoun et al., 2005). More recent studies have also shown that high GI and GL foods can contribute to metabolic syndrome features such as IR (Perala et al., 2011), postprandial glycaemia (Kochan et al., 2012) and dyslipidemia (T. M. Wolever & Mehling, 2003). For example, one study in healthy men and women found that intakes of high GL foods were associated with increased dyslipidemia markers (e.g., increased triglyceride levels and reduced HDL levels) (Finley et al, 2010). In terms of GL, one cross-sectional study showed that high GL was associated with visceral fat accumulation in men (Sahyoun et al., 2005). In addition, the Nurses' Health Study (S. Liu et al., 2001; Salmeron et al., 1997) showed that high GI and GL intakes were positively associated with hypertriglyceridemia, and hyperglycemia. Overall it is suggested that dietary GI and GL are related to the development of chronic diseases such as T2DM, CVD, and some types of cancer (Barclay et al., 2008).

1.4.3 High glycemic index/glycemic load and overweight

High GI diets have been associated with obesity in adults and adolescents (Ma, Y. et al., 2005; Papadaki, 2010; (D. S. Ludwig et al., 1999). Other studies in adults have found that low GL diets are associated with decrease in visceral adiposity (Sahyoun, 2005), BMI (Mendez, 2009), but this effect was not seen with

GI. Another study with children and adolescents found a positive association between GI/GL and body fat percentage as assessed with skinfold measurements. These associations were stronger among adolescents than children (Nielsen, 2005). In contrast, a longitudinal study reported that total sugar intake was a stronger predictor of BMI and fat mass than GI/GL intake among overweight adolescents with strong background of T2DM (Davis, 2007). However, in the study conducted by Davis and colleagues, the intake of GI was relatively lower (GI =59) than what had been reported in previous studies (GI >60).

1.4.4 High glycemic index/glycemic load and the risk of cardiovascular disease

Studies have found that diets high GI and GL can lead to a risk of cardiovascular disease. For example, studies of overweight women in Japan found important associations between GI/GL and cardiovascular risk markers such as IR, hypertriglyceridemia and low levels of HDL (Amano et al., 2004). The study found that rice consumption really increased dietary GI. Other epidemiological studies have found similar connections between high GI and GL in the diet and CVD risk factors in obese adults and those with DM2. These risk factors include low HDL levels (S. Liu et al., 2001), high triglyceride levels (T. M. Wolever & Mehling, 2003), high CRP levels (Liu, et al., 2002), and insulin resistance (Amano et al., 2004). Liu and collaborators also found that high GI is associated with increased risk of coronary heart disease in women (S. Liu et al., 2001). Similarly the risk of stroke increased when participants with higher BMI (≥25) consumed high GI and GL foods (Oh, 2004).

1.4.5 High glycemic index/glycemic load and insulin resistance

Not all the studies conducted in humans have found that dietary GI and GL lead to insulin resistance (McKeown, 2004). For example one cross-sectional study in healthy adults found no connection between GI and HOMA-IR; rather GL seem to be most related to IR, suggesting that large portion sizes of carbohydrates could contribute to metabolic dysfunction and potentially be more important than the 'quality of carbohydrate' (Domínguez et al, 2010). In contrast, one feeding trial compared low and high GI meals and found important

connections between high GI meals and insulinemia (Perala et al., 2011). Studies with animals have found positive associations of high dietary GI and IR (Wiseman, Higgins, Denyer, & Miller, 1996). Studies conducted in children and adolescents are limited. For example, so far, one study found an important connection between dietary GI and HOMA-IR, ALT, and GGT in adolescent withouth, but in this study GL was not significantly related to these metabolic markers (Goletzke et al, 2013).

1.4.6 High glycemic index/glycemic load and NAFLD

Dietary glycemic index and glycemic load also seem to be associated with the development of NAFLD, as shown in one cross-sectional study regarding the effect of high GI and liver disease (Valtuena et al., 2006). This study found a significant positive relationship between dietary GI and the degree of steatosis in healthy subjects, but that dietary GL did not seem to contribute to steatosis (Valtuena et al., 2006). In addition, this same author found that the degree of steatosis was higher in subjects that consumed high GI foods and impaired HOMA-IR (Valtuena et al., 2006). In terms of the pediatric population, high GI and GL values were found among obese children with IR and high levels of aminotransferases (Mager et al., 2010). These studies suggest that high GI foods could contribute to hepatic fat accumulation, especially in IR subjects via an increased flux of fat from portal vein and up regulation of hepatic de novo lipogenesis. However, it is unclear whether these are the implicated mechanisms by which GI could contribute to steatosis.

1.5 Treatment of non-alcoholic fatty liver disease

There is no specific treatment for adult and child patients with NAFLD; however, research on this area is emerging every day. To date, recommendations have focused on weight reduction (Chalasani et al., 2012). However, there are no evidenced-based guidelines about weight-loss diets for children with NAFLD (Chalasani et al., 2012). While studies show that weight loss reduces hepatic enzymes, dyslipidemia, fasting insulin levels, inflammation, histologic parameters (steatosis and fibrosis), and overall quality of life (Hickman et al., 2004; Nobili & Manco, 2007; A. A. Patel, Torres, & Harrison, 2009), the exact mechanisms by

which changes in weight elucidate all these improvements are not clear. Many strategies are used to achieve weight loss. These include pharmacology therapy, bariatric surgical interventions, and lifestyle changes such as diet and physical activity.

Most lifestyle modification treatments focus on education about lifestyle changes to promote weight loss, as there is strong evidence that weight loss improves hepatic fat deposition and overall liver health, particularly in adults with NAFLD (Hickman et al., 2004; Promrat et al., 2010). For example, a lifestyle-change intervention in adults with NAFLD resulted in significant weight loss, ALT improvements, and steatosis reduction; but fibrosis or inflammation reductions were not observed (Promrat et al., 2010).

Evidence from lifestyle management studies that focused on dietary modification (hypocaloric diets, total fat, saturated fat and carbohydrate restriction) were consider for this literature review.

1.5.1 Calorie restriction

One common nutritional approach is to induce weight reduction by reducing calories in overweight and obese patients. However, caloric restriction does not have consistent results. For example, in obese adults, body weight loss induced with caloric restriction diets (500-1400kcal/day) for 5-6 months improved steatosis (Drenick, Simmons, & Murphy, 1970; M. A. Huang et al., 2005). In contrast, short-term studies of high-fat diets (10-12weeks) followed by low-calorie diets (LCD) for 2 weeks decreased body weight, abdominal adipose tissue, and liver index, and improved ALT levels and hepatic steatosis. However, the LCD worsened lipid profile markers (except triglycerides), necrosis, and inflammatory markers (M. A. Huang et al., 2005). These results revealed that dramatic weight loss could worsen the overall NAFLD scenario. Therefore, researchers have suggested that weight loss rates should be moderated (Chalasani et al., 2012).

In children, losing weight through low calorie diets (LCD) has improved glucose, insulin, and free fatty acids (Deschamps, Desjeux, Machinot, Rolland, & Lestradet, 1978). In terms of gradual weight loss, nutritional recommendations to achieve caloric reduction (1200 to 1500kcal/d) contributed to a mean weight loss of 8.2%, which was associated with improvements in insulin

sensitivity, ALT, and AST levels and a reduction of about 61.6% of intrahepatic lipid content (Vitola et al., 2009). Reducing carbohydrates from the diet may inhibit lipogenesis and alter the way fat is used in the body. A recent short-term study compared a LCD with a reduced carbohydrate diet (45-50%), obtaining similar values in weight reduction and serum TG, and no changes in ALT or insulin when compared with low fat diets (20-25%). However, the reduced carbohydrate diet led to a greater reduction in the accumulation of hepatic triglycerides (Browning et al., 2011).

Combining caloric restriction with either a carbohydrate or fat restriction diet is reportedly as an effective weight loss strategy for obese adults (A. J. Nordmann et al., 2006). Therefore, diets with carbohydrate or fat restriction could have the same effect on the liver. Randomized control trials among overweight and obese adults suggest that both diets are equally effective for reducing weight, IHL, and abdominal visceral and subcutaneous fat; and that both improved insulin resistance, ALT and TGB-β. Patients who had more IHL showed a greater reduction (≈7 fold) independent of visceral fat loss (Haufe et al., 2011). By contrast, another study found restricting total fat was more effective than a low carbohydrate diet at lowering liver enzymes (de Luis, Aller, Izaola, Gonzalez Sagrado, & Conde, 2010). A long-term moderate hypo-caloric (total-fat restricted) diet in adults with NAFLD significantly decreased body weight and BMI. However, it did not significantly improve liver enzymes, glucose, and insulin (Catalano, Trovato, Martines, Randazzo, & Tonzuso, 2008).

It is necessary to perform more long-term clinical trials in adults in order to understand the potential benefits of these approaches to lifestyle management in adults with NAFLD. Information about dietary changes among children with NAFLD is limited and non-conclusive. Some but not all data suggest that children with NAFLD consume similar amounts of fat and total calories compared to other obese children (de Piano et al., 2007) and yet the non-NAFLD, obese child continues to have no signs of NAFLD. This could explain why the current studies have different results. It might be important for future studies to consider first characterizing the current diet of the children and then to randomize children to different dietary approaches.

1.5.2 Low-fat diets

It is not clear what contributes more to decreased intrahepatic lipid (IHL) accumulation: a restriction in calories or a specific restriction in macronutrients. One of the objectives in treating children with NAFLD is to reduce body weight slowly. Animal studies have showed that low-fat diets led to a greater decrease in weight, hepatic steatosis and inflammation than high-fat diets that merely restricted calories (Q. Wang et al., 2011). However, not all studies conducted in obese adults have found body weight loss. For example, one study with overweight adults tested the effect of a low-fat diet for two weeks and showed that participants decreased liver fat accumulation and insulin levels, regardless of body weight changes (Westerbacka et al., 2005).

The impact of total fat versus individual fatty acids intake has been difficult to address, due to highly different outcomes in both short-term and long-term clinical trials. Saturation index of fatty acids has been highlighted as playing a key role in the development of chronic diseases (Fernandez & West, 2005; Hirabara, Curi, & Maechler, 2010). For example, saturated fatty acids (SFAs) are recognized as playing a major role in the development of atherosclerosis by altering lipid profile (high LDL particles) in people who are healthy, obese and have DM2 (Hirabara, Curi, & Maechler, 2010). Replacing SFAs in the diet with monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) have been shown to improve lipid markers, insulin levels, HOMA-IR, and may improve body composition (Haghighatdoost, Hosseinzadeh-Attar, Kabiri, Eshraghian, & Esmaillzadeh, 2012). However, there have been confounding outcomes, and different ratios have been used in the studies. These limitations make it difficult to develop guidelines for people with chronic diseases. The role of trans fatty acids (TFAs) in the diet has been associated with CVD, IR and DM2 (Lichtenstein, Ausman, Jalbert, & Schaefer, 1999; Thompson, Minihane, & Williams, 2011). However, data about TFAs and liver disease is limited. Therefore, little is known about the amount and type of fat that a person with NAFLD should consider in their diet. As reported by Mager and colleagues, children with NAFLD seem to consume more SFAs (20% from calories) than n-3 and n-6 fatty acids. This study found a correlation between low n-6 and n-3 intake and inflammation and severity of disease, assessed by levels of TNF-α and ALT variables (Mager et al., 2010). More recently, Mager and colleagues have

shown that acute intakes of high saturated fat meals (low in GI and GL) have been associated with prolonged hyperinsulinemia, lipemia and altered lipoprotein expression, all features associated with an increased risk for hepatic fat deposition (Mager et al 2013).

1.5.3 Low fructose diets

Currently, there is no dietary reference intake for the consumption of total and commercially added fructose. Recognized international agencies have declared that there is no substantial evidence to establish that a higher limit of sugars, including fructose, should be recommended. However, the Agency for European Food Safety had suggested that sugar intake should contribute to less than 25% of the total energy intake (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010). The American Heart Association has suggested limiting the intake of added sugar to less than 150kcal/day (35g/day) for men and 100kcal/day (25g/day) for women (R. Johnson K. et al., 2009). The American Academy of Pediatrics recommends limiting juice and sweetened beverages intake in children (Committee on Nutrition, 2001). The World Health Organization recommends consuming less than 10% of total calories from sugar (Waxman & Norum, 2004).

Madero conducted the first study assessing the effect of a reduced fructose diet on MS features (Madero, et al., 2011). Specifically, the study was conducted with overweight or obese participants that received either a low fructose diet (<20 g/d) or a moderate level that approximates the level found in diets that contain naturally-occurring food sources of fructose (50-70 g/d) for 6 weeks. At the end of the study both diets significantly reduced body weight and blood pressure, but the moderate natural-occurring fructose diet resulted in higher weight reduction. The moderate natural-occurring fructose diet also achieved a greater significant reduction of triglycerides, IR and blood glucose than the low-fructose diet (Madero, et al., 2011).

No low-fructose dietary interventions have been done with overweigth and obese children children or in those with NAFLD. Only one pilot study assessed how reducing sugar-sweetened beverages affected weight changes in healthy weight and overweight adolescents. In fact, the overweight adolecents

significantly reduced their body weight from baseline. However, this study did not compare the effect of sugar-sweetened beverages with HFCS-sweetened beverages (Ebbeling et al., 2006). A pilot study assesses the effect of a low total fructose (10% of calories) diet vs. a low fat diet (30% from calories) in children with NAFLD for 6 months. In this study, the low fructose diet resulted in lower levels of LDL when comapred to the low fat diet. However, in that study, no changes were seen in body weight or other metabolic markers at the end of the study, for any of the treatments (Vos, M.B, 2009).

At this moment there is little information about restricting total and commercially added fructose in children's diets. The evidence so far suggests that restricting fructose itself may be less effective than reducing the fructose that comes from processed foods (e.g., HFCS). It is not known whether such an approach may help children with NAFLD to reduce their weight and improve their overall health.

1.5.4 Low glycemic index and low glycemic load diets

In 1999, Ludwig et al. proposed that low GI foods might help subjects to lose weight by decreasing food intake and increasing satiety (D. S. Ludwig et al., 1999). However the evidence that supports this argument comes mainly from short-term and observational studies and in contrast long-term studies have shown unclear results. For example, one study in adults examined whether low GI meals increased satiety and decreased energy intake in subsequent meals (Kristensen et al., 2010). The results showed that low GI meals with high fiber content prolonged satiety but there was no effect on the next energy intake. Glucose and insulin levels may affect satiety: in one study, a low GI breakfast resulted in low glycemic and insulin levels. The glycemic response was positively associated with energy intake (Flint et al., 2006). The insulin response was positively associated with satiety, and low breakfast energy intake was associated with lower lunch energy intake (Flint et al., 2006).

Short-term randomized crossover studies in children have assessed subjective satiety and reduction of calories with low GI diets. These studies found a decrease in insulin levels and prolonged satiety in terms of time before the next meal, and significantly decreased energy intake during lunchtime (Warren et al,

2003). In terms of GL, hunger was assessed using a 5-point scale in children, but the amount of food and energy intake (during lunch) did not differ after consuming either the low-GL or high GL breakfast (LaCombe & Ganji, 2010). Finally, in an observational study, children aged 2-7 who consumed high GI breakfasts reduced their caloric intake through the rest of the day. When a morning snack was consumed, the satiety effect was prolonged (Buyken, Trauner, Gunther, Kroke, & Remer, 2007).

Low-GI diets have been associated with satiety on the grounds that they modulate leptin and ghrelin levels. However, the related mechanisms are not clear (Niwano et al., 2009). Reynolds and collaborators compared the effect of low GI with high GI meals over 10 hours. Although they found no differences in satiety between the diets, the low GI diet significantly reduced postprandial glucose, insulin, and ghrelin (GHR) and increased cholecystokinin (CCK) during the first seven hours (Reynolds, Stockmann, Atkinson, Denyer, & Brand-Miller, 2009). Intake prior to the study day was not reported. Different contents of fiber and macronutrients may have affected the results. The content and type of fiber is thought to induce satiety by reducing the levels of leptin. Studies conducted in obese adults with and without insulin-resistant, low-GI enriched diets have shown greater reduction in fasting leptin concentrations than isocaloric diets (Z. Zhang et al., 2011). More recently, it was shown that low-GI meals compared with high-GI meals decreased subjective appetite ratings, but leptin and ghrelin levels were not different between meals. However, low-GI meals showed a postprandial lowering effect on glucose and insulin levels (Krog-Mikkelsen et al., 2011). Another study did not find any significant result on satiety or appetite after a low-GI meal compared to a high-GI meal, and again the only effect was that low-GI meals induced low glucose and insulin levels (Makris et al., 2011).

In terms of changes in body composition, some but not all studies in adults have found that low GL diets are associated with a decrease in visceral adiposity (Rossi et al., 2010; Sahyoun et al., 2005), and in BMI (Mendez, Covas, Marrugat, Vila, & Schroder, 2009). This effect was not seen with GI diets. In contrast, a longitudinal study reported that total sugar was a stronger predictor of BMI and fat mass than GI/GL diets (J. N. Davis et al., 2007). In a long-term Brazilian study with women, a low-GI diet had a significant influence on weight

loss only in the first months of the study. At the end of the 12-month diet, the groups regained weight (Sichieri, Moura, Genelhu, Hu, & Willett, 2007).

Studies in children have found that low GI/GL diets are more effective than low-fat diets in decreasing weight (Esfahani, Wong, Mirrahimi, Villa, & Kendall, 2011). A six-week intervention study in children found that a low GI diet significantly reduced body fat percentage, waist-to-hip ratio and metabolic parameters, but did not influence overall body weight or BMI (Fajcsak, Gabor, Kovacs, & Martos, 2008). More recently, Siegel and collaborators compared a low GI approach to a low-fat diet and found that 95% of the children in the low GI program significantly decreased their BMI (Siegel, Neidhard, & Kirk, 2011). However, some research has identified that other components from the diet are more effective at inducing weight loss. For example, an important cohort study, the Donald study, showed that fiber (Buyken et al., 2008) but not low GI/GL diets, was associated with lower BMI and lower body fat percentage in children and adolescents (G. Cheng et al., 2009). However, more long-term studies in children are needed.

In terms of metabolic and cardiovascular markers, it is suggested that low GI diets can reduce dyslipidemia and improve blood glucose levels. A recent study found that consuming low GI fruits and breads improved HbA1c, HDL, and blood pressure; this suggests that low GI diets can be used to control glycemia and to reduce the risk of CVD (D. J. Jenkins et al., 2011). Another recent study found that low GL diets have greater impact than low fat diets in decreasing the risk of CVD (C. J. Chiu et al., 2011). In addition, other studies also suggested that low GI diets significantly decrease blood pressure, HA1c (T. M. Wolever & Mehling, 2002), free fatty acids, and triglycerides (T. M. Wolever & Mehling, 2003) in subjects with impaired glucose tolerance (IGT). However, it is not clear if these improvements are caused only by low dietary GI or by reductions in the intake of total carbohydrate calories. For example, one randomized control trial compared low GI diet versus conventional nutrition education. Both had the same effect -- decreasing fasting glucose, total cholesterol, LDL, weight and energy intake -- but the low GI diet led to a significantly higher decrease in HA1c (Amano et al., 2004). A more recent study showed that GI of foods contributes more to glycemia control than restrictions in the total amount of carbohydrates (Kochan et al.,

2012). Despite all the epidemiological evidence, the role of GI/GL as a treatment for DM2 and CVD still needs to be clarified due to confounding variables and lack of long-term studies (Bajorek & Morello, 2010).

The evidence relating low-GI diets to DM2, CVD, and obesity treatment suggests that low-GI diets may be also helpful in treating NAFLD. The lowering effect of low-GI meals on hyperglycemia and hyperinsulinemia also could contribute to reducing postprandial accumulation of both hepatical and intestinal-derived triacylglycerol-rich lipoproteins in obese subjects with insulin resistance (Harbis et al., 2004). These suggest, that improving insulin sensitivity may reduce hepatic triacylglycerol and inflammatory markers. These results may limit the progression of NAFLD before it leads to more serious consequences (D. J. Jenkins et al., 2006). For example, an Italian study suggested that high GI diets are related to a high degree of liver steatosis in adults with IR (Valtuena et al., 2006). The role of total carbohydrates, simple sugars, fiber and glycemic load on fatty infiltration in the liver is not clear.

The application of low GI diets in daily life is quite controversial. Some people are unfamiliar with the GI concept and therefore do not seem interested in it. In the case of its use in children, there is not a lot of information. However, in Australia and United Kingdom, the concept of GI is applied in food labeling and advertising and in consequence the consumption of healthy food choices has improved (H. L. Mitchell, 2008).

1.5.5 Physical activity interventions in children

There is a lack of nutritional and physical activity guidelines to promote weight loss in children with NAFLD, because few studies have been conducted in children. Current recommendations for treating childhood NAFLD recommend increasing physical activity and decreasing sedentary activities (Chalasani et al., 2012). However, the exact approach by which this is to be achieved in children with NAFLD is not described. As previously mentioned, research has shown that weight reduction is associated with improvements in insulin sensitivity, blood lipid levels, hepatic fat accumulation, fibrosis, and hepatic inflammation (A. A. Patel, Torres, & Harrison, 2009). However, the exact mechanisms by which weight loss could reverse NAFLD are not clear, and the different treatment

approaches (e.g. Dietary Interventions) are not always consistent in their results. Results from different studies assessing changes in exercise and metabolic syndrome features are summarized in **Table 1.6.**

In terms of physical activity, Magalotti conducted a study with obese children and place them in either nutritional counseling plus physical activity, diet alone or metformin (6 months). Magolotti observed that children in the first group significantly reduced more weight, improved liver transaminases, and improved hepatic insulin resistance when compared to the other treatments (Magalotti et al., 2004). Other studies with obese adolescents with NAFLD combining diet and physical activity also have resulted in greater weight loss when compared to studies prescribing only physical activity (Nobili & Manco, 2007; Nobili et al., 2008). In a three-month intervention diet, 31 overweight patients with chronic liver disease also engaged in physical activity (aerobic exercise150min/week) and weekly visits for nutritional counseling, followed by 12 months of maintenance (visits 1/month). The results showed a mean weight reduction of 3.4% at 3 months and 4% at 15 months, which corresponded to a decrease in ALT and fasting insulin levels (Promrat et al., 2010). In contrast, Hickman prescribed only physical activity changes in 14 obese adults with NAFLD, and at the end of the study a liver biopsy showed improvements in the degree of steatosis and stage of fibrosis (Hickman, 2004). A study of obese adolescents, some with and some without NAFLD, prescribed a balanced diet and physical activity (multidisciplinary treatment) during 12 weeks. The adolescents with NAFLD achieved important reductions in visceral and subcutaneous fat content and improved glucose levels (de Piano et al., 2007).

Table 1.6 Effect of physical activity interventions on metabolic syndrome

Author	Findings
Worburton et al. 2006	Physical activity decreases by 5% the risk of cardiovascular diseases
Cleland et al 2008, Sleap and Tolfrer 2000, Erikssen G. 2001, Bijnen et al 1999 , Erikseen et al 1998, Blair et al 1995	The increase of physical activity (PA) reduces the risk of premature death
Ronald et al 2004, Cuff et al 2003	Exercise decreases insulin resistance in subject with type 2 Diabetes
Ortega et al 2007	PA and cardio respiratory fitness levels are associated with total and abdominal adiposity
Spomer at al 2007	Home environment affects the PA and dietary patterns of preschool children
Ruíz et al 2004	C-reactive protein and C3 are negatively associated with cardiovascular Fitness
Ortega et al 2007	Low levels of total PA and especially vigorous PA play an important role in the development of overweight and excess central adiposity in children and adolescents
Dencker et al 2008, Ekelund et al 2007	A decrease in PA is strongly correlated with an increase in DEXA-measured abdominal adiposity
Boet et al 2006	There is an inverse relationship between PA, fitness and overweight in adolescents
Baker et al 2008	12 week walking intervention decreases BMI, % body fat, blood pressure and HDL.
Platalet et al 2005	Inverse relationship between PA and waist circumference
Savva et al 2003, Taylor et al 2000	Decreased PA is associated with increased risk for overweight/obesity
Nobili and Manco 2007, Yunianingtias and Volker 2006,	An increase in PA and diet reduces degree of steatosis in liver patients
Hickaman et al 2004	15 month lifestyle intervention (including PA) improved serum ALT levels in patients with hepatic steatosis
Huang et al 2005	One year of intensive dietary counseling and an increase in PA improved liver histology in patients with NASH
Tudore-Locke and Basset 2004, Swartz et al 2003, Hay 1997, Weston et al 1997	PA and diet intervention should be considered as the main strategy in order to prevent or even treat.

1.6 Role of the parent and family in lifestyle modification.

Parents/caregivers have an important role in the dietary intake of their children (Lindsay, Sussner, Kim, & Gortmaker, 2006). Actually, they may be an

important influence during weight loss interventions (Guilfoyle SM et al, 2010). There are many different factors that health professionals should take into account during the nutritional assessment of children, particularly overweight and obese children. For example, health professionals should investigate in depth if a child is making his/her own food choices or if the family is influencing or determining those food choices. Parents are important role models in influencing children's decisions with regard to food consumption. This means that the parent's role is important in determining a child's eating behavior (Blake, 2011). For example, one study found that parents might be a useful target to ensure that young children and adolescents are consuming fruit and vegetables (Melbye E L et al, 2011).

Other factors are parental nutritional knowledge and their level of education. For example, one study found that parents that know the correct nutritional concepts related to sodium intake, fats and carbohydrates were the ones with higher education levels, but that higher education level did not always mean the parents were following these healthy recommendations (Pomerleau J et al, 2000). In terms of parental role modeling, Phyllis and colleagues found that parental role modeling contributes to higher parental self-efficacy to induce healthy dietary habits in their children. This was specially achieved when the children were less than 12 years (Phyllis A et al, 2009). Other important factors are parental stress (Guilfoyle SM et al, 2010), family income, parental self-confidence, and parental emotional and physical health (Chang MW et al, 2008).

There are many suggestions for future dietary intervention programs with children and adolescents. For example, it is more common to associate children's eating behaviors with their mothers' eating habits, as suggested by Campbell and colleagues. Campbell and colleagues also proposed that intervention programs should target parental self-efficacy to role model healthy eating habits. (e.g., limiting fast food and sugary drinks, and sedentary behaviors in their children) (Campbell K et al, 2010).

Another proposed strategy is to target the whole family rather than only the child (Jones et al, 2010). Actually, this approach seems to be more effective with overweight and obese children (Kitscha CE etal, 2009). A recent Cochrane review concluded that future nutritional interventions with children should also encourage parental support (Waters E et al, 2011). It will be also important to consider if in the family there is a background of obesity and chronic diseases.

Families with this background may have a different perception of children's overweight and health risks (Phyllis A et al, 2009).

1.6.1 Influence of parental weight and childhood obesity

Parents play a crucial role in childhood obesity and lifestyle behaviors (L. Zhang & McIntosh, 2011), as they confer genetic and environmental factors on their children (Abdelkafi Koubaa et al., 2012; L. Zhang & McIntosh, 2011). Different studies show that parental BMI predicts childhood BMI at birth (Kleiser, Schaffrath Rosario, Mensink, Prinz-Langenohl, & Kurth, 2009) and parental obesity increases the prevalence of childhood overweight and obesity (Abdelkafi Koubaa et al., 2012). Maternal weight and gestational weight gain have been also associated with child BMI (Hinkle et al., 2012) and it is also proposed that there is a higher risk of childhood obesity when the same-sex parent and/or both parents are obese (Jaaskelainen et al., 2011; Perez-Pastor et al., 2009a).

1.6.2 Parental influence on environmental factors

Scaglioni and colleagues suggest that environmental exposure modifies children´s genetic predispositions to obesity and chronic diseases (Scaglioni, 2008). Parents shape their children's environment and eating behaviors that persist for generations (Birch, 2001). The psychosocial determinants that link parents with their children´s diet are: parental feeding practices, perception of their children's weight, role modeling and knowledge of nutrition. Family functioning, socioeconomic status and parent´s medical conditions can also be associated with children´s weight (Birch, 1990).

1.6.2.1 Parental feeding practices

Some of the eating behaviors that parent influence to their children are feeding practices. For example, parents of younger children, in particular, are the ones who typically decide what food items to purchase when grocery shopping, what foods are provided during their child's lunch and snacks, and meal frequency and content, particularly within the home environment (Steinbeck, 1998). This means that parents of younger children have control over the child´s nutrition

intake at early ages (Johnson and Birch, 1994). Sometimes these practices can have negative outcomes. For example, parents may restrict access to food and keep certain kinds of foods hidden, or conversely may role model the consumption of energy-dense food items. This may negatively affect the way overweight children respond to food (Faith, et al., 2003). One study that compared parental feeding practices of overweight and non-overweight (control) 5-year-old girls found that restrictive eating patterns are associated with increases in energy consumption, particularly in overweight girls (Birch, Fisher, Davidson, 2003). Some other negative practices include rewarding children with sugar-dense foods and pressuring the child to finish all the foods on his/her plate (Birch, 1982). This is a problem because parents determined the child´s food portions and in consequence children loose the ability to self regulate his/her dietary intake (Fisher, 2007). This will increase his/her energy intake. The potential long-term consequences may result in increased weight in childhood, especially during puberty (Villa-Caballero, 2009).

Some other ways that parents influence their children's eating behavior is by providing the food and supervising their food choices (Blake, 2011). However, some researchers have observed that positive practices such as monitoring the types of food and shopping for low-fat products does not seem to significantly influence children´s body weight (Johnson and Birch, 1994). Positive practices that link healthy eating behaviors in children are: increasing exposure to healthy foods at home (Wardle, 2003), making these foods accessible to children (Cullen, 2003), and eating meals as a family (Videon and Manning, 2003). Therefore it is suggested that parental involvement in food preparation and providing a family environment facilitates children to eat a healthy diet (Nemet et al, 2005). For example, children that have meals with their parents have greater intakes of fruits, vegetables, and dairy products, and decreased intake of soft drinks (Videon & Manning, 2003).

1.6.2.2 Parental role modeling

Parents influence their children's eating behaviors and food choices by being role models (Golan and Weizman, 2001). Studies have shown that parents that follow healthy habits such as eating fruits and vegetables are more likely to inspire their children to develop these healthy habits (Fisher, 2002). Therefore,

nutritional education should be provided to parents to ensure that children and adolescents are consuming fruit and vegetables (Melbye E L et al, 2011). In contrast, when parents follow unhealthy eating patterns and have negative attitudes, the result is unhealthy eating behaviors, mainly in adolescent girls (Yañez, 2007). Furthermore, Phyllis and colleagues found that stronger parental role modeling translated to a greater parental self-efficacy to induce healthy dietary habits in their children. This was particularly true when the children were less than 12 years (Phyllis A et al, 2009).

1.6.2.3 Parental nutrition knowledge

Parental nutritional knowledge is linked with parental level of education. For example, Pomerlau (2000) found that parents with more nutritional knowledge (e.g., about sodium, fats and carbohydrate intakes) were those with a higher education level. But parents with higher education level do not always follow healthy recommendations (Pomerleau J et al, 2000). Moreover, one study that assessed healthy lifestyle behaviors among children with NASH found that these children had good knowledge about physical activity and healthy eating, but were not consuming an adequate intake of fruits and had low levels of sedentary habits. This study also addressed that these children had a low level of quality of life factors that could contribute to their unhealthy lifestyle patterns (Hattar LN et al, 2011).

1.6.3 Family functioning

Parents also influence eating behaviors through interactions within the family and through different parenting styles (Rhee, 2008). Some authors suggest that parents that effectively manage their roles and have good verbal and emotional communication with the rest of the family members have a positive influence on the development of healthy behaviors in their children (Lin J, 2004). Baumrinds et al. has classified parenting styles as authoritarian, authoritative, permissive, uninvolved and neglectful. This classification has helped researchers to identify the parenting style that may be more related to healthy weight among children. For example, parents that are authoritative are warmer, more involved in food choices and are more able to establish limits. These parents have the lowest prevalence of overweight children (Carper JL, 2008). An authoritarian parenting

style is associated with increased energy consumption in children, especially during the first years of life (Wake, 2007). Permissive styles also have negative outcomes. For example, preschool-aged children with permissive parents had higher BMIs than children of parents with authoritative parenting styles (Carper JL, 2008). This was also corroborated by Bourdeaudhuij et al., who found that permissive parents more frequently allow their 10-year-old children to eat sweet foods and processed foods (Bourdeaudhuij & Oost, 1998).

1.6.4 Influence of parents on childhood weight management.

The influence of parents on children´s diet also plays a key role in children's weight loss interventions (Guilfoyle SM et al, 2010). Therefore, one strategy in children´s weight management is to include the parents in the weight loss programs (Garn and Clark, 1976). Epstein and colleagues have worked since the early 80s comparing the effect of targeting parents and children, targeting only children or targeting only parents through nutritional education, physical activity and behavioral changes. Epstein has found that targeting parents and children led to a significantly greater decrease in overweight percentage compared with targeting only a single group. Other studies have reported that when nutritional education is given separately to both children and parents, children have greater weight reductions when the focus of this education includes the child and the parent, rather than targeting the child alone (Shapiro, 1977; Brownell KD, 1983; Jones et al, 2010). Similar results are found when comparing targeting only the parents and targeting the whole family (Golan M, 1994; Janick & Tanas, 2007). Recently it has been suggested that a family-based weight loss program with obese children and adolescents should be considered the gold standard in treating pediatric obesity (Skelton JA, 2012). Data from one meta-analysis also concluded that most of the family-based interventions with obese children resulted in moderate and sustainable changes in weight in the long-term (Jerica M, 2011).

Not all parental factors help children to lose weight. Some facilitators suggested in weight management in children are: discussing about nutrition with children, and planning meals and physical activities together (Cottrell, 2012). In order to promote healthy changes, it is important that parents acknowledge their child's disease or weight condition (Rodriguez-Oliveiros, 2011). One of the

factors associated with effective weight loss among children is parental involvement. Recently a Cochrane review concluded that future nutritional interventions with children should also encourage parental support (Waters E et al, 2011). For example, the children with diabetes and obesity who were more likely to lose weight were those whose parents were positive role models and frequently monitored their food choices (Gellar, 2007; Heinberg, 2010).

Some authors have observed that parents of children who follow a dietary intervention may face a lot of barriers. Some are related to the design of the management program -- the inflexibility of appointments, length of the program, transportation, and quality of care provided (Grimes-Robinson, 2008). Other barriers that affect the outcomes of dietary interventions are parenting styles, and parental lack of knowledge about nutrition and food preparation practices (Rodriguez- Oliveiros, 2011). It is also important to address parental stress (Guilfoyle SM et al., 2010) because it is known that having a child with a chronic disease creates extra stress for the parents (Zeller et al 2007).

1.7 Conclusion

The increasing worldwide prevalence of childhood obesity is associated with the increasing prevalence in childhood NAFLD (Schwimmer et al., 2006). The etiology of NAFLD is not clear but multiple factors seem to contribute to its development (B. W. Smith & Adams, 2011). The leading cause for NAFLD in children may be unhealthy physical activity and dietary patterns characterized by high intakes of simple sugars (GI/GL/fructose) and saturated fat (Mager et al 2010, Jin et al 2012). Specifically, high fructose corn syrup seems to be important in the development of NAFLD and therefore is a possible target in the treatment. Current treatment guidelines suggest a gradual weight loss through lifestyle modification (diet and exercise) (Nobili et al., 2008; Reinehr, Schmidt, Toschke, & Andler, 2009; C. L. Wang et al., 2008). However, there are still no evidence-based guidelines regarding specific dietary recommendations for treating childhood NAFLD, and few studies have examined the effectiveness of specific dietary strategies. There is still a gap in the literature regarding dietary strategies that focus on reducing dietary fructose, GI and GL in childhood NAFLD.

Lack of adherence and high rates of patients that do not return to clinic appointments consistently influences the health care team's ability to evaluate the efficacy of intervention modalities on patient care outcomes. These challenges and low parental self-efficacy to role model healthy behaviors affect family´s ability to incorporate healthy lifestyle patterns. Recent evidence indicates that when parents are involved during weight loss programming, their children are likely to make greater and sustainable lifestyle changes (Guilfoyle SM et al, 2010). Very little is known about the extent to which these factors influence patient care outcomes in children with NAFLD. One purpose of this thesis was to examine the impact of a dietary intervention (low GI/GL/fructose) on body composition, liver function and metabolic markers of dyslipidemia, inflammation and lipoprotein expression in children and adolescents with NAFLD. The second purpose is to explore parental perceptions, barriers and facilitators regarding lifestyle management in their children.

Chapter 2 Research plan

2.1 Study rationale

Childhood overweight and obesity has increased globally in the last decades. For example, in Canada, the prevalence of childhood overweight and obesity is observed in 26-30% of the population (Roberts, 2012). Pediatric obesity coincidently is related with highly co-morbidities associated with metabolic dysregulation. These co-morbidities include the increasing rates of diabetes, polycystic ovarian syndrome and kidney and liver disease. Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in children and adults that has been associated with this increase in childhood obesity (Nobili et al., 2009). NAFLD is a liver disease that consists of liver abnormalities that range from histological changes such as simple steatosis (fat accumulation in the liver) to the development of steatosis with/without inflammation and fibrosis (also known as non-alcoholic steatohepatitis or NASH) to cirrhosis (Ko et al., 2009; Tamura & Shimomura, 2005). Although cirrhosis due to NAFLD in childhood is rare, some cases have been reported globally (M. H. Fishbein, Miner, Mogren, & Chalekson, 2003).

The etiology of NAFLD is not well recognized, but the presence of obesity, visceral fat accumulation, insulin resistance, and dyslipidemia are the main factors associated with the development of this liver disease (Pacifico, Nobili, Anania, Verdecchia, & Chiesa, 2011). Lifestyle factors such as unhealthy dietary intake patterns characterized by high intakes of fat and simple sugars, and sedentary lifestyle behaviors have been associated with a high risk for NAFLD in children and adults (Mager et al., 2010). Recent evidence has also shown that diets high in saturated fat, simple sugars (e.g. fructose) that are higher in glycemic index/load and are low in vitamins and minerals has been associated with inflammation and hepatic fibrosis in NAFLD (Assy et al., 2008; Cannizzo et al., 2012; Mager et al., 2010; Nobili et al., 2006; Valtuena et al., 2006) suggesting that dietary factors may play an important role in the etiology of this disorder.

Current treatment for childhood NAFLD is focused on lifestyle modification to induce weight loss in obese individuals and the use of some medications to improve insulin sensitivity (e.g. metformin) (Alisi, Feldstein, Villani, Raponi, & Nobili, 2012). Currently, it is unknown to what extent diet and

exercise may contribute to improvements in overall liver function in childhood NAFLD and what are the most effective approaches to promote lifestyle modification. Most studies have focused on reductions in energy intake to promote weight loss in childhood NAFLD (Vitola et al., 2009) and it is unclear whether changes in dietary quality (eg reductions in GI or GL or fat intake) or reductions in caloric intake leading to weight loss, contribute to improvements in overall liver function (Vitola et al., 2009) and what the short term verses long term benefits are in terms of overall liver function. Reducing dietary glycemic index (GI)/glycemic load (GL) has shown to be a promising strategy in inducing weight loss in obese adolescents (Ebbeling CB, Leidig MM, Sinclair KB, Hangen JP,Ludwig DS, 2003), but little is known whether dietary modification in GI or GL intake is an effective strategy to treat childhood NAFLD. Reductions in dietary fructose (including naturally occurring and commercially added fructose sources) and its interrelationship to disease onset and disease severity have not been extensively studied in children with NAFLD.

Few studies in children with NAFLD have examined the potential contribution of psychosocial environmental factors (such as parental attitudes and beliefs regarding food intake) that may influence patient care outcomes (S. Scaglioni, Salvioni, & Galimberti, 2008). Parental and caregiver feeding practices, perceptions about body weights and nutrition and role modelling are all known affect food intake in children (Birch & Davison, 2001; Savage, Fisher, & Birch, 2007). Some authors have highlighted that parents of children that follow a dietary intervention may face a lot of barriers such as lack of time, emotional stress and lack of nutritional knowledge (Cottrell et al., 2012; Grimes-Robison & Evans, 2008; Rodriguez-Oliveros et al., 2011); all of which may influence the success of dietary intervention strategies of children and their families.

Currently there are no evidence-based guidelines for the treatment of childhood NAFLD using lifestyle management. Although lifestyle modification remains the mainstay of therapy, very little is actually known regarding the effectiveness of dietary interventions in children with NAFLD or what the specific dietary approach should be used to promote this. Understanding the knowledge, attitudes and beliefs of parents/caregivers of children with NAFLD regarding nutrition education and its efficacy in terms of promoting lifestyle change is a

critical component to long-term success of nutritional treatment in childhood. This information would facilitate a starting point for dieticians and other health professionals to develop effective nutrition programming for families of children with NAFLD. It will also provide an important insight into what parents think about the sustainability of current interventions. While current evidence indicates that intake patterns in children with NAFLD are characterized by high intakes of simple sugars (e.g. fructose, GI and GL) (Mager et al., 2010), no data is available regarding the impact of dietary interventions that focus on reductions in fructose and GI/GL in children with NAFLD and whether or not this may result in improvements in markers of metabolic dysregulation and liver function.

Therefore, the overall objective of this thesis was to examine the effectiveness of a dietary therapy aimed at reducing GI and GL and fructose intake on markers of liver function, body composition and metabolic dysregulation in children with NAFLD. To evaluate the potential influence of parental influences on lifestyle modification, we also studied the perceptions of parents and caregivers of children with NAFLD regarding lifestyle modification.

2.2 Hypothesis and objectives

2.2.1 Study 1. Dietary intervention: FRAGILE

Study 1. Hypothesis*:* A low GI/GL/fructose (emphasis on reducing sources of commercially added fructose) dietary intervention over 6 months will result in significant improvement in body composition, liver function and metabolic parameters in children with non-alcoholic liver disease (NAFLD).

Study 1. Objectives. (The outcomes of this study are discussed in Chapter 3).

Objective 1: To assess the effect of a low GI/GL/fructose diet on body composition in children and adolescents with NAFLD over 6 months.

Objective 2: To assess the effect of a low GI/GL/fructose diet on liver function (AST, ALT, GGT in children and adolescents with NAFLD over 6 months

Objective 3: To assess the effect of a low GI/GL/fructose diet on metabolic parameters (insulin, HOMA-IR, triglycerides, glucose, cholesterol, HDL, LDL and NEFA) in children and adolescents with NAFLD over 6 months.

Objective 4: To assess the effect of a low GI/GL/Fructose diet on adipocytokines (TNF-α, IL-6, IL-10, adiponectin and leptin) and markers of lipoprotein expression (apob100, apob48, apoc3) in children and adolescents with NAFLD over 6 months.

2.2.2 Study 2: Parental perceptions regarding lifestyle modification in children with NAFLD.

Study 2. Objectives. (Chapter 4).

Objective 1: To explore the perception of parents/caregivers of children with NAFLD regarding nutrition education

Objective 2: To explore the experience and identify barriers and facilitators of parents/caregivers of children with NAFLD when helping their children to follow a healthy diet.

Objective 3: To compare these findings with parents/caregivers of healthy lean children who have received nutritional education regarding lifestyle management (Control group).

Chapter 3- Fragile study

3.1 Introduction

Childhood obesity increases the risk of chronic diseases such as cardiovascular disease, diabetes mellitus, kidney disease and liver disease (Wang et al., 2011). Nonalcoholic Fatty Liver Disease (NAFLD) is one of the most common liver diseases, particularly in overweight and obese individuals. NAFLD is a liver disease describes a spectrum of liver dysfunction. In the earlier stages of the disease, there is excessive fat accumulation in hepatocytes (simple steatosis) and it can progress to more serious liver damage characterized by inflammation with/without fibrosis (Nonalcoholic Steatohepatitis or NASH) (Alisi, Cianfarani, Manco, Agostoni, & Nobili, 2011; Ko et al., 2009). Although, rare in childhood, adults with NASH have been known to develop cirrhosis; the most serious expression of the disease (Kopec & Burns, 2011b). NAFLD is typically diagnosed in overweight and obese children, but some children (5-25%) have healthy body weights within normal reference ranges (Yap et al 2011). The treatment for children with NAFLD, should be focused on lifestyle management (Alisi, Feldstein, Villani, Raponi, & Nobili, 2012; Gossard & Lindor, 2011).

The trends of high fructose consumption worldwide and its association with high prevalence of chronic diseases such as NAFLD, has led to interest in investigating the role of total and commercially added fructose in the etiology of NAFLD (Angelopoulos et al., 2009; Ostos et al., 2002; Perez-Pozo et al., 2010). Recent data suggests that mean intakes of total fructose among US adolescents (12-18 years) ranges between 9-23% of total energy intake; an intake that has been strongly associated with the onset of obesity and metabolic syndrome (MS) (Sun, Anderson, Flickinger, Williamson-Hughes, & Empie, 2011b). All of these factors suggest that high fructose intakes might be a significant contributor to MS in obesity and NAFLD; particularly at intakes that exceed 10% of total energy intake.

Short clinical human studies (<10 weeks) that used high fructose doses (16-20% daily calories from total fructose) found that fructose increases the expression of certain MS features, liver function enzymes and intra hepatic lipid content (IHL) (K. A. Le et al., 2006; Perez-Pozo et al., 2010; Sheludiakova, Rooney, & Boakes, 2012). In contrast, a recent epidemiological study did not find

positive associations between fructose intake at much lower levels of intake (9% of daily calories) and MS in adults and children (Sun, Anderson, Flickinger, Williamson-Hughes, & Empie, 2011a). All of this suggests that there may be a threshold of fructose intake that needs to be reached before adverse effects of fructose on metabolism occurs; particularly in individuals who are overweight and/or obese.

High fructose corn syrup (HFCS) may be one of the important factors influencing MS because HFCS has been associated with increased expression of cardiovascular disease risk factors such as insulin resistance and dyslipidemia (Haley, 2012). In addition, in a feeding study with children, investigators found fructose beverages (9% calories from fructose or glucose beverages) increased postprandial triglycerides levels in healthy children and children with NAFLD. But this negative effect was higher in the NAFLD group (Bray, Nielsen, & Popkin, 2004; Jin et al., 2012; K. L. Stanhope et al., 2009). Taken all together, this information suggests that reducing fructose intake (<9-10% of energy intake) could be part of a dietary treatment strategy for children with NAFLD.

The impact of the quality (GI) and quantity (GL) of carbohydrates in the diet has been investigated in terms of hyperglycemia in patients with diabetes (C. J. Chiu et al., 2011). Studies have associated GI and GL with an increase of IR (Perala et al., 2011), postprandial glycaemia (Kochan et al., 2012) and dyslipidemia (T. M. Wolever & Mehling, 2003). Low GI diets seem to improve glucose, insulin, HbA1c, HDL, blood pressure, free fatty acids, and triglycerides levels (T. M. Wolever & Mehling, 2003). In terms of body composition studies in adults and some in children found that low GL diets are associated with weight loss and reduction in visceral adiposity (Rossi et al., 2010; Sahyoun et al., 2005).

Currently, there are no evidence-based guidelines that focus on specific dietary strategies for the treatment of childhood NAFLD. While lifestyle modification aimed at promoting weight loss has been the mainstay of therapy, little is known the extent to which changes in diet nutrient quality versus energy restriction leading to weight loss will evoke favorable changes in the liver in children with NAFLD. Very few studies have examined the efficacy of a dietary strategies focused on reductions in fructose, glycemic index (GI) or glycemic load (GL) from the diet of children with NAFLD. Hence, the purpose of this study was

to examine the impact of a dietary intervention (low GI/GL/fructose) on body composition, liver function and metabolic markers in children and adolescents with NAFLD. The study hypothesis is that a low GI/GL/fructose dietary intervention (in the absence of energy restriction) over 6 months would result in significant improvement in body composition, liver function and metabolic parameters in children with NAFLD.

3.2 Subjects and methods

The data presented in this chapter, is a subset of a larger prospective study examining the influence of diet and lifestyle modification in children with NAFLD (Diet modification only). The study is a 6-month pilot intervention study examining the impact of a low GI, GL and fructose diet on changes in liver function (ALT, AST, GGT and CRP); metabolic biochemical parameters (glucose, insulin, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), non-esterified free fatty acids (NEFA) and uric acid; lipoprotein metabolism (apolipoprotein B-48 (Apo B-48), apolipoprotein B-100 (Apo B-100) and apolipoprotein C-III (Apo C-III)); adipocytokines (interleukin-10 (IL-10), interleukin-6 (IL-6), adiponectin and TNF-α) and body composition in children and adolescents diagnosed with NAFLD (8-18 years). Data was compared to healthy lean control children (8-18 years). Subjects were followed at baseline, 3 and 6 months **Figure 3.1**.

Informed consent and/or assent were obtained from all parents/caregivers of participants prior to subject enrolment. Ethics approval was obtained from the Human Research Ethics Board, University of Alberta. Operational and Administrative approval was obtained from Alberta Health Services via Northern Alberta Clinical Trials Centre (NACTRC).

Children and adolescents diagnosed with NAFLD (n=12) were recruited from the Pediatric Gastroenterology Clinic at Stollery Children´s Hospital, Edmonton, Alberta. Healthy lean controls (n=14) were recruited with the help of recruitment fliers at University of Alberta/Stollery Children´s Hospital.

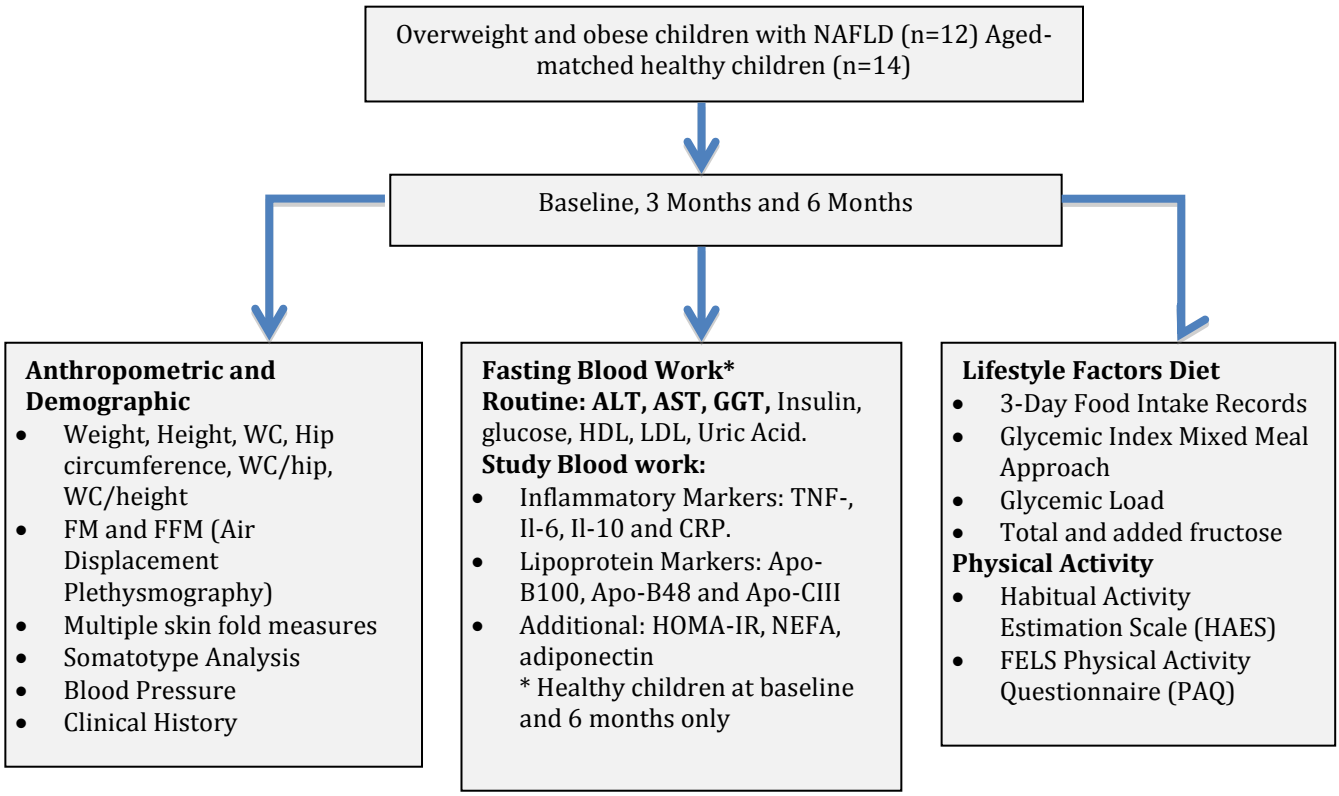

Figure 3 . 1 Study Flow Chart

3.2.1 Inclusion and exclusion criteria

Children diagnosed with NAFLD (8-18 years) and healthy lean control participants (8-18 years) were recruited for this study. All patients underwent a screening (serological and blood work) to rule out the other competing causes of fatty liver disease in order to prevent the potential inclusion of children with other known primary liver diseases associated with steatohepatitis (e.g. Wilson disease, other metabolic disorders), **(Chapter 1, Table 1.3).**

All patients with a known primary diagnosis of Type 2 Diabetes or those taking insulin; patients on medications known to cause steatosis and/or patients with limited mobility due to joint injury or concurrent chronic disease resulting in limited joint mobility (e.g. rheumatoid arthritis) or inability to participate in age-appropriate physical activities were also excluded from the study.

3.2.2 Diagnosis of NAFLD

The diagnosis of NAFLD is suspected in obese children with elevated levels of liver enzymes (AST, ALT and GGT), the presence of severe insulin resistance (by HOMA-IR), hypertriglyceridemia and excessive visceral adiposity (Vajro et al., 2012a) and is confirmed by exclusion of other secondary fatty liver

related diseases (Mager, Ling, & Roberts, 2008) and by evidence of steatosis as assessed with abdominal ultrasound or liver biopsy. Liver biopsy in children is typically performed to exclude other secondary diseases, in case of suspected advanced liver disease and before pharmacological/surgical treatment (Chalasani et al., 2012; Vajro et al., 2012b). In this study all children were diagnosed using biochemical and ultrasound evidence of fatty liver with/without liver biopsy.

3.2.3 Assessment and anthropometric measurements

Body composition assessment included the following measurements: weight, height, body circumferences and diameters, skin folds, determination of body fat percentage (BF%). Subjects were encouraged to void before initiating the body composition assessment and all the body composition measurements were taken under fasting conditions (Typically 10-12 overnight fasting hours).

3.2.3.1 Blood pressure

The blood pressure was measured at the clinical research unit (CRU) according to the AHA guidelines described below (Pickering et al., 2005). Participants rested for 15 minutes upon arrival to the unit. **(For cloths specification see section 3.2.3.4).** Children were instructed to be seated with the back supported, legs uncrossed, right arm supported over a table in 90-degree angle position and to not talk during the measurement. Middle line of the cuff was placed on the upper arm at the level of the right atrium (2.5 cm above the elbow case). If blood pressure readings were high a second reading was performed after 5 minutes.

3.2.3.2 Weight and height

The weight of participants was assessed using the air displacement plethysmography (Bod Pod, COSMED Chicago, IL, USA, Inc. calibrated scale, which has been validated in children and adolescents (Lockner et al., 2000; McCrory et al., 1995). Weight to the nearest 0.5kg was assessed without shoes and with minimum clothing worn **(For clothing specification see 3.2.4.4).** Height was measured without shoes to the nearest 0.5 cm with the use of a commercial stadiometer (Charder HM200PW, Medical Supplies, North Blend, WA, USA). Body mass index (BMI) was calculated as: Weight (kg) / Height (m^2). Children were classified as normal weight or obese according to International Obesity Task

Force (IOTF) and Centre of Disease Control (CDC) criteria for age and sex-specific BMI cut-off points (overweight >85th percentile, obese >95th percentile (Cole, Bellizzi, Flegal, & Dietz, 2000; Onis et al., 2007).

3.2.3.3 Body circumferences

Body circumferences were measured using a steel flexible tape (Rosscraft Innovations Incorporated Toronto, Canada). Waist circumference (WC) was measured using the WHO criteria: Midpoint between the lower border of the rib cage and the iliac crest (Rudolf et al., 2007). WC percentiles for age and gender were used to assess for the presence of visceral adiposity (Katzmarzyk, 2004). Hip circumference was measured at the maximum protuberance of the buttocks (Taylor R et al., 2000). The mid arm circumference was measured at the mid-point between the tip of the shoulder and the olecranon process and the acromion union of the left arm of participants and it has been validated it use in children (M. White, Davies, & Murphy, 2008). The calf girth was taken at the level of the largest circumference of the calf (International Society for the Advancement of Kinanthropometry, 2001).

3.2.3.4 Diameters and skinfolds

Humerus and femur diameter were measured using a small bone caliper (Calibres Argentinos, Rosario, Argentina). The skinfolds (triceps, biceps, subscapular, supraspinal, iliac, abdominal and calf) were measured using a Lange skinfold caliper (Beta technology, Santa Cruz, CA, USA) (International Society for the Advancement of Kinanthropometry, 2001).

The skinfolds measurements were also used to calculate the distribution between the truncal and extremity ratio (Leppik et al; 2004) as follows: Σ trunk skinfolds (Subscapular+Supraspinal+Ileac+Abdominal)/Σ extremities skinfolds (Bicep+ Triceps +Calf).

3.2.3.5 Determination of body fat percentage

Body composition was determined using air displacement plethysmography (Bod-Pod) using the Human Nutrition Research Unit (HNRU) protocol. Bod-Pod was warmed-up and calibrated (calibration occurred before each test). Body fat percentage and body free fat mas percentage was evaluated using a validated methodology: Air Displacement Plethismography (Bod-Pod,

COSMED, Chicago, IL, USA) (D. A. Fields, Goran, & McCrory, 2002). The Siri equation was used to determine body fat percentage (% Fat= (4.95/Db-4.50) *100) (Siri, 1961). This method has been used previously in this group to determine absolute fat mass and BF% in obese children with and without NAFLD (Mager, Yap et al., 2012).

Children were asked to void before the body composition assessment, to not exercise in the previous 2 hours and to remove all jewelry. In order to minimize inaccurate measurements of volume, children were required to wear the least clothing possible (P. Higgins, Fields, Hunter, & Gower, 2001). (For boys it was required to use form-fitting Speedo or Lycra/Spandex-type swimsuit, or single-layer compression shorts and wear a swim cap to compress the hair; for girls it was required to use form-fitting Speedo or Lycra/Spandex-type swimsuit, or single-layer compression shorts and single layer jog bra and wear a swim cap to compress the hair). In some cases for children that felt uncomfortable with the clothing specifications outlined, they were allowed to wear a thigh length t-shirt. Most of the children in this cohort were comfortable to use the required clothing for the bod-pod test. However, 3 children in the NAFLD group were allowed to wear a tight t-shirt and tight short for the bod-pod test.

Then, children were asked to enter to the Bod-Pod chamber and to not move during the test. Thoracic gas volume (TGV) was predicted (Crapo, Morris, Clayton, & Nixon, 1982) and two tests were performed and if movement was detected a third test was performed.

3.2.3.6 Somatotype

The Somatotype was calculated using the Somatotype 1.2 software (Swear technologies, Australia). In order to determine the somatotype the following measurements were entered in the software: height, weight, triceps, subscapular, supraspinal, calf, girths (flexed arm and calf) and bone breadths (humerus and femur) (Carter & Heath, 2003). The software uses the following equations:

Endomprphy = $-0.718+0.1451\ (X) - 0.00068\ (X^2) + 0.0000014\ (X^3)$

Where X = (sum of triceps, subscapular and supraspinal skinfolds) multiplied by (170.18/height in cm).

Mesomorphy = 0.858 x humerus breadth + 0.601 x femur breadth + 0.188 x corrected arm girth + 0.161 x corrected calf girth – height 0.131 + 4.5

Ectomorphy = Calculated depending on height-weight ratio (HWR)

If HWR $\geq$ 40.75, then = 0.732 (HWR) – 28.58

If HWR < 40.75 but > 38.25, then = 0.463 (HWR) – 17.63

If HWR $\leq$ 38.25, then = 0.1

3.2.4 Biochemical measures

Participants were asked to fast for 12 hours overnight at baseline, 3-month and 6-month visits. Children and adolescents with NAFLD had blood work collected at all three visits; healthy lean controls at baseline and six months only. Fasting blood work included liver biochemistry parameters; metabolic parameters and extra 5 ml of plasma were used to analyze adipocytokines and inflammation, and lipoprotein metabolism markers. Extra plasma was centrifuged for 15 minutes at 1000-x g within 30 minutes of collection. Aliquots of plasma were made and stored at -80ºC until analysis. Blood for this study was collected as part of routine clinical blood work for children with NAFLD.

3.2.4.1 Liver biochemistry parameters

Liver biochemistry parameters included: ALT, AST, GGT and were analyzed by Alberta Health Services (AHS) following standardized protocols from the Core Laboratory (Synchron LX Systems Analyzer; Beckman Coulter, Fullerton, CA). This represents routine clinical blood work.

3.2.4.2 Metabolic parameters

The metabolic parameters included were fasting glucose, insulin, triglycerides, total cholesterol, HDL, LDL, CRP and uric acid. Metabolic parameters were analyzed by laboratory services Alberta Health Services (AHS) following standardized protocols from the Core Laboratory (Synchron LX Systems Analyzer; Beckman Coulter, Fullerton, CA). The homeostasis model of assessment of insulin resistance (HOMA-IR) was calculated as follows: [Fasting Insulin fasting insulin (μU/mL)/22.5 x glucose (mmol/L)]; HOMA-IR values less than 3 are considered within normal ranges (Gungor, Saad, Janosky, & Arslanian, 2004; Keskin, Kurtoglu, Kendirci, Atabek, & Yazici, 2005)Adipose tissue insulin resistance index (AI-IR) was calculated as follows: [Fasting insulin (uU7ml) x

NEFA (mEq/L)] (Mager, Mazurak et al., 2012). All these parameters were routine clinical blood work.

Plasma concentrations of NEFA were analyzed using commercial Enzyme-linked Immuno-Sorbent Assay (ELISA) kits (WAKO Pure Chemical Industries, Ltd, Richmond, and USA). For NEFA, NAFLD samples were diluted 1:30 with distilled water and used 10µl whereas control samples were not diluted. NEFA detectable level range was 0.01- 4mEq/L. This method had been used by this research group to measure NEFA in both obese children with and without NAFLD and in healthy children (Mager et al 2012). Blood collected for these assays were in addition to routine clinical blood work.

3.2.4.3 Adipocytokines and Markers of Inflammation

Adipocytokines and markers of inflammation included pro inflammatory markers such as CRP, tumor necrosis factor-α (TNF-α ELISA kit; R&D Systems, Minneapolis, USA), interleukin-6 (IL-6 ELISA kit; R&D Systems, Minneapolis, USA). As part of the anti inflammatory markers interleukin-10, (IL-10 ELISA kit; R&D Systems, Minneapolis, USA) and adiponectin (an adipocytokine) (Okamoto et al., 2001) (Human Adiponectin ELISA kit; Millipore Corporation, Missouri, USA) were included.

For TNF-α, the detectable range is 0.038-0.191 pg/mL in 200µl of sample. For IL-10 the detectable range is 3.9-7.8 pg/mL in 200µl. For IL-6, the detectable range is 0.016-0.110 pg/mL in 100µl sample. Adiponectin samples were diluted 1:500 (Assay Diluent, Phosphate base buffer). The detectable adiponectin range is 1.56-100 ng/mL in a 20µl of diluted sample. Blood collected for these assays were in addition to routine clinical care.

3.2.4.4 Lipoprotein metabolism markers

Markers of lipoprotein metabolism included assay of Apolipoprotein B-48 (Apo-B48 ELISA kit; Shibayagi, Gunma, Japan), Apolipoprotein B-100 (Apo-B100 ELISA kit; Kamiya Biomedical Company, Seattle, USA) and Apolipoprotein C-III (Apo-CIII ELISA kit; Abnova, Taiwan) using standardized commercial kits. For Apo B-48, the detectable range is of 2.5-160ng/ml in a 10µl sample size; NAFLD and lean samples were diluted 1/200 (Buffer Solution (C)). For Apo B-100, the kit detectable range was 13.7 to 10 000 ng/mL in a 100µl diluted sample size; NAFLD samples were diluted 1/4000 and lean samples

1/1000 (PBS). Apo C-III detectable range was 0.002-2 μg/ml in a 50 μl diluted sample (EIA diluent, buffered protein base); NAFLD and lean samples were diluted 1/800. Blood collected for these assays were in addition to routine clinical care.

3.2.5. Dietary intake analysis

Dietary intake was assessed in each visit using 3-day food records (two weekdays and one weekend day) prior to the study visit. Children and Parents/caregivers received hard copies/electronic copies of the three-day food records before the study visit. The energy, macronutrient (protein, carbohydrates and cats) and micronutrient (vitamin E and minerals: Ca, Mg, Mo, P, K and Na) of the diet were analyzed using The Food Processor SQL 10.8.0 ESHA Research 2011.

3.2.5.1 Glycemic index and glycemic load analysis

GI and GL of the diet were calculated using the values from the international table of GI (Foster-Powell. Atkinson, 2002 and 2008). The content of fat, protein and fiber content of a meal can influence the GI/GL of a meal. The following equations (Galgani, Aguirre, & Díaz, 2006; T. M. S. Wolever & Bolognesi, 1996) were used for the GI and GL analysis and example of calculations are found in the **Appendix A, Table A.3.**

GI mix meal: $\sum$ (food item carbohydrate g × GI)/total carbohydrate meal g

The GL: $\sum$ (food item carbohydrate g × GI of each food)/100

3.2.5.2 Dietary fructose analysis

In order to calculate total fructose, free fructose and sucrose values of each individual food items in grams were taken from the Canadian nutrient file, the USDA database (National Nutrient Database for Standard Reference, Release 25 Software v.1.2.2) and food manufacturers webpages. Then, total fructose for each individual food item was calculated as follows: free fructose (g) + (sucrose (g) /2): **See example in Appendix A, Table A.2**. Then, the total fructose in grams was classified by fructose sources: fruits, vegetables, dairy products, grain products (whole and refined products), sweetened beverages, other products (cookies, donuts, pizza) and HFCS (Only food products that the food label reported HFCS within the ingredients) **(Appendix A, Table A.1).** The grams of

sweetened beverages, other products and HFCS, were added to compare the intake of these products, because these are considered unhealthy sources of fructose (Madero et al., 2011). Finally free fructose, total fructose per day was calculated as follows: adding the total grams of three days and getting the average **(Appendix A, Table A.2).**

3.2.6 Diet intervention

Within the nutritional education provided the main focus was to improve the quality of the diet by encouraging the consumption of low fructose/GI/GL foods. The purpose of the FRAGILE diet was to provide dietary education that would focus on dietary reductions in commercially added fructose, GI and GL and promote age appropriate intakes of energy in children with NAFLD and in the lean healthy controls.

Children were provided with sample menus with a macronutrient distribution as follows: 15-20% of calories from protein, 25-30% calories from fat and 45-50% calories from carbohydrates (Wolover et al 2006, 1991, Stewart et al 2005, Ebbeling et al 2005, 2003, Pereira et al 2004, Johnson-Down et al 2003, Relly et al 2003, Ludwing 2002, Spieth et al 2000). Example menus consisted of 3 meals and 2 snacks that ranged from 1600-2300kcal. The number of servings for each study participant was prescribed according to age, gender and energy requirements. Energy requirements for each child was based an assessment of basal metabolic rate (BMR) with an activity factor of 1.5. This activity factor was chosen because it has been previously demonstrated that children with NAFLD spend more than 60-65% of their daytime activities in sedentary activities (Mager et al 2010). Therefore it was not expected that children would perform highly intense activities during the study and so an activity factor of 1.5 was used as this factor reflects this level of activity (RINGWALD-SMITH et al., 1999). BMR was calculated according to the WHO equations (Schofield, 1985) using ideal body weight (Phillips, Edlbeck, Kirby, & Goday, 2007).

In the first visit nutritional education included brochure with basic information about fructose, GI and GL. Participants also received a list of foods ranked according to low-high GI/GL/Fructose foods and they were encouraged to consume low GI/GL/fructose foods (legumes, fruits, vegetables and whole grains) and to reduce high GI/GL/fructose (sweetened beverages, fruit juices, bakery

goods). A sheet with practical tips to reduce the GI (e.g. not over cook pasta, combine carbohydrates with protein sources or healthy fats etc.) was given as part of the educational tools. A set of low GI/GL/fructose recipes was also given to participants. These recipes included breakfast, lunch, dinner recipes and snack ideas. Examples of HFCS foods were given and dietary counseling focused on reducing consumption of HFCS-containing foods. Emphasis was placed on increased consumption of fruits and vegetables. The purpose of the first clinic visit was to evoke sufficient dietary changes in total fructose (less than 7 % of calories) by focusing on reductions in intake of commercially added sources of fructose, GI and GL. The main aim of this first visit was to ensure that the child and family became familiarized with a series of educational tools that were aimed to promote a reduction in GI/GL and total fructose intake. These included educational tools such as list of foods with low, medium and high GI/GL and frucotose contents, sample menus with specific examples about age appropriate samples sizes and multiple snack ideas, **(See 3.2.6.3).** Participants were encouragde to select low GI/GL/fructose foods on a daily basis and to moderate their selections of medium-high containing GI/GL and fructose foods as follows: select 1 item of each food group of medium content of GI/GL/fructose foods from 3-5 days per week, select 1 non-processed foods of high content of GI/GL/fructose from 3-5 days per week and select 1 processed food or treat once per week. With these dietary changes the diet provided a low GI (<55), low GL (<80) and low fructose content (<7%) **(See 3.2.6.3).**

In the second visit (3 month), the concepts of dietary GI, GL and the contribution of commercially added fructose to foods were reinforced by comparing the content of added sugar and added fructose from different products. Participants were provided with instructions about reading food labels. The goal of this exercise was to encourage participants to identify commercially added fructose sources in food products and encourage them to reduce the frequency of intake of products with commercially added fructose (>11gr) and HFCS sources.

The goal of this visit regarding GI and GL was to reinforce the concepts, by providing tips of how to modify their own preferred dishes (e.g. If participant preferred waffles, suggestions on how to increased the content of fiber or protein to the mix, or combinations with other food items in order to decrease the GI were given). In each visit participants received different recipes and snack ideas that

None of these tools in nutrition education changed the overall focus in terms of GI/GL/fructose reductions, but were additional tools to help patients translate the nutritional concepts taught and to reinforce these with simple and practical tools for the child and their family. In order to assess compliance to the diet follow up Phone calls and emails were made once a month to each client. In each follow up phone call 24hr recall was performed to review potential dietary sources of HFCS in particular. Dietary adjustments and information reinforcement such as snack ideas, recipes were given if necessary. The goals of each visit of GI, GL and fructose are specified below **(See 3.2.6.1 and 3.2.6.2).**

3.2.6.1 Glycemic index and glycemic load dietary changes

Children and their families were encouraged to increase consumption of low GI foods such as fruits, vegetables, whole grains and legumes and to reduce intakes of high GI foods such as processed grains, potatoes and sugars. To help parents and children with their food choices, low GI foods (45-55) were labeled as choose more often; medium GI foods (55-60) as choose sometimes and high GI foods (>60) as choose least often. The aim was to achieve a low GI consumption (45-55), which is associated with increase satiety in children (Buyken, Trauner, Gunther, Kroke, & Remer, 2007). Subjects were counseled about GL and were advised to monitor their food portions. Larger food portions of a low GI food can still increase the GL. The aim was to consume per day a GL below 80 per day.

3.2.6.2 Fructose dietary changes

The major dietary goal was to encourage intakes of total fructose up to a maximum of 7% of calories as it has been showed that fructose intakes that range of a minimum of 9% to 25% or more calories from total fructose is associated with IR, dyslipidemia and increased visceral adiposity (K. Stanhope, 2008). For example, reducing the frequency of intake of foods that had commercially added fructose and HFCS products (carbonated beverages, juice, bakery goods, refined breakfast cereals) reduces about 20% of calories from total fructose. Advising families to have a variety of small servings (according to nutrition guidelines) of low to medium content of fructose fruits and vegetables during the week to help keep fructose intake to a maximum of 7% calories.. Therefore, in this study the overall quality of fructose intake was improved by reducing industrialized sources of added fructose and encouraging the consumption of naturally occurring

fructose sources (fruits and vegetables) as it has been suggested that naturally occurring fructose is not associated with the expression of MS dysregulation (Madero et al., 2011).

3.2.6.3 Nutritional education materials

The nutritional education material included a brochure (FRAGILE brochure) explaining the concepts of glycemic index, glycemic load and fructose, a list of foods, an example menu that contained a table with their individual number of food servings and portion sizes, a sheet with tips to decrease the glycemic index of the foods, a set of recipes and snack ideas and a set of sheets on how to read food labels.

Fragile brochure

The Fragile brochure explained why we were doing the study, the concepts of the dietary changes in the study such as glycemic index, glycemic load and fructose and what things the participants could expect in the study **See appendix A, page 209.** The brochure was giving to participants at recruitment and in the first visit the dietary concepts of the study were reinforced by giving examples of meals in their 3 day food record that could be high in fructose or in glycemic index, explaining how this may influence in their liver health and tips on how they can improve those meals.

List of food exchanges: low glycemic index, glycemic load and fructose foods

This list contained foods ranking the foods according to low to high glycemic index, glycemic load and fructose. This list divided foods by choose more often, choose sometimes and choose least often.

Choose more often list

The choose more often foods combined foods with low glycemic index (<55), low glycemic load <80 and was combined with fruits and vegetables with low content of total fructose (7gr per portion). Children and parents were adviced to prefer these foods during the week (e.g. choose these fruits and vegetables in your daily snacks or side dishes) and were reminded that larger portions of these foods (e.g. strawberry) would increase the content of fructose and the GI in their diet.

Choose sometimes list

The choose sometimes foods contained medium glycemic index foods (55-60) and glycemic load foods (80-120), and it also contained naturally occurring foods with medium to high content of fructose (7-11gr per portion). Children and parents were advice to spread the intake of these food ítems during the week (e.g. you can choose one fruit of this list every day or combine these food ítems with the ones in the choose more often list). This list also contained information about being aware of portion sizes of fruits and bakery products in this list, and contained tips on how to combine these foods with other foods in order to increase satiety and decrease the GI.

Choose least oftent list

The choose least often foods contained high glycemic index foods (>60) and high glycemic load foods (>120), and fructose (>11 gr). Specifically contained added fructose, sucrose, HFCS food products (e.g. fruit juice, candy and carbonated beverages). Children and parents were adviced to choose small portions of the fruits and vegetables in this list, to choose small servings of processed foods in this least one day per week or after exercise (e.g. you can have small slice of cake in the weekend). They were also advice to reduce the portions or restrict as much as posible the intake of carbonated beverages.

Menu sample sheet

This sheet contained a menu sample with 3 meals and 2 snacks. Each menu contained the food servings according to calories that range from 1400-2400 kcal. This sheet also contained a table with food servings and portion size servings.

How to decrease the glycemic index

This sheet contained tips to decrease the glycemic index of the foods (e.g. do not over cook rice and pasta) and how to combine carbohydrate-containing foods to decrease the glycemic index (e.g. combine a cup of fruit with slices of cheese).

Recipes and snack ideas

The set of recipes of low glycemic index, glycemic load and fructose, included breakfast, lunch, and dinner and snack examples.

3.2.7 Physical activity assessment

Habitual physical activity was assessed using two validated questionnaires **See appendix A, Questionnaires 1 and 2**. Children and parents/caregivers were instructed on how to answer the physical activity questionnaires at the first study visit and were provided with hard copies or an electronic copy was sent via e-mail. At each visit, children completed the Habitual Activity Estimation Scale (HAES) (Hay & Cairney, 2006) and the FELS questionnaire (Treuth, Hou, Young, & Maynard, 2005).

Regarding the HAES questionnaire children were asked to report a typical weekday and weekend day of a previous week before the study visit. HAES results are presented as percentage of hours spent in different activity intensity levels (inactive, somewhat inactive, somewhat active and active) (Hay & Cairney, 2006). **See appendix A, Questionnaires 2.**

The FELS physical activity questionnaire for children (PAQ) assess the habitual physical activity since the previous visit and it asks how frequently children participated in physical activities that are ranked according to intensity levels which are converted to sport index, leisure index and core index (Treuth, Hou, Young, & Maynard, 2005). **See appendix A, Questionnaires 1 and 2** and **Table 3.1 for example calculations.**

Table 3.1 FELS Calculations

Index	Activities are ranked:	Frequency	Likert Scale
Sport Index	**Low Intensity,** ≤4.5 MET[1] = 0.76 (biking, walking and bowling). **Moderate Intensity**, 4.5-7.9 MET = 1.26 (aerobics, jogging, basketball and skateboard). **High Intensity,** ≥8.0 MET = 1.76 (running, football and field hockey).	Regularly (4.5) Often (2.5) Sometimes (0.5)	No sports listed = 1 0.01-<4 = 2 4-<8 = 3 8-<12 = 4 ≥ 12 = 5
Leisure Index	Mean score of Leisure questions	Very often (5) Often (4) Sometimes (3) Seldom (2) Never (1)	
Work Index	**Low Intensity,** ≤3 MET = 0.76 (cleaning kitchen, carrying laundry baskets, watering flowers, feeding pets, picking up trash). **Moderate Intensity,** 3-4.9 MET = 1.26 (cleaning bathroom, carrying food bags, weeding garden, walking large animals, sweeping, picking up sticks). **High Intensity,** ≥5.0 MET = 1.76 (cleaning barn, mowing lawn, heavy lifting).	Regularly (4.5) Often (2.5) Sometimes (0.5)	No sports listed = 1 0.01-<4 = 2 4-<8 = 3 8-<12 = 4 ≥ 12 = 5
Total Score	Sport Index + leisure Index + Work Index		

[1]Abbreviations: Metabolic Equivalent of Task (MET) .

Reference: (Treuth, Hou, Young, & Maynard, 2005).

3.2.8 Statistical analysis

Repeated measures ANOVA was used to assess the effect of the dietary intervention on primary outcomes of interest (e.g. laboratory parameters, anthropometric and dietary intake variables: GI/GL/fructose intake) and to test the interaction between time and group interactions. Post-hoc pair-wise comparisons within and between groups were performed using Bonferroni correction for variables demonstrating normal distributions. Bonferroni correction is a simple method calculates a new alpha level (0.05 or lower) that will be use to evaluate each comparison test. Therefore, it counteracts the problem of multiple comparisons by controlling error rate.

Non-parametric analyses were performed using the Mann Whitney test that asserts that the populations of two samples have the same probability distribution (Hart A, 2001) and this test was followed by the post hoc Dunn test, which is used when the variables demonstrate skewed distributions. Significance was considered at a p value of <0.05. All statistic analyses were performed using SAS version 9.2.

To examine the potential for the dietary intervention to normalize biochemical variables, the following variables were dichotomized into normal/abnormal: ALT (above and below 20 U/L), AST (above and below 40 U/L), HOMA-IR (above and below 3), triglycerides (above and below 1.5mmol/L) and insulin (above and below 20 mU/L). Dietary variables were categorized as GI (<55 for low GI foods; 55-60 for medium GI foods and >60 for high GI foods) and GL (<80 for low GL foods; 80-120 for medium GL foods and >120 for high GL foods) (Foster-Powel, 2002) and then analyzed using a repeated measures analysis of variance (ANOVA) using a Bonneferonni correctin for post-hoc pair wise comparisonsTo assess the potential interrelationships of dietary modification on primary outcome variables (metabolic, cardiovascular, liver related functions), multivariate analysis between these factors was performed.

3.3 Results

3.3.1 Demographic and body composition assessment

The Demographic characteristics and body composition assessment of subjects are presented in **Table 3.2**. The skinfolds measurements are presented in **Table 3.3.** The Somatotype results are presented en **Table 3.4**.

3.3.1.1 Demographic and body composition changes at baseline, 3 and 6 months of dietary intervention

A total of 12 children and adolescents with NAFLD (n=8 children with Simple Steatosis and n=4 children with NASH were recruited for this study. From those children with NAFLD n=7 had an ultrasound (US) and only and n=5 had a liver Biopsy (in addition to US to diagnose NAFLD). An additional group of lean, healthy children (n=14 children) for the control group. No significant differences in ages between the groups were observed over the entire study intervention (**Table 3.2**); but significant differences in the proportion of male children in the NAFLD group (n=11 out of 12 children) versus the healthy control group (n=5 males out of 14 children) ($p<0.001$) **(Table 3.2)** were observed.

a) Blood pressure

Baseline: Children and adolescents with NAFLD had greater Systolic Blood Pressure (SBP) when compared with healthy lean children at baseline ($p<0.001$) and during the entire intervention ($p<0.05$.) **Table 3.2.** Three out of twelve of the children with NAFLD were above the 95^{th} percentile for SBP according to age, gender height percentiles (NHLBI, 2007). In addition children with NASH had clinically higher SBP levels at baseline when compared to children with SS **Appendix A, Table A.15**. In contrast, no significant differences between groups were observed in diastolic blood pressures (DPB) at baseline ($p>0.05$).

Dietary intervention: One of the major findings is that children with NAFLD significantly reduced SBP from baseline to 3 months ($p=0.02$) and there was a trend towards the reduction of SBP from baseline to 6 months **Table 3.2**. When the Percentage of change (Delta percentage Δ%) of SBP was calculated, a significant reduction from baseline to 3 months and to 6 months was found **Figure 3.2.** At 6 months children with NASH reduced their SBP by 18% and children with SS by 11% **Appendix A, Table A.15.** There were no changes in SBP within

the control group over the intervention. No changes in DBP were noted between groups over the intervention period ($p<0.05$).

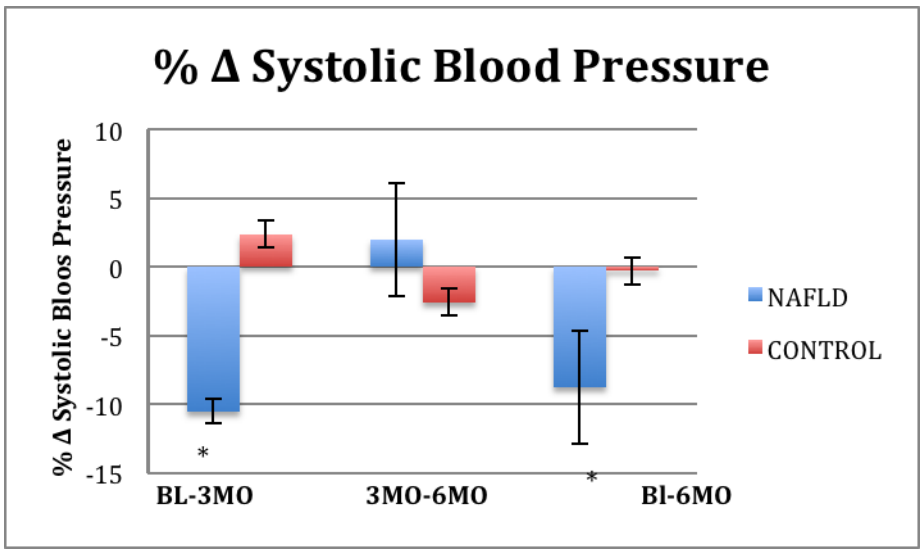

Figure 3.2 Percent of change in systolic blood in children and adolescents with NAFLD and lean children during the intervention.
From Baseline to 3months (BL-3MO), from 3 months to 6 months (3MO-6MO) and from Baseline to 6 (BL-6 MO) months. Bars with a star represent significant percentage of change from baseline ($p<0.05$).

b) Anthropometric variables: weight, height, BMI and waist circumference

Baseline: Children with NAFLD had significantly greater weight, height, and BMI than the control group over the entire dietary intervention ($p<0.001$) **Table 3.2.** All children with NAFLD had BMI-z scores in excess of 2 standard deviations according to CDC growth standards (CDC, 2000) **Table 3.2.** Eleven out of twelve children with NAFLD were above the 95th percentile of WC for age/gender (Katzmarzyk, 2004). All children in the control group except two, had BMI's that were <85th percentile (BMI) **Table 3.2.** However, according to the IOTF cut off values these two children had BMI-z scores that were under 1.6 BMI z-scores and a percentage of body fat that were within normal reference ranges (Ogden, Li, Freedman, Borrud, & Flegal, 2011). Within the control group there were 3 children with BMI-z scores below -2. However their body fat percentage was within healthy ranges indicating that body composition was within normal reference ranges (Ogden, Li, Freedman, Borrud, & Flegal, 2011). All healthy children had WC within normal reference ranges (Katzmarzyk et al 2004).

Dietary intervention: There were no significant changes in weight, height, BMI or WC (including percentiles and z-scores) in any of the two groups over the entire intervention period **Table 3.2.**

c) Body composition

Baseline: Children with NAFLD had a higher absolute and percentage of body fat mass, and smaller absolute and percentage fat free mass when compared to the control group at baseline ($p<0.001$) **Table 3.2.** The body fat percentages of all the children in the control group were $<5^{th}$ for age and gender, meaning healthy ranges (Ogden, Li, Freedman, Borrud, & Flegal, 2011)**Table 3.2.**

Dietary intervention: In terms of Body Fat (BF%) changes a significant time group effect ($p=0.03$) was found **Table 3.8**. Children with NAFLD showed a significant reduction in BF% from baseline to 6 month ($p=0.03$) **Table 3.2.** When the Δ% of BF% was calculated, a significant reduction from baseline to 6 months was found **Figure 3.3.** The BF%, BF (kg), Fat Free Mass (FFM%) and FFM (kg) of lean children did not change significantly in the study **Table 3.2.**

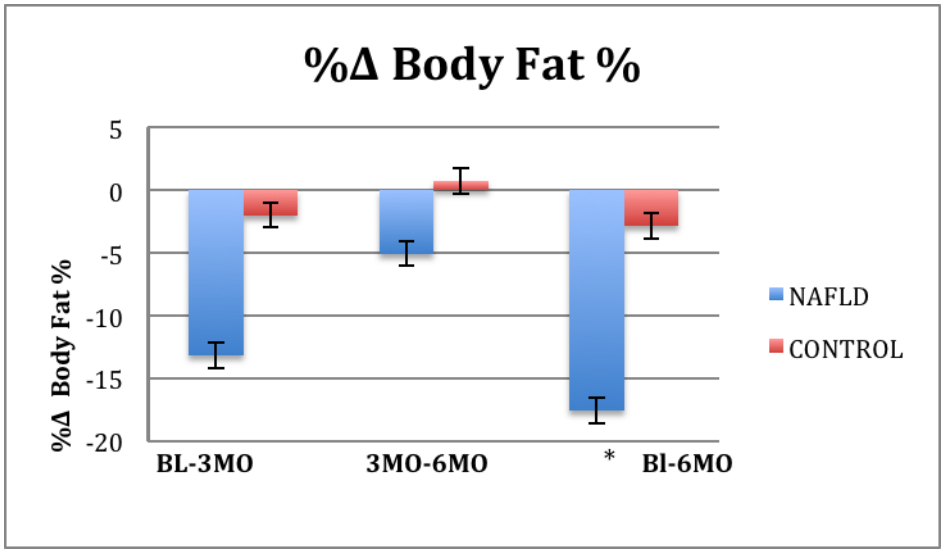

Figure 3.3 Percent of change in body fat in children and adolescents with NAFLD and lean children (controls) during the intervention

Baseline to 3months (BL-3MO), from 3 months to 6 months (3MO-6MO) and from Baseline to 6 months (BL-6MO). Bars with a star represent significant percentage of change from baseline ($p<0.05$).

d) Skinfolds and somatotype:

Baseline: Children with NAFLD had greater absolute measures of skinfolds (Triceps, Biceps, Abdominal, Subscapular, Supraspinal, Ileac Crest), mid-arm circumference and diameters (Femur, Humerus) when compared to the control group at baseline and during the entire study ($p<0.001$). Children with NAFLD had higher sum of trunk skinfolds, sum of extremities skinfolds and TER when compared with lean children **See Table 3.3.** Children with NAFLD had triceps and subscapular skinfolds >95th percentile for age and sex Ado & Himes, 2010). Children in the control group had triceps and subscapular skinfolds between 5 to <85th percentile for age and sex (Ado & Himes, 2010). Children with NAFLD had greater endomorphic shape when compared to the control group (characterized with ectomorphic shapes) at all times during the study **See Table 3.4.**

Dietary intervention: In terms of skinfolds changes there were important reductions reflecting changes in subcutaneous fat distribution (Abdominal, Ileac crest and Supraspinal). Time and Time*Group effect are shown in **Appendix A, Table A.8.** Children with NAFLD significantly reduced abdominal skinfold from baseline to 3 months ($p=0.03$), significantly reduced Ileac crest skinfold from baseline to 3 months ($p=0.04$), from baseline to 6 months ($p=0.02$), and significantly reduced Supraspinal skinfold from baseline to 3 months ($p=0.004$) and from baseline to 6 months ($p=0.005$). Children with NAFLD significantly reduced sum of trunk from baseline to 3 months ($p=0.005$) and from baseline to 6 months ($p=0.001$), and sum of extremities from baseline to 3 months ($p=0.02$) and from baseline to 6 months ($p=0.005$). Children in the control group did not experienced changes in skinfolds or TER over the time during the intervention.

Somatotype analysis

There were no significant changes in terms of the somatotype shape of lean children in the study **Table 3.4.** Children with NAFLD improved their body shape by reducing their endomorphic values significantly form baseline to 3 months ($p=0.02$) **Table 3.4.** The rest of the somatotype components did not change significantly during the study. However some children with NAFLD (n=3) achieved a body shape distribution comparable to children with healthy body

weights at 3 months. **Figure 3.4** shows the absolute somatotype changes during the dietary intervention in both groups.

Table 3.2 Demographic and body composition

Variable	NAFLD F=1 M=11			Control F=9 M=5		
Demographic	**Baseline** (n=12)	**3 Months** (n=8)	**6 Months** (n=7)	**Baseline** (n=14)	**3 Months** (n=13)	**6 Months** (n=13)
Age (yrs.)	13.6 ± 2.6	14.9 ± 2.1	14.9 ± 2.4	12.5 ± 2.7	12.6 ± 2.7	12.9 ± 2.7
Systolic BP (mmHg)	123 ± 11[a]	110 ± 10[c]	113 ± 14[ac]	95 ± 15[b]	97 ± 12[b]	94.6 ± 8.3[b]
Diastolic BP (mmHg)	70 ± 10	70 ± 9	66 ± 8	66 ± 8	63 ± 9	63 ± 7
Weight (kg)	94.9 ± 26.6[a]	101.3 ± 25.4[a]	100.6 ± 21.5[a]	41.4 ± 10.1[b]	41.7 ± 9.2[b]	42.5 ± 9.2[b]
Weight-z	2.6 ± 0.5[a]	2.6 ± 0.7[a]	2.6 ± 0.6[a]	-0.2 ± 1.2[b]	-0.2 ± 1.2[b]	-0.2 ± 1.1[b]
Height (cm)	167 ± 13[a]	175 ± 10[a]	176 ± 10[a]	152 ± 11[b]	152 ± 10[b]	155 ± 10[b]
Height-z	0.9 ± 1.0[a]	1.1 ± 0.9[a]	1.1 ± 0.9[a]	0.4 ± 0.8[b]	0.5 ± 0.9[b]	0.7 ± 0.9[b]
BMI (kg/m^2)	33.5 ± 7.1[a]	33.1 ± 8.2[a]	32.4 ± 6.8[a]	17.7 ± 2.9[b]	17.8 ± 2.9[b]	17.2 ± 2.7[b]
BMI z score	2.3 ± 0.3[a]	2.2 ± 0.4[a]	2.2 ± 0.4[a]	-0.5 ± 1.4[b]	-0.5 ± 1.3[b]	-0.9 ± 1.3[b]
% Body Fat (BF)	36.4 ± 6.4[a]	31.6 ± 7.6[c]	30.0 ± 8.4[ac]	12.6 ± 6.0[b]	15.1 ± 5.5[b]	16.2 ± 6.3[b]
% Fat free mass (FFM)	63.6 ± 6.4[a]	68.4 ± 7.6[a]	70 ± 8.4[a]	87.4 ± 6.0[b]	84.9 ± 5.5[b]	83.8 ± 6.3[b]
Waist circumference (cm)	102 ±11[a]	101 ± 13[a]	100 ± 13[a]	61 ± 7[b]	61 ± 7[b]	62 ± 6[b]
Hip circumference (cm)	111 ± 10[a]	114 ± 13[a]	112 ± 11[a]	79 ± 8[b]	80 ± 7[b]	80 ± 7[b]
Waist to hip	0.92 ± 0.06[a]	0.88 ± 0.04[a]	0.89 ± 0.08[a]	0.78 ± 0.07[b]	0.77 ± 0.08[b]	0.77 ± 0.04[b]
Waist to height	0.61 ± 0.06[a]	0.58 ± 0.08[a]	0.57 ± 0.07[a]	0.40 ± 0.05[b]	0.40 ± 0.05[b]	0.39 ± 0.03[b]

[1] Values are mean ± SD. Values with different superscripts (e.g. a, b or c) indicate significant differences between groups or time ($p < 0.05$). Values with superscript "a" represent comparisons between children with NAFLD and the control group (lean children) identified with superscript "b". Values with superscript "c" represent comparison between different time intervals. Repeated measured analysis of variance (ANOVA) was used to assess effect of dietary intervention over the 6-month intervention. Differences between groups were tested using a one-way ANOVA and by using a post-hoc pair wise comparison following a Boneferonni comparison of the data (for variables demonstrating normal distributions). [2] Obese children with nonalcoholic fatty liver disease (NAFLD) with body weights as defined by Centre for Disease Control (CDC) and healthy children with body weights within normal reference ranges (Lean Control). (Ogden C, Flegal K; 2010). [3] As determined by CDC growth standards using Epi-Software..[4] Blood Pressure (BP), Body Mass Index (BMI), Body Fat (BF) and Fat-free Mass (FFM) as determined by air displacement plethysmography. Waist circumference; (NAFLD n=11 at baseline, n=8 at 3mo and n=7 at 6mo), (Lean n=14 at baseline, n=13 at 3mo, n=13 at 6mo)

Table 3.3 Skinfolds and diameters

Variable	NAFLD F=1 M=11			Control F=9 M=5		
Skinfolds [3]	**Baseline** (n=12)	**3 Months** (n=8)	**6 Months** (n=7)	**Baseline** (n=14)	**3 Months** (n=13)	**6 Months** (n=13)
Triceps (mm)	29.9 ± 5.2^{a}	24.4 ± 9.1^{ac}	22.1 ± 6.0^{ac}	13.4 ± 4.5^{b}	12.6 ± 4.3^{b}	12.9 ± 4.1^{b}
Biceps (mm)	29.5 ± 6.9^{a}	23.1 ± 10.3^{ac}	22.7 ± 7.8^{ac}	11.1 ± 4.2^{b}	11.5 ± 3.7^{b}	10.6 ± 3.2^{b}
Subscapular (mm)	34.6 ± 9.6^{a}	29.1 ± 7.6^{ac}	27.0 ± 4.8^{ac}	10.9 ± 3.1^{b}	10.6 ± 3.3^{b}	10.9 ± 3.0
Supraspinal (mm)	32.9 ± 6.1^{a}	25.9 ± 5.2^{a}	24.1 ± 5.3^{ac}	10.4 ± 4.9^{b}	11.5 ± 4.4^{b}	11.2 ± 3.8^{b}
Ileac crest (mm)	33.5 ± 6.6^{a}	27.1 ± 6.7^{ac}	26.3 ± 7.9^{ac}	12.1 ± 6.2^{b}	12.6 ± 5.8^{b}	11.9 ± 5.3^{b}
Abdominal (mm)	35.8 ± 6.3^{a}	29.7 ± 4.8^{ac}	27.3 ± 6.5	12.9 ± 6.5^{b}	12.4 ± 5.3^{b}	12.8 ± 4.5^{b}
Media Calf (mm)	32.8 ± 7.4^{a}	27.8 ± 9.5^{a}	25.9 ± 7.8^{a}	15.4 ± 4.9^{b}	16.5 ± 5.5^{b}	16.6 ± 5.7^{b}
TER	1.5 ± 0.2^{a}	1.6 ± 0.3^{b}	1.5 ± 0.2^{b}	1.1 ± 0.2^{b}	1.2 ± 0.2^{b}	1.2 ± 0.3^{b}
Σ trunk	137 ± 23^{ac}	110 ± 20^{a}	105 ± 22^{ac}	46 ± 19^{b}	47 ± 17^{b}	47 ± 16^{b}
Σ extremities	92 ± 15^{ac}	75 ± 25^{a}	71 ± 18^{ac}	40 ± 12^{b}	41 ± 12^{b}	40 ± 12^{b}
Diameters (cm)						
Humerus	6.0 ± 1.5^{a}	5.5 ± 0.4^{a}	6.0 ± 0.6^{a}	4.8 ± 0.4^{b}	4.9 ± 0.4^{b}	5.1 ± 0.4^{b}
Femur	8.8 ± 1.8^{a}	10.1 ± 1.4^{a}	9.5 ± 1.4^{a}	8.2 ± 0.6^{b}	8.4 ± 0.6^{b}	8.9 ± 0.5^{b}
Circumferences (cm)						
Mid-arm	33.1 ± 3.5^{a}	34.0 ± 3.4^{a}	34.6 ± 3.6^{a}	22.8 ± 3.5^{b}	22.7 ± 2.7^{b}	23.1 ± 2.5^{b}
Calf	38.7 ± 2.6^{a}	40.0 ± 5.3^{a}	39.3 ± 3.1^{a}	29.3 ± 4.4^{b}	29.9 ± 3.9^{b}	29.3 ± 3.7^{b}

[1] Values are mean ± SD. Values with different superscripts (e.g. a, b or c) indicate significant differences between groups or time ($p < 0.05$). Values with superscript "a" represent comparisons between children with NAFLD and the control group (lean children) identified with superscript "b". Values with superscript "c" represent comparison between different time intervals. Repeated measured analysis of variance (ANOVA) was used to assess effect of dietary intervention over the 6-month intervention. Differences between groups were tested using a one-way ANOVA and by using a post-hoc pair wise comparison following a Boneferonni comparison of the data (for variables demonstrating normal distributions). [2] Trunk-extremity ratio (TER) Σ trunk skinfolds (Subscapular+Supraspinal+Ileac+Abdominal)/Σ extremities skinfolds (Bicep+ Triceps +Calf) [3] (NAFLD n=11 at baseline, n=7 at 3mo and n=7 at 6mo), (Lean n=14 at baseline, n=13 at 3mo, n=13 at 6mo)

Table 3.4 Somatotype

Variable	NAFLD F=1 M=11			Control F=9 M=5		
Somatotype	**Baseline** (n=12)	**3 Months** (n=8)	**6 Months** (n=7)	**Baseline** (n=14)	**3 Months** (n=13)	**6 Months** (n=13)
Endomorphic	8 ± 0.7^{a}	6.9 ± 0.9^{bc}	6.7 ± 1.1^{bc}	3.3 ± 1.2^{c}	3.3 ± 1.1^{c}	3.4 ± 1.0^{c}
Mesomorphic	4.4 ± 1.4^{a}	4.3 ± 1.3^{a}	4.5 ± 1.0^{a}	2.2 ± 1.3^{b}	2.4 ± 1.3^{b}	2.2 ± 1.2^{b}
Ectomorphic	0.3 ± 0.3^{a}	0.6 ± 0.5^{a}	0.4 ± 0.4^{a}	4.1 ± 1.7^{b}	3.8 ± 1.7^{b}	4.1 ± 1.7^{b}

[1] Values are mean ± SD. Values with different superscripts (e.g. a, b or c) indicate significant differences between groups or time ($p < 0.05$). Values with superscript "a" represent comparisons between children with NAFLD and the control group (lean children) identified with superscript "b". Values with superscript "c" represent comparison between different time intervals. Repeated measured ANOVA was used to assess effect of dietary intervention. Differences between groups were tested using a one-way ANOVA and by using a post-hoc pair wise comparison following a Boneferonni comparison of the data (for variables demonstrating normal distributions). [2] Values calculated with the Somatotype Software 2001 Sweat technologies. The following measurements were entered in the software: height, weight, triceps, subscapular, supraspinal, calf, girths (flexed arm and calf) and bone breadths (humerus and femur) (Carter & Heath, 2003). The software uses the following equations: Endomprphy = $-0.718+0.1451\ (X) - 0.00068\ (X^2) + 0.0000014\ (X^3)$ Where X = (sum of triceps, subscapular and supraspinal skinfolds) multiplied by (170.18/height in cm). Mesomorphy = 0.858 x humerus breadth + 0.601 x femur breadth + 0.188 corrected arm girth + 0.161 x corrected calf girth – height 0.131 + 4.5 Ectomorphy = Calculated depending on height-weight ratio (HWR), If HWR $\geq$ 40.75, then = 0.732 (HWR) – 28.58, If HWR < 40.75 but > 38.25, then = 0.463 (HWR) – 17.63, If HWR $\leq$ 38.25, then = 0.1. Somatotype; (NAFLD n=11 at baseline, n=7 at 3mo and n=7 at 6mo), (Lean n=14 at baseline, n=13 at 3mo, n=13 at 6mo)

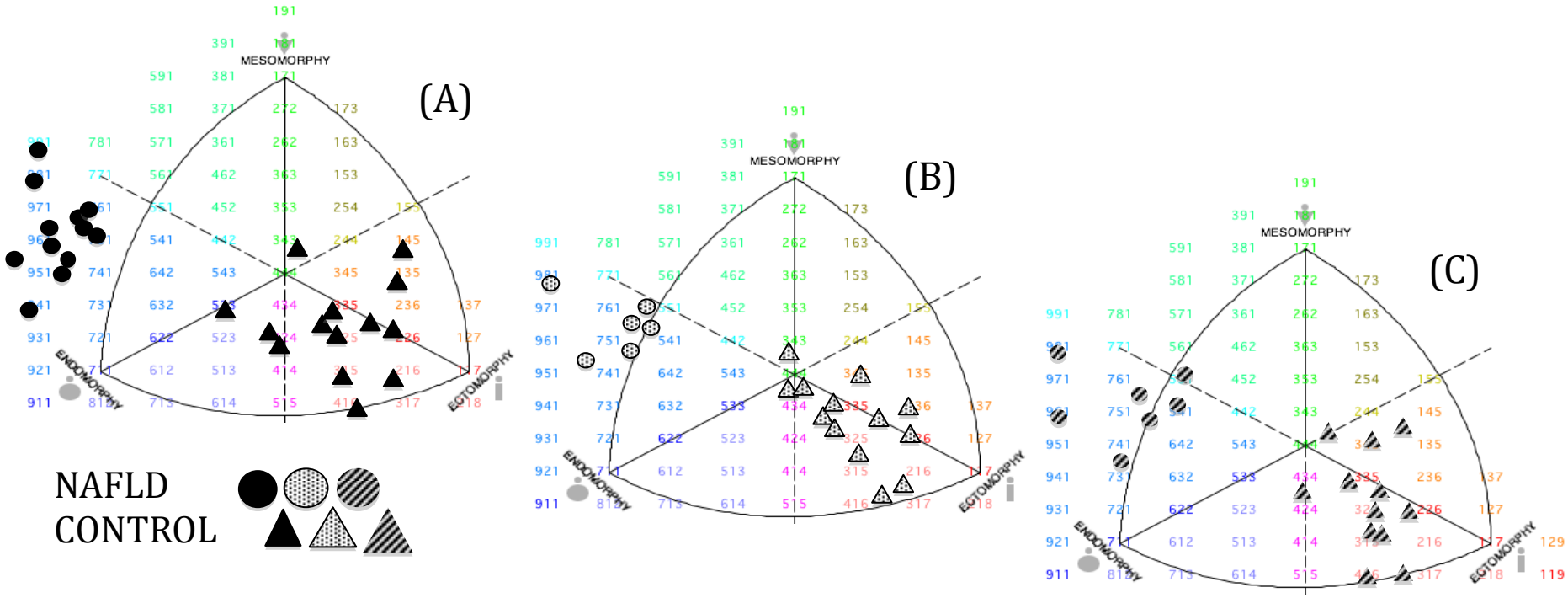

Figure 3.4 Absolute somatotype changes during the dietary intervention in children and adolescents with NAFLD and lean

Figure A Baseline (NAFLD ●, Control ▲); Figure B 3 Months (NAFLD ◍, Control ◬) and Figure C 6 Months (NAFLD ◪, Control ◭).

3.3.2 Fasting biochemical parameters at baseline and during dietary intervention

The Fasting Biochemical parameters at baseline and during the dietary intervention of subjects are presented in **Table 3.5** and **Table 3.6.**

a) Liver function parameters

Baseline: Children and adolescents with NAFLD had significant higher plasma levels in ALT, AST, and GGT when compared to lean controls (p=<0.001, p=0.003, p=<0.001, p=0.03 respectively) at baseline and during the dietary intervention **Table 3.5.**

Dietary intervention: Children in the control group did not experience changes in liver function parameters during the study. There were no major changes in liver biochemistry parameters among children with NAFLD during the study **Table 3.5.** However, it is clinically important to know if children with NAFLD improved their liver enzymes to normal levels as this is part of routine clinical assessment **(See Table 3.5 for normal ranges).** Therefore, ALT was treated as a categorical variable above and below 20, and there were significantly more children with NAFLD that reduced ALT levels below 20 U/L from baseline to 3 months (p=0.02) **Table 3.5 and Appendix A, Table A.9** and from 3 months to 6 months (p=0.04) **Table 3.5.** Moreover, elevated levels of ALT and AST are quite sensitive, but other conditions (heart and skeletal muscle injuries) can present as well elevated transaminases levels. Therefore, in children it was proposed that the cut of value should be the 95th percentile for age, which is below 20 U/L and provides better sensitivity without less specificity to detect accurately chronic liver damage (Schiwmmer J.B et al., 2010).

a) Metabolic parameters

Baseline: Children with NAFLD had significantly higher values of insulin (p<0.001), triglycerides (p<0.001), HOMA-IR as absolute and categorical variable (p=0.002, p<0.001) and uric acid (p<0.001) and significant lower level of HDL (p<0.001) when compared to the healthy control group at baseline and during the dietary intervention **Table 3.5.** No significant differences in the rest of the metabolic parameters between groups at baseline.

Dietary intervention: There were no significant changes within the control group in any of the metabolic parameters studied over the entire dietary intervention.

During the dietary intervention, children with NAFLD significantly reduced NEFA values from baseline to 6 months (p=0.01) **Table 3.5 and Table 3.9** with a trend towards the reduction of NEFA from baseline to 3 months (p=0.08). Significant decrease of HOMA-IR as categorical variable during the first 3 months (p=0.001) and from 3 months to 6 months (p=0.005) were found; and a trend towards the reduction of HOMA-IR from baseline to 3 months (p=0.07) when HOMA-IR was treated as continuous variable **Table 3.5.**

c) Adipocytokines, lipoproteins and markers of inflammation

Baseline: Children with NAFLD had significant higher values of adiponectin (p=0.02), IL-6 (p=0.003), CRP (p=0.02) Apo-B100 (p<0.001) and Apo-B48 (p<0.001) when compared to lean children at baseline **Table 3.6.** No significant differences in IL-10 or Apo-CIII were noted between groups at baseline (p>0.05).

Dietary intervention: No significant changes in TNF-α, IL-6, IL-10, adiponectin or Apo-B48 were noted within and between groups over the six months intervention **(Table 3.6).** However, significant changes in Apo-B100 from baseline to 6 months (p=0.001) and Apo-CIII from baseline to 3 months (p=0.03) among children with NAFLD only were observed **Table 3.6.**

Table 3.5 Metabolic and biochemical characteristics of children with NAFLD and healthy lean children during the study.

Variable	NAFLD F=1 M=11			Control F=9 M=5		
	Baseline	**3 Months**	**6 Months**	**Baseline**	**6 Months**	Normal Reference Range
ALT (U/L)	62 ± 46[a]	40 ± 16[a]	59 ± 46[a]	16 ± 3[b]	17 ± 5[b]	<40
AST (U/L)	38 ± 17[a]	30 ± 5[a]	38 ± 17[a]	26 ± 3[b]	25 ± 5[b]	<40
GGT (U/L)	22 ± 11[a]	17 ± 11[a]	18 ± 10[a]	5 ± 0.3[b]	6 ± 1[b]	<45
Glucose (mmol/L)	5.3 ± 0.3	5.0 ± 0.3	4.9 ± 0.7	5.0 ± 0.3	4.9 ± 0.4	3.3-6.1
Insulin (mU/L)[4]	45 ± 44[a]	24 ± 12[a]	31 ± 12[a]	8 ± 4[b]	16 ± 21[b]	5-20
HOMA-IR[24]	10.8 ± 11.3[a]	5.3 ± 2.7[ac]	6.6 ± 2.3[ac]	1.7 ± 0.9[b]	2.0 ± 0.8[b]	<3
AI-IR [3]	21.9 ± 20.6[a]	8.6 ± 5.5[a]	8.7 ± 2.6[a]	3.1 ± 2.1	2.6 ± 2.3	-
Triglyceride (mmol/L)	1.8 ± 0.8[a]	1.3 ± 0.6[a]	1.7 ± 0.5[a]	0.8 ± 0.3[b]	0.9 ± 0.5[b]	<1.5
NEFA[4] (mmol/L)	0.46 ± 0.23[ac]	0.33 ± 0.09[ab]	0.30 ± 0.15[b]	0.36 ± 0.13[ac]	0.26 ± 0.16[b]	-
Total Cholesterol (mmol/L)	4.3 ± 1.4	4.1 ± 1.3	4.1 ± 1.1	4.1 ± 0.7	4 ± 0.8	<4.4
HDL (mmol/L)	0.97 ± 0.11[a]	0.97 ± 0.12[a]	1.04 ± 0.17[a]	1.46 ± 0.15[b]	1.42 ± 0.22[b]	>1
LDL (mmol/L)	2.51 ± 1.03	2.57 ± 1.17	2.33 ± 0.99	2.28 ± 0.63	2.21 ± 0.81	<2.8
CRP (μg/L)	2.3 ± 1.5[a]	1.8 ± 1.5[a]	1.5 ± 1.6[a]	1.0 ± 2.6[b]	0.4 ± 0.3[b]	<8.0
Uric Acid	416 ± 103[a]	388 ± 80[a]	397 ± 54[a]	267 ± 37[b]	271 ± 22[b]	135-510

[1] Values are mean ± SD. Values with different superscripts (e.g. a, b or c) indicate significant differences between groups or time ($p<0.05$). Values with superscript "a" represent comparison between children with NAFLD and the lean children identified with superscript "b". Values with superscript "c" represent significant differences using repeated measures ANOVA. Multivariate repeated-measures analysis of variance (ANOVA) was used to test the interaction between time and group and potential group-time interactions. When a significant interaction was found between factors, differences across groups were analyzed by ANOVA followed by Bonferroni´s corrections if the variables were normally distributed. When variables were found to be non-parametric, the Mann-Whitney U test followed by the post-hoc Dunn test was used..[2] HOMA-IR = Fasting Insulin (μU/mL)/22.5*glucose (mmol/L) AI-IR: Adipose tissue-IR = Fasting Insulin (μU/mL)*NEFA (mEq/L). Abbreviations: Non-esterified free fatty acids (NEFA), C-reactive protein (CRP). Uric acid; NAFLD (n=9 at baseline, n=7 at 3 mo, n=7 at 6 mo), Lean (n= 14 at baseline, NA at 3mo, n=12 at 6 mo).

Table 3.6 Markers of inflammation, lipoprotein and adipocytokine expression in children and adolescents with NAFLD and in healthy lean children

Variable	NAFLD F=1 M=11			Control F=9 M=5		
	Baseline	**3 Months**	**6 Months**	**Baseline**	**6 Months**	Normal Reference Range
IL-6	0.8 ± 0.2[a]	0.8 ± 0.4[a]	1.0 ± 0.4[a]	0.4 ± 0.4[b]	0.4 ± 0.4[b]	-
IL-10	4.4 ± 1.7	5.1 ± 0.8	4.4 ± 1.1	4.0 ± 1.0	4.3 ± 1.9	-
Adiponectin (ng/mL)	8 ± 2.2[a]	7.3 ± 2.4[a]	7.5 ± 2.0[a]	10.3 ± 2.2[b]	NA	>10
TNF-α	1.5 ± 0.3	1.6 ± 0.3	1.6 ± 0.3[c]	1.3 ± 0.2	1.6 ± 0.2	-
Apo-B100 (μg/mL)	529 ± 136 [a]	263 ± 134 [b]	344 ± 133 [bc]	226 ± 100 [b]	81 ± 26 [c]	-
Apo-B48 (μg/mL)	5.3 ± 1.4 [a]	4.9 ± 1.1[a]	4.9 ± 0.9 [a]	3.1 ± 1.1 [b]	2.7 ± 0.7 [b]	-
Apo-CIII (μg/dL)	16.4 ± 11	31.4 ± 25.2	25.2 ± 16.2 [c]	16.1 ± 6.2	18.2 ± 7.0	-

[1] Values are mean ± SD. Values with different superscripts (e.g. a, b or c) indicate significant differences between groups or time ($p<0.05$) ($p<0.05$). Values with superscript "a" represent comparison between children with NAFLD and the lean children identified with superscript "b". Values with superscript "c" represent significant differences using repeated measures ANOVA. Multivariate repeated-measures analysis of variance (ANOVA) was used to test the interaction between time and group and potential group-time interactions. When a significant interaction was found between factors, differences across groups were analyzed by ANOVA followed by Bonferoni´s corrections if the variables were normally distributed. When variables were found to be non-parametric, the Mann-Whitney U test followed by the post-hoc Dunn test was used.[2] Abbreviations: Interleukin factor-6 (IL-6), Interleukin factor-10 (IL-10), Apolipoprotein-B100 (Apo-B100), Apolipoprotein-B48 (Apo-B48), Apolipoprotein-CIII (Apo-CIII) and Tumor necrosis factor-alpha (TNF-α). [3] IL-6; NAFDL (n=11 at baseline, n=7 at 3mo, n=6 at 6mo), Lean (n=5 at baseline, n=4 at 6mo). IL-10; NAFDL (n=11 at baseline, n=7 at 3mo, n=6 at 6mo), Lean (n=14 at baseline, n=6 at 6mo). Adiponectin; NAFDL (n=10 at baseline, n=7 at 3mo, n=6 at 6mo), Lean (n=13 at baseline, n=0 at 6mo). TNF-α; NAFDL (n=12 at baseline, n=7 at 3mo, n=6 at 6mo), Lean (n=12 at baseline, n=10 at 6mo). Apo-B48; NAFDL (n=11 at baseline, n=7 at 3mo, n=6 at 6mo), Lean (n=12 at baseline, n=9 at 6mo). Apo-B100; NAFDL (n=10 at baseline, n=7 at 3mo, n=5 at 6mo), Lean (n=11 at baseline, n=9 at 6mo). Apo-CIII; NAFDL (n=9 at baseline, n=5 at 3mo, n=5 at 6mo), Lean (n=13 at baseline, n=9 at 6mo)

3.3.3 Dietary intake

The dietary intake of subjects during at baseline and over the dietary intervention is presented in **Table 3.9**.

3.3.3.1 Baseline dietary intake

a) Energy and macronutrient intake:

The baseline energy intake as Kcal/kg and Kcal/FFM differed between groups but not as absolute kcal (kcal/day). Regarding macronutrient intake, protein intake (grams and as percentage from calories) differed between groups during the intervention (p=0.001, p=0.006) respectively **Table 3.9.** Carbohydrate intake as percentage from calories also differed between groups during the intervention (p=0.02)**.** Fat intake (grams and as percentage from calories) did not differ between groups at baseline**. (**In the study some children with NAFLD (n=6) and lean children (n=2) underreported their dietary Energy Intake. (Energy Intake (EI)/Basal Metabolic Rate (BMR), <1.06) (Livingstone & Black, 2003) **Table 3.9**).

b) Glycemic index and glycemic load

The Dietary GI and GL were not significantly different between groups at baseline (p=0.28) and (p=0.68) respectively **Table 3.9**.

c) Fructose intake

Children with NAFLD significantly had a higher consumption of fructose (total fructose in grams; p=0.01; total fructose as percentage of calories; p=0.02 at baseline when compared to lean children **Table 3.9.**

Other micronutrients: Micronutrients such as omega3/omega, cholesterol, fiber, vitamin E and potassium did not differ between groups at baseline, except for sodium, which differed between groups at baseline and over the entire 6 months (p=0.04) **Appendix A, Table A.11.**

3.3.3.2 Changes in dietary intake over the intervention

a) Energy and macronutrient intake:

There were no significant changes in dietary intake of lean children regarding macro-and-micronutrients during the entire dietary intervention. In contrast, children with NAFLD significantly reduced energy during the first 3 months (p=0.01) but the reduction was only a trend during the overall intervention (p=0.2) **Table 3.9**. Children with NAFLD change their carbohydrates intake (as a

percentage of total calories) (Baseline to 6 months (p=0.001) and from 3 Months to 6 Months p=0.04). In terms of dietary fat, children with NAFLD changed the absolute amount of total fat in grams consumed from baseline to 3 months (p=0.005) and from baseline to 6 months (p=0.01) **Table 3.9.** Children with NAFLD changed the Saturated fat grams intake from baseline to 3 months (p=0.01) **Table 3.9.** Children and adolescents with NAFLD and lean children underreported their dietary intake at 3 months (NAFLD n=6, Lean n=4) and 6 months (NAFLD n=6, Lean n=5) (EI/BMR <1.06) (Livingstone & Black, 2003) **Table 3.9**.

b) Glycemic index and glycemic load:

There were significant reductions in dietary GI intake in both groups. Lean children significantly reduced GI only from 3 months to 6 months (p=0.02). However, Children with NAFLD had the greatest reductions of GI over 6 months. (Baseline to 3months (p=0.009); Baseline to 6 months p=0.005) **See Table 3.9**. Time/group effects are shown in **Appendix A, Table A.13 and A.14.** The next table shows the number of children that changed their consumptions of high GI foods (>60) to medium GI foods (55-60) or to low GI foods (<55) during the dietary intervention **Table 3.7.**

Table 3.7 Number of children that consume low-high glycemic index foods during the study

GI values NAFLD	**Baseline**	**3 Months**	**6 Months**
Low GI	n=2	n=7	n=7
Medium GI	n=1	None	n=none
High GI	n=9	n=1	n=none
GI values control	**Baseline**	**3 Months**	**6 Months**
Low GI	n=12	n=13	n=11
Medium GI	n=1	None	n=
High GI	n=1	None	n=2

Children with NAFLD presented a significant overall reduction in dietary GL during the dietary intervention. (Baseline to 3months p<0.001; Baseline to 6 months p=0.03). **See Table 3.9.** Time/group effects are shown in **Appendix A, Table 1A.3 and A.14.** The next table shows the number of children that reduced GL from high GL to low GL during the dietary intervention **Table 3.8.**

Table 3.8 Number of children that consume low-high glycemic load foods during the study

GL values NAFLD	**Baseline**	**3 Months**	**6 Months**
Low GL	n=1	n=7	n=3
High GL	n=11	n=1	n=4
GL values control	**Baseline**	**3 Months**	**6 Months**
Low GL	n=5	n=5	n=6
High GL	n=9	N=8	n=7

c) Fructose intake:

There were no significant changes in dietary fructose (total fructose or HFCS) within lean children over the entire intervention. Children with NAFLD significantly reduced the absolute intake of total fructose (p=0.001) and free fructose (p=0.005) during the first 3 months **Table 3.9.** There was also a trend towards the reduction of total fructose as percentage of calories from baseline to 3 months (p=0.05). Regarding the sources of dietary fructose children with NAFLD significantly reduced their intake of HFCS products during the first 3 months of the intervention (p=0.004) **(Figure 3.5).** Time/group effects are shown in **Appendix A, Table A.13 and A.14.** No other significant changes in fructose sources (absolute grams) were found.

Other micronutrients: No significant changes in fiber, omega-3/omega-6, cholesterol, vitamin E or potassium intake were noted over the entire study (p>0.05). There was a trend towards a reduction in sodium over time (p=0.09). **Appendix A, Table A.11.**

Table 3.9 Dietary intake of children before and during the dietary intervention

Variable	NAFLD[4] F=1 M=11			Control F=9 M=5		
Dietary intake	**Baseline** (n=12)	**3 Months** (n=8)	**6 Months** (n=7)	**Baseline** (n=14)	**3 Months** (n=13)	**6 Months** (n=13)
Energy (Kcal/d)	1958 ± 652[a]	1416 ± 492[bc]	1660 ± 429[a]	1709 ± 338[a]	1544 ± 351[b]	1507 ± 362[b]
Energy (kcal/kg)	21.8 ± 9.2[a]	15.1 ± 6.4[a]	16.9 ± 5.2[a]	43.3 ± 13.7[b]	38.4 ± 10.1[b]	37.8 ± 14.2[b]
Energy (kcal/FFM)	33.8 ± 13.7[a]	21.6 ± 8[a]	24.4 ± 6.6[a]	49.5 ± 15.2[b]	44.9 ± 10.7[b]	44.7 ± 15[b]
Protein (g/d)	73.8 ± 32.8[a]	77.9 ± 16.6[a]	76.2 ± 23.8[a]	61.7 ± 15.9[b]	64.5 ± 11.9[b]	60.5 ± 10.9[b]
Protein %	17.5 ± 4.5[a]	18.5 ± 3.6[a]	18.4 ± 2.4[a]	15.0 ± 2.8	16.0 ± 2.8	15.8 ± 3.4
Fat (g/d)	66.9 ± 41.4[a]	58.6 ± 23[b]	50.1± 20.1[b]	58.6 ± 19.4[a]	52.2 ± 19.4[b]	56.3 ± 22.4[b]
Fat (%)	37.1 ± 10.4[a]	31.8 ± 5.9[b]	26.7± 5[b]	31.7 ± 5.2[a]	28.5 ± 7.9[b]	29.9 ± 6.6[b]
Saturated fat (g/d)	22.3 ± 13[a]	25.2 ± 16.5[b]	15.7 ± 4.7[b]	21.5 ± 7.4[a]	18.9 ± 7.2[b]	17.4 ± 5.9[b]
Saturated fat (%)	12.1 ± 3.4[a]	12.9 ± 5.6[a]	8.5 ± 1.4[b]	11.7 ± 2.6[a]	10.3 ± 3.2[a]	9.6 ± 2.8[b]
MUFA (g/d)	21.3 ± 18.3[a]	16.5 ± 4.2[b]	17.1 ± 9.1[b]	19.0 ± 8.5[a]	17.8 ± 7.6[b]	20.9 ± 11.9[b]
MUFA (%)	11.2 ± 4.4	9.4 ± 2.1	8.9 ± 2.6	10.2 ± 3.6	9.6 ± 3.2	10.8 ± 4.0
PUFA (g/d)	11.5 ± 7.1	9.9 ± 2.9	8.9 ± 3.7	9.2 ± 5.3	8.9 ± 5.1	10.3 ± 5.8
PUFA (%)	6.9 ± 3.8	5.6 ± 1.7	4.9 ± 1.6	4.9 ± 1.9	4.7 ± 2.1	5.3 ± 1.9
Carbohydrate (g/d)	192 ± 84	208 ± 72	230 ± 43	222 ± 39	228 ± 52	220 ± 43
Carbohydrate (%)	45.4 ± 9.8	49.7 ± 7.8	55.8 ± 6[c]	53.9 ± 4.8	55.5 ± 6.0	54.3 ± 5.5
Fiber (g/d)	20.4 ± 9.7	19.5 ± 8.7	23.7 ± 12	16.7 ± 7.2	15.9 ± 5.4	17.7 ± 5.6
Soluble Fiber (g/d)	0.42 ± 1.1	0.43 ± 0.76	0.30 ± 0.63	1.44 ± 1.89	0.39 ± 0.91	0.06 ± 0.15
[3]Total fructose (g/d)	31.2 ±18.5[a]	14.0 ± 5.8[bc]	22.7 ± 11.6[a]	18.0 ± 9.7[a]	16.8 ± 6.6[b]	14.0 ± 8.9[b]
Total fructose (%)	6.4 ± 3.6[a]	4.1 ± 1.9[a]	6.0 ± 3.2[a]	4.4 ± 2.3[b]	4.3 ± 1.7[b]	3.6 ± 2.1[b]
[3]Free fructose (g/d)	22.4 ± 14.6[a]	11.2 ± 4.9[bc]	18.3 ± 9.8[a]	9.7 ± 6.4[b]	13.3 ± 6.3[b]	8.3 ± 4.4[b]
Free fructose (%)	4.7 ± 3.0 [a]	3.3 ± 1.5[a]	4.7 ± 2.5[a]	2.3 ± 1.5[b]	3.7 ± 2.2[b]	2.2 ± 1.1[b]
Glycemic Index	60 ± 10[a]	39 ± 16[ac]	42 ± [7ac]	44 ± 11[b]	39 ± [7bc]	51 ± 21
Glycemic load	116 ± 39[b]	61 ± 27[ac]	87 ± 23[bc]	92 ± 24[b]	87 ± 23[b]	83 ± 28[b]
TEE/BMR	1.1 ± 0.3	0.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1.07 ± 1.0

[1] Values are mean ± SD. Values with different superscripts (e.g. a, b or c) indicate significant differences between groups ($p < 0.05$). Values with superscript "a" represent comparisons between children with NAFLD and the control group (lean children) identified with superscript "b". Values with superscript "c" represent comparison between different time intervals. Repeated measured ANOVA was used to assess effect of dietary intervention. Differences between groups were tested using a one-way ANOVA and by using a post-hoc pair wise comparison following a Boneferronni comparison of the data (for variables demonstrating normal distributions).[2] Used Glycemic Index (GI) and Glycemic load (GL) values from the Foster-Powel international tables. GI calculated as ∑ (carbohydrate content of each food item (g) × GI)/total amount of carbohydrate in meal (g) and GL calculated as ∑(carbohydrate content of each food item (g) × GI)/100. [3] Free fructose: values from Canadian nutrient file, USDA database and food manufacturers webpages. Total fructose calculated as fructose (g)+ (sucrose (g)/2). [4]Underreporting was considered EI/BMR <1.06 (Livingstone & Black, 2003).

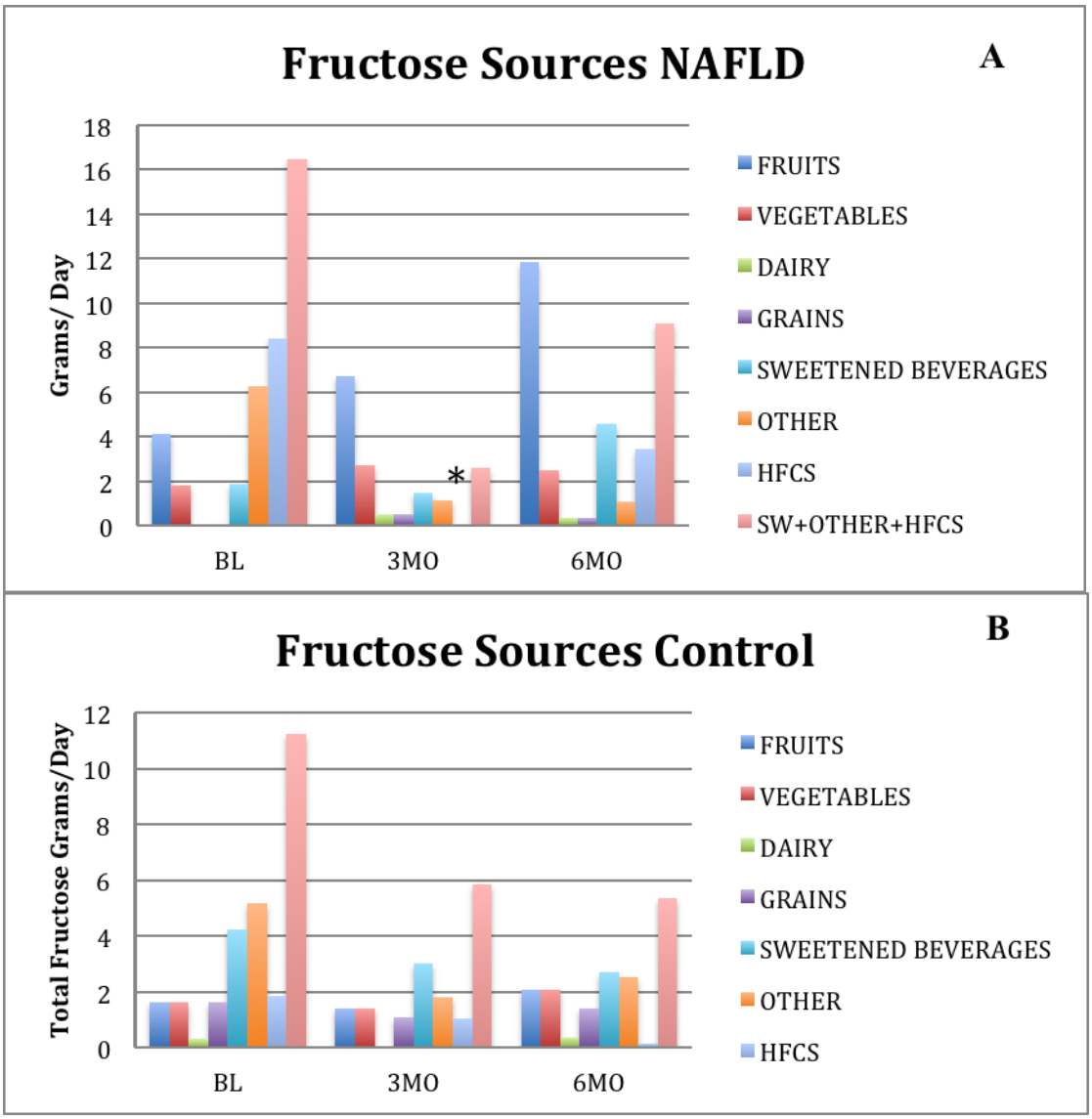

Figure 3.5 Comparison of dietary fructose sources between children and adolescents during the study

Children and adolescents with NAFLD (A) and healthy children (B) during the study. Baseline (BL), three months (3MO) and six months (6MO) on a low GI/GL/fructose over 6 months. Y-axis represents Total fructose grams/day and the X-axis represents the different study visits. Bars with a star represent significant changes from baseline ($p=0.004$).

3.3.4 Physical activity

3.3.4.1 Physical activity at baseline

The components of the FELS questionnaire (Leisure index, Work index, Sport Index, Total Index) or the HAES questionnaire (Inactive, Somewhat inactive, Somewhat active and active) did not differ between groups at baseline **Figure 3.6-3.7**

3.3.4.2 Physical activity changes during the intervention

No major differences regarding the components of the FELS questionnaire was found between groups or over the intervention. There was only a non-significant trend ($p=0.05$) towards the reduction of leisure index **Figure3.6.**

There were no differences in physical activity changes when assessed with the HAES questionnaire in any of the groups during the dietary intervention. However the activity levels were significantly different between weekdays and weekend days ($p<0.001$) **Figure 3.7.** Children with NAFLD were 7% more active during the weekend days than weekdays and Children in the control group were 9% more active during the weekdays than weekend days.

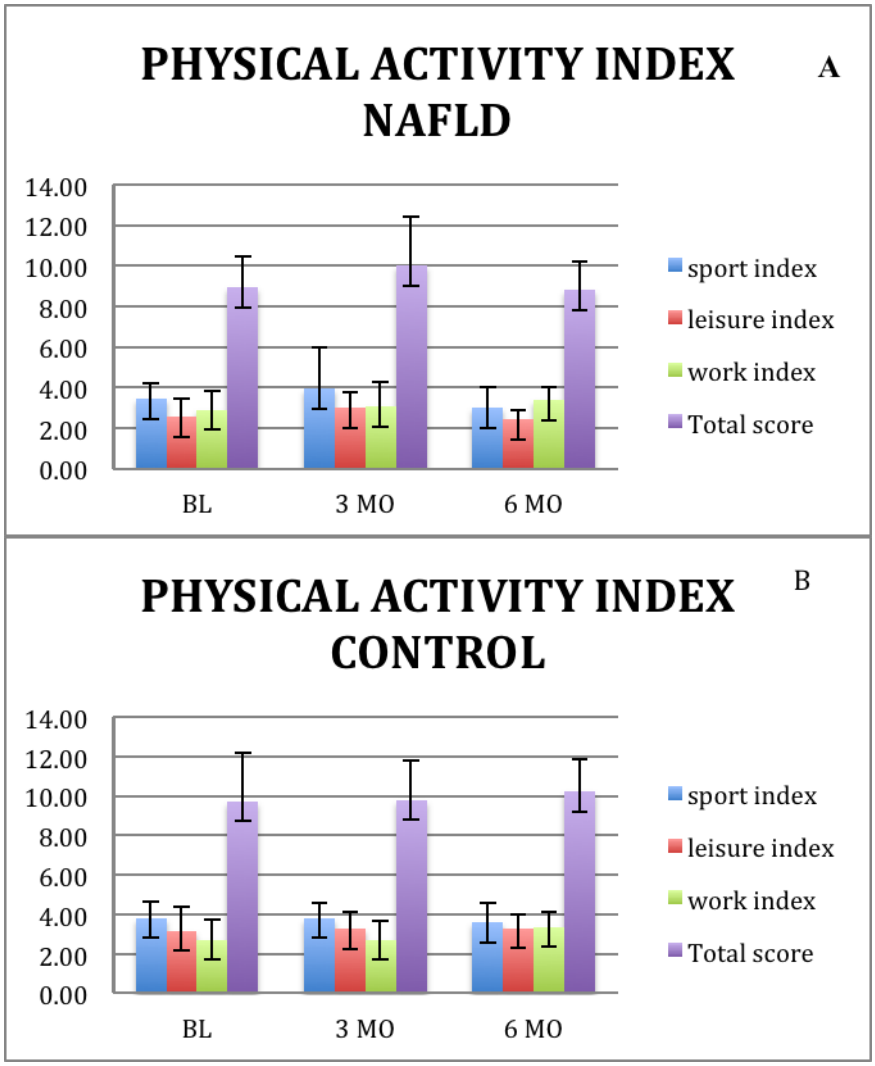

Figure 3.6 Comparison of physical activity index of children and adolescents during the study

Children with NAFLD (A) and healthy children (B). Baseline (BL), three months (3 MO) and six months (6 MO) on a low GI/GL/fructose diet. The sports index (frequency of different activities converted to Likert Scale) The leisure index is calculated (Average of the frequency of different activities). The work index (Frequency of different activities converted to Likert Scale) the total score represents the sum of the sports, leisure and work index (Treuth, Hou, Young, & Maynard, 2005).

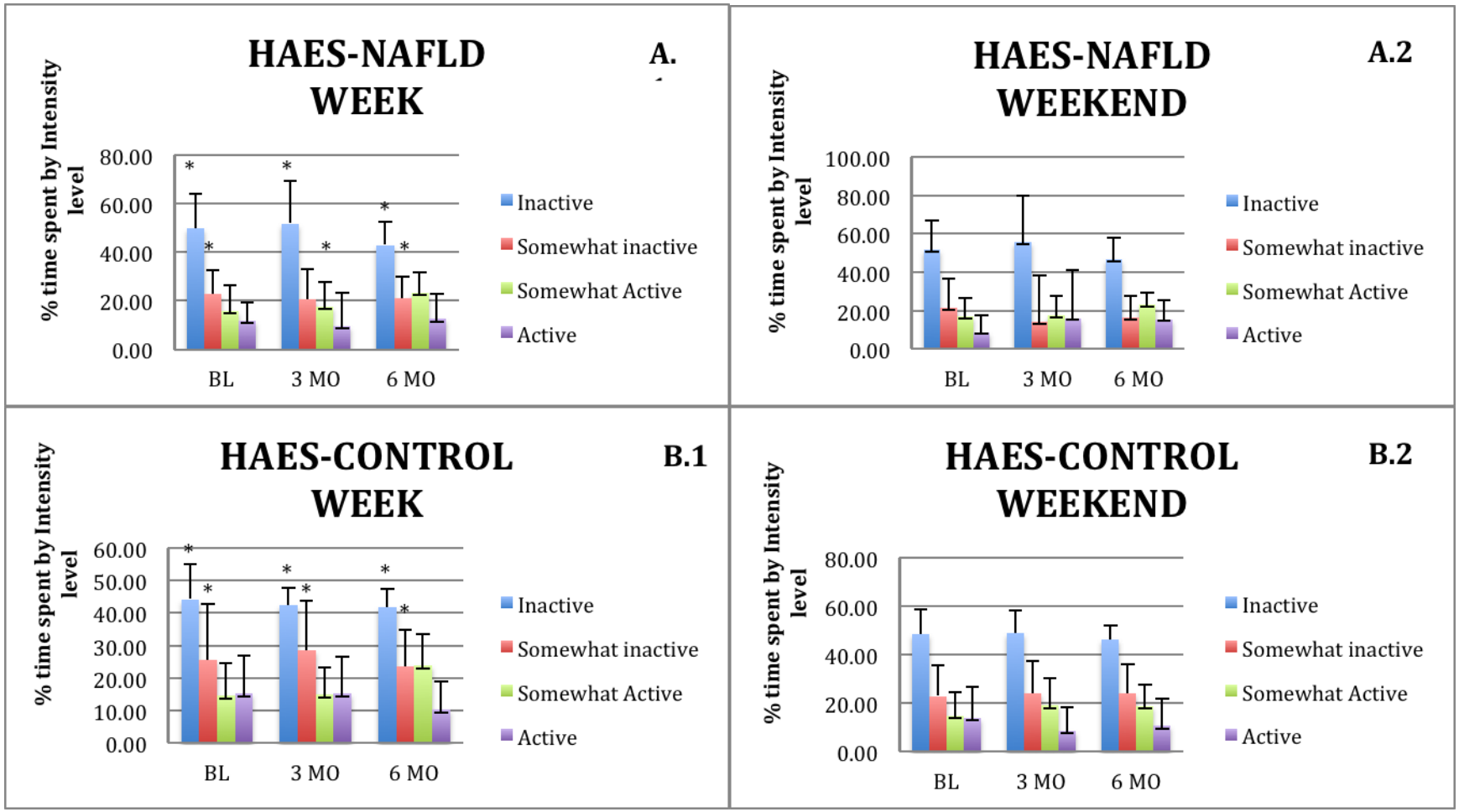

Figure 3.7 Comparisons of percentage of hours and intensity levels of physical activity during the study
Intensity levels (Inactive, Somewhat inactive, Somewhat active and Active) during the week (Children and adolescents with NAFLD **A.1**, Control group **B.1**) and during the weekend (Children and adolescents with NAFLD **A.2,** Control group **B.2**) at baseline (BL), three months (3MO) and six months (6 MO) using a validated questionnaires: Habitual Estimation Activity Scales (HAES). Bars with a star are significant different from weekend Bars **($p<0.001$)** (Hay & Cairney, 2006)**.**

3.3.5 Interrelationships of dietary modification (GI/GL and fructose reductions) and study outcomes (demographic, body composition and blood pressure, biochemical markers of liver function, insulin resistance and inflammation)

3.3.5.1 Glycemic index interrelations

GI categorized as <55; 55-60 and >60 was related to the following variables:

a) Gender and age: There were no significant relationships between dietary GI with gender or age in any of the two groups during the dietary intervention **Appendix A, Table A.5 and A.6.**

b) Body composition and blood pressure: There were no relationships between dietary GI with body composition variables (weight, height, BMI, WC, BF%, skinfolds or somatotype) during the dietary intervention. However, dietary GI intake was related with SBP (p=0.01) **Table 3.10.**

c) Liver functions markers: GI intake was related only to GGT (p=0.04) but not to AST or ALT during the dietary intervention **Table 3.10.**

d) Metabolic markers: GI intake was related to plasma concentrations of Insulin (p=0.04) but not to the rest of the metabolic markers studied (HOMA-IR, glucose, Triglycerides, NEFA, HDL, LDL or Total cholesterol) during the dietary intervention **Table 3.10.**

e) Adipocytokines, inflammation and lipoprotein markers: GI intake was not related to markers of inflammation, adipocytokines or lipoprotein expression except that GI was positively associated with Apo-B100 (p=0.01) **Table 3.10.**

3.3.5.2 Glycemic load interrelations

Gender and age: There were no relationships between dietary GL with gender or age in any of the two groups during the dietary intervention **Appendix A, Table A.5 and A.6.**

a) Body composition and blood pressure: Dietary GL was not related with body composition variables:(weight, height, BMI, WC, BF%, skinfolds or somatotype) or blood pressure during the dietary intervention **Table 3.11.**

b) Liver functions markers: Dietary GL was related to serum levels of AST (above and below 40 U/L) p=0.04 but not to ALT **Table 3.11.**

c) Metabolic markers: Dietary GL intake was not related to any metabolic markers **Table 3.11.**

d) Adipocytokines, Lipoproteins and Markers of Inflammation: Dietary GL was not related to any of the adipocytokines, lipoproteins or markers of inflammation **Table 3.11.**

3.3.5.2 Relationship of dietary fructose with outcome variables

Total fructose

a) Gender and age: There were no relationship between total fructose intake with gender or age in any of the two groups during the dietary intervention **Appendix A, Table A.5 and A.6.**

b) Body composition and BP: There were important relationships between total fructose intake (absolute gm basis) with SBP (p=0.04). Total fructose was not related to any body composition variables (weight, height, BMI, WC, BF%, skinfolds or somatotype) **Table 3.12**.

c) Liver functions markers: Total fructose intake (absolute gm basis) was related with plasma levels of AST (p=0.003) and when adding BF% in the model the relationships were still significant (p=0.003); meaning that children with higher BF% and higher fructose intake had higher plasma levels of AST. Regarding the rest of liver parameters, there was only a trend towards the relation of total fructose intake with plasma levels of ALT (treated as continuous variable) but not to GGT **Table 3.12.**

d) Metabolic markers: Total fructose intake (absolute gm basis) was also related with MS markers such as AI (p=0.03), HOMA-IR (p=0.04) and Insulin (p=0.003) **Table 3.12.**

e) Adipocytokines, inflammation and lipoprotein markers: Total fructose intake (absolute basis) was not related to plasma adipocytokines except for ApoB-100 (p=0.02) **Table 3.12.**

High fructose corn syrup interrelationships: HFCS was not related to any of the following study variables: gender, age, body composition, liver function markers, metabolic markers, adipocytokines, lipoproteins or inflammation markers. HFCS was only related with SBP (p= 0.002) **Table 3.13.**

Table 3.10 Multivariate analysis between glycemic index and outcome variables

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
SBP	GI[1]	0.391	<0.001	**0.016**
	BF %			<0.001
ALT	GI[1]	0.538	<0.001	0.276
	BF %			<0.001
AST	GI[1]	0.115	0.049	0.743
	BF %			0.019
GGT	GI[1]	0.079	0.041	**0.041**
HOMA-IR	GI[1]	0.221	0.011	0.127
	BF %			0.039
Insulin	GI[1]	0.549	<0.001	**0.046**
	BF %			<0.001
Apo-B100	GI[1]	0.367	0.004	**0.011**
	BF %			0.019
Apo-B48	GI[1]	0.365	0.003	0.189
	BF %			0.001

[1] Categorical variable: GI (<55; 55-60 and >60)
[2]Abbreviations: Systolic Blood Pressure (SBP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), homeostasis model of assessment of insulin resistance (HOMA-IR), Apolipoprotein B-100 (Apo-B100) and Apolipoprotein B-48 (Apo-B48).

Table 3.11 Multivariate analysis between glycemic load and outcome variables

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
SBP	GL	0.332	<0.001	0.284
	BF %			**<0.001**
ALT	GL	0.531	<0.001	0.441
	BF %			**<0.001**
AST[1]	GL[1]	0.193	0.003	**0.041**
	BF %			**0.002**
AST[1]	GL[1]	0.270	0.001	0.568
	BF %			0.005
	GL[1]+BF %			**0.047**
HOMA-IR	GL	0.225	0.009	0.112
	BF %			**0.015**
Insulin	GL	0.510	<0.001	0.659
	BF %			**<0.001**
TG	GL	0.207	0.002	0.302
	BF %			**0.003**
Apo-B100	GL	0.264	0.008	0.246
	BF %			**0.001**
Apo-B48	GL	0.352	0.004	0.281
	BF %			**<0.001**

[1] Categorical variable: AST (above and below 40 U/L) and GL (<80; 80-120 and >120).
[2]Abbreviations: Systolic Blood Pressure (SBP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), homeostasis model of assessment of insulin resistance (HOMA-IR), Triglycerides (TG), Apolipoprotein B-100 (Apo-B100) and Apolipoprotein B-48 (Apo-B48).

Table 3.12 Multivariate analysis between total fructose and outcome variables

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
SBP	T Fructose BF %	0.389	<0.001	**0.004** **<0.001**
ALT	T Fructose BF %	0.544	<0.001	**0.064** **<0.001**
AST	T Fructose BF %	0.223	0.003	**0.003** **0.019**
AST	T Fructose BF % T Fructose + BF %	0.324	<0.001	0.093 0.223 **0.003**
HOMA-IR	T Fructose BF %	0.220	0.003	**0.042** 0.015
AI	T Fructose BF %	0.174	0.017	**0.034** 0.102
Insulin[1]	T Fructose BF %	0.566	<0.001	**0.003** <0.001
Apo-B100	T Fructose BF %	0.300	0.009	**0.028** 0.002
Apo-B48	T Fructose BF %	0.319	0.003	0.485 <0.001

[1] Categorical variable: Insulin (above and below 20 mU/L)
[2]Abreviations: Systolic Blood Pressure (SBP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), homeostasis model of assessment of insulin resistance (HOMA-IR), Apolipoprotein B-100 (Apo-B100), Apolipoprotein B-48 (Apo-B48).

Table 3.13 Multivariate analysis between HFCS and blood pressure

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
HFCS	SBP	0.137	0.002	0.002
	DBP	0.029	0.169	0.169

[1]Abreviations: High Fructose Corn Syrup (HFCS), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)

Table 3.14 Multivariate analysis between sodium, potassium with sitolyc blood pressure

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
SBP	Sodium Group	.435	<.0001	0.207 <.0001
SBP	Sodium Group Sodium + Group	.460	<.0001	0.508 0.722 0.093
SBP	Potassium Group	.428	<.0001	0.381 <.0001
SBP	Potassium Group Potassium + Group	.431	<.0001	0.364 0.003 0.556

[1]Abreviations: High Fructose Corn Syrup (HFCS), Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP)

3.4 Discussion

Currently there are no evidence-based guidelines that focus on specific lifestyle strategies (diet and/or exercise) for the treatment of NAFLD. The main approach to therapy includes lifestyle modification (diet and physical activity); with a focus on weight reduction to promote improvements in liver function (Alisi, Feldstein, Villani, Raponi, & Nobili, 2012; Chalasani et al., 2012), but current evidence regarding the specifics of what these interventions should entail are limited. Weight reduction has been associated with improvement of plasma ALT levels and reductions in the extent of steatosis in children and adults with NAFLD (Chalasani et al., 2012; Nobili et al., 2008). Typically weight management strategies in children with NAFLD have focused on calorie restriction (fat and carbohydrate reduction) and increasing physical activity (Pacifico et al., 2012; Reinehr, Schmidt, Toschke, & Andler, 2009; Wang et al., 2008). Few studies have examined the effectiveness of dietary interventions in the treatment of children and adolescences with NAFLD; particularly when dietary interventions do not focus on promoting caloric restrictions.

There is evidence that children with NAFLD with higher intakes of simple sugars (GI/GL/fructose) and saturated fat have an increased risk for insulin resistance, dyslipidemia and inflammation; all factors known to increase the risk for liver damage in childhood NAFLD (Mager et al 2010, Jin et al 2012). This suggests that dietary intervention strategies that focus on reductions in GI and GL, along with fructose may be potentially beneficial in the treatment of childhood NAFLD. Recently, low GI/GL diets have been proposed as a possible dietary target among obese children to promote weight loss (Ebbeling CB, Leidig MM, Sinclair KB, Hangen JP, Ludwig DS, 2003; Kong, Chan, Nelson, & Chan, 2011). However, little is known regarding the efficacy of this approach in children with NAFLD. The purpose of this study was to evaluate whether a lifestyle intervention with a focused at lowering glycemic index, glycemic load and fructose (FRAGILE) will affect body composition, insulin resistance, dyslipidemia, lipoprotein expression, inflammation and liver function in children and adolescents with NAFLD. We hypothesized that a low GI/GL/fructose dietary intervention (in the absence of energy restriction) over 6 months would result in significant improvement in body composition, liver function and metabolic parameters in children with Nonalcoholic liver disease (NAFLD).

In the present study, no significant changes in body weight over the course of the study were observed. In contrast significant reductions in body fat% and SBP and

changes in body fat distribution as assessed with skinfold measures (specially related to subcutaneous central adiposity) and somatotype shape were noted in children with NAFLD, particularly in the first three months of therapy where the most significant reductions in dietary GI/GL/fructose were observed. Other important changes noted over the study period were significant reductions in the number of children with abnormal serum values of TG, ALT, GGT and AST and HOMA-IR, as well as significant reductions in plasma non-esterified free fatty acids and ApoB-100 concentrations in children with NAFLD. No other changes in markers of inflammation and lipoprotein expression were found in these children. Within the overall intervention period, reductions in fructose, GI and GL were related to changes in consumption of foods high in HFCS (sweetened beverages, processed foods) rather than from naturally occurring food sources such as fruits and vegetables. In terms of GI/GL, children with NAFLD reduced the intake of baked goods, candy, sugar and ice cream. They also reduced the frequency of intake of sweetened beverages or change their intake of reduced sugar beverages or preferred beverages with no caloric sweeteners. The intake of fruits and vegetables slightly increased and this did not influence the dietary GI or GL overall fiber intake. All of these changes (metabolic and dietary) occurred in the absence of any changes in physical activity patterns; which were characterized by more inactive hours during the week and low score sport and leisure values. In contrast, while the lean healthy children experienced similar changes in diet intake patterns, no significant changes in body composition, blood pressure, physical activity patterns and/or biochemical variables were noted over the intervention.

The results of this study (FRAGILE) are similar to other lifestyle interventions studies in children with NAFLD, where the main findings have been related to reductions in body fat%, dyslipidemia, insulin resistance and hepatic transaminases along with a modest reduction in body weight (3-10%) over 3-12 months (Pacifico et al., 2012; Reinehr, Schmidt, Toschke, & Andler, 2009; Wang et al., 2008). The main difference between these studies and FRAGILE is that their dietary intervention approach have focused on hypocaloric diets aiming to reduce overall macronutrient intake (refined sugars, fat) and the amount of food intake where the primary focus of the intervention has been on weight loss (Santomauro et al., 2012). These studies as well focused on including increasing physical activity recommendations (Pacifico et al., 2012; Reinehr, Schmidt, Toschke, & Andler, 2009; Santomauro et al., 2012) and

some of them even supervised and implemented specific exercise regimens (Wang et al., 2008). These differences in lifestyle modification approaches are important because until now the separate effect of dietary changes verses physical activity changes has not been assessed previously in childhood NAFLD, particularly in relation to modifying GI/GL/fructose intake. In contrast, FRAGILE was focused on a specific dietary approach (Reduction of the intake of high GI/GL/fructose foods) without any strong emphasis on energy intake reduction. The focus in this study was on age-appropriate energy intake (BMR x 1.5); which for some children may have represented reductions in energy intake.

Some of the limitations of the previous lifestyle studies cited within the literature also are that most of the study participants had a diagnosis of NAFLD made with the use of ultrasonography and blood work only, and therefore had the potential to underestimate the actual prevalence of NAFLD from the obese controls within their studies (Shannon et al., 2011). Detecting changes in disease severity (SS verses NASH) would have also been challenging in many of these cohorts. This is important as children with NASH in particular, often have more severe metabolic syndrome expression and may respond differently to dietary interventions (Mencin & Lavine, 2011; Vos & McClain, 2008). In this cohort, children with NASH (diagnosed via liver biopsy) exhibited some important clinical differences. For example, these children presented with higher systolic blood pressure, HOMA-IR, insulin and ApoB-100 values at baseline, and also experienced the most significant reductions in these parameters over the dietary intervention. This is likely due to the fact that these children had the highest intakes of fructose (particularly from HFCS) at baseline. These results suggest that modest changes in dietary intake may be highly beneficial in children with NAFLD, even in the absence of weight loss. Hence, having the ability to diagnose disease severity or to specifically measure changes in hepatic fat content in response to dietary therapy is important. Unfortunately, the design of this study did not include the use of specific measures of hepatic fat, a factor that does limit the ability to determine how the changes in dietary intake influenced this important outcome.

Some studies have shown that the absence of weight loss has occurred in studies focused on both dietary and physical activity (over 3-8 months) in obese children and adolescents, but they found significant reductions of body fat percentage, SBP and improved insulin sensitivity as major outcomes (Marcano et al., 2011,

Balagopal, 2005); similar to the findings in the current study. Physical activity consisted in supervised aerobic exercised and parent involvement during exercise (Marcano et al., 2011, Balagopal, 2005). However, it is not clear whether changes in physical activity or diet influenced their results The dietary changes were focused on hypocaloric diets and aimed at reducing the frequency of hypercaloric snacks with high fat and sugar contents (Balagopal, 2005). These results could be potentially influenced by the small sample size and non-adherence to the diet. In FRAGILE, the sample size was sufficient to detect changes in the primary outcome variables (such as body composition), but possibly other factors may have influenced the results (non-adherence to the diet, lack of more frequent follow-ups and lack of physical activity program). The effect of more focused dietary changes and its relationships to metabolic markers of disease risk and underlying disease mechanism in children with NAFLD are still needed.

Studies with specific dietary approaches such as improving the quality of the carbohydrates intake (GI/GL/fructose) are limited or only in obese adults with different chronic diseases. For example, low GI diet over 6 months significantly reduced SBP and the risk of CVD in adults with type 2 Diabetes (Jenkins et al., 2010). Even less is known about adults and children with NAFLD. In terms of dietary fructose changes, a 50% reduction of fructose intake over 6 months in adults with NAFLD resulted in significant reductions in liver enzymes, insulin and HOMA-IR (Volynets et al., 2012). In contrast, a more recent study has shown that fructose intakes ranging between 8-30% of total energy intake is not associated with increased hepatic and muscle fat deposition (Bravo et al 2013) in obese adults. The main difference with these studies and the current study is that baseline intakes of fructose were much higher in the adults with NAFLD in these studies than the intakes of children in the FRAGILE study (15-33% of the fructose intake vs. 6% respectively) and that dietary adherence to the interventions was a potential limiting factor in the ability to study outcomes. In addition, these studies did not include any information regarding change in GI or GL intake or report specifically on the micronutrient compositions of these diets. All factors that potentially could have confounded overall study findings. In obese adults the literature does suggest that fructose intakes exceeding 10% of total energy intake are more consistent with an increased risk for MS, whereas fructose intakes below 9-10% of total energy intake is associated with significant improvements in metabolic parameters in adults with NAFLD (Madero et

al., 2011). This finding is consistent with the current study results. However, the mechanisms by which reductions in both dietary fructose and GI/GL could modulate the progression of NAFLD in children remain unclear.

Potential explanations for how changes in dietary fructose could influence the results in the present study include fructose intake seems to increase the synthesis of hepatic triglycerides by up regulation of de-novo lipogenesis, and to increase the flux of non-esterified free fatty acids to the liver and this may concurrently increase hepatic lipid content which interferes with hepatic insulin signaling (K. L. Stanhope et al., 2011). This hypothesis was previously supported by human feeding trials in which sugar sweetened beverages and high fructose diets given to obese adults increased serum non-esterified free fatty acids, IR and liver fat content suggesting an up regulation of de-novo lipogenesis (Assy et al., 2008; Sevastianova et al., 2012). In FRAGILE, a reduction in the number of children with NAFLD with elevated triglycerides, non-esterified free fatty acids and IR after the intervention occurred. This suggests that perhaps the flux of fat from the periphery (via NEFA) to the liver was reduced to some extent and which may have resulted in improved insulin sensitivity (A. J. Sanyal et al., 2001).

Another possible explanation could be associated with improvements in hepatic triglyceride clearance as it is showed in meal challenge studies with adults in which high intakes of fructose (rich fructose drinks with 22gr of fructose and meals with 2.5 gr if fructose) resulted in reductions of lipoprotein lipase activity and consequently impaired the clearance of triglycerides and Apo-B levels (Chong, 2007). During the FRAGILE study, reductions in Apo-B100 occurred. This may be important as there is evidence that 200gr of fructose supplemented for 7 days in the form of beverages in healthy adults with risk of Type 2 DM resulted in high levels of VLDL triglyceride rich particles associated with high levels of Apo-B100 particles (Tappy & Le, 2010). Whether these changes could be exerted by changing intakes primarily related to naturally occurring sources of fructose (such as fruits and vegetables) or whether these would be induced by changes in HFCS is unclear since most of these studies included the use of drinks that include monosaccharides (fructose vs. glucose meal challenges) to deliver fructose.

There is evidence that HFCS may have greater negative effects than other sources of fructose (Angelopoulos et al., 2009; Melanson et al., 2007; Tetri, Basaranoglu, Brunt, Yerian, & Neuschwander-Tetri, 2008). For example, cross-

sectional studies in children and control trials in adults have found that high intakes of HFCS rich beverages (administered 200g/d of fructose in a 10% bottle water) were associated with high levels of SBP and uric acid (Perez-Pozo et al., 2010). HFCS also seems to lead into greater synthesis of glycated metabolites and oxidative stress that could be associated with liver damage (Assy et al., 2008). Furthermore, HFCS could also induce a greater up regulation of phosphofructokinase, which consequently induces ATP depletion and inflammation in the liver (Ouyang et al., 2008) and HFCS may have greater increases of de novo lipogenesis (K. L. Stanhope & Havel, 2010). In this study, the greatest reductions in fructose intake included HFCS. Changes in HFCS were the most strongly associated with the reductions in TG, insulin, HOMA-IR, ALT, ApoB-100 and the changes in body composition observed. Hence, it is possible that HFCS reduction could impact de novo regulation of fat synthesis.

Finally, high fructose intake have been associated with increased visceral adiposity, factors known to be associated with IR and increased plasma levels of free fatty acid levels (Nomura & Yamanouchi, 2012). In addition increased VAT is associated with high Apo-B100 levels. In FRAGILE, important reductions in subcutaneous central adiposity, which were associated with reductions of fructose were observed. All of this suggests that changes in body fat distribution and reduction in overall body fatness in response to dietary manipulation (i.e. low added fructose/GI/GL intake) may be more important than the actual weight lost to reverse or delay progression of NAFLD in children. Potentially, there may be a threshold of both relative body fatness and fructose intake whereby individuals over a spectrum of body weights may become more susceptible to the effects of higher intakes of fructose. Interestingly, the lean children in this cohort were consuming processed foods high in HFCS, but did not experience significant changes to the VAT, body fat percentage and/or their metabolic parameters studied over the entire intervention. This finding is similar to other studies that have shown the increasing fructose intake is with increasing visceral adiposity in obese adults (K. L. Stanhope et al., 2009) and suggests that potentially there may be a threshold of 'relative adiposity' that may influence the ability of the body to adapt to a higher fructose intake without any metabolic dysregulation (K. L. Stanhope & Havel, 2010).

3.5 Strengths and limitations

This study has several strengths and limitations. Sample size was relatively small, although it was sufficiently powered to detect changes in the primary outcome variables of interest during the dietary intervention (Appendix 1, Table 4). Sample size was affected by patient drop out (2 children with NAFLD dropped out at 3 months, and 2 more do not show up at 3 and 6 months visits respectively); a characteristic that is not uncommon within pediatric weight management clinics (Kitscha et al 2009).

Another potential limitation in this study was the inability to gender-match healthy controls to children with NAFLD. However, it is unlikely that these differences were a major factor influencing study outcomes, as there were no significant relationships between gender and primary outcomes of interest. Another potential weakness in this study was the lack of specific measures of pubertal status (such as Tanner staging) in the children participating in this study. This may have influenced the ability to determine the effects of pubertal development on some primary outcome measures such as insulin resistance. For this reason, a statistical analysis was performed in which we stratified the data for children above and below 13 years of age. When this analysis was performed, no significant differences between these two groups in terms of the outcome variables (such as insulin resistance) were observed. Finally, the extent to which steatosis was influenced by the dietary strategy was not possible to determine as we did include any measures (such as MRI) to assess this.

The majority of children with NAFLD tended to underreport their energy intake more than the control group, and this was consistent among the same participants during the dietary intervention as showed in Table 3.9 and Appendix 1 Table 16. Dietary underreporting has been previously described as potential issue among children with NAFLD (Mager et al., 2010) Therefore, dietary underreporting may have influenced energy intake. However, it is important to highlight that children with NAFLD maintained their weight during the intervention and the extent of how dietary underreporting may affect specific macronutrients is difficult to distinguish. Moreover, other studies suggest that social and personal characteristics of participants could increase the frequency of dietary underreporting (Mirmiran, 2006; Garriguet, 2008). The psychosocial variables of energy underreporting and how this may influence macro-and-micronutrient in children with NAFLD have not been clarified

yet. In order to minimize the influence of dietary underreporting, dietary intake was adjusted (Garriguet, 2008). Lack of adherence to the diet could also be a limitation in this study. Some participants did not follow the recommendations in the last three months of the study. However, this might be influenced by other psychosocial variable such as the influence of the parents and the family dynamic (Blake, 2011). Parents can influence decisions regarding food choices and the availability of healthy food choices, all of which are important during dietary interventions (Guilfoyle SM et al, 2010). One of the major reasons for lack of adherence in weight loss programs has also been the high rate of non-return to clinic (Kitscha et al 2009). Evaluation of these potential factors will be in Chapter 4.

A final limitation is that we are unable to distinguish between the individual effects of dietary fructose reduction strategies and decreased GI/GL reduction strategies and how this may be specifically influencing study outcomes. In general the focus in this dietary intervention strategy was to increase naturally occurring sources of fructose via an increase in naturally occurring sources of fructose (fruits and vegetables), along with an emphasis on GI reduction in the diet, it is difficult to distinguish whether the reduction in either one of these approaches is the main variable responsible for the changes noted. However, one important feature is that no significant changes in micronutrient intakes were noted over the course of the intervention which does suggest the primary 'effect' variables were related specifically to these changes in dietary intake in the children with NAFLD.

While this study has limitations, it is important to mention that many variables were used in the study such as skinfold measurements and somatotype analysis in addition with liver function, inflammation and metabolic markers. Recent studies have also suggested other anthropometric measurements of subcutaneous fat may be an important tool to assess risk for CVD, MS and NAFLD in the clinical setting (Mager, Yap et al., 2012). Another strength in this study is children with NAFLD were diagnosed using liver biopsy, which facilitated the ability to detect cases with simple steatosis or NASH. This provides important information about the efficacy of this dietary intervention in children and adolescents with NAFLD across a spectrum of disease expression. In FRAGILE, having a control group of children with normal healthy body weights provided the opportunity to assess whether or not changes in the quality of carbohydrate could have a greater impact in obese children when compared to lean children or if metabolic markers could be improved within the healthy

population. In contrast, many of previous studies have not been able to compare their results with healthy children. Finally, the dietary intervention provided was focused on evoking dietary intake patterns in the absence of energy restriction. The method employed in the study consisted of simple, easy to follow materials; palatable recipes and snack ideas that help children and their families modify their diet.

3.6 Conclusions and Clinical Implications

In summary, results from this study suggest that improvements in markers of MS and liver function, as well as in body composition and blood pressure related to disease risk in childhood NAFLD can be achieved with modest changes in dietary carbohydrate quality (GI/GL/fructose), even in the absence of weight loss or dietary energy restrictions. The strongest relationships were observed when children reduced their intake of fructose; particularly from foods that are high in high fructose corn syrup, rather than natural occurring sources of fructose. These changes all occurred in the absence of any changes in activity patterns in children with NAFLD.

No major changes in biochemical or body composition in children with a healthy body weight were observed in response to reductions in GI/GL/fructose intake in this group. All of this suggests that modest reductions in fructose intake, particularly of HFCS, may result in significant reductions in metabolic dysregulation (K. L. Stanhope & Havel, 2010) and hepatic damage in children with NAFLD. Targeting reductions in fructose intake particularly processed foods characterized by high levels of HFCS, may be a potential therapeutic target for children with NAFLD.

Chapter 4. Exploring parents' attitudes and perceptions about nutrition and lifestyle management in children and adolescents with nonalcoholic fatty liver disease (NAFLD)

4.1 Introduction

NAFLD is estimated to occur in up to 30% of overweight and obese children globally (Schwimmer et al, 2006). The causes are thought to be multifactorial, related to obesity and lifestyles characterized by sedentary activity and high intakes of energy, simple sugars and fat (Gaemers, 2006). Therefore it is not surprising that the major treatment strategy in children with NAFLD is to promote gradual weight loss with changes in diet and physical activity (Alisi & Nobili, 2012; Gossard & Lindor, 2011). However, there is still a lot of controversy over the best way to achieve and sustain weight loss over the long term in overweight and obese children, particularly within the context of providing interdisciplinary approaches to care.

Recent evidence indicates that including the family´s perspectives and participation in weight loss programming is very important to ensure program effectiveness and to promote lifestyle changes that are sustainable for the child and adolescent (Guilfoyle SM et al, 2010). According to the literature, the biggest concerns with pediatric nutrition weight management programs include poor compliance with health professional recommendations, high rates of no-return to clinics, and lack of overall family lifestyle change (Kitscha, 2009). Much of the literature about pediatric weight management has shown that for sustained and long-term success in weight management to occur in overweight children, parental involvement in the overall treatment plan is critical (Howard, 2007; Ball, 2012). In children with NAFLD, very little is known about what parents think about current approaches to lifestyle management and how this may influence the efficacy of lifestyle management to treat childhood NAFLD.

In order to develop effective lifestyle intervention strategies for obese children with NAFLD, it is important to understand what the parents know about nutrition and whether they can influence their child and family to adhere to lifestyle recommendations (Nsiah-Kumi, 2009). The purpose of this study was to describe and explore the attitudes and beliefs of parents and caregivers of children with NAFLD and lean healthy children regarding the barriers and facilitators that influence the parent's and child's ability to sustain and incorporate lifestyle recommendations

provided during nutrition counseling about healthy eating and physical activity.

4.2 Subjects and methods

4.2.1 Study design

We conducted an explorative study using both qualitative (focus groups) and quantitative (validated questionnaire) methodology in parents and caregivers of obese children with NAFLD (cases) and parents/caregivers of children with body weights within the healthy range (controls). Using focus groups is a validated methodology that explores knowledge, attitudes, perceptions and beliefs about different concepts (Jenny Kitzinger, 1995). It provides the unique opportunity to explore and probe concepts in more detail. Focus group generates information by promoting interaction between the participants (Stewart & Shamdasani, 1990).

A validated questionnaire called the Lifestyle Behavioral Checklist (quantitative tool) was also used to assess parental perceptions and confidence in dealing with their children´s lifestyle behavior problems (e.g., eating too quickly) (West, 2009). The study design included a plan to evaluate the knowledge of parents from three groups: parents/caregivers of children with NAFLD who had received nutrition education in the past six months (Case 1 group), parents/caregivers of children with NAFLD who had not received nutrition education in the past six months (Case 2 group) and parents/caregivers of children of lean children who had received nutrition education in the past six months (Controls). The results of this study only include findings from Case 1 and the Controls.

4.2.2 Inclusion and exclusion criteria

In the study were included parents/caregivers of children diagnosed with NAFLD (4-18 years) and parents/caregivers of lean children (4-18 years) who had received nutrition education focused on healthy lifestyles regarding their child in the past six months. Parents/caregivers of children with NAFLD and/or parents of healthy lean children (<4 or >18years) were excluded from the study. The BMI of parents/caregivers should be < 50kg/m^2. Parents/caregivers who were less than 18 years of age or who were unable to provide informed consent were excluded from the study.

4.2.3 Subject recruitment

We used a purposive sampling approach (participants were selected according to the research question) (Charmaz, 2006). We selected parents of children with NAFLD and parents of healthy lean children in order to explore their knowledge, attitudes, beliefs and their experience when helping their children to follow a healthy diet. Parents/caregivers of children with NAFLD (n=4) were recruited from the Pediatric Liver Clinics at the Stollery Children's Hospital, Edmonton Alberta. The families of children with NAFLD had participated in the FRAGILE study (Chapter 3) within the past six months. Parents/caregivers of children with healthy body weights were recruited from the university and general community (n=8) via the use of recruitment fliers. Some of the families of healthy body weight children (n=3) also participated in the FRAGILE study as controls. Informed consent was obtained from all study participants prior to study enrollment. The Human Research Ethics Board, University of Alberta, approved this study. Operational and Administrative Approval was obtained from Stollery Children's Hospital and the Northern Alberta Clinical Trials Centre.

4.2.4 Objectives

The study objectives were to explore and describe barriers and facilitators experienced by parents/caregivers of NAFLD and lean children, to learn how those barriers and facilitators influence the parents' ability to support healthy lifestyle changes in their children.

Primary Outcomes

1. Nutritional education: To explore the source of nutrition education parents have been provided, the perception of this information and what other information these parents would like to receive.
2. Barriers: To explore the experience and identify barriers faced by parents/caregivers of children with NAFLD when helping their children to follow a healthy diet.
3. Facilitators: To explore the experience and identify facilitators that allows parents/caregivers of children with NAFLD to help their children follow a healthy diet.

Secondary outcomes

1. To describe demographic information related to parental/child age, weight and height, as there is significant evidence to show that parental BMI is a major determinant of a child's BMI.
2. To assess parental self-efficacy/parental perception of child's behaviors regarding food intake and meal environment using a validated questionnaire.

4.2.5 Demographic assessment and anthropometric measurements (parent and child)

Information regarding parental age, and child's age, weight and height were determined by parental self-report. The weight and height of parents/caregivers was assessed in the study, as there is significant evidence to show that parental BMI is a major determinant of a child's BMI (Ajala et al., 2012; Perez-Pastor et al., 2009b). Weight was assessed without shoes and with minimum clothes to the nearest 0.5 kg (Pelstar scale, model 752KL from Health o meter Professional, Bridgeview, Illinois, USA). The height was measured without shoes to the nearest 0.5 cm with the use of a commercial stadiometer (Charder HM200PW, Medical Supplies, North Blend, WA, USA). Body mass index (BMI) was calculated as: Weight (kg)/Height (m^2). Participants were classified as normal weight (18.5 and <25kg/m^2), overweight (25-29kg/m^2) or obese (≥30kg/m^2) (Douketis et al., 2005).

4.2.6 Focus group

A total of four focus groups were conducted within the Clinical Research Unit at the University of Alberta. Two of the focus groups were conducted with parents/caregivers of overweight/obese children with NAFLD (Case: Group 1) who had received nutrition education in the Fragile Study within the past six months. The control group represents parents of healthy lean children who had received nutrition education within the past six months from either their participation in the Fragile Study or at routinely scheduled visits with their family physician. Studying the general perspective of parents/caregivers with children within healthy body weight ranges provides important information regarding nutritional education, healthy eating behaviors and what factors might influence their ability to support health eating behaviors in their child, independent of a focus on weight loss. Data was generated through focus groups conducted at the Clinical Research Unit (CRU) at the University of Alberta, and through key field notes taken during the focus group. An in-depth interview guide was develop to help the moderator of the focus groups guide the

participants. The focus group interview guide content was based on review of the literature and included nine open-ended questions that addressed concepts such as parental perceptions and attitudes towards nutrition education; and parental perceptions, beliefs, barriers and facilitators about nutrition and healthy eating and how parents encourage their children to follow healthy lifestyle behaviors **(Appendix B, Table B.1).** External reviewers examined the focus group interview guide content to ensure accuracy and recommended a shorter version (6 open-ended questions **(Figure 4.1)** to be used in order to create a discussion to explore parents' perceptions regarding nutrition information they may have received in the past and explore the barriers and facilitators encountered when helping their children to eat a healthy diet (e.g., Can you describe how you encourage your child to eat a variety of healthy foods?). This was the guide that was used within the focus groups. Probe questions were used to gain an in-depth understanding of each answer. (e.g., Can you tell me more about it? Or, Can you give me any examples?) **(Figure 4.1).**

1.- Have you received nutritional education in the past?, Can you tell me more about the nutritional education that you received?

Probe: What type of health professional?

Probe: Was it general nutrition recommendations? Was it a specific dietary recommendation?

2.- Now I want to know if there was anything you found helpful about the nutrition education provided?

Probe: Was there any tool or information that you found helpful?

3.- Can you tell if you were able to understand all of the terms used. Can you think of any examples?

Probe: Was it given examples to apply those terms?

4.- Is there any other nutritional information that you would like to receive from health professionals?

Probe: Menus, snack ideas, more constant interviews with dieticians, books or web pages recommendation

5.- Now I would like to know what has been the most difficult for you in helping your child to eat healthy?

Probe: Can you describe those difficulties in more detail?

6.- Can you describe how you encourage your child to eat a variety of healthy foods?

Probe: Can you tell me more about it? Can you give me more examples?

Figure 4.1 Interview guide used in focus group with parents of children with NAFLD and parents of children with healthy body weights

Three experienced moderators conducted the focus group and moderator assistants (graduate/undergraduate students) took notes during the actual focus group. The focus groups were recorded (Sony IC recorder, ICD-PX312) and lasted from 45-60 minutes. A debriefing session was held at the end of each focus group, at which time the moderator assistants and moderators reviewed the notes and discussed important quotes and "body language" that they identified during the focus group. Body language enhances the data analysis, because it helps to clarify the context and meaning of the participant's quotes (Krueger & Casey, 2008). Then, the 4 focus groups were transcribed verbatim, independently, by two graduate students who compared and verified the transcripts in order to assess accuracy. All the participants'

identities were coded to keep confidential identifiers in the transcriptions. The transcribed focus groups were organized by themes using Microsoft Excel. First, each line of the transcription was reviewed in order to code important concepts and words such as (e.g. snacks, recipes, recommendations). Then, responses were arranged by each research question (e.g., have you received nutritional education in the past?) and emerging themes were identified (e.g., Source of Nutritional Education/Information). Finally, the themes were classified by the following outcomes: Nutritional education/Information, barriers and facilitators. A data spreadsheet was created in order to compare findings among groups (Control group vs. NAFLD group). A report was generated that summarized key findings and themes. Quotes for each theme identified were included in the table and presented to the research group for feedback and external validation.

4.2.7 Lifestyle behavior checklist questionnaire

West and Sanders created the Lifestyle Behavior Checklist (LBC) (West & Sanders, 2005). The LBC questionnaire is structured by 25 items that assess 3 areas of children´s lifestyle behaviors (Eating behaviors, questions 1-15; Activity behaviors, questions 16-19; and Image self-stem behaviors, questions 20-25) **(Appendix B, Questionnaire 1).** The purpose of the LBC questionnaire is to assess parental perceptions and confidence in dealing with their children´s lifestyle behavior problems (e.g. eating too quickly) (West, 2009). Therefore, the questionnaire includes two scales: the problem scale and the confidence scale.

The problem scale asks participants to rate whether or not the behavior represents a problem for them (7-point Likert Scale, 1=Not at all, 7=Very Much). The confidence scale asks participants to rate how confident they are in dealing with the stated behavior of their child (10-point Likert Scale, 1=Certain I can do it, 10=I cannot do it). In this study, participants were asked to fill the LBC questionnaire before starting the focus groups at the Clinical Research Unit (CRU). This questionnaire has been validated for use in parents/caregivers of both overweight and obese children and parents of children with healthy body weights (West, 2009). The clinical cut of values for the problem scale is >50 and for the confident scale is <204 (West, 2009).

4.2.8 Statistical analysis: LBC scale

To assess the potential interrelationships of demographic and anthropometric variables including child age and child BMI, and parent age and parent BMI, and the scales (problem and confidence) in the LBC questionnaire, a multivariate analysis between these factors was performed. Interrelationships between parental demographic characteristics (age) and anthropometric characteristics (BMI and weight) were analyzed. Interrelationships between children demographic characteristics (age) and anthropometric characteristics (BMI and weight) were also analyzed. Significance was considered at a p value of <0.05. Results were expressed as mean ± SD. All statistic analysis was performed using SAS version 9.2.

4.3 Results

In this study a total of four focus groups were conducted. These included two focus groups with parents/caregivers of children with NAFLD (n=2 parents/focus group) and two focus groups of parents of children with healthy body weights (n=4 parents/focus group).

4.3.1 Anthropometric and demographic information

Demographic and Anthropometric data of study participants is presented in **Table 4.1.** No significant differences in parental and child age were present between groups ($p>0.05$). However, parental and child weight and BMI was significantly greater in the NAFLD group when compared to the children and parents in the control group **(Table 4.1).** Parental BMI was related to childhood BMI **(Table 4.2).**

Table 4.1 Demographic and anthropometric characteristics

Characteristic	NAFLD n= 4 Female n=3 Male n=1	CONTROL Female n=7 Male n=1	p-value
Age	43.5 ± 9.6	41.9 ± 8.4	0.529
Parental weight	88.1 ± 19.6	62.8 ± 8.0	**0.03**
Parental BMI	30.4 ± 5.6	21.9 ± 2.5	**0.004**
Children´s age	13 ± 4.5	10.2 ± 5.7	0.300
Children´s weight	89.2 ± 27.7	33.4 ± 15	**0.03**
Children´s BMI	32.6 ± 5.2	16.4 ± 3.1	**0.01**
Number of Children in the household	1.6 ± 0.7	1.3 ± 0.5	0.26

Table 4.2 Parental BMI correlation with children BMI

Dependent Variable	Independent Variable	r^2	p-value
Parental BMI	Children BMI	0.41	**0.032**

4.3.2. Qualitative methodology: focus groups' parental perceptions about factors influencing lifestyle changes in their children

The results are presented according to the primary outcomes: Nutrition information, barriers and facilitators, and parental self-efficacy. The results of each outcome are then presented by themes that emerged during the focus groups.

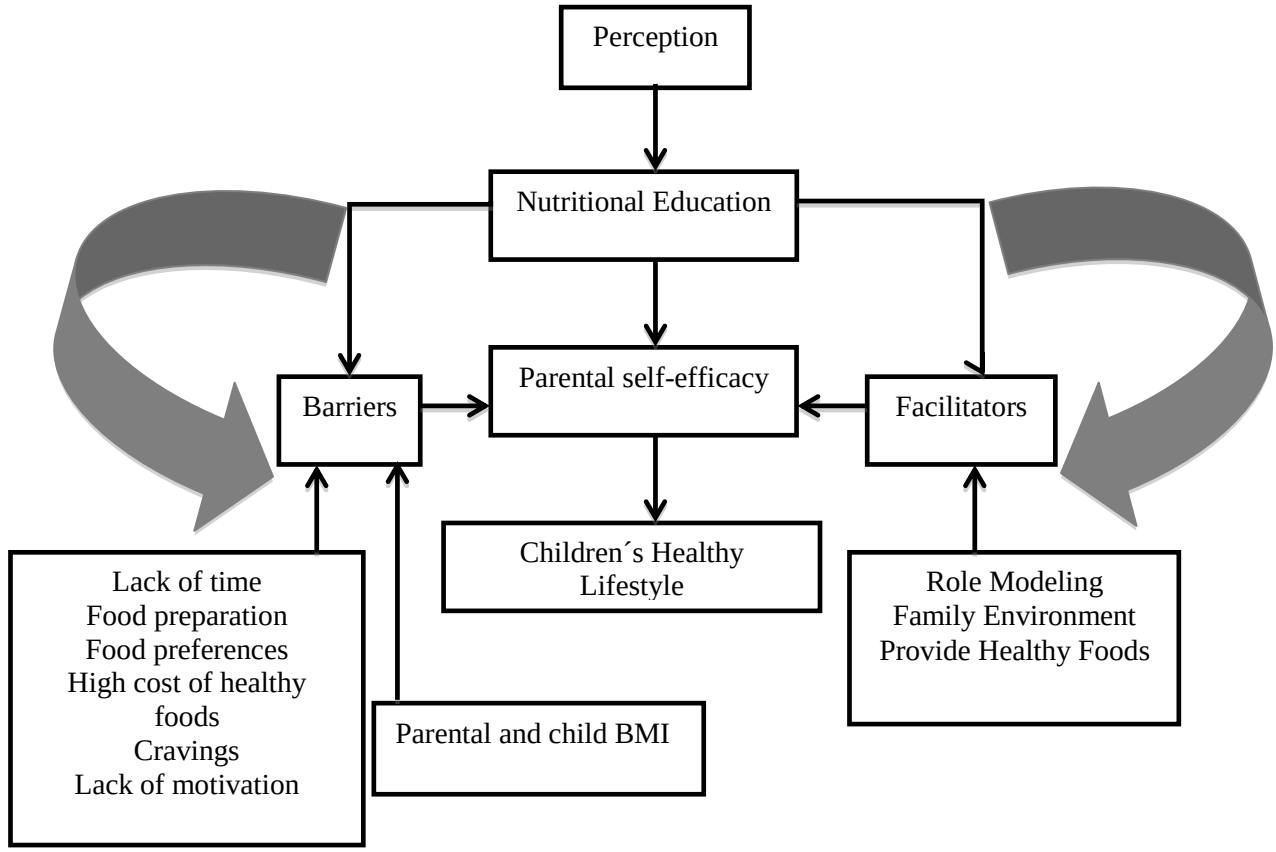

Figure 4.2 Factors that influence parental self-efficacy to help their children to have a healthy lifestyle

4.3.2.1 Nutrition education

The focus groups started by asking participants if they had received nutritional education in the past (both the last six months and in general). Participants affirmed that they had received nutritional information in the past. There were five important

themes regarding nutritional education: the source of nutritional education, type of nutritional education, perception of nutritional education, feelings about nutritional education and nutritional education parents would like to receive **(Table 4.3).**

The major sources of nutrition education cited in both groups were health professionals (the pediatrician and nurse were the most common source), followed by Internet. Parents of children with NAFLD also specified that they received nutritional education from The FRAGILE study (Chapter 3) (In this study, lean children and children with NAFLD were put on a low glycemic index (GI), glycemic load (GL) and fructose diet). The most common nutritional education/information parents had received in the past was related to infant feeding/child development and general healthy eating. Parents of children with NAFLD added that they had received specific information related to a gluten-free diet, liver health and the dietary components of the FRAGILE study **(Table 4.4)**. Table 4.4 summarizes the data regarding the types of nutrition education as well as the perceptions shared by parents in both groups relating how useful they found the information.

Table 4.3 Nutritional Education

Key Theme	Controls	Quotes	NAFLD	Quotes
Source of nutritional education	Physicians/ Pediatrician	*"Yeah, I have received information from physicians when I take my children for check-ups."* "*When they are babies and you are going to the pediatrician."*	Physicians/ Pediatrician	*"When our kids were born and we were talking to the clinics."*
	Public health nurses	*"I´ve received nutritional education from the public health nurses."*	Dietitians	*"Well, I mean we talked to the Dietitian, I think two weeks ago, I think, and she was actually really good."*
	Internet	*"In my case it just has been the Internet searches, going to web pages of health institutes and find some recommendations."*	Internet	*"I went online and I found a site that it gives you the glycemic index for just about anything. But I haven´t used it since I found it is also important the amount"* *"All the information we ever got is by going online and reading and figuring out what to do."*
			Media	*" And a little bit of Dr. O."*
			School	*"I kind of have a lots, between school and being doing research online and another child dietitian as well."* *"Other than high school and things like that, I don´t think we have much."*
			Fragile Study	*"The only dietary information that I received pertinent to this its from Fragile study."*

Key Theme	Control	Quotes	NAFLD	Quotes
Type of Nutritional Education	Nutrition education related to first years of life.	"*You know, about solids, and weaning and all that kind of things."* *"I was told foods to avoid during the first year of my child, I think they said no honey, no peanut butter, how often to give children or not give children meals and the appropriate size and the variety of foods."*	Nutrition education related to first years of life.	*"I googled what to feed a newborn? or what to feed at six months? or what reactions to look for."* *"They would tell us what kind of diet or what kind of foods they would have as little infants."*
	Principles of healthy eating in terms of chronic disease prevention	*"Yeah, a good example of that is if you are told to keep your sodium to 1500 milligrams… to the average person what does that mean."*	General nutrition education	*"They always talked about Canada's food guide: Make sure that you'd eat so many servings of fruits and vegetables and things like that." (School)*
			Gluten free diet.	*"I have met with a dietitian a couple of times, but it was taught more about eating gluten free."*
			NAFLD related recommendations	*"It was all home remedies … if you eat a lot of broccoli your enzymes go down, if you eat lemons or limes your enzymes go down."* *"Pretty much was diabetic diet, they all said: do the diabetic diet, get ride of the sugars."*
			FRAGILE (Glycemic Index)	*"They talked a lot about the GI values of certain foods and so you just look at different tables and different information that is on that."*

Key Theme	Control	Quotes	NAFLD	Quotes
Perceptions about nutritional education	Lack of practical nutritional recommendations	*"Things like you know, have so many grams of such and such per day, but you know to actually show it on a plate what kind of portion looks like."* *"There is health education and health promotion and certainly you know, we all know what we kind of should be doing but actually putting these things into practice is, very different."*	Lack of practical nutritional recommendations	*"The information is out there but it is not simplified and therefore, it is just not been followed."*
	Lack of Trusted Online Information	*"We have think about the kind of information we´re reading and see if it make sense or not."*	Lack of Trusted Online Information	*"I found that every site you went they almost all contradict themselves in one way or another and there is just different theories behind certain eating habits. So, different sites say different things so it is almost like a try and error and say ok so lets try this to see if it works."* *"Some webpages had too much of reading information."*
			Glycemic index Information is helpful (Fragile Study)	*"The little chart, the eat most often, eat two to three times a week and eat once a week chart and I have that taped on my fridge. So, when I´m try to think of... when I need to refer to it is right in front to my face every time I got to the fridge. So, for me that has been the most helpful thing."* *"I mean that is the biggest deal that I found was all the GI index."*

Key Theme	Control	Quotes	NAFLD	Quotes
Parental attitudes	Lack of support from health professionals	*"And some pediatricians say: as long as they´re healthy weight it does not matter what they´re eating."* *"The thing I don´t like of the health care is that you wait for about 40 minutes to get to the doctor and then once you get in there, he barely looks at you and in five minutes you are out of the room. I have to be honest I hate that, because I just don´t feel they put enough attention to the specific and individual needs of the patient. I don´t know if that is the case with nutritionist."*	Lack of support from health professionals	*"Realistic goals, if we talk to a dietitian there have to be some you know understanding out of it and really work on something that is going to work for us not only make an unfeasible goal that is going to be ok lets do this and in a week we say: --heck that--."* *"You don't want to be discourage, you don´t want to go see a nutritionist and be just completely discouraged, I mean I don't want to go see a stranger and be told I´m doing everything wrong, I get that already, I mean If you´re going to get the information you want to know, what you´re doing right and you can make small changes at a time, because you can´t do complete drastic overall of everything what they´re doing, because the kids are going to revel to you."*
			Frustration and surprised related to serving sizes and glycemic index	*"The hardest things to grasp are things like serving sizes."* *"I don´t remember all, I only know all that increased my frustration overall because… (Laughs), yeah, knowing that a bagel was 4 servings, that was surprising, yeah it shocked me."* *"I just didn´t really know about the glycemic index, so I was kind of surprised there is some higher fruits and higher vegetables."*

Key Theme	Control	Quotes	NAFLD	Quotes
Nutritional Education Parents need	**Recipes**	*"Recipes, because everybody knows like fruit is good, everybody knows vegetables are good, everybody knows that we are not supposed to eat too much fat, everybody knows that we are not supposed to eat too much carbohydrates, but not everybody knows how to combine those."* *"Some things that are easy to follow and also it has to taste good."* *"I think is going to be better if we have an information on how to combine meals to make it better for our kids."*	**Recipes**	*"I´m really, you know struggling with recipes and they have to be fast, like the ones that take 2 or 3 hours of preparation, I´m not going to do them, cause I like to eat by you know 7 at night."* *"Different ideas for what to pack for snack ideas, those types of things.*" *"I think, because we work such a long schedule, on some say we don´t get home until 8 o´clock at night sometimes. You know, we need better ideas of what we can have that are late and that are not going to cause to gain more weight because he´s eating late around night."*
	Online resources	*"I think something that kids get to know themselves, that they can go online and tape what they eat that day and that they can tell them all the things they shouldn´t eat or they should be eat less and try to eat more of this and that they can do that themselves."* *"Just give them an app to figure it out what they ate and they can figure what they are supposed to eat."*	Online resources	*"Easy step-by step", "I would like to look more specifically information from somebody that will be credit or published for something."* *"If the pages have a lot of colorful pictures or tables or charts that would be like the easiest way to do it."*
	Practical Tips	*"I think practical tips rather than just kind of the head knowledge and how to applied is always very good."*	Shopping list	*"Something that we can work or get so when we go shopping, like a list, so then you are not kind of side-track by everything else."* *"Yeah, a list, like this is something we have*

				to focus on remember to get this or remember to pick up this."
	More access to registered dieticians	*"Yeah, more access to dietitians."*	Motivation techniques	*"Motivation techniques."* *"And anything that gives hope."*
	School Nutrition Programs/ Policies	*"In my opinion there should be more strict obligations for the schools, not to give processed foods."* *"Reinforce policies in schools and like daycares to implement for example um… today is a fruit tasting day."* *"A class at school that teaches them or they can show him how to bake something healthy and that they can… you know if he makes it, maybe it´ll be better than mommy made vegetables."*		

4.3.2.2 Parental challenges and barriers to promote healthy lifestyles

Table 4.4 shows a summary of the themes and concepts cited by parents as the major challenges facing them in supporting healthy lifestyles within the context of the family. The major theme that emerged from the focus groups in both groups were environmental factors such as exposing children to "junk/fast foods" through the media and school, the high cost of healthy foods vs. the affordable cost of junk foods, children´s food preferences and a lack of time. However, parents of children with NAFLD share additional barriers such as children´s lack of motivation to follow healthy lifestyles, children´s constant cravings, children`s lack of involvement in food preparation, and lack of time to be role models for their children.

Table 4.4 Parental challenges and barriers to promote healthy lifestyles

Key themes Control	**Quotes**	**Key themes NAFLD**	**Quotes**
Food Preferences	*"My kids are very picky, they are in that age right now that we don´t like vegetables or fruits…sometimes I have to mask the vegetables."*	Food preferences	*"The only problem with my son is that, he´s very picky eater you have to cook things in a specific ways. Hamburger you can´t just cook the hamburger, you have to put this taco seasoning on top, because he doesn´t like plain hamburger."* *"My son doesn´t like fruit so I found it very challenged to, I still don´t find a fruit that he eats."* *"I´m just so happy that he´ll eat any fruit and any vegetables, I don´t think he even like carrots at all, you know."*
High cost of healthy foods	*"I think something that is really a challenge is that you know, to buy the healthy fruit and veggies etc. it´s very expensive."* *"I think the challenge is that you can buy those big litres of soda, and the fast foods, and the cookies and the chips, they´re so much less expensive than the healthy options and I think you know, we need to look at making the healthy options much more affordable for, if we´re going to see a real change."*	High cost of healthy foods	*"Because healthy is more expensive."* *"I found that like when we only have one income on its own, it's cheaper to buy unhealthy foods than the healthy foods."* *"Here is a good example: hotdog is one of the cheapest things in the earth. But I mean they´re one of the unhealthiest things you can buy."*
Parental Stress and Lack of time	*"The lack of time."* *"I mean for me time is a big barrier too so, I don´t have a lot of time to figure it out*	Parental Stress and Lack of time	*"No, I need to work, unless we meet in the evening or something, or on a Saturday morning, I don´t know what are you*

	different things." *"My kids are older… it´s everybody's extremely busy schedules."* *"How we make sure that we follow the guidelines? I would say only part of the time, because with all the stress of work, limited time, sometimes I got to be honest with you, we just give up."*		*schedules but I just cant do day time. It´s very annoying with people get cranky cause they think is too much time for appointments or health visits."* *"We´ve programed ourselves that Saturday be a kind of stay home. We are not going to schedule anything so it means we schedule movie in the theater or we schedule lunch or something, that´s fine."* "*I know that I should be his role model and you know, should be doing that too, I mean I don´t exercise either, so I don't even showing him... I don´t have time to exercise."*
Junk Food exposure	*"This is my major concern, because she is in a daycare which provide her lunch and snacks and everything is processed, although on the menu it says so whole grain bun with a margarine and etc. this is just full with ketchup and everything is processed or rice with chicken but these are processed chicken nuggets and just a bowl of rice. This is not nutritional."* *"My major concern is that outside home whether not supervised or there is neither me or my husband there is like still no real good choice."* *"Is the finance of junk foods right? And*	Food preparation	*"The food is pretty boring, you know, he only eats so much hummus and raw vegetables and then is just really boring, and I´m sick to death of making green salads like I just can not get the motivation to even making green salads, cause I was doing and green salads and couscous, quinoa salad and you know always having like 2 or 3 different salads on the go, and went away from baking and I just can not face either making it, I get all bored and giving up, it is very boring."* *"I buy those greens in the container and then put the dressing, and you have there (laughs),*

	advertising, I mean it´s because they want to have hamburgers all the time, cause their friends have hamburgers all the time and they see just all the advertising for all different foods, restaurants and whatever."		"*Struggle with snacks."* *"When mom has not chopped vegetables, because I figured he should do that right and guess what there´s no raw veggies in the snack".*
		Constant cravings	*"I don't have sweet cravings, but my son does."*
		Food portions	*"My servings are about the right size, but my son´s servings are double. So it is very difficult to get him to mmm take less."*
		Lack of motivation	*"My son lack´s of motivation"* *"A poor social life."*
		Negative influence by other family members and Parenting styles	*"So his dad feeds him with frozen express precooked stuff, he buys fast food a lot, almost daily, and he argues about whether he has certain conditions or not and then with that, that ignorance, you know and there´s no much I can do."* *"We gave in to him a little to much. That´s the problem I mean he´s got that face that…. He would kind of sneak in and say oh dad: Mom didn´t give me a potato with my dinner. And you know he would say: oh mom didn't give you one? ok her is your potato."*

4.3.2.3 Parental facilitators to promote healthy lifestyle changes

Table 4.5 summarizes the themes and concepts that parents cite as the major facilitators in helping them to foster healthy lifestyles in their children. The major facilitators identified in the control group included parents role-modeling healthy lifestyle behaviors for their children, being involved in food preparation and planning, encouraging children to try new foods, providing healthy foods at home, and monitoring portions (quantity and quality). This created a family environment, which seems to allow these parents the opportunity to talk about healthy food options and ways their children can improve their nutrition. The parents of lean healthy children value the concept of including their children in meal planning, food shopping and food preparation.

In contrast, parents of children with NAFLD did not utilize these strategies to the same extent. For example, food portion was more focused on quantity control, related to sugary and high fat snacks, rather than improving the quality of snacks. Although role modeling was cited as an important concept to support healthier food choices, parents of children with NAFLD perceived the "art of negotiation" to be a more feasible approach to promoting healthier food choices in their child. Less focus was spent on meal preparation and/or integrating the child into this process.

Table 4.5 Parental facilitators to promote healthy lifestyles

Key themes control	Quotes control	Key themes NAFLD	Quotes NAFLD
Role Modeling	"Children copy us, *If we have some sort of food habits, they would pretty much copy us."* *"A lot of it´s by example. We eat healthy, so."* *"I mean so often we go to the grocery store, and I tell him he has to pick a fruit and has to pick vegetables, so at least he is picking some things."*	Role Modeling	*"My son and I are on we fit and that´s part of our weekly regimen, its to go on fit and look how much we are loosing and keep the track on that. So I mean we´re… we´re both on our goal. He is actually stayed pretty stable and I´ve lost a little bit of weight so. I just told him that we´re doing good because the point is not to drastically lose weight; it's to not gain any."*
Provide Healthy Foods	*"It is keeping with a big variety, available foods at home."* *"And it´s what foods I keep in the house so you know if there is a lot of junk food around then if it´s going to be eaten and if it´s not there then something else has to be eaten."*	Provide Healthy Foods	*"As long as we have a lot of fruits and vegetables at all times, there is always something in there to peace them. I mean we bought a box of five pounds mandarin oranges the other day and my son loves oranges so that´s part of you know if you are hungry and you want a treat, grab an orange and I mean he does it."*
Food portions and treats	*"Portion control."* *"I have to keep reminding my nineteen year old about that you know… if he grabs something and starts eating out of it. I will say to him: use a bowl… you have no idea how much of that are you eating."* *"With mine you know it would always be … you´ll have two, you can have two cookies and that´s it."* *"So I guess we´ve always tried to… you have treats but… and so not say "no you can*	Food portions	*"We kind of cut down the sort of the starchy foods that he eats so when we have dinner his potatoes are much smaller than it was before."* *"So I try you know to put more vegetables in it. So if he takes more then it´s more vegetables or it´s brown rice, well at least is brown rice and he is not taking that much potatoes."* *"Now everything has to come in a bowl or*

	never have them but just kind of moderate it." *"If I know that she had a slush today or something of the sort, you know… we´ll just try to balance it out, and that it´s not a daily occurrence."*		*nothing that comes as snack unless is in a bowl. In that way he just has his portion and that´s all he gets. So he understands because if he brings the bag, he´ll eat till the bag is completely empty but if they pour the bowl and then when they are done there is not a desire of keep looking in the bowl from more because it is empty. So we found that works a lot better for us too."* *"He is a juice drinker also. So he likes juice and what we´ve been doing now is sort of supplementing his juice with half water-half juice."*
Family involvement in food preparation	*"Most of the time it is home made food. So it involves a lot of preplanning you know… like at least a day a head of time and every night at supper is usually ok what are we having for supper tomorrow. So that I can… you know… actually make it. Sometimes I´m cooking supper at four in the afternoon so that we can bag it and take it… Things like that definitely makes it a lot harder, it would be much easier just to grab a quick something at a fast food place."* *"I don´t mind cooking for six hours, even I do it every weekend to have food ready for the whole week, so it´s all about how the family is, I don´t know… how important for the family is to have good food or to have*	Food Negotiation	*"Usually it´s a negotiation." " Well if he already tried one new thing today, then don´t worry about it." " I mean, we do try it, the negotiation, and it works."* "*I don´t know that if this actually matters but… "Can I have more meat? And then I said: after you eat your half cup of vegetables then you can have more meat."* *"Sometimes I get home late myself and they already had dinner and then he´ll say what he eat or didn´t eat and then I´ll say well… we can have ice cream after we play and fit for a while."* *"He really loves potatoes so… for he to have another baked potato or mashed*

	something good." "*We have to be creative for example our son doesn´t like fruits if he eats them alone but if we mix them with yogurt then it is eatable for him or If we prepare milkshakes… He would not eat the strawberries per se but if we make a strawberry milkshake, he would eat it."* "*Packing your kid a lunch so they´re not going to the seven eleven or the fast food… then you can have a good idea of what they´ve been eating during the day."*		*potato he has too have his meat portion and then we make the deal with him and he stick t it in the most part and he understands if he doesn´t eat the meat he doesn´t get the potato."*
Encourage to try new healthy foods	*"What is important it´s just to show them how to eat, how I don´t know, how does it taste and everything comes with the time and be consistent."*	Meal Preplanning	*"If we are out and we know we´re going to be out for the whole entire day, we usually pack snacks. Like I got a bag that has apples or oranges, the healthy snacks my son can eat, the snacks that his brother has to have; his granola bars and things."* *"I mean when we were going for fast food it used to be McDonalds, because I mean the youngest loved… that´s the only burgers that we can actually get into him. But now fast food facilities is off the table because I mean that´s a lot of fat, that´s a lot of calories."*
Talking about Nutrition	*"I always have a discussion with my daughter; about how much food did she have today that had color in it that´s kind of how it have help me to identify what she is eating and what she is using for snacks and its sort*	Read Food Labels	*"We really look at the nutritional value in everything we buy now, everything. We don´t put anything in the car until we look at it and if its some kind of a new trendy you know breakfast cereal or something*

	of association of ok you didn´t have this much color, now you need to have this much color with your meal at dinner time to kind of make up for it. Many other foods that are sort of the filler are the ones without color and so we identified them in that way."		*that its you know some snack food, before putting it in the car, we look at how much sugar is in the bag, I mean we never would done that years ago. We just would buy the cheapest one or the one that has the most attractive package."*
Family Environment	"*I´ve always tried to make a point of as much as possible, all of us eating supper together which means some nights it´s at 5, some nights it´s at 6:30, you know it´s all over the place."*	Motivation	*"I said to him repeatedly, is living a life where you have to eat healthy and exercise is not a bad life to live, you´ll look better, you´ll feel better, and you´ll live a long healthy life and not drinking, never drinking alcohol. Those are bad lifestyle to have in your life ohm, so you know I that try repeat that to… this is a positive thing, you are going to feel better, and you are going to a be very drop and gorgeous and loose you know you´re already good looking kid and if you loose 30 pounds, you just going to be gorgeous, you know start to exercise and get enough body you´ll be gorgeous."*

4.3.3. Quantitative methodology: Lifestyle behavior checklist (Parental self-efficacy)

Results from the Lifestyle behavior checklist are illustrated in **Tables 4.6 and 4.7**

Table 4.6 LBC questionnaire total score results

Characteristic	NAFLD n= 4 Female n=3 Male n=1	CONTROL n=8 Female n=7 Male n=1	P value
Problem Scale	82 ± 17	48 ± 17	**0.008**
Confidence Scale	148 ± 92	204 ± 38	0.168

4.3.3.1 Problem scale results

Parents of children with healthy body weights had a significantly lower total problem score than parents of children with NAFLD (48 vs. 82; $p<0.05$). The major differences in problems identified by the parents of children with NAFLD were in relation to challenges related to child eating behaviors (eating too quickly and too much), sedentary physical activity levels (watching too many video games, refusing to participate in non-sedentary activities) and child energy level to participate in these activities.

Correlations of demographic and anthropometric data with problem scale

a) **Age of children:** In the study, there was a group effect between the age of the children and the problem scale items. These findings indicated that in the control group, parents of younger children had identified having more problems related with their children's behavior. There was a trend towards parents of lean children and children with NAFLD to find item 18, as a big problem ($p=0.05$). Significant associations were found with items 17 ($p=0.02$) and 19 ($p=0.003$), indicating that when children in the NAFLD group are older, their parents seem to struggle a lot with this item **(Appendix B, Table B.5).**
b) **Children's BMI:** In the study, it was found that the BMI of children from both groups was related to items such as 8, argues about food; item 9, demands extra helpings at meals; item 17, spends too much time playing video or computer games; and Item 19, refuses to do physical activity **(Appendix B, Table B.3).**

c) **Age of parent:** The influence of parental age on the problem scale items suggests that this is an issue for parents in the NAFLD group. The older the parents in the NAFLD group, the more the behavior becomes a problem for them. Specifically these correlations were, "significantly complains about doing physical activity" and "refuses to do physical activity" (p=0.02, p=0.004 respectively). In terms of children´s food behaviors there were no associations with parental age **(Appendix B, Table B.4).**

d) **Parental BMI:** In terms of the effect of parental BMI on the problem scale behaviors, this was found to affect both groups. BMI was significantly associated with items such as eats too much food (p=0.002); spends too much time playing video or computer games (p=0.04); complains about doing physical activity (p=0.003); and refuses to do physical activity (p=0.02). Also, there was a trend towards the association of parental BMI and their children food behavior, argues about food (p=0.09) **(Appendix B, Table B.2).**

4.3.3.2 Confidence scale results

Parents of children with healthy body weights had higher total confidence scores than parents of children with NAFLD (204 vs. 148). However, these differences were not significant **(Table 4.3).** The major differences were that parents of children with NAFLD had significant higher problem scores and lower confidence scores connected to food-related behaviors (eats too much; p=0.001, argues about food; p=0.006 and demands extra helpings at meals; p=0.02) and physical activity-related behaviors (spends too much time playing video games or computer games; p=0.005, complains about doing physical activity; p=0.001 and refuses to do physical activity; p=0.02) **(Table 4.4).** The parents of children with NAFLD also tended to have higher scores on the problem scale and lower scores on the confidence scale regarding food-related items such as eats too quickly (p=0.09) and yells about food (p=0.08), and weight self-stem behaviors such as complains about being unfit or feeling low in energy (p=0.08) **(Table 4.4).**

Correlations of demographic and anthropometric with confidence scale

a) **Age of children:** The child's age does not seem to influence the confidence of parents in either group in terms of dealing with a child's food-related behaviors. However, parents in the NAFLD group had the lowest levels of confidence when handling behaviors related to physical activity and weight self-stem behaviors. These findings were significant with items 17 (p=0.02) and 19 (p=0.003) and were only a trend with items 18 (p=0.05) and 20 (p=0.05) **(Appendix B, Table B.9).**

b) **Children BMI:** The BMI of children did not affect the confidence of the parents in dealing with their children's behavior problems. However, there were trends towards children's BMI being an issue for parents of children with NAFLD. These trends were found in items such as arguing about food (p=0.06), spending too much time playing video games (p=0.09), complaining about doing and refusing to do physical activity (p=0.05) **(Appendix B, Table B.7).**

c) **Age of Parent:** The age of the parents in both groups did not seem to influence any of their children´s behaviors related to food. However, there were some instances where parental age influenced physical activity. For example, older parents in the NAFLD group tended to have lower confidence scores than younger parents of children in the control group in regards to children spending too much time playing video games (p=0.05). Older parents in the NAFLD group seemed to have significantly lower self-confidence when trying to promote change in their child's physical activity level and the behaviors exhibited by their child. The specific behaviors included items such as complaining about doing physical activity (p=0.02); refusing to do physical activity (p=0.004); and complaining about being unfit or feeling low in energy (p=0.03) **(Appendix B, Table B.8).**

d) **Parental BMI:** Parents with higher BMI seemed to have significantly lower levels of confidence when dealing with their children's food-related behaviors, such as eats too much (p=0.007. This trend was greater among parents in the NAFLD group with higher BMI (p=0.002). In both groups, parental BMI was also found to be associated with item 5, yells about food (p=0.02) and a trend towards the association of higher parental BMI of parents of children with NAFLD and item 1, eats too quickly (p=0.06); and item 8, argues about food

(p=0.09). Parents with high BMI were also less confident in handling physical activity behaviors such as spends too much time playing video games (p=0.04), complains about doing physical activity (p=0.003) and refuses to do physical activity (p=0.02). There was a trend towards parents in the NAFLD group with higher BMI and higher children complaining about being unfit or feeling low in energy (p=0.07) **(Appendix B, Table B.6).**

Table 4.7 Differences among groups by each lifestyle behavior

Lifestyle Behaviors	Problem Scale		Confident Scale		
	NAFLD	**Control**	**NAFLD**	**Control**	**p**
1. Eats too quickly	5.0 ± 2.4	2.5 ± 2.1	5.2 ± 4.4	8.2 ± 1.8	**0.097**
2. Eats too much food	6.0 ± 1.1	2.3 ± 1.4	4.7 ± 4.1	8.2 ± 1.4	**0.001**
3. Eats unhealthy snacks	4.5 ± 0.5	3.6 ± 1.8	5.7 ± 4.0	7.0 ± 1.5	0.386
4. Whines or whines about food	3.7 ± 1.8	2.7 ± 1.9	6.7 ± 4.2	8.2 ± 1.1	0.411
5. Yells about food	3.2 ± 1.7	1.7 ± 1.0	6.7 ± 4.2	9.1 ± 1.3	**0.083**
6. Throws a tantrum about food	2.5 ± 1.0	1.7 ± 1.7	7.2 ± 4.2	8.5 ± 1.5	0.452
7. Refuses to eat certain foods	4.7 ± 0.9	3.3 ± 2.1	6.0 ± 3.9	6.2 ± 1.1	0.267
8. Argues about food (e.g., when you say No more)	4.7 ± 1.2	1.6 ± 0.9	5.5 ± 4.2	8.2 ± 1.3	**0.006**
9. Demands extra helpings at meals	5.0 ± 1.8	2.3 ± 1.5	5.2 ± 4.0	8.1 ± 1.4	**0.023**
10. Requests food continuously between meals	2.5 ± 1.2	1.7 ± 1.3	5.5 ± 4.2	8.7 ± 1.3	0.389
11. Demands food when shopping or on outings	3.7 ± 2.7	2.7 ± 1.6	7.0 ± 4.2	7.7 ± 1.8	0.445
12. Sneaks food when they know they are not supposed to	3.5 ± 2.5	2.5 ± 1.6	5.7 ± 4.9	6.8 ± 2.3	0.415
13. Hides food	1.2 ± 0.5	1.0 ± 0.0	7.7 ± 4.5	8.2 ± 2.1	0.166
14. Steals food (e.g., from other children's lunch boxes)	1.0 ± 0.0	1.1 ± 0.3	7.7 ± 4.5	7.3 ± 2.5	0.505
15. Eat food to comfort themselves when feeling let down or depressed	2.0 ± 1.4	1.5 ±1.0	5.2 ± 3.7	8.0 ± 1.9	0.505
16. Watches too much television	3.5 ± 2.6	3.0 ± 1.7	7.0 ± 3.8	6.6 ± 1.3	0.702
17. Spends too much time playing video or computer games	5.2 ± 2.3	2.0 ± 0.9	4.7 ± 3.7	7.8 ± 1.8	**0.005**
18. Complains about doing physical activity (e.g., This is boring, I'm too tired, My leg hurts)	5.5 ± 1.7	1.2 ± 0.7	4.5 ± 4.3	9.0 ± 1.4	**0.001**
19. Refuses to do physical activity	3.7 ± 2.6	1.2 ± 0.4	4.5 ± 4.3	8.6 ± 1.3	**0.020**
20. Complains about being unfit or feeling low in energy	3.0 ± 2.8	1.1 ± 0.3	4.2 ± 4.2	9.0 ± 1.4	0.080
21. Complains about being overweight	1.7 ± 0.9	1.6 ± 0.7	4.5 ± 4.3	8.8 ± 1.8	0.807
22. Complains about being teased	2.5 ± 1.7	1.3 ± 0.7	6.7 ± 4.2	8.1 ± 2.9	0.136
23. Complains about not having enough friends	1.0 ± 0.0	1.5 ± 1.0	6.7 ± 4.2	8.0 ± 3.1	0.328
24. Complains about being unattractive	1.0 ± 0.0	1.1 ± 0.3	6.5 ± 4.3	8.3 ± 3.0	0.505
25. Complains about not fitting into clothes	1.5 ± 0.5	1.1 ± 0.3	6.5 ± 4.3	8.7 ± 2.0	0.187

4.3.3.3 Interrelationships between parental concern/self-efficacy and barriers and facilitators that parents perceive as affecting their ability to integrate lifestyle changes within their children

a) **Control group:** These parents showed higher levels of self-confidence when dealing with their children´s food, physical activity and image-self-stem behaviors **(Table 4.5).** This translated into the parent having an increased number of strategies to include their child in terms of developing a home environment that promoted healthy eating. For example, during the focus group, these parents gave more examples of facilitators (including their child in meal preparation, meal choices, acting as a role model for healthy eating behaviors) and fewer barriers (such as time constraints) than parents of children with NAFLD. These parents were characterized as being younger than the parents of children with NAFLD and having body weights within healthy reference ranges. Only one parent in this group was overweight and scored higher in the problem scale and lower in the confidence scale than the other parents in this group, but overall this parent experienced the same facilitators and barriers as the rest of the lean group. In addition, overall there was no different between comments from parents in the control group who were in the FRAGILE study and the parents who were not in the study. Therefore, it seems that similar experiences regarding barriers and facilitators of promoting healthy lifestyle behaviors were similar amongst the parents who did and did not participate in the FRAGILE Study.

Table 4.8 Focus group and LBC comparisons (parents of children with healthy body weights)

Focus group results		**LBC results**
Facilitators	**Barriers**	**Behaviors of child related to food intake**
Role modeling healthy eating behaviors Encouraging child to try new foods Family involvement in food preparation Provide healthy foods Portion control Monitoring t**r**eats Family environment Talking about nutrition	Junk food exposure High cost of healthy foods Food preferences Lack of time	All parents of healthy weight children: **(Problem score = 2.1, Confidence score = 8.4)** (Eats too quickly, eats too much food, yells about food, refuses to eat certain foods and demands extra helpings at meals) [a] **(Problem score = 3.4, Confidence score = 7.4)**
Facilitators	**Barriers**	**Behaviors of child related to physical activity**
Did not mention facilitators or barriers related to physical activity behaviors		All parents of healthy weight children: **(Problem score = 1.5, Confidence score = 8.5)** (Spends too much time playing video or computer games, complains about doing physical activity, and refuses to do physical activity)
Facilitators	**Barriers**	**Behaviors of child related to self image**
Did not mention facilitators or barriers related to children´s image self-stem behaviors		All parents of healthy weight children: **(Problem score = 1.1, Confidence score = 9)** Complains about being unfit or feeling low in en**e**rgy
Demographic and anthropometric characteristics		
These parents were characterized as being younger than the parents of children with NAFLD and being normal weight. [a] One parent was overweight		

b) Parents of children with NAFLD

All parents of children with NAFLD used food negotiation as a strategy to influence their children to eat healthy, and all had similar barriers such as children´s food preferences, lack of time to prepare food, and high cost of food. Two parents in this group used other strategies as facilitators. These included reducing food servings, meal preplanning, and providing healthy foods at home. They had much greater self-confidence in dealing with their children´s food behaviors (problem score = 4, confidence score = 8.5) **Table 4.6.** These same parents also encouraged physical activity behaviors by being role models for their children; similarly, in the LBC for these parents, their children´s physical activity behaviors did not represent a big problem and the parents were confident enough to deal with the behaviors (problem score = 3, confidence score = 9) **Table 4.6.** The other two parents that did not use role modeling as a facilitator mentioned that this was mainly due to the lack of time for exercise. Similarly, in the LBC questionnaire these same parents seemed to perceive the physical activity behaviors as big problems (problem scale mean score = 6.6) and were not confident enough to deal with them (confident scale mean score = 1.3) **Table 4.6**

Table 4.9 Focus group and LBC comparisons (parents of children with NAFLD)

Focus group results		LBC results
Facilitators	**Barriers**	**Behaviors of child related to food intake**
Food Negotiation All parents of children with NAFLD [a] Two parents of children with NAFLD used other strategies: **Food servings** **Meal preplanning** **Healthy foods at home**	All parents of children with NAFLD: **Food preferences** **Lack of time** **Food preparation** **High cost of healthy foods** [c] **Cravings**	All parents of children with NAFLD: **(Problem score = 4.8, Confidence score = 5.5)** [a] Parents of children with NAFLD with more confidence in dealing with their children´s food behaviors **(Problem score = 4, Confidence score = 8.5)** [b] Parents of children with NAFLD with more confidence in dealing with their children´s food behaviors **(Problem score = 5.5, Confidence score = 2.5)** (Eats too quickly, eats too much food, yells about food, refuses to eat certain foods and demands extra helpings at meals)
Facilitators	**Barriers**	**Behaviors of child related to physical activity**
[a] **Role model**	[b] **Lack of time/role model**	All parents of children with NAFLD: **(Problem score = 4.8, Confidence score = 4.5)** [a] Parents of children with NAFLD with more confidence in children´s physical activity behaviors. **(Problem score = 3, Confidence score = 9)** [b] Parents of children with NAFLD with less confidence in children´s physical activity behaviors. **(Problem score = 6.6, Confidence score = 1.3)** (Spends too much time playing video or computer games, complains about doing physical activity, and refuses to do physical activity)
Facilitators	**Barriers**	**Behaviors of child related to self image**
[c] **Motivation**	[b] **Lack of motivation**	[c] One parent in the NAFLD group perceived this item as a big problem and did not feeling enough confidence to deal with it: Complains about being unfit or feeling low in energy

[a] These parents were characterized as being younger than the other parents in the NAFLD group but with higher BMI in the entire cohort.
[b] Parents were characterized as being older than the control group and the rest of the parents in the NAFLD group.
[c] This parent was normal weight.

4.4 Discussion

The increasing worldwide prevalence of childhood obesity has induced a rise in related comorbidities such as nonalcoholic fatty liver disease (NAFLD). The optimal treatment for childhood NAFLD is unclear. Current clinical guidelines recommend promoting gradual weight loss though lifestyle modification in children (Chalasani et al., 2012; Nobili et al., 2008; Nobili et al., 2008; Reinehr, Schmidt, Toschke, & Andler, 2009; C. L. Wang et al., 2008; C. L. Wang et al., 2008). However, it is well known that carrying out dietary and physical activity changes is not easy and can be quite challenging for both the child and the whole family. Parents and/or caregivers play an important role in promoting changes in a child's dietary intake (Guilfoyle SM et al, 2010). Therefore, it is important to explore the experience of parents of children with NAFLD when helping their children to follow a healthy diet and to study how it may differ from that of parents and caregivers of children with healthy body weights. Examining these issues may provide important insights into developing effective diet and lifestyle interventions for children with NAFLD. The purpose of this study was to describe and explore the attitudes and beliefs of parents and caregivers of children with NAFLD and lean healthy children regarding the barriers and facilitators that influence the parent's and child's ability to sustain and incorporate lifestyle recommendations provided during nutrition counseling about healthy eating and physical activity.

Both groups in this study have received children´s age-appropriate nutritional information in the past from pediatricians and nurses during check-up visits. It seems that contact with dieticians has not occurred that frequently. Some participants in both groups were enrolled in the FRAGILE study, which consisted of nutrition education regarding a low GI/GL/fructose diet for 6 months. In the FRAGILE study, parents and children received sample menus, recipes, snack ideas and instructions on how to decrease GI/GL/fructose. Many parents of children with NAFLD commented that they found the information about the FRAGILE study very helpful and easy to follow. However, it is possible that these parents felt more confident and involved during the first three months of the

study, but their low self-confidence regarding barriers affected their ability to help their children to sustain the dietary changes throughout the rest of the study. Participants in the control group did not share any comments regarding the FRAGILE study during the focus group even though some of them participated in this study. Parents whose children participated in the FRAGILE study and those whose children did not, identified the same barriers to and facilitators for promoting lifestyle change in their children.

Parents in both groups agreed that the information they received during regularly scheduled health visits did not satisfy their needs and concerns because of a lack of information about practical nutritional recommendations, which consequently leads to non-adherence. These finding are similar to perceptions assessed in parents of children with cancer; studies have showed that parents in that cohort expressed a need for easier nutrition concepts (Montgomery et al., 2013). Concepts such as weight status of children are not always well translatable between health professionals and families, leading to parents underestimating their children's weight status (Chiang, 2009; Jansen, 2006). For example, in an interview study with parents of overweight children, the parents received nutrition information from pediatricians but they didn't understand such concepts as weight percentiles (Garret-Wright, 2012). It is possible that other nutrition concepts are confusing for parents.

In this study, both groups of parents would like health professional teams to be more involved. They would like more access to dietitians and more practical tips. In addition, parents expressed a strong desire for the health care professionals to utilize motivational techniques to promote their ability to initiate and sustain lifestyle changes in their children. Parents of children with NAFLD made it clear that they do not like to be judged. They expressed concerns that this had occurred previously. Similarly, parents of overweight or obese children perceive that health professionals often demonstrate a lack of compassion and/or support to the parents and families with overweight and obese children (Holt et al., 2008). Other studies with parents of overweight children and adolescents commented that their experiences with health professionals range from positive to

negative, because parents perceive that some health care professionals blame mothers or show a lack of interest in parental concerns, while other health professionals have been shown to be very supportive (Edmunds, 2005; Turner, Salisbury, & Shield, 2011). In addition, Campbell and colleagues have proposed that using motivational interviewing can enhance weight management programs in children because it increases the confidence of both parents and children about making lifestyle changes (Campbell et al., 2011).

Access to health information on the Internet is increasing. The Internet offers parents the opportunity to search more quickly and efficiently for the information that they really want. However, it requires specific skills such as navigation and objective evaluation of the information (Cline & Haynes, 2001). The parents in this study stated that the information on the web is not always understandable and reliable; therefore, trusted online resources are needed. Similarly one qualitative study with adolescents found that the Internet is an easy way to find nutrition information. However, the extensive amount of information can lead people to quit a search (Larsen & Martey, 2011).

It is possible that a lack of motivation, practical recommendations or the contradictory and complicated information from health professionals or the Internet consequently leads to non-adherence. For example, in terms of motivation it is known that adults and children with NAFLD had lower scores in the quality-of-life category as compared to their healthy counterparts (David et al, 2009; Kistler et al, 2010). However, in a recent study examining quality of life of children with-and-without NASH found that quality of life did not change in the children after six months of weight loss counseling. (Kerkar et al., 2013). This study also addressed that children with NASH had a greater score of depression and lower body self-esteem than obese children without NASH (Kerkar et al., 2013). Therefore, targeting motivational techniques that address the concerns of both the child and parent could induce children with NAFLD to adhere to lifestyle modification strategies. However, the efficacy of this approach may really depend on the individual parent and child, as other studies have found that people merely ignore the information provided by health care professionals because they

want to keep enjoying the food they like to eat. In fact, studies have found that when parents like the taste of a certain fruit or vegetable, it is more likely that their children will eat that food (Jeyanthi and Ziebland, 2002). People already know what they should be eating, but a lack of motivation also seems to be a major problem (Goode et al., 1996). For example, in one study, low-income mothers expressed awareness of healthy eating recommendations for their children, and acknowledged the consequences of not following a healthy diet, but external barriers such as family low income influenced their decisions about following nutritional recommendations (L. K. Kelly & Patterson, 2006).

It seems that children with fatty liver disease are also aware of the healthy lifestyles they should be embracing, as this was demonstrated in a study that compared attitudes about healthy eating and exercise patterns among children and adolescents with NASH. Some of the children were obese, and others were lean (Hattar, Wilson, Tabotabo, Smith, & Abrams, 2011). In that study, surprisingly 55% (sometimes) and 35% (always) of children with NASH affirmed to read food labels and 80% of those children thought that sometimes they were consuming healthy foods. However, children with NASH reported the lowest consumption of fruits and milk products, and higher sedentary patterns when compared to the obese and lean controls. These authors also commented that 95% of the children with NASH in their cohort were trying to lose weight at the time of study (Hattar, Wilson, Tabotabo, Smith, & Abrams, 2011). Therefore, it could be that the children with NAFLD were receiving nutritional education at that time, but still not following the recommendations. However, this study did not address whether the families of these children were influencing the children's food choices, nor did it address how nutrition education influenced the behaviors of the parents and the families. Therefore, exploring the role of parents of children with NAFLD during lifestyle modification programs in children would be important for future development of interventions in children with NAFLD.

In this study, parents cited several barriers that influenced their ability to incorporate healthy lifestyle patterns in their children. These included lack of time, high cost of healthy foods, and their children´s specific food preferences.

These findings are similar to previous focus group studies. For example, low-income parents of healthy children shared their barriers to preparing healthy foods at home. These barriers included their children´s food preferences, highly and inexpensive access to fast food, and time constraints (L. K. Kelly & Patterson, 2006). However, in this study parents in the control group seemed to be more confident about handling these barriers by trying to be more involved in food preparation, pre-planning of meals, and including the child in all phases of these processes. Even when the control group cited a lack of time as a barrier influencing the ability to include these factors into their daily activities, they still strongly emphasized the importance of food preparation. These parents place a high value on having good food and available healthy food at home all the time. Parents in the control group also seemed to be concerned about exposing their children to junk food advertisements. However, they try to manage this problem by talking about nutrition with their children, and giving their children ideas about how to control food portions, especially in terms of treats. Encouraging parents to introduce positive patterns, such as having conversations with children at an early age about healthy foods, is associated with increases in intake of healthy foods among children (Lerner and Parlakian, 2006). In this study, children in the control group were younger than the children in the NAFLD group. This age disparity could have influenced the parents' ability to successfully elicit behavioral change with regard to dietary intake. The children's age may have been one of the reasons that parents of children with NAFLD demonstrated lower confidence scores than parents of lean children; developmentally, adolescents would be looking to their peers, rather than their parents, as the main role models for eliciting change (Rhee, 2005).

In contrast, for parents of children with NAFLD lack of time (No time to make healthy changes or meet dietitians seems to be a big problem. Moreover, their children seem to prefer and demand more dense energy and starchy foods and do not like fruits and vegetables. Some ways that parents of children with NAFLD try to help their children is by negotiating food portions, treats and preferred foods. Food negotiation has been identified as a strategy among parents

with different backgrounds such as low-income mothers (L. K. Kelly & Patterson, 2006), parents of kindergarten children (DuBois, 2010), and parents of obese children and adolescents (Uzark, Becker, Dielmen, Rocchini, & Katch, 1988). However this strategy is associated with increases in weight, because with this strategy, parents are not always able to establish limits and rules about food rewards (Cook, 2009). In this study, parents increasingly used food negotiation, as their children grew older. Similarly, the children and parents in the case group were older than those in the control group, and parents of children with NAFLD affirmed frequently used food negotiation. This means that potentially older parents have less confidence to handle lifestyle behaviors, (especially when their children are adolescents and the easiest way to deal with the conflict is with rewards rather than provide healthy ideas, compromise in terms of food preparation or motivate their adolescents). However, studies show that this strategy is not the best option (Ehmke, 2012; Cook, 2009). Evidence has shown that children of mothers who were more permissive regarding junk food were at greater risk of becoming obese (as assessed with child´s waist circumference) (Ehmke, 2012). In contrast, parents who embrace an authoritarian parenting style (similar to the control group in this study) do not use negotiation as a primary strategy, but rather focus on monitoring what their children eat (Cook, 2009). Cook also implies that certain phrases favored by some mothers of obese children such as "clean your plate, then you can have dessert," must not be used, because this will only increase a child's desire for a treat. When food negotiation does not work, parents of children with NAFLD use other strategies, such as controlling serving sizes.

There was only one parent in the NAFLD group that was actively trying to be a role model for his son in terms of promoting physical activity. However, the rest of the parents differed about this. One possible explanation is that the parents who affirmed that they were not going to be role models in terms of exercise were older parents. This finding was also confirmed through the LBC questionnaire, which showed that older parents have less confidence when handling their children's physical activity behavior problems.

Parental lack of time also emerged as a big problem for parents of overweight children, in terms of not providing a family environment that supports the family eating together at meal-time (Goode et al., 1996). Providing a family environment has been suggested as a good opportunity to influence children to develop healthy eating behaviors (among other things, it increases the intake of fruits and vegetables), enhance communication skills, and improve family functioning (Hamilton, 2009). This finding is consistent with the answers among the control group. It is important to highlight that children of parents in the control group were younger and perhaps this facilitates parental role modeling of healthy patterns. This has been addressed previously in a longitudinal study. In this study, health behaviors and parenting styles of parents of school-aged children and adolescents were assessed and consistently found to have a greater influence of parents among younger children (Lohaus, Vierhaus, & Ball, 2009). Not providing a family-based meal environment could make it more difficult for parents to be role models. Similarly to the present focus group study, parents of overweight children also struggle to have a positive influence on their children, in terms of physical activity and nutrition behavior (Holt et al., 2008).

4.5 Strengths and limitations

The present study has some limitations. It was a challenge to recruit parents of children with NAFLD, because they expressed that they did not have time to participate or that they had already had to make time for multiple clinic visits. This reflects the common challenges that many weight-management treatment centers face, where patient non-return rates exceed 50% (Kitscha et al 2009). All of the parents in the case group represent parent whose children were in the FRAGILE study (Chapter 3). Hence, perceptions and factors that influence the efficacy of dietary interventions may not translate to the experiences of other families with children with NAFLD. In addition, many of the parents of the children with healthy body weights were also enrolled in the FRAGILE study. Despite this, the theoretical saturation of the main themes was reached in all major concepts within this study in both groups. In addition, parents whose

children participated in the FRAGILE study and those whose children did not identified the same barriers to and facilitators for promoting lifestyle change in their children. This suggests that these perceptions and attitudes are consistent with those of the general population.

In the study it was not possible to assess the weight and height of the participant's children on the day that the focus group was held. However, many of the children in both groups were also participating in the FRAGILE study, meaning that it was possible to compare the anthropometrics characteristics of both parents and children and the study outcomes. In the study, no information was collected regarding the participants' education level, household income, or dietary intake. Therefore it is not possible to determine the extent to which lower incomes/education or parental eating patterns may influence a child's eating patterns or food choices. Finally it is not possible to assess the parenting styles (e.g., permissive vs. authoritarian) of the participants in the study and how this might be a factor influencing study findings, because we did not include a validated tool to assess this characteristic within the study design. In the future, we could include a validated research tool that assesses a parenting style.

Some of the strengths of this study are that it was possible to have a control group. This provides the opportunity to compare the experiences of parents of children with NAFLD with the experiences of parents of lean children. In addition, we were able to explore some of the important factors (such as parental self-efficacy) and how this may influence a parent's ability to support a child in lifestyle changes. This is important since there is substantial evidence that parental influences for promoting long-term changes in children occur within the first decade of life (S. Scaglioni, Salvioni, & Galimberti, 2008). Hence, understanding the associated barriers and facilitators from the parent's perspective is important. This study also combined a mix method approach (qualitative and quantitative) that allowed a more in-depth exploration of parental experience about their children lifestyle modification.

This study's findings add highly relevant information to the current literature about parents' perceptions regarding nutrition, particularly the

perceptions of parents of children with NAFLD. Specifically, this is the first time that a study has explored the perception of nutritional education of parents of children with NAFLD and addressed the barriers and facilitators that these parents face when helping their children to follow a healthy diet.

4.6 Conclusion and clinical implications

In conclusion, the findings of this pilot study suggest that parents of children with NAFLD seem to face more barriers when encouraging their children to follow a healthy lifestyle and feel less confident about handling their children´s behavioral problems. This appears to contribute to low self-confidence, making it a challenge for these parents to role model healthy lifestyle behaviors for their children. The findings also suggest that nutritional programs and interventions should focus on strategies to increase parental self-efficacy to promote healthy patterns in their communication skills and motivation techniques. These results will help health professionals who treat overweight children, and specifically those who work with families of overweight children with NAFLD**.** Additional research to examine these factors in childcare and school settings is also warranted. Understanding the factors that influence a parents ability to promote and sustain lifestyle change in a child is critical to ensure the development of effective treatment strategies for the children and adolescents with NAFLD.

Chapter 5. Integration chapter

5.1. Summary and main of findings

Currently guidelines for lifestyle management and treatment of childhood NAFLD recommend a gradual weight loss through lifestyle modification (diet and exercise) to improve a chld's quality of life and reduce the risk of cardiovascular and liver morbidity and mortality (Chalasani et al., 2012; Nobili et al., 2008; Reinehr, Schmidt, Toschke, & Andler, 2009; C. L. Wang et al., 2008). However, these recommendations do not include any specific recommendations on how this should be achieved; particularly in the child with NAFLD. Few studies have examined the effectiveness of specific dietary strategies or whether or not dietary modification in the absence of weight loss can promote body composition changes or improvements in cardio metabolic risk factors or liver function in childhood NAFLD. Therefore it is currently unknown what specific dietary regimen may promote significant improvement in disease expression in childhood NAFLD.

The diets of children with NAFLD has been characterized with higher intakes of simple sugars (GI/GL/fructose) and saturated fat (Mager et al 2010, Jin et al 2012), which are associated with increasing indices of dyslipidemia, inflammation, insulin resistance and altered lipoprotein expression (Nobili & Manco, 2007). In addition, high intakes of fructose have been associated with an up regulation of de novo lipogenesis in animal models (Chong, Fielding, & Frayn, 2007a; Crescenzo et al., 2012). All these dietary factors are thought to be associated with an increased risk for a fatty liver (Zelber-Sagi et al., 2007). Low GI/GL diets are used in weight management in children and effectively reduce hyperinsulinemia and insulin resistance; all factors known to be associated with risk for NAFLD (Schwimmer et al., 2003). However, little is known regarding the efficacy of this approach to promote weight loss and/or altered liver function in children with NAFLD. This suggests that dietary intervention strategies that focus on reductions in GI and GL, along with fructose, may be potentially beneficial in the treatment of childhood NAFLD.

However, lifestyle changes are not easy to achieve and the role of the family in their children dietary modifications seems to be a fundamental key in

children´s adherence to any forms of lifestyle changes (Guilfoyle SM et al, 2010). Therefore it is not only important to assess the effect of specific dietary treatments for childhood NAFLD. It is also important to explore what is the experience of parents of children with NAFLD and their child when helping their children to follow a healthy diet and to study how this may differ from the parents and caregivers of children with healthy body weights. Examining these issues may provide important insight into developing effective diet and lifestyle interventions for children with NAFLD.

5.1.1 Dietary intervention

The key findings in the Fragile Study **(Chapter 3)** are associated with significant reductions in cardio-metabolic risk factors such as systolic blood pressure, body fat percentage, changes in body fat distribution (particularly in those related to subcutaneous adiposity) in children and adolescents with NAFLD. These all occurred in the absence of weight loss, a key feature of this study. In addition, all children experienced significant reductions in plasma levels of non-esterified free fatty acids, ApoB-100, HOMA-IR and insulin levels; while many children normalized their plasma levels of ALT, AST, GGT and triglycerides values. These changes occurred predominantly in the first three months of the study when the most significant reductions in dietary GI/GL and fructose were observed. Interestingly, although lean healthy children also experienced similar reductions in dietary intake of GI/GL and fructose, this had no major impact on these biochemical variables or on body composition. **(Table 3.9 in Chapter 3).**

Reduction of GI/GL and fructose were achieved in the study. Children reduced their intakes of baked goods, candy, sugar and ice cream and the intake frequency of sweetened beverages, and increased their intake of fruits and vegetables. All of this occurred without any significant changes in fiber intake. Reductions in total fructose intake were achieved with reductions in HFCS foods (sweetened beverages, processed foods) rather than from naturally occurring food sources such as fruits and vegetables. Changes in HCFS were the most strongly associated with the reductions in TG, insulin, HOMA-IR, ALT, ApoB-100 and

the changes in body composition (subcutaneous central adiposity) observed in the children with NAFLD. All of these changes (metabolic, anthropometric and dietary) occurred in the absence of changes of physical activity. All of this suggests that reductions in HCFS are an important strategy to reduce cardio metabolic risk in overweight and obese children of NAFLD.

Lean children consumed similar intakes of commercially added fructose (e.g. sweetened beverages and bakery products) but lower intake of HFCS when compared with children with NAFLD. However, healthy weight children did not experience significant changes to the VAT, body fat percentage and/or their metabolic parameters studied over the entire intervention. This suggests that there is a threshold for the level of adiposity and body composition that may influence the ability of the body to adapt to a higher fructose intake before any derangements in cardio-metabolic risk factors occur. Therefore, changes in body fat distribution and body fat percentage in response to dietary manipulation (i.e. low fructose/GI/GL intake) may be more important than the actual weight loss to reverse or delay progression of NAFLD in children. However, the controversy between BMI, waist circumference, body fat distribution, and body fat percentage as predictors of decreased risk of cardio- metabolic risk factors still are under discussion. For example, some studies have suggested that overall body fat distribution improvements in obese children is a better predictor of decreased risk of cardio-metabolic disorders (Daniels, Morrison, Sprecher, Khoury, & Kimball, 1999); while others have found that changes in BMI among overweight girls and boys are important (Lawlor et al., 2010). More randomized studies that address the effect of diet on body composition and metabolic markers in children with NAFLD are needed to examine these factors.

Possible dietary fructose associated mechanisms with cardio-metabolic risk factors reductions in children with NAFLD include reductions in the synthesis of hepatic triglycerides via de-novo lipogenesis and concurrently improve hepatic insulin signaling (K. L. Stanhope et al., 2011). In animal models fructose is associated with increase activity of de novo lipogenesis mediators such as carbohydrate response element binding protein (CHREBP), and sterol

regulatory element binding protein 1c (SREBP-1c) and lipogenic genes fatty acid synthase (FAS), stearoyl coenzyme –A desaturase-1 and acetyl co-A carbocxilase (ACC) (Lim, Mietus-Snyder, Valente, Schwarz, & Lustig, 2010; Nomura & Yamanouchi, 2012). Preliminary studies in humans have shown that really high fructose sweetened beverages (15 to 25% of energy from fructose) given to men and women for 10 weeks not only up regulates hepatic de novo lipogenesis, but also promotes IR, dyslipidemia, and increased visceral adiposity (K. Stanhope, 2008).

In terms of HFCS consumption, is proposed that HFCS could lead to greater up regulation of de novo lipogenesis and consequently lead to a worst metabolic dysfunction (K. L. Stanhope, 2012). This was demonstrated after administration of 12-15% concentration of HFCS drinks to rats for 10 weeks that led to greater altered lipid profiles, weight gain and liver dysfunction (Figlewicz et al., 2009). HFCS is also suggested to have greater negative cardio metabolic factors in children (Perez-Pozo et al., 2010), greater synthesis of glycated metabolites and oxidative stress associated with liver damage (Assy et al., 2008), greater up regulation of phosphofructokinase, which consequently induces ATP depletion and inflammation in the liver (Ouyang et al., 2008).

All this suggests that higher intakes of foods that have commercially added sources of fructose as a sweetener , could have a more negative impact on the metabolism of obese children than in lean children. However, the extent to which HFCS may induce hepatic steatosis is still a mater of debate. A recent study has shown that consumption of both sucrose and high fructose corn syrup (between 8-30% of total energy intake) may not increase liver fat or ectopic fat muscle fat deposition in overweight and obese adults (Bravo et al 2013). More research to evaluate the interrelationships between commercially added fructose (in the form of HFCS) to total fructose intake in children and adults with NAFLD is clearly warranted.

In conclusion, the dietary intervention (low glycemic index/glycemic load/fructose) promoted significant reductions in cardio-metabolic risk factors in children with NAFLD in the absence of a weight loss. These changes could be

related to reductions of the synthesis of hepatic triglycerides or improved triglyceride clearance associated with reductions of HFCS. In order to verify these hypothesis more randomized control trials in childhood NAFLD are needed. Specifically it would be interesting to assess the effect of different doses of HFCS in the fasted and postprandial state or in the long-term among healthy weight, obese (non-NAFLD) and NAFLD children. However, these types of studies can be challenging to perform, and often require invasive methodologies to assess the impact on the liver.

5.1.2 Exploratory Study: Parental Perceptions regarding Nutrition Education, Barriers and Facilitators to promoting lifestyle changes in the child.

Both groups in this study have received prior nutrition education about children´s age appropriate nutritional information. In addition, many children's families (NAFLD and lean) also participated in the FRAGILE study (NAFLD n=4, Lean n=2). One major barrier that parents cited regarding the prior nutrition education they received was related to the perceived lack of practical translation of recommended lifestyle changes into their daily routines and the lack of available routine follow up with knowledgeable health care professionals who understand and know about childhood nutrition This was cited as one of the major reasons for non-adherence to prior nutrition education by health care professionals This finding has previously reported in studies with parents of overweight children (Montgomery et al., 2013). Therefore parents would like a greater involvement of the health professional team, more access to dietitians, practical nutrition tips and motivation techniques that has been suggested to increase the confident of the whole family to make changes (Campbell et al., 2011). The increasing access to health information through the Internet gives opportunity to these parents to search in a faster way the information that they really want. However the information is not always understandable and reliable by the parent and family. Access to credible nutrition information on the Internet, was hence an important request for families and are givers to promote lifestyle change within their child.

The barriers that influenced parents of children with NAFLD and the parents of children with healthy body weights' ability to incorporate healthy lifestyle patterns among their children cited included lack of time, high cost of healthy foods and their children´s specific food preferences. These findings are comparable to other studies with low-income mothers (L. K. Kelly & Patterson, 2006). However, parents in the control group seemed to be more confident in handling these barriers by incorporating daily facilitators such as family involvement in all phases of food preparation and acting as role models by incorporating this into their own daily routines. For parents in the control group lack of time was a big barrier. However, having good food and available healthy food at home all the time was perceived as highly valuable, particularly as they recognized that their children could be exposed to inducements to eat 'junk' food through the media and school environments. Therefore, they try to manage this problem by talking about nutrition with their children, giving their children ideas on how to control food portions and encouraging them to try new healthy foods. In overall these parents promote a family meal environments that include participatory actions in meal preparation, meal selection and role modeling; all factors known to increase healthy eating behaviors among children, enhance communication skills and improve family functioning (Lerner and Parlakian, 2006; Hamilton, 2009).

In contrast, for parents of children with NAFLD lack of time influences their ability of food preparation and their children seem to prefer unhealthy foods rather than fruits and vegetables. Similarly, parents of overweight children have reported that lack of time influences their availability to role model healthy eating behaviors for their children (Goode et al., 1996). Therefore, one strategy used by these parents is negotiation of food portions and treats. Low-income mothers and parents of obese children and adolescents also use this strategy (Uzark, Becker, Dielmen, Rocchini, & Katch, 1988). When food negotiation does not work, these parents use other strategies such as control serving sizes.

Lack of time also influences the opportunity of being role model in terms of physical activity behaviors for parents of children with NAFLD. Similarly to

this study, the parents of overweight children also struggle to influence in positive ways their children, in terms of physical activity and nutrition behavior (Holt et al., 2008). Older parents in this group seemed to struggle more than younger parents to role model physical activity behaviors and therefore have less confidence when handling physical activity behavior problems of their children.

We speculate that younger parents efficiently handle physical activity behavior problems of their children because they may feel with more energy and motivation to influence their children. In contrast, on average "older parents" often have children at different stages of development and would be more likely to be the parent of adolescent children. Given that the literature indicates that adolescence is a time to look to peers for role models (rather than the parent), the issue is probably more likely to be related to this (Gebremariam, M.K et al., 2013).

In conclusion, this exploratory study suggest that families with NAFLD could be more prone to non-adherence in the last 3 months of the Fragile Study because these families showed low levels of self-efficacy to role model and sustain healthy lifestyle behaviors. Future research, focused on incorporating positive parenting styles into nutrition interventions for children with NAFLD, would facilitate the evaluation of the factors that influence effective lifestyle modification in children and their families with NAFLD and potentially contribute to the development of more effective treatment strategies for the child with NAFLD.

5.2 Clinical Implications and Future Research

Overall the findings of this study demonstrate that a diet that combines a low GI/GL/fructose approach could be a good dietary strategy for children with NAFLD. Specifically teaching families of children with NAFLD on how to reduce HFCS food sources from their diet was associated with important clinical cardio-metabolic improvements and liver function. Although dietary changes were predominantly achieved in the first three months, the overall metabolic improvements were observed as well at six months.

One possible explanation of greater dietary changes during the first three months in this study could be because families were highly motivated at the beginning of the study. Particularly, parents are a key part of childhood lifestyle modifications because they facilitate food options, access to physical activity and role model lifestyle behaviors and support (Campbell, 2011). In addition, it has been seen previously that parents of overweight children are highly worried about their children´s weight (Lampard, 2008). Therefore, possibly parents of children with NAFLD were more involved at the beginning of the dietary intervention because they were concerned about their child´s disease. However, even when these parents were highly concerned about their children health problem, issues related to decreased parental self-efficacy were reported. For example, the strategies that parents of children with NAFLD used to influence their children to eat healthy such as food negotiation and restricting food portion sizes are related with parenting feeding practices of restriction and pressure to eat, which are known to be associated with obesogenic behaviors (Blisset, 2012). Therefore dietary interventions could also include teaching positive parenting skills in order to increase adherence to the dietary treatment beyond the time their families are participating in it. For example a twelve month weight program with obese children combined lifestyle education and parenting skills and achieved a reduction of 10% BMI z score of children at the end of the study (Golley, 2007). In addition, Sanders and colleagues showed that when parents receive positive parental skills they increase their capacity to be self-efficacy to encourage healthy eating among their family (Sanders, 2008).

It could be also possible that age and BMI of parents could affect their confidence to handle their children behavior. For example in the study, age of both parents and children seems to affect their ability to make lifestyle changes. Parents in the NAFLD group were older than the control group and the majority of children with NAFLD were in adolescent age, so perhaps it is even more difficult for these parents to be role models and influence their children. So perhaps programs that address peer counseling among children with NAFLD could be more effective as proposed by a weight reduction short-term study (18

weeks) where older children in a school advice younger children and resulted in 5% reduction of weight (Foster, Wadden, & Brownell, 1985). But still parents are a big part in the whole scenario; so maybe showing them how to motivate their children in a non-directive way could also be helpful. Another possibility could be targeting on dietary and physical activity changes among parents while their children are in the study as it was suggested after 3 year follow up among families of school age obese children and showed that when parents reduced BMI <25kg/m^2 their children significantly reduce more weight and metabolic markers than the control group (Kanda & Kawaguchi, 2004).

A particular barrier for parents of children with NAFLD was related to food preparation. These parents stated that they do not have time to prepare healthy foods all the time. In the FRAGILE study participants received several nutrition tools such as menus, recipes and snack ideas and specific information related to dietary GI and sources of both naturally occurring and commercially added fructose, which these parents affirmed were helpful for them. However it might be important to not only to provide dietary tools but also self management tools to promote changes in both parental and child/adolescent lifestyles. For example, showing parents and children how to set their own goals, suggestions on how to organize their schedules and overall increase their empowerment related the dietary changes could also be helpful. This aspect is especially important because children with NASH have shown to have greater negative attitudes towards following healthy dietary and physical activity changes than obese children without NASH (Hattar, Wilson, Tabotabo, Smith, & Abrams, 2011). More research about teaching parenting skills and children empowerment regarding their dietary treatment should be assessed. Specially target the barriers of parents and improve positive facilitators.

5.3 Final conclusion and clinical implications

In conclusion the findings of this study suggest that reducing GI/GL/ fructose from the diet of children with NAFLD are associated with improvements of the metabolic dysregulation that characterized NAFLD, regardless of dramatic changes in weight. These findings open the possibility to clinicians and dietitians

to set more realistic goals (e.g. weight changes, specific dietary changes that the family could not feel ready to perform) and to assess during check up visits the readiness and confidence of the families of children with NAFLD regarding their lifestyle changes. Specifically parents of children with NAFLD played an important part of the FRAGILE study. Therefore the exploratory findings about these parent´s perception of nutrition education and their barriers and facilitators about their children lifestyle behaviors are important for future dietary interventions and should be addressed as part of the existing clinical guidelines.

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Appendix A. Fragile Study

U N I V E R S I T Y O F A L B E R T A
Form A-1 Assent form (Healthy children and Adolescents)

Title of Project: Impact of lifestyle intervention in Non Alcoholic Fatty Liver Disease (NAFLD) in children.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687

Co-Investigators: Dr. Susan M. Gilmour, MD Telephone: 780-248-5409
Dr. Jason Yap, MD Telephone: 780-248-5420

We would like you to participate in a research study that will help us understand what the best type of diet and what level of physical activity would be good for children with liver disease.

What will you have to do?:
If you and your parents agree that it is okay to take part in this study, we will ask you to come to clinic three times (First visit, 3, 6 and 12 months) after you start this study.
When you come to The Clinical Research Unit, CRU we will ask you to:
1) We will measure your weigh, height, waist and some arm measurements. We will measure your waist by putting a tape measure around your waist and take a measurement of your skin from the back of your arm, thigh, and calf and behind the back and on the side of the waist with a caliper. Calipers look like tongs. It will look like you will be getting a little pinch but it will not feel this way. This does not hurt.

2) We will also measure the amount of muscle in your body with a special machine called the Bod Pod. This test takes about 5 minutes. We will ask you to wear a swimsuit and a swim cap when you do this test. You will not get wet. This test is very safe. We will ask you to do this in each visit: once when you start the study and at 6 months later.

3) **Diet and Physical Activity.**
We will put you into one of the three different groups. You cannot decide which group you will be in. The way we will decide what group you will be in will be done randomly (i.e. like tossing a coin).

- ✓ **Group 1:** Healthy Eating
- ✓ **Group 2**: Healthy Eating and (less sugar)
- ✓ **Group3:** Healthy Eating and (less sugar) + Physical Activity

A. Diet Plan
If you are placed into **Group 1**, you will be taught a diet that focuses on healthy eating and food portion sizes. We will explain how to follow this type of meal plan and we will help you chose foods to eat that you already like to eat. If you are placed into **Groups 2 or 3** you will be taught a diet that focuses on healthy eating but will also avoid lots of sugary foods. We think these types of foods could make your liver get fat in it. We will provide you with a list of foods to choose from. We will explain how to follow this type of meal plan and we will help you chose foods to eat that you already like. We will ask you to write down what you have eaten while you are on the diet before you come back for each clinic visit.
B. Physical Activity

If you participate in this study you will ask you to answer some questions about the type of physical activity you are already doing. We will ask you to wear a pedometer so we can see how steps you take every day. A pedometer is a small lightweight instrument that will record the number of steps you take in a day; the pedometer will be attached to the waistband of your pants. You will be asked to wear the pedometer for 4 days (2 weekdays and 2 weekend days) every week. We will also ask you to write down everyday how many steps you took.

Physical Activity Intervention: You will only participate in this part of the study if you are put into Group 3. In this group, we will give you advice on how to increase the number of steps you takes on the four days per week that you wear the pedometer. It is important to us that you are comfortable wearing the pedometer. It is okay if you do not want to do this on any one day. You can wear it on another day.

4) Medical Records
We would also like to look at your medical chart to see the results from other tests you have had.

5) Blood work
We will order your regular blood work at the Stollery Children's Hospital. You will be asked not to eat anything after your supper the night before you come to the hospital. We will take an extra half of a teaspoon of blood to measure some liver tests and other tests we think are important. We will ask you to have blood work two times in the study. The first time is when you first start the study. We will ask you to do this after 6 months.

Will it help?: We don't know if this will help to have a healthy liver. We think this type of diet will help children with liver disease. You will learn about healthy eating and physical activity, which will definitively help you to become healthier. When you are in this study, you will try new activities that are fun, and hopefully find some favorites that you can do every day.

Will it hurt? None of the additional tests that we want to do in this study will hurt. You must tell your mom or dad or your doctor about anything you think is different.

Can you quit?: You don't have to take part in the study, and you can quit at any time. No one will be mad at you if you decide you don't want to do this, or if you decide to stop part way through. You should tell the doctor or dietitian or the research team that you want to quit.

Who will know?: No one except your parents and the doctor will know you're taking part in the study unless you want to tell them. Your name and your chart won't be seen by anyone except the doctors and nurses and dietitians and research team.

Your signature: We would like you to sign this form to show that you agree to take part. Your mom or dad will be asked to sign another form agreeing for you to take part in the study.
Do you have more questions? You can ask your mom or dad about anything you don't understand. You can also talk to Dr. Susan M. Gilmour, Dr. Jason Yap or Dr. Diana Mager (Researcher). Dr. Gilmour's telephone number is 780-248-5409, Dr. Jason Yap´s telephone number is 780-248-5420 and Dr. Mager's telephone number is 780-492-7687.

I agree to take part in the study.
Signature of research participant:_____Date: _____
Signature of witness:_____Date: _____
Signature of investigator:_____Date:_____

U N I V E R S I T Y O F A L B E R T A

Form A-2 Assent Form (Children and adolescents with NAFLD)

Title of Project: Impact of lifestyle intervention in Non Alcoholic Fatty Liver Disease (NAFLD) in children.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687

Co-Investigator: Dr. Susan M. Gilmour, MD Telephone: 780-407-3339

You have a fatty liver. We would like you to participate in a research study that will help us understand what the best type of diet and what level of physical activity would be good for your liver.

What will you have to do?:

If you and your parents agree that it is okay to take part in this study, we will ask you to come to clinic three times (3, 6 and 12 months) after you start this study. When you come to clinic we will ask you to:

1) We will measure your weigh, height, waist and some arm measurements. We will measure your waist by putting a tape measure around your waist and take a measurement of your skin from the back of your arm, thigh, and calf and behind the back and on the side of the waist with a caliper. Calipers look like tongs. It will look like you will be getting a little pinch but it will not feel this way. This does not hurt.
2) We will also measure the amount of muscle in your body with a special machine called the Bod Pod. This test takes about 5 minutes. We will ask you to wear a swimsuit and a swim cap when you do this test. You will not get wet. This test is very safe. We will ask you to do this twice: once when you start the study and 6 months later.
3) **Diet and Physical Activity.**
   We will put you into one of the three different groups. You cannot decide which group you will be in. The way we will decide what group you will be in will be done randomly (i.e. like tossing a coin).
   - ✓ **Group 1:** Healthy Eating
   - ✓ **Group 2**: Healthy Eating (less sugar)
   - ✓ **Group3:** Healthy Eating (less sugar) + Physical Activity

A. Diet Plan

If you are placed into **Group 1**, you will be taught a diet that focuses on healthy eating and food portion sizes. We will explain how to follow this type of meal plan and we will help you chose foods to eat that you already like to eat. If you are placed into **Groups 2 or 3** you will be taught a diet that focuses on healthy eating but will also avoid lots of sugary foods. We think these types of foods might be making your liver get fat in it. We will provide you with a list of foods to choose from. We will explain how to follow this type of meal plan and we will help you chose foods to eat that you already like. We will ask you to write down what you have eaten while you are on the diet t before you come back for each clinic visit.

B. Physical Activity

If you participate in this study you will ask you to answer some questions about the type of physical activity you are already doing. We will ask you to wear a pedometer so we can see how steps you take every day. A pedometer is a small light instrument that will record the number of steps you take in a day; the pedometer will be attached to the waistband of your pants. You will be asked to wear the pedometer for 4 days (2 weekdays and 2 weekend days) every week. We will also ask you to write down everyday how many steps you took.

Physical Activity Intervention: You will only participate in this part of the study you are put into Group 3. In this group, we will give you advice on how to increase the number of steps you takes on the four days per week that you wear the pedometer. It is important to us that you are

comfortable wearing the pedometer. It is okay if you do not want to do this on any one day. You can wear it on another day.

4) Medical Records
We would also like to look at your medical chart to see the results from other tests you have had.

6) Blood work
Your doctor will order your regular blood work at the Stollery Children's Hospital. You will be asked not to eat anything after your supper the night before you come to the hospital. We will not poke you for an extra blood test; the only thing that will happen is that we will take an extra half of a teaspoon of blood to measure some liver tests and other tests we think are important.

7) Liver Biopsy
Your doctor will decide whether you need a liver biopsy in order to understand better, what is happening with your liver Your doctor will explain all of these risks to you and your parents BEFORE they do the liver biopsy. If you agree that you will participate in this study, then we will ask the doctor who performs the liver biopsy to share the results of the liver biopsy with our research team. This will help us to understand how much fat is in your liver. We will also ask you to have a second liver biopsy one year after the first one. We want to do this so we can see if the diet and physical activity plan helped your liver. If you decide you do NOT want to have this second liver biopsy for the study this is okay. You can talk to your doctor about this.

Will it help?: We don't know if this will help your liver. You will learn about healthy eating and physical activity, which will definitively help you to become healthier. When you are in this study, you will try new activities that are fun, and hopefully find some favorites that you can do every day

Will it hurt? None of the additional tests that we want to do in this study will hurt. If your doctor decides that you need a liver biopsy, you may feel a little uncomfortable. The doctor will explain all of this to you, and your mom and dad. You must tell your mom or dad or your doctor about anything you think is different.

Can you quit?: You don't have to take part in the study, and you can quit at any time. No one will be mad at you if you decide you don't want to do this, or if you decide to stop part way through. You should tell the doctor or dietitian or the research team that you want to quit.

Who will know?: No one except your parents and the doctor will know you're taking part in the study unless you want to tell them. Your name and your chart won't be seen by anyone except the doctors and nurses and dietitians and research team.
Your signature: We would like you to sign this form to show that you agree to take part. Your mom or dad will be asked to sign another form agreeing for you to take part in the study.
Do you have more questions? You can ask your mom or dad about anything you don't understand. You can also talk to Dr. Gilmour or Diana Mager (researcher). Dr. Gilmour's telephone number is 780-407-3339 and Dr. Mager's telephone number is 780-492-7687

I agree to take part in the study.
Signature of research participant:_____Date: _____
Signature of witness:_____Date: _____
Signature of investigator:_____Date:_____

U N I V E R S I T Y O F A L B E R T A

Form A-3 Parent consent form (Healthy children and adolescents)

PARENT CONSENT FORM (Healthy Children & Adolescents)

Title of Project: Impact of lifestyle intervention in Non Alcoholic Fatty Liver Disease (NAFLD) in children (FRAGILE)

Principal Investigator: Diana Mager, PhD RD **Phone Number:** 780-492-7687

Co-Investigator: Dr. Susan M. Gilmour, MD **Phone Number:** 780-248-5409
Dr. Jason Yap MD **Phone Number**: 780-248-5409

	Yes	**No**
Do you understand that your child has been asked to participate in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Do you understand the benefits and risks involved for your child in taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to withdraw your child from the studyat any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
Do you understand who will have access to your child's records, including personally identifiable health information?	☐	☐
Do you want the investigator(s) to inform your child's family doctor or pediatrician that your child is participating in this research study?	☐	☐

Doctor's name _____

Who explained this study to you? _____

Child's Name _____

I agree for my child to take part in this study: YES ☐ NO ☐

Signature of Parent or Guardian _____

Date & Time _____

(Printed Name) _____

Signature of Investigator or Designee _____

Date & Time _____

U N I V E R S I T Y O F A L B E R T A

Form A-4 Parent consent form (Children and adolescents with NAFLD)

PARENT CONSENT FORM (Children & Adolescents with NAFLD)

Title of Project: Impact of lifestyle intervention in Non Alcoholic Fatty Liver Disease (NAFLD) in children (FRAGILE)

Principal Investigator: Diana Mager, PhD RD **Phone Number:** 780-492-7687

Co-Investigator: Dr. Susan M. Gilmour, MD **Phone Number:** 780-248-5409
Dr. Jason Yap MD **Phone Number**: 780-248-5409

	Yes	**No**
Do you understand that your child has been asked to participate in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Do you understand the benefits and risks involved for your child in taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to withdraw your child from the studyat any time, without having to give a reason and without affecting your child's future medical care?	☐	☐
Do you understand who will have access to your child's records, including personally identifiable health information?	☐	☐
Do you want the investigator(s) to inform your child's family doctor or pediatrician that your child is participating in this research study?	☐	☐

Doctor's name _____

Who explained this study to you? _____

Child's Name _____

I agree for my child to take part in this study: YES ☐ NO ☐

Signature of Parent or Guardian _____

Date & Time _____

(Printed Name) _____

Signature of Investigator or Designee _____

Date & Time _____

U N I V E R S I T Y O F A L B E R T A

Form A-5 Information letter (Healthy control group)

Title of Project: Impact of lifestyle intervention in Non Alcoholic Fatty Liver Disease (NAFLD) in children (FRAGILE)

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687

Co-Investigator: Dr. Susan M. Gilmour, MD Telephone: 780-248-5409
Dr. Jason Yap, MD Telephone: 780-248-5420

This information letter is intended for the study subject. If you are signing on behalf of your child, the words 'you' and 'your' should be read as your 'your child'.

Purpose of this study

We would like you to participate in a research study that will help us understand what the best type of diet and what level of physical activity would be good for children with liver disease. Our previous work suggests that simple sugars (such as fructose) and foods high in glycemic index (e.g. white bread) might be one of the reasons why some children get fatty livers especially if large amounts of these foods are eaten. We have also shown that increasing physical activity levels (e.g. walking) may also help the liver, but we still need to understand better exactly how much physical and what type of diet is healthy for the liver.

Procedure(s) of the study

1. Anthropometric Measurements

We will measure your weight and height and take some other body measurements during each study visit. We will measure your waist circumference by putting a tape measure around your waist and take a measurement of your skin from the back of your arm, thigh, and calf and behind the back and on the side of the waist with a caliper. Your wrist and elbow diameter will be measured will a small bone caliper. Calipers look like tongs. It will look like a little pinch but will not feel like one. **These measurements will be done at the Clinical Research Unit (CRU) at the University of Alberta when you start the study and then again at 3, 6 and 12 months after you start the study.**

We will also measure the amount of muscle in your body with a special machine called the Bod Pod machine. The Bod Pod is a machine that measures the amount of muscle in a child's body by measuring body weight and by detecting the difference in volume of the air before and after a child sits in the machine. This allows the machine to calculate how much of your body is muscle and how much is fat. The Bod Pod consists of two chambers: the test chamber, where you will be sitting and the reference chamber which is mainly the seat. The Bod Pod test takes about 5 minutes, and you will be asked to wear a swimsuit and a swim cap. You need to wear these instead of regular clothes so the machine can measure your muscles correctly. You will not get wet and the test is not uncomfortable at all and it is completely safe. The Bod Pod test will be done at the CRU, at time of entry into our study and 3, 6 and 12 months later. We will pay for parking costs at the university.

2. Diet Plan

We will teach you and your child what kinds of foods are healthy for your child's body. If you choose to participate in our study you will be put into one of three groups. The type of diet treatment you will be put into will be determined by chance (like the toss of a coin or dice).
There are three different diet treatments we would like to study are:

- ✓ **Group 1:** Standard Dietetic counseling,
- ✓ **Group 2**: low Glycemic and fructose diet
- ✓ **Group 3:** low Glycemic and fructose diet + Physical Activity intervention.

If you are placed into **Group 1**, you will be taught a diet that focuses on healthy eating and food portion sizes. This is the diet counseling that is typically provided to children and their families in the clinic by the registered dietitian.

If you are placed into **Groups 2 or 3** you will be taught a diet that focuses on healthy eating but will also focus on including foods with a low glycemic index and fructose content. We will provide you with a list of foods to choose from. We will explain how to follow this type of meal plan in a way that will make it easy to follow. We will call you to assess your diet, give you tips about how to follow the diet and any question you may have about it, one week after your first visit, and once each of the following months prior to your next visit.

Everyone participating in this study (regardless of which group you are put into) will be asked to come to clinic at three, six and twelve months after you start this study. We will ask you to fill out a three day food record based on what you eat before you come to these clinic visits. For the three-day food records we will ask you to write down what you have eaten for the three days (2 weekdays and 1 weekend day). It will take about 10 minutes to fill out the food record on each of the three days. We will ask you to do this three times. The first time will be when you start this study and three more times (the week before each study visit). We will remind you about these food intake records

3. Physical Activity

If you participate in this study you will first be asked to fill out an activity questionnaire, (this will be at the CRU). The activity questionnaire will ask you some questions about the type and amount of physical activity you are already doing.

Measuring physical activity with a pedometer: We will ask you to wear a pedometer so we can see how much physical activity you do every day. A pedometer is a small light weight instrument that will record the number of steps you take in a day; the pedometer will be attached to the waistband of your pants. You will be asked to wear the pedometer for 4 days (2 weekdays and 2 weekend days) every week. **At the end of each of these days we will ask you to record the total number of steps that is recorded on the pedometer. We will give you a physical activity record to write this information down. It will only take a few minutes to write this down on the sheet.**

Physical Activity Intervention: You will only participate in this part of the study you are put into Group 3. In this group, we will give you advice on how to increase the number of steps you takes on the four days per week that you wear the pedometer. It is important to us that you are comfortable wearing the pedometer. It is okay if you do not want to do this. You can wear it on another day. We will call you at home every week to review the pedometers records with you for the first six weeks of the study. We will answer any questions you might have about your diet or physical activity regardless of what group you are in. If your are in **Group 3** we will give you advice on how to increase the number of steps you take every day. These phone calls with take only about 10 minutes.

4. Medical Records

We would also like to look at your medical records to find out about medications, relevant lab work (for example at the amount of fat and sugar that is present in the blood), weight, height and history of injuries over the 12 months. This will help us understand if you have had any medical problems that might prevent you from participating in your regular physical activities.

5. Blood work

We will ask you to have blood work done at the beginning of the study and after 6 months after you start this study. The reason we would like you to do this, is that we would like to measure your blood fat and sugar levels and other factors that tell us about your liver. You will be asked not to eat anything after your supper the night before you come to the hospital. We will make sure that you know the results of this test. If there are any concerns about the results, we will refer you to a liver doctor.

Benefits

Your participation in this study will help us learn more about the diets of children with liver disease, to understand what diet and amount of physical activity is necessary in order to improve children´s health with liver disease. There is very little information about this and we need to understand this so we can provide the best nutritional information to children with liver disease. By participating in this program, you will try new activities that are fun, and hopefully find some favorites that you can do every day. No matter in which group you are placed in, you will learn about healthy eating and physical activity, which will definitively help you to become healthier.

Risks

All of the ***additional*** tests used in this study are harmless. The exercise-diet program is safe. The pedometer does not hurt, it is attached to their pants, and it can be hidden with a shirt if you don't want anyone to see it. We will only ask you to participate in activities that you already enjoy and are able to do. With regards to the diets, both of them are completely safe and balanced and you will get all the nutrients that you need.

Confidentiality: We will not share any information in your personal health record with anyone. Any research data collected about you during this study will not identify you by name, only by your initials and a coded number. Your name will not be shared with anyone outside the research clinic and your name will not be in any reports published from this research.

The personal health information collected in this study may need to be checked by the Health Research Ethics Board (HREB) at the University of Alberta. This may be necessary so the HREB can make sure that the data collected in the study is accurate. By signing the consent form you give permission for the collection, use and sharing of information from your medical records for purpose of this research. In the University of Alberta, study information is required to be kept for 7 years. Even if you withdraw from the study, the medical information, which is obtained from you, will not be destroyed. You have a right to check your health records and request changes if your personal information is incorrect.

Voluntary Participation

You don't have to take part in the study at all, and you can quit at any time. No one will be mad at you if you decide that you don't want to do this, or if you decide to stop part way through. You should tell the dietitian that you want to quit.

Compensation for Injury*:* If you become ill or injured as a result of participating in this study, necessary medical treatment will be available at no additional cost to you. By signing this consent form you are not releasing the investigator(s) or institution(s) from their legal and professional responsibilities.

Do you have more questions?

You can ask your dietitian about anything you don't understand. You can also talk to Diana Mager, Susan M. Gilmour or Jason Yap. Diana Mager's phone number is 492-7687. Susan Gilmour's telephone number is 248-5409. Jason Yap's telephone number is 248-5420. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Susan M. Gilmour, MD Telephone: 780-248-5409
Dr. Jason Yap. MD Telephone: 780-248-5420

UNIVERSITY OF ALBERTA

Form A-6 Infromattion letter (Children and adolescents with NAFLD)

Title of Project: Impact of lifestyle intervention in Non Alcoholic Fatty Liver Disease (NAFLD) in children (FRAGILE)

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Susan M. Gilmour, MD Telephone: 780-407-3339
Dr. Jason Yap, MD Telephone: 780-248-5420

This information letter is intended for the study subject. If you are signing on behalf of your child, the words 'you' and 'your' should be read as your 'your child'.

Purpose of this study

We would like you to participate in a research study that will help us understand what the best type of diet is and what level of physical activity would be good for your liver. Our previous work suggests that simple sugars (such as fructose) and foods high in glycemic index (e.g. white bread) might be one of the reasons why some children get fatty livers especially if large amounts of these foods are eaten. We have also shown that increasing physical activity levels (e.g. walking) may also help your liver.

Procedure(s) of the study

1. **Anthropometric Measurements**

We will measure your weight and height and take some other body measurements during each clinic visit. We will measure your waist circumference by putting a tape measure around your waist and take a measurement of your skin from the back of your arm, thigh, and calf and behind the back and on the side of the waist with a caliper. Your wrist and elbow diameter will be measured will a small bone caliper. Calipers look like tongs. It will look like a little pinch but will not feel like one. **These measurements will be done at the hospital clinic when you start the study and then again three; six months and 12 months after you start the study. This will happen during your regular clinic appointments.**

We will also measure the amount of muscle in your body with a special machine called the Bod Pod machine. The Bod Pod is a machine that measures the amount of muscle in a child's body by measuring body weight and by detecting the difference in volume of the air before and after a child sits in the machine. This allows the machine to calculate how much of your body is muscle and how much is fat. The Bod Pod consists of two chambers: the test chamber, where you will be sitting and the reference chamber which is mainly the seat. The Bod Pod test takes about 5 minutes, and you will be asked to wear a swimsuit and a swim cap. You need to wear these instead of regular clothes so the machine can measure your muscles correctly. You will not get wet and the test is not uncomfortable at all and it is completely safe. The Bod Pod test will be done at the Human Nutrition Research Unit, HNRU, University of Alberta, at time of entry into our study and 6 months later during your regularly scheduled clinic appointment. Because this test is not happening at the same time as your clinic appointment we will pay for parking costs at the university. We will try and schedule the second appointment at the same time at your 6 month clinic appointment with the doctor. If we cannot do this, then we will cover your parking costs at the university as well when your child comes back for the Bod Pod test again

2. **Diet Plan**

Your child is currently seeing a dietitian at the Gastroenterology Clinics, Pediatrics, Stollery Children's Hospital; the dietitian will teach you and your child what kinds of foods are healthy for

your child's body. If you choose to participate in our study you will be put into one of three groups. The type of diet treatment you will be put into will be determined by chance (like the toss of a coin or dice). You will continue to receive the same type of care by a registered dietitian even if you do not want to participate in this study. There are three different diet treatments we would like to study are:

- ✓ **Group 1:** Standard Dietetic counseling,
- ✓ **Group 2**: low Glycemic and fructose diet
- ✓ **Group 3:** low Glycemic and fructose diet + Physical Activity intervention.

If you are placed into **Group 1**, you will be taught a diet that focuses on healthy eating and food portion sizes. This is the diet counseling that is typically provided to children and their families in this clinic by the registered dietitian. If you are placed into **Groups 2 or 3** you will be taught a diet that focuses on healthy eating but will also focus on including foods with a low glycemic index and fructose content. We will provide you with a list of foods to choose from. We will explain how to follow this type of meal plan in a way that will make it easy to follow. Everyone participating in this study (regardless of which group you are put into) will be asked to come to clinic at three and six months after you start this study. We will ask you to fill out a three day food record based on what you eat before you come to these clinic visits. For the three-day food records we will ask you to write down what you have eaten for the three days (2 weekdays and 1 weekend day). It will take about 10 minutes to fill out the food record on each of the three days. We will ask you to do this three times. The first time will be when you start this study and three more times (the week before each clinic visit). We will call you to remind you about these food intake records one week before you come back to clinic.

3. Physical Activity

If you participate in this study you will first be asked to fill out an activity questionnaire, (this will be in clinic). The activity questionnaire will ask you some questions about the type and amount of physical activity you are already doing.

Measuring physical activity with a pedometer: We will ask you to wear a pedometer so we can see how much physical activity you do every day. A pedometer is a small lightweight instrument that will record the number of steps you take in a day; the pedometer will be attached to the waistband of your pants. You will be asked to wear the pedometer for 4 days (2 weekdays and 2 weekend days) every week. **At the end of each of these days we will ask you to record the total number of steps that is recorded on the pedometer. We will give you a physical activity record to write this information down. It will only take a few minutes to write this down on the sheet.**

Physical Activity Intervention: You will only participate in this part of the study you are put into Group 3. In this group, we will give you advice on how to increase the number of steps you takes on the four days per week that you wear the pedometer. We will call you at home every week to review the pedometers records with you for the first six weeks of the study. We will answer any questions you might have about your diet or physical activity regardless of what group you are in. If your are in **Group 3** we will give you advice on how to increase the number of steps you take every day. These phone calls with take only about 10 minutes. It is important to us that you are comfortable wearing the pedometer. It is okay if you do not want to do this. You can wear it on another day.

4. Medical Records

We would also like to look at your medical records to find out about medications, relevant lab work (for example at the amount of fat and sugar that is present in the blood), weight, height and history of injuries over the 12 months. This will help us understand if you have had any medical problems that might prevent you from participating in your regular physical activities.

5. Blood work

Your doctor will order your regular blood work at the Stollery Children's Hospital. You will be asked not to eat anything after your supper the night before you come to the hospital. This is part of routine patient care. We will not poke you for an extra blood test; the only thing that will

happen is that we will take an extra half of a teaspoon of blood to measure some liver enzymes, serum virology, glucose, and insulin and lipid profile. **The Blood work would be done at the Hospital at when you first start the study, and then again six and 12 months later.**

6. Liver Biopsy

Your doctor will decide whether you need a liver biopsy in order to understand better what is happening with your liver. This is the standard part of clinical care to diagnose NAFLD in our clinics and will be done whether or not you participate in this study. Your doctor will explain the medical risks associated with you having a liver biopsy. A liver biopsy requires the use of medications for pain and carries a risk for bleeding. Your doctor will explain all of these risks to you and your parents BEFORE they do the liver biopsy. If you agree that you will participate in this study, then we will ask the doctor who performs the liver biopsy to share the results of the liver biopsy with our research team. This will help us to understand how sick your liver is. If you agree to be part of this study, we will also ask you to have a second liver biopsy (one year after you start this study). This second liver biopsy is NOT part of the routine clinical care of the patients within our clinics. This second liver biopsy will help us understand if the diet and physical activity plan helped your liver. If you decide you do NOT want to have this second liver biopsy for the study this is okay. You can talk to your doctor about this.

Benefits

We are not sure of the best way to children get healthier and more active. Your participation in this study will help us to understand what diet and amount of physical activity is necessary in order to improve children's health with liver disease. By participating in this program, you will try new activities that are fun, and hopefully find some favorites that you can do every day. No matter in which group you are placed in, you will learn about healthy eating and physical activity, which will definitively help you to become healthier.

Risks

All of the ***additional*** tests used in this study are harmless. The exercise-diet program is safe. The pedometer does not hurt, it is attached to their pants, and it can be hidden with a shirt if you don't want anyone to see it. We will only ask you to participate in activities that you already enjoy and are able to do. With regards to the diets, both of them are completely safe and balanced and you will get all the nutrients that you need.

Voluntary Participation

You don't have to take part in the study at all, and you can quit at any time. No one will be mad at you if you decide that you don't want to do this, or if you decide to stop part way through. You should tell the dietitian that you want to quit. This will not affect the type of care you will receive from the dietitian. You can still continue to see the dietitian at any time without participating in this study.

Who will know?

No one except you, the dietitian and the graduate student will know you're taking part in the study unless you want to tell them. Your name, food records, and activity records won't be seen by anyone except the researcher, dietitian, and student during the study.

Do you have more quetions?

You can ask your dietitian about anything you don't understand. You can also talk to Diana Mager or Susan M. Gilmour. Diana Mager's phone number is 492-7687. Susan Gilmour's telephone number is 407-3339. If you have any problems or concerns about any part of this study please call the Patient Relations Office of Capital Health at (780)-492-8080.This office has no connection with the study researchers.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Susan M. Gilmour, MD Telephone: 780-248-540
Dr. Jason Yap. MD Telephone: 780-248-542

Table A.1 Common fructose sources within the cohort

Fruits	**Vegetables**	**Dairy products**	**Grain products**	**Sweetened beverages**	**Other products**	**HFCS**
Apple Banana Blueberries Grapefruit Grapes Mango Oranges Pear Strawberries	Green beans Broccoli Corn Carrots Cucumber Lettuce Onion Potato Tomato	Milk Yoghurt Cheese	Bagel White Bread Whole grain bread Pita bread Porridge Rice Pasta Home made muffin Home made waffles	Apple juice Cranberry juice Grapefruit juice Orange Juice Chocolate milk	Cornflakes™ Cheerios™ Froot Loops™ Sugary cookies Ice cream Candy Chocolates Honey Sugar Cake Muffin Waffles Donuts Corn chips Pizza	Cola soft drink Orange soft drink Lemon Soft drink Gatorade Candy

Table A-2 Example total fructose calculation

Total Fructose = Free fructose gr + Sucrose gr/2				
Food item	Food item gr	Free Fructose gr	Sucrose gr	Total fructose
Apple[1] medium size (7cm)	182 gr	10.7	3.7	12.5
[1]Apple values of fructose and sucrose taken from USDA database. [2] Fructose calculation (Babwik, Additional Nutrients to database, Unpublished, 2012)				

Table A.3 GI/GL example calculations

Food Item	(g)	GI	Carb in food	GI mix meal	GL
CRACKER, SALTINE/OYSTER/SODA/SOUP	120	63	85.09	43.48	53.60
CHEESE, CHEDDAR, DICED	70	0	0.9	0	0
COOKIE, CHOCOLATE CHIP, COMMERCIAL, 18-28% FAT, 8.9-10.2 CM	25	62	16.01	8.05	9.92
APPLE, RAW, W/ SKIN, 7 CM MEDIUM	154	34	21.27	5.86	7.23
Total	369	159	123.27	**57.40**	**70.76**
GL = ∑ (carbohydrate content of each food item (g) × GI of each food)/100 Mix meal = ∑ (carbohydrate content of each food item (g) × GI)/total amount of carbohydrate in each meal (g)					

Table A.4 Power statistical analysis

Variable	Power
Systolic BP	0.97
Diastolic	0.25
Body Fat %	0.99
Weight	0.99
Weight z	0.99
BMI z	0.99
Ht z	0.22
Ht	0.99
TER/sum extremities/sum of trunk	>0.99
ALT categorical (> and < 20) and or continuous	>0.99
AST continuous	0.67
GGT continuous	0.99
HOMA-IR (continuous > and < 3)	0.78/0.77
TG (categorical > and < 1.5 mmol/L)	0.81
ApoB-100	0.99
ApoB-48	0.99
ApoC-3	0.45
IL-6	0.77
Il-10	<0.2
TNF –alpha	0.37
HDL	0.90
LDL	<0.2

Table A.5 Multivariate analysis of age effect

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
Time	SBP	0.033	0.346	0.150
	Age			0.669
Time	Weight z-score	0.010	0.724	0.465
	Age			0.694
Time	Height z-score	0.010	0.706	0.445
	Age			0.665
Time	Waist Circumference	0.014	0.622	0.392
	Age			0.462
Time	BF%	0.014	0.620	0.359
	Age			0.543
Time	Abdominal Skinfold	0.054	0.176	0.071
	Age			0.318
Time	NEFA	0.136	0.031	0.011
	Age			0.644
Time	Apo-B100	0.015	0.595	0.433
Time	Apo-B48	0.039	0.426	0.356
	Age			0.374
Time	ALT	0.010	0.760	0.542
	Age			0.595
Time	AST	0.008	0.812	0.600
	Age			0.704
Time	GGT	0.020	0.600	0.349
	Age			0.545
Time	HOMA-IR	0.018	0.658	0.364
	Age			0.746
Time	Triglycerides	0.005	0.870	0.710
	Age			0.685
Time	Insulin	0.001	0.961	0.787
	Age			0.896
Time	TNF-alpha	0.098	0.101	0.049
	Age			0.761
Time	IL-6	0.018	0.757	0.485
	Age			0.964
Time	IL-10	0.012	0.765	0.577
	Age			0.684
Time	CRP	0.050	0.288	0.135
	Age			0.594
Time	Kcal	0.064	0.117	0.041
	Age			0.591
Time	Diet Fat%	0.139	0.008	0.002
	Age			0.402
Time	Diet Sat Fat%	0.119	0.017	0.004
	Age			0.597
Time	Protein%	0.014	0.628	0.367
	Age			0.805
Time	Total fructose (gr)	0.071	0.093	0.031
	Age			0.458

Time	GI Age	0.016	0.595	0.337 0.725
Time	GL Age	0.076	0.077	0.025 0.644

[1] Categorical variables: Age (above and below 13 years)
[2]Abreviations: Systolic Blood Pressure (BP), Waist Circumference (WC), Body Fat (BF), Fat Free Mass (FFM), Mid-Arm Circumference, Trunk-Extremity Ratio (TER)Triglycerides (TG), Non-esterified free fatty acids (NEFA), homeostasis model of assessment of insulin resistance (HOMA-IR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), High-density lipoprotein (HDL), Total Cholesterol/HDL Ratio (TC/HDL Ratio) C-reactive protein (CRP), Interleukin-6 (IL-6), Apolipoprotein B-48 (Apo-B48), Apolipoprotein B-100 (Apo-B100).

Table A.6 Multivariate analysis of gender effect

Dependent Variable	**Independent Variable**	**r^2**	**p-value of the Model**	**p-value/Indep. Variable**
Time	SBP Gender	0.315	0.364	0.168 0.776
Time	Weight z-score Gender	0.007	0.781	0.588 0.968
Time	Height z-score Gender	0.012	0.672	0.441 0.594
Time	WC Gender	0.006	0.814	0.584 0.935
Time	BF% Gender	0.009	0.737	0.522 0.870
Time	Abdominal Skin fold Gender	0.041	0.268	0.114 0.695
Time	NEFA Gender	0.145	0.025	0.009 0.416
Time	Apo-B100 Gender	0.273	0.002	0.006 0.096
Time	Apo-B100 Gender Apo-B100+Gender	0.361	0.006	0.009 0.080 **0.027**
Time	Apo-B48 Gender	0.026	0.573	0.312 0.649
Time	ALT Gender	0.005	0.864	0.679 0.867
Time	AST Gender	0.006	0.855	0.662 0.839
Time	GGT Gender	0.012	0.721	0.467 0.985
Time	HOMA-IR Gender	0.035	0.447	0.271 0.350
Time	Triglycerides Gender	0.003	0.917	0.821 0.808
Time	Insulin Gender	0.012	0.757	0.682 0.484
Time	TNF-alpha	0.111	0.073	0.028

	Gender			0.394
Time	IL-6	0.022	0.709	0.490
	Gender			0.718
Time	IL-10	0.018	0.687	0.588
	Gender			0.540
Time	CRP	0.043	0.342	0.152
	Gender			0.898
Time	Kcal	0.060	0.134	0.051
	Gender			0.896
Time	Diet Fat%	0.130	0.011	0.003
	Gender			0.982
Time	Diet Sat Fat%	0.121	0.015	0.004
	Gender			0.485
Time	Total Fructose gr	0.063	0.122	0.046
	Gender			0.958
Time	Diet Protein%	0.019	0.526	0.299
	Gender			0.520
Time	GI	0.015	0.605	0.371
	Gender			0.763
Time	GL	0.073	0.085	0.030
	Gender			0.933

[2]Abreviations: Systolic Blood Pressure (BP), Waist Circumference (WC), Body Fat (BF), Fat Free Mass (FFM), Mid-Arm Circumference, Trunk-Extremity Ratio (TER)Triglycerides (TG), Non-esterified free fatty acids (NEFA), homeostasis model of assessment of insulin resistance (HOMA-IR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), High-density lipoprotein (HDL), Total Cholesterol/HDL Ratio (TC/HDL Ratio) C-reactive protein (CRP), Interleukin-6 (IL-6), Apolipoprotein B-48 (Apo-B48), Apolipoprotein B-100 (Apo-B100).

Table A.7 Multivariate analysis of demographic and body composition variables with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
Gender	Group	0.402	<0.001	**<0.001**
	Time			0.997
SBP[1]	Group	0.435	<0.001	**<0.001**
	Time			0.376
Weight (kg)	Group	0.734	<0.001	**<0.001**
	Time			0.791
Weight (z-score)	Group	0.685	<0.001	**<0.001**
	Time			0.988
Weight (Percentile)	Group	0.512	<0.001	**<0.001**
	Time			0.991
Height	Group	0.438	<0.001	**<0.001**
	Time			0.246
BMI (z-score)	Group	0.646	<0.001	**<0.001**
	Time			0.927
BMI (Percentile)	Group	0.567	<0.001	**<0.001**
	Time			0.901
WC[1]	Group	0.830	<0.001	**<0.001**
	Time			0.980
Hip	Group	0.772	<0.001	**<0.001**
	Time			0.692
WC/HT[1]	Group	0.731	<0.001	**<0.001**
	Time			0.516
WC/Hip[1]	Group	0.488	<0.001	**<0.001**
	Time			0.498
BF%[1]	Group	0.659	<0.001	**<0.001**
	Time			0.941
BF (kg)	Group	0.661	<0.001	**<0.001**
	Time			0.978
FFM%[1]	Group	0.659	<0.001	**<0.001**
	Time			0.941
FFM (kg)	Group	0.667	<0.001	**<0.001**
	Time			0.464
MAC[1]	Group	0.756	<0.001	**<0.001**
	Time			0.689
Femoral	Group	0.222	0.001	**0.004**
	Time			0.098
Humerus	Group	0.309	<0.001	**<0.001**
	Time			0.477
Triceps	Group	0.601	<0.001	**<0.001**
	Time			0.096
Biceps	Group	0.607	<0.001	**<0.001**
	Time			0.195
Subscapular	Group	0.765	<0.001	**<0.001**
	Time			0.164
Abdominal	Group	0.728	<0.001	**<0.001**
	Time			0.119
Supraspinal	Group	0.738	<0.001	**<0.001**

	Time			0.147
Ileac Crest	Group Time	0.658	<0.001	**<0.001** 0.259
Med Calf	Group Time	0.497	<0.001	**<0.001** 0.575
TER[1]	Group Time	0.423	<0.001	**<0.001** 0.557
Sum Trunk	Group Time	0.773	<0.001	**<0.001** 0.089
Sum Extremities	Group Time	0.636	<0.001	**<0.001** 0.169
Endo	Group Time	0.785	<0.001	**<0.001** 0.226
Meso	Group Time	0.422	<0.001	**<0.001** 0.962
Ecto	Group Time	0.641	<0.001	**<0.001** 0.968

[1]Abreviations: Systolic Blood Pressure (BP), Waist Circumference (WC), Waist to Height Ratio (WC/HT), Waist to Hip Ratio (WC/Hip), Body Fat (BF), Fat Free Mass (FFM), Mid-Arm Circumference, Trunk-Extremity Ratio (TER)

Table A.8 Multivariate aalysis of demographic and body composition variables with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
SBP	Group	0.476	<0.001	**<0.001**
	Time			0.218
	Group + Time			0.101
BF%	Group	0.695	<0.001	**<0.001**
	Time			0.749
	Group + Time			**0.034**
Femural	Group	0.249	0.003	**0.003**
	Time			0.065
	Group + Time			0.352
Triceps	Group	0.633	<0.001	**<0.001**
	Time			**0.036**
	Group + Time			0.084
Biceps	Group	0.634	<0.001	**<0.001**
	Time			0.095
	Group + Time			0.110
Subscapular	Group	0.785	<0.001	**<0.001**
	Time			0.064
	Group + Time			0.071
Abdominal	Group	0.753	<0.001	**<0.001**
	Time			**0.039**
	Group + Time			0.054
Supraspinal	Group	0.783	<0.001	**<0.001**
	Time			**0.026**
	Group + Time			**0.003**
Ileac Crest	Group	0.682	<0.001	**<0.001**
	Time			0.128
	Group + Time			0.118
Sum Trunk	Group	0.804	<0.001	**<0.001**
	Time			**0.018**
	Group + Time			**0.013**
Sum Extremities	Group	0.671	<0.001	**<0.001**
	Time			**0.017**
	Group + Time			**0.048**
Endo	Group	0.803	<0.001	**<0.001**
	Time			0.096
	Group + Time			0.077
Meso	Group	0.426	<0.001	**<0.001**
	Time			0.974
	Group + Time			0.824
Ecto	Group	0.643	<0.001	**<0.001**
	Time			0.977
	Group + Time			0.803

[1]Systolic Blood Pressure (BP), Waist Circumference (WC), Waist to Height Ratio (WC/HT), Waist to Hip Ratio (WC/Hip), Body Fat (BF), Fat Free Mass (FFM), Mid-Arm Circumference, Trunk-Extremity Ratio (TER)

Table A.9 Multivariate analysis of fasting laboratory markers with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
TG	Group Time	0.373	<0.001	**<0.001** 0.249
NEFA	Group Time	0.173	0.030	0.143 **0.029**
HOMA-IR	Group Time	0.279	0.002	**0.002** 0.352
ALT	Group Time	0.367	<0.001	**<0.001** 0.241
ALT[1]	Group Time	0.785	<0.001	**<0.001** 0.076
AST	Group Time	0.238	0.003	**0.003** 0.270
GGT	Group Time	0.497	<0.001	**<0.001** 0.580
HDL	Group Time	0.670	<0.001	**<0.001** 0.917
TC/HDL Ratio	Group Time	0.352	<0.001	**<0.001** 0.943
CRP	Group Time	0.152	0.049	**0.026** 0.393
IL-6	Group Time	0.282	0.020	**0.003** 0.571
Uric acid	Group Time	0.569	<0.001	**<0.001** 0.751
Apo-B48	Group Time	0.520	<0.001	<0.001 0.500
Apo-B100	Group Time	0.692	<0.001	**<0.001** **<0.001**
Adiponectin	Group Time	0.270	0.016	**0.022** 0.782

[1] Categorical variables: ALT (above and below 20 U/L).

[2]Abbreviations: Triglycerides (TG), Non-esterified free fatty acids (NEFA), homeostasis model of assessment of insulin resistance (HOMA-IR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), High-density lipoprotein (HDL), Total Cholesterol/HDL Ratio (TC/HDL Ratio) C-reactive protein (CRP), Interleukin-6 (IL-6), Apolipoprotein B-48 (Apo-B48), Apolipoprotein B-100 (Apo-B100).

Table A.10 Multivariate analyss of fasting laboratory markers

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
Insulin	Group Time Group + Time	0.265	0.008	**0.002** 0.465 0.160
HOMA-IR*	Group Time Group + Time	0.765	<0.001	**<0.001** **0.005** 0.087
ALT*	Group Time Group + Time	0.792	<0.001	**0.045** **<0.001** 0.386

[1] Categorical variables: ALT (above and below 20 U/L).
[2] Abbreviations: homeostasis model of assessment of insulin resistance (HOMA-IR), alanine aminotransferase (ALT),

A.11 Multivariate analysis of dietary variables with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
Kcal	Group Time	0.112	0.056	0.348 **0.044**
Pro (gr)	Group Time	0.189	0.004	**0.001** 0.410
Pr %	Group Time	0.210	0.001	**0.006** 0.104
Cho %	Group Time	0.183	0.005	**0.010** 0.061
Fat (gr)	Group Time	0.180	0.005	0.159 **0.006**
Fat %	Group Time	0.170	0.007	0.195 **0.008**
Sat Fat (gr)	Group Time	0.154	0.013	0.366 **0.009**
Sat Fat %	Group Time	0.117	0.047	0.722 **0.023**
Mufa (gr)	Group Time	0.116	0.049	0.283 **0.044**
Diet Cholesterol	Group Time	0.125	0.036	**0.014** 0.370
Potassium	Group Time	0.017	0.762	0.557 0.652
Sodium	Group Time	0.009	0.009	**0.004** 0.366

[1] Abbreviations: Proteins (Pr), Carbohydrates (Cho), Saturated Fat (Sat Fat), Mono unsaturated fat (Mufa)

Table A.12 Multivariate analysis of dietary variables with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
Pro (gr)	Group	0.203	0.014	**0.001**
	Time			0.373
	Group + Time			0.587
Cho %	Group	0.252	0.002	**0.020**
	Time			**0.018**
	Group + Time			0.067
[1] Abbreviations: Proteins (Pr) and Carbohydrates (Cho)				

Table A.13 Multivariate analysis of GI/GL/Fructose with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
GI	Group	0.138	0.023	0.288
	Time			**0.017**
GL	Group	0.137	0.024	0.689
	Time			**0.011**
T Fructose (gr)	Group	0.172	0.007	0.018
	Time			0.053
HFCS	Group	0.143	0.020	**0.042**
	Time			0.079
Free Fructose (gr)	Group	0.172	0.007	**0.001**
	Time			0.484
[1]Abbreviations: Glycemic Index (GI), Glycemic Load (GL), T Fructose, (Total Fructose), High Fructose Corn Syrup (HFCS)				

A.14 Multivariate analysis of GI/GL/fructose with group and time

Dependent Variable	Independent Variable	r^2	p-value of the Model	p-value/Indep. Variable
GI	Group	0.263	0.001	0.518
	Time			**0.007**
	Group + Time			**0.008**
GL	Group	0.244	0.003	0.900
	Time			**0.002**
	Group + Time			**0.017**
T Fructose (gr)	Group	0.245	0.003	**0.027**
	Time			**0.021**
	Group + Time			0.059
Free Fructose (gr)	Group	0.281	0.009	**0.002**
	Time			0.295
	Group + Time			**0.013**
[1]Abbreviations: Glycemic Index (GI), Glycemic Load (GL), T Fructose, (Total Fructose), High Fructose Corn Syrup (HFCS)				

Table A.15 Comparison of children with simple steatosis and nonalcoholic steatohepatitis

	Children with SS n=8			Children with NASH n=4		
Variable	**Baseline n=8**	**3 Months n=5**	**6 Months n=5**	**Baseline n=4**	**3 Months n=3**	**6 Months n=2**
Weight	86.9 ± 21.4	93.3 ± 20.5	91.7 ± 15.0	110.6 ± 32	117 ± 28.2	122.8 ± 21
BMI-z score	2.2 ± 0.2	2.1 ± 0.3	2.0 ± 0.2	2.4 ± 0.4	2.3 ± 0.6	2.5 ± 0.5
WC	100.8 ±12.1	99.6 ± 11.8	94.1 ± 10.8	105.8 ± 8.7	109 ± 13.2	113 ± 2.1
Body Fat%	36.0 ± 5.4	31.2 ± 6.8	28.3 ± 7.3	37.0 ± 9.0	36.2 ± 7.6	33.9 ± 12.6
SBP	121 ± 2	108 ± 8	115 ± 12	128 ± 12	113 ± 13	105 ± 21
HOMA-IR	6.5 ± 2.1	4.1 ± 1.8	7.1 ± 2.4	19.3 ± 17.6	7.4 ± 3.5	5.1 ± 0.5
Insulin	29 ± 9	19 ± 7	33 ± 13	77 ± 69	34 ± 13	24 ± 2
ALT	71 ± 54	35 ± 12	68 ± 53	42 ± 14	53 ± 20	37 ± 5
AST	43 ± 18	27 ± 4	42 ± 18	28 ± 4	34 ± 0.7	26 ± 2
GGT	24 ± 9	11 ±2	13 ± 6	16 ± 13	32 ± 6	30 ± 7
Triglycerides	1.7 ± 0.3	1.6 ± 0.5	1.7 ± 0.3	1.6 ±1.5	1.3 ± 0.6	1.3 ± 0.9
HDL	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	0.8 ± 0.0	0.9 ± 0.1	0.8 ± 0.0
LDL	2.6 ± 0.8	2.0 ± 0.7	2.0 ± 0.5	2.2 ± 1.4	3.5 ± 1.8	3.0 ± 1.7
NEFA	0.47 ± 0.28	0.28 ± 0.02	0.25 ± 0.15	0.44 ± 0.05	0.45 ± 0.01	0.38 ± 0.12
Apo-B100	471 ± 99	234 ± 66	323 ± 144	613 ± 149	385 ± 220	NA
Apo-B48	5.6 ± 1.3	5.8 ± 1.0	5.3 ± 0.8	4.9 ± 1.6	3.9 ± 0.1	4.0 ± 0.3
TNF-alpha	1.6 ± 0.3	1.7 ± 0.3	1.4 ± 0.2	1.3 ± 0.3	1.6 ± 0.0	1.7 ± 0.2
IL-6	0.7 ± 0.3	0.4 ± 0.3	0.9 ± 0.5	0.8 ± 0.3	1.2 ± 0.2	1.0 ± 0.3
IL-10	3.6 ± 1.7	4.6 ± 0.9	4.9 ± 0.9	5.6 ± 0.7	5.3 ± 0.5	3.3 ± 0.4
Total fructose (gr)	32.6 ± 19.7	14.8 ± 3.7	22.2 ± 13.6	28.3 ± 18.3	13.4 ± 9.4	24.2 ± 8.5
HFCS (gr)	4.9 ± 7.7	0.0 ± 0.0	1.3 ± 2.9	15.4 ± 19.1	0.0 ± 0.0	8.7 ± 12.3
GI	60.8 ± 10.5	30.0 ± 14.2	44 ± 6	58.0 ± 9.5	43.7 ± 19.6	34 ± 2
GL	125 ± 32	76 ± 34	96 ± 19	98 ± 50	55 ± 17	65 ± 20

Abbreviations: Body Mass Index (BMI), Waist Circumference (WC), Systolic Blood Pressure (SBP), Non-esterified free fatty acids (NEFA), homeostasis model of assessment of insulin resistance (HOMA-IR), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), High-density lipoprotein (HDL), Los density lipoprotein (LDL), Interleukin-6 (IL-6), Apolipoprotein B-48 (Apo-B48), Apolipoprotein B-100 (Apo-B100), High fructose corn syrup (HFCS), Glycemic index (GI) and Glycemic load (GL).

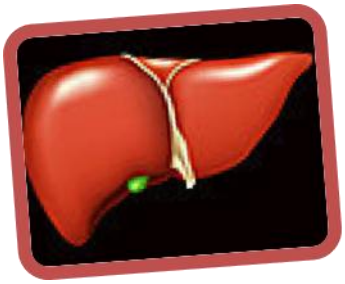
FRAGILE Study Recruitment Flier

HELP US UNDERSTAND WHAT IS THE BEST DIET FOR CHILDREN WITH LIVER DISEASE

WE NEED PARTICIPANTS FOR THE FRAGILE STUDY

Sugars and fats eaten in excess are accumulated as fat in your body, and can damage your liver…
…specially foods like processed food, fast food, sodas, juices and bakery goods.

In the FRAGILE diet we teach you and your child how to choose a diet low in sugars and fats, in order to have a healthy liver.



FRAGILE does not prevent children from eating what they like – we will teach you to know how often they can have each food in their diet, and the diet will not be difficult to follow.

We would like to talk to you if your child:

- Does not have any medical conditions

Participation in FRAGILE is really simple

FOR MORE INFORMATION,
PLEASE CONTACT US
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Quesstionnaire A.1 FELS Physical Activity Questionnaire for Children (PAQC)

Thank you for being here today and for helping us by answering this questionnaire. We would like you to work through this questionnaire and answer all the questions set out here as best you can. Please write your name on the questionnaire. We will not show the answers to this questionnaire to anyone outside our study. This is not a test, there are no right or wrong answers. We want you to answer all questions if you can. So take your time in answering the questions. We will be happy to answer any questions you might have, so please ask if you are not sure about what something means or how to fill out the questionnaire. You can answer your questions directly in the box that is below each question. There are four questions to this questionnaire. Each question is on a separate page. Please write directly on the paper the question is listed in the box below the question.

Name: _____ Date: _____

1. In the last year, what sports did you play in school?

I played_____ 3 Regularly 2 Often 1 Sometimes
I played_____ 3 Regularly 2 Often 1 Sometimes
I played_____ 3 Regularly 2 Often 1 Sometimes
I played_____ 3 Regularly 2 Often 1 Sometimes

2. In the last year, what sports or physically active games did you play outside of school?

I played_____ 3 Regularly 2 Often 1 Sometimes
I played_____ 3 Regularly 2 Often 1 Sometimes
I played_____ 3 Regularly 2 Often 1 Sometimes
I played_____ 3 Regularly 2 Often 1 Sometimes

3. When I play sports or games I sweat?

Very often 5 often 4 sometimes 3 seldom 2 never 1

4. During leisure time I play sports

Very often 5 often 4 sometimes 3 seldom 2 never 1

5. During leisure time I watch television or read:

Very often 5 often 4 sometimes 3 seldom 2 never 1

6. Do you walk and/or bicycle to and from school?

Very often 5 often 4 sometimes 3 seldom 2 never 1

7. What chores do you do at home, that are physically active and how often do you do them?

Chore _____ regularly 3 often 2 sometimes 1
Chore _____ regularly 3 often 2 sometimes 1
Chore _____ regularly 3 often 2 sometimes 1

8. When I do chores I sweat

Never 5 Seldom 4 Sometimes 3 Often 2 Very Often 1

Appendix 1 Questionnaire A.2 The Habitual Activity Estimation Scale (HAES)

This questionnaire will ask you questions about your daily activities. Please read all of the instructions carefully and answer each question as truthfully as you can.

Subject Number: _____ Date: _____

INSTRUCTIONS (please read!)

Please recall the activities of *one typical weekday* (choose from Tuesday, Wednesday or Thursday) and *one typical Saturday* within the past 2 weeks. For each given time period, please estimate the percentage of time that you spent in each of 4 different activity levels. For each of the time periods, the total time spent in all activity levels must add up to 100%.

The different activity levels are described below:

ACTIVITY LEVEL DESCRIPTIONS

These descriptions give you examples of activities that are typical of each activity level. You should refer back to these descriptions as often as you need when completing your estimates.

a) **inactive** – *lying down*, sleeping, resting, napping
b) **somewhat inactive** – *sitting*, reading, watching television, playing video games, time in front of the computer, playing games or activities which are mostly done sitting down
c) **somewhat active** – *walking*, shopping, light household chores
d) **very active** – *running*, jumping, skipping, bicycling, skating, swimming, games that require lots of movement and make you breathe/sweat hard

Following is a sample of a completed time period:

SAMPLE: From when you finished breakfast until when you started lunch, please estimate the percentage of time that you spent in each of the following activity levels:

a) inactive	5% (i.e., having a nap)
b) somewhat inactive	60% (i.e., watching TV)
c) somewhat active	25% (i.e., shopping)
d) very active	10% (i.e., riding a bicycle)
TOTAL	100%

WEEKDAY ACTIVITY For *one typical weekday in the past 2 weeks*, (choose from one of Tuesday, Wednesday or Thursday), please estimate the percentage of time that you spent in each activity level.

1. After getting out of bed until starting breakfast:

a) inactive _____ %
b) somewhat inactive _____ %
c) somewhat active _____ %
d) very active _____ %

TOTAL	100%

2. After finishing breakfast until starting lunch:

a) inactive _____ %
b) somewhat inactive _____ %
c) somewhat active _____ %
d) very active _____ %

TOTAL	100%

3. After finishing lunch until starting supper:

a) inactive _____ %

b) somewhat inactive _____ %

c) somewhat active _____ %

d) very active _____ %

TOTAL	100%

4. After finishing supper until bedtime:

a) inactive _____ %

b) somewhat inactive _____ %

c) somewhat active _____ %

d) very active _____ %

TOTAL	100%

For the *typical weekday* that you are referring to, please answer the following questions as accurately as possible in the spaces provided.

5. At what time did you get out of bed in the morning? _____

6. At what time did you start eating breakfast? _____

7. How long did you spend eating breakfast? _____ minutes

8. At what time did you start eating lunch? _____

9. How long did you spend eating lunch? _____ minutes

10. At what time did you start eating supper? _____

11. How long did you spend eating supper? _____ minutes

12. At what time did you go to bed that evening? _____

13. For the *typical weekday* that this questionnaire has asked you about, please rate your overall level of activity (please circle one response only):

a) very inactive

b) inactive

c) somewhat inactive

d) somewhat active

e) active

f) very active

14. Is this "typical" Tuesday, Wednesday or Thursday that you described in this questionnaire (please circle one response only):

a) a lot like most weekdays

b) a little bit like most weekdays

c) a little bit different from most weekdays

d) a lot different from most weekdays

SATURDAY ACTIVITY

For *one typical Saturday in the past 2 weeks*, please estimate the percentage of time that you spent in each activity level.

15. After getting out of bed until starting breakfast:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

16. After finishing breakfast until starting lunch:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

17. After finishing lunch until starting supper:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

18. After finishing supper until bedtime:

a) inactive	_____ %
b) somewhat inactive	_____ %
c) somewhat active	_____ %
d) very active	_____ %
TOTAL	100%

For the *typical Saturday* that you are referring to, please answer the following questions as accurately as possible in the spaces provided.

19. At what time did you get out of bed in the morning? _____

20. At what time did you start eating breakfast? _____

21. How long did you spend eating breakfast? _____ minutes

22. At what time did you start eating lunch? _____

23. How long did you spend eating lunch? _____ minutes

24. At what time did you start eating supper? _____

25. How long did you spend eating supper? _____ minutes

26. At what time did you go to bed that evening? _____

27. For the *typical Saturday* that this questionnaire has asked you about, please rate your overall level of activity (please circle one response only):

a) very inactive
b) inactive
c) somewhat inactive
d) somewhat active
e) active
f) very active

28. Is the "typical" Saturday that you described in this questionnaire (please circle one response only):

a) a lot like most Saturdays
b) a little bit like most Saturdays
c) a little bit different from most Saturdays
d) a lot different from most Saturdays

29. If you have any comments about your activity patterns that you think are important, please mention them on the back of this page. Thank-you.

Appendix B Focus Group Study

UNIVERSITY OF ALBERTA

Form B-1 Consent Form (Control Group)

Title of the Project: Exploring Parent's knowledge, attitudes and beliefs about nutrition and lifestyle management in children and adolescents with nonalcoholic fatty liver disease (NAFLD).

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Jason Yap, MD Telephone: 780-248-5420
Study Coordinator: Ingrid Rivera, Msc Student Telephone: 780-991-1962

Please circle YES or NO to the statements below related to the information in the information sheet.

Do you understand that you have been asked to be in a research study?	YES NO
Have you read and received a copy of the attached Information Sheet?	YES NO
Do you understand the benefits and risks involved in taking part in this research study?	YES NO
Have you had an opportunity to ask questions and discuss this study?	YES NO
Have your questions been answered by the Information Sheet?	YES NO
Do you understand that you are free to withdraw from the study at any time, without having to give a reason?	YES NO
Has confidentiality been explained to you on the Information Sheet?	YES NO
Do you understand that only the research team will have access to the data?	YES NO
Do you consent to being digitally recorded (no names will be identified)?	YES NO
Do you consent to being interviewed as part of a focus group?	YES NO

Participant Signature: _____ Date & Time_____

(Printed Name)_____

Signature of Witness_____ Date & Time_____

Signature of Investigator or Designee_____ Date & Time_____

U N I V E R S I T Y O F A L B E R T A

B-2 Consent Form (Parents of childen with NAFLD)

Title of the Project: Exploring Parent's knowledge, attitudes and beliefs about nutrition and lifestyle management in children and adolescents with nonalcoholic fatty liver disease (NAFLD).

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Jason Yap, MD Telephone: 780-248-5420
Study Coordinator: Ingrid Rivera, Msc Student Telephone: 780-991-1962

Please circle YES or NO to the statements below related to the information in the information sheet.

Do you understand that you have been asked to be in a research study?	YES NO
Have you read and received a copy of the attached Information Sheet?	YES NO
Do you understand the benefits and risks involved in taking part in this research study?	YES NO
Have you had an opportunity to ask questions and discuss this study?	YES NO
Have your questions been answered by the Information Sheet?	YES NO
Do you understand that you are free to withdraw from the study at any time, without having to give a reason?	YES NO
Has confidentiality been explained to you on the Information Sheet?	YES NO
Do you understand that only the research team will have access to the data?	YES NO
Do you consent to being digitally recorded (no names will be identified)?	YES NO
Do you consent to being interviewed as part of a focus group?	YES NO

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Participant Signature: _____ Date & Time_____

(Printed Name)_____

Signature of Witness_____ Date & Time_____

Signature of Investigator or Designee_____ Date & Time_____

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U N I V E R S I T Y O F A L B E R T A

B-3 Infromation letter (Healthy control group)

Title of the Project: Exploring Parent's knowledge, attitudes and beliefs about nutrition and lifestyle management in children and adolescents with nonalcoholic fatty liver disease (NAFLD).

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Jason Yap, MD Telephone: 780-248-5420
Study Coordinator: Ingrid Rivera, Msc Student Telephone: 780-991-1962

Purpose of the study:

We would like to gain an understanding of what your general knowledge is about nutrition and what nutrition information you think is important to be provided, if you were to receive nutrition education about your child's diet.

Procedures of the study, what do I have to do?

We would like to conduct a focus group with you, to get your experience, opinions and thoughts about what you know about general nutrition and the type of nutrition education you think should be provided about your child's diet. We will ask you to come to the clinical research unit (CRU) at the University of Alberta to participate in a focus group with other parents. We will ask you and other participants in the group to answer some questions. To make sure we can fully understand your answers, we will record your answers with the use of a digital recorder. The focus group will take a maximum of 45-60 minutes.

At the end of the focus group, we will also ask you two other things.

Questionnaire:

The first thing will be to fill out a questionnaire about how your child feels about eating and participating in physical activity. This will help us to understand what factors may influence what your child likes to eat. This will take you about 10 minutes. You can fill this out in a room by yourself in the Clinical Research Unit.

Height and Weight: We will ask you to let us measure your height and weight in the Clinical Research Unit. We will also ask you tell us what your child's weight, height and age is. We will do this in a private room where only the graduate student and yourself will be present. This will only take about 5 minutes to complete.

Benefits of the study.

The results of this study will help us to understand how to create better nutrition programs that will be easy for parents and children to understand and follow.

Risks of the study:

All of the additional tests used in this study are harmless.

Confidentiality:

We will not share any information with anyone. Any research data collected about you during this study will not identify you by name, only by your initials and a coded

number. The research team will not share your name with anyone outside the research clinic and your name will not be in any reports published from this research. We will ask all the other participants in the group not to share any of the information that was talked in the focus group.

The personal information collected in this study may need to be checked by the Health Research Ethics Board (HREB) at the University of Alberta. This may be necessary so the HREB can make sure that the data collected in the study is accurate. In the University of Alberta, study information is required to be kept for 7 years. Even if you withdraw from the study, the information, which is obtained from you the research, will not be destroyed.

Voluntary participation:

You don't have to take part in this study at all or you can quit at any time. No one will be upset with you if you decide that you don't want to do this or if you decide to stop part way through. You should tell your doctor or study investigators if you do not want to participate in this study. This will not affect the care that you and your child will receive by anyone within the clinic or at the Stollery Children's Hospital. You can still continue to see the dietitian, nurse and doctor without participating in this study.

Reimbursement:

We will reimburse you up to a maximum of $20 dollars for the cost of parking on the day of the focus group.

Do you have more questions?

You can ask the principal investigator, study coordinator about anything you don´t understand.

You can also talk to Dr Yap. Dr Jason Yap´s phone number is 780-248-5420. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Study Coordinator: Ingrid Rivera, Msc Student Telephone: 780-991-1962
e-mail: irivera@ualberta.ca

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U N I V E R S I T Y O F A L B E R T A

B-4 Information letter (Parents of children with NAFLD)

Title of the Project: Exploring Parent's knowledge, attitudes and beliefs about nutrition and lifestyle management in children and adolescents with nonalcoholic fatty liver disease (NAFLD).

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Co-Investigator: Dr. Jason Yap, MD Telephone: 780-248-5420
Study Coordinator: Ingrid Rivera, Msc Student Telephone: 780-991-1962

Purpose of the study:

We want to explore the perspective of parents/caregivers of children with NAFLD about whether or not the nutrition education you might have received about your child's diet was helpful or not. We would also like to gain an understanding of what your general knowledge is about nutrition and what you think about the nutrition education (if any) you have been provided about your child's diet .

Procedures of the study, what do I have to do?

We would like to conduct a focus group with you, the parents/caregivers of children with fatty liver, to get your experience, opinions and thoughts about the type of nutrition education you have been provided about your child's diet. We will ask you to come to the clinical research unit (CRU) at the University of Alberta to participate in a focus group with other parents of children who have a fatty liver. We will ask you some questions and ask you and the other participants in the group to answer these questions. To make sure we can fully understand your ansewrs, we will record your answers with the use of a digital recorder. The focus group will take a maximum of 45-60 minutes. At the end of the focus group, we will also ask you to two other things:

Questionnaire:

The first thing will be to fill out a questionnaire that asks you some questions about how your child feels about eating and about participating in physical activity. This will help us to understand what factors influence what your child likes to eat and how this might influence your ability to help your child to follow the nutrition advice that you are given in clinic. This will take you about 10 minutes to fill out. You can fill this out in a room by yourself in the Clinical Research Unit.

Height and Weight: The second thing we will ask you to do is to let us measure your height and weight in the Clinical Research Unit. We will also ask you tell us what your child's weight and height and what your age is. We will do this in a private room where only the graduate student and yourself will be present. This information will help us understand the type of dietary advice you may have been provided and whether or not your child's weight is within a healthy weight range. This will only take about 5 minutes to complete.

Benefits of the study.

The results of this study will help us to understand how to create better nutrition programs that will be easy for parents and children to understand and follow.

Risks of the study: All of the additional tests used in this study are harmless.

Who will be interviewed?
You and other parents of children with fatty liver disease will participate in the focus group together. The questionnaire and your height and weight will be done in private in a separate room. Only the graduate student and you will be present for your height and weight measurement.

Confidentiality:
We will not share any information with anyone. Any research data collected about you during this study will not identify you by name, only by your initials and a coded number. Your name will not be shared with by the research team with anyone outside the research clinic and your name will not be in any reports published from this research. We will ask all the other participants in the group not to share any of the information that was talked about in the focus group. However, we cannot promise that the other parents will not talk about what was discussed in the focus group with other people.

The personal information collected in this study may need to be checked by the Health Research Ethics Board (HREB) at the University of Alberta. This may be necessary so the HREB can make sure that the data collected in the study is accurate. In the University of Alberta, study information is required to be kept for 7 years. Even if you withdraw from the study, the information, which is obtained from you the research, will not be destroyed.

Voluntary participation:
You don't have to take part in this study at all or you can quit at any time. No one will be upset with you if you decide that you don't want to do this or if you decide to stop part way through. You should tell your doctor or study investigators if you do not want to participate in this study. This will not affect the care that you and your child will receive by anyone within the clinic or at the Stollery Children's Hospital. You can still continue to see the dietitian, nurse and doctor without participating in this study.

Reimbursement:
We will reimburse you up to a maximum of $20 dollars for the cost of parking on the day of the focus group.

Do you have more questions?
You can ask the principal investigator, study coordinator about anything you don´t understand. You can also talk to Dr Yap. Dr Jason Yap´s phone number is 780-248-5420. If you have any problems or concerns about any part of this study please call the Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

Principal Investigator: Diana Mager, PhD RD Telephone: 780-492-7687
Study Coordinator: Ingrid Rivera, Msc Student Telephone: 780-991-1962
e-mail: irivera@ualberta.ca

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Questionnaire B.1 Lifestyle Behavior Checklist

Below is a list of behaviours parents with children often have to manage. For each item: (1) circle the number that best describes how much of a behaviour has been with your child in the last month, and (2) rate how confident you are in dealing with it. If that behaviour is not currently occurring, rate how confident you are that you could successfully deal with your child´s behaviour if it did occur. Remember to put a confidence rating for every item.

Rate your confidence from 1 (Certain I can't do it) to 10 (Certain I can do it).

		TO WHAT EXTENT HAS THIS BEHAVIOUR BEEN A PROBLEM FOR YOU WITH YOUR CHILD?							HOW CONFIDENT ARE YOU IN DEALING WITH IT?
		Not at all	A little		Somewhat		Much	Very much	
1.	Eats too quickly	1	2	3	4	5	6	7	☐
2.	Eats too much	1	2	3	4	5	6	7	☐
3.	Eats unhealthy snacks	1	2	3	4	5	6	7	☐
4.	Whinges or whines about food	1	2	3	4	5	6	7	☐
5.	Yells about food	1	2	3	4	5	6	7	☐
6.	Throws a tantrum about food	1	2	3	4	5	6	7	☐
7.	Refuses to eat certain foods (i.e. fussy eating)	1	2	3	4	5	6	7	☐
8.	Argues about food (e.g. when you say *No more*)	1	2	3	4	5	6	7	☐
9.	Demands extra helpings at meals	1	2	3	4	5	6	7	☐
10.	Requests food continuously between meals	1	2	3	4	5	6	7	☐
11.	Demands food when shopping or on outings	1	2	3	4	5	6	7	☐
12.	Sneaks food when they know they are not supposed to	1	2	3	4	5	6	7	☐
13.	Hides food	1	2	3	4	5	6	7	☐
14.	Steals food (e.g. from other children's lunchboxes)	1	2	3	4	5	6	7	☐
15.	Eats food to comfort themselves when feeling let down or depressed	1	2	3	4	5	6	7	☐
16.	Watches too much television	1	2	3	4	5	6	7	☐
17.	Spends too much time playing video or computer games	1	2	3	4	5	6	7	☐

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	TO WHAT EXTENT HAS THIS BEHAVIOUR BEEN A PROBLEM FOR YOU WITH YOUR CHILD?							HOW CONFIDENT ARE YOU IN DEALING WITH IT?
	Not at all	A little		Somewhat		Much	Very much	
18. Complains about doing physical activity (e.g. *This is boring, I'm too tired, My leg hurts*)	1	2	3	4	5	6	7	☐
19. Refuses to do physical activity	1	2	3	4	5	6	7	☐
20. Complains about being unfit or feeling low in energy	1	2	3	4	5	6	7	☐
21. Complains about being overweight	1	2	3	4	5	6	7	☐
22. Complains about being teased	1	2	3	4	5	6	7	☐
23. Complains about not having enough friends	1	2	3	4	5	6	7	☐
24. Complains about being unattractive	1	2	3	4	5	6	7	☐
25. Complains about not fitting into clothes	1	2	3	4	5	6	7	☐

Table B.1 Interview guide-focus group

Questions	
1.- Have you received nutritional education in the past?, Can you tell me more about the nutritional education that you received? **Probe: what type of health professional?**	Perception
2.- Now I want to know if was there anything you found helpful about the nutrition education provided? **Probe: Was there any tool or information that you found helpful?**	Attitude
3.- Can you tell if you were able to understand all of the terms used. Can you think of any examples? **Probe: was it given examples to apply those terms?**	Understandability
4.- I want to know in what ways you help your child with his/her diet Probe 1: Making his/her meals	Behavior: Role modeling
5.- Currently what do you know about the nutrition for your children or adolescent? Probe 1: What types of food choices are better for him/her?	Knowledge Beliefs
6.- In your opinion, how it should be a healthy diet for children with NAFLD? **Probe 1: How many food servings of each food group per day are necessary for your child?** **Probe 2: How restrict it should be?**	Beliefs/Perception
7.- Is there any other nutritional information that you would like to receive from health professionals? **Probe: Menus, snack ideas, more constant interviews with dieticians, books or web pages recommendation**	Facilitators
8.- Now I would like to know what has been the most difficult for you in helping your child to eat healthy? **Probe: can you describe more those difficulties?**	Barriers
9.- Can you describe me in what ways or how do you encourage your child to eat a variety of healthy foods? **Probe: Can you tell me more about it? Can you give me more examples?**	Behavior: Role modeling

B.2 Multivariate analysis of problem scale items with parental BMI

Question	Problem Scale			
	Independent variable	**r^2**	**p-Model**	**p-Independent variable**
2. Eats too much food	Parent BMI Group Parent BMI*Group	0.90	0.002	0.079 0.007 0.002
8. Argues about food (e.g. when you say No more)	Parent BMI Group Parent BMI*Group	0.80	0.003	0.288 0.036 0.093
9. Demands extra helpings at meals	Parent BMI Group	0.45	0.064	0.426 0.300
17. Spends too much time playing video or computer games	Parent BMI Group Parent BMI*Group	0.74	0.009	0.030 0.018 0.048
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Parent BMI Group Parent BMI*Group	0.94	<0.001	0.765 0.005 0.003
19. Refuses to do physical activity	Parent BMI Group Parent BMI*Group	0.78	0.004	0.170 0.007 0.028
Total Score	Parent BMI Group	0.53	0.032	0.437 0.185

Table B.3 Multivariate analysis of problem scale with child BMI

Question	Problem Scale			
	Independent variable	**r^2**	**p-Model**	**p-Independent variable**
8. Argues about food (e.g. when you say No more)	Child BMI Group Child BMI * Group	0.81	0.006	0.204 0.140 0.063
9. Demands extra helpings at meals	Child BMI Group Child BMI * Group	0.60	0.075	0.370 0.101 0.063
17. Spends too much time playing video or computer games	Child BMI Group Child BMI * Group	0.76	0.012	0.050 0.109 0.095
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Child BMI Group	0.89	0.001	0.063 0.139
19. Refuses to do physical activity	Child BMI Group Child BMI * Group	0.85	0.002	0.008 0.048 0.053
Total Score	Child BMI Group	0.43	0.100	0.752 0.447

B.4 Multivariate analysis of problem scale items with parental age

Question	Problem Scale			
	Independent variable	r^2	p-Model	p-Independent variable
2. Eats too much food	Parent Age Group	0.68	0.005	0.473 0.002
8. Argues about food (e.g. when you say No more)	Parent Age Group	0.71	0.003	0.817 0.001
9. Demands extra helpings at meals	Parent Age Group	0.43	0.076	0.578 0.027
17. Spends too much time playing video or computer games	Parent Age Group Parent Age*Group	0.77	0.006	0.064 0.185 0.051
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Parent Age Group Parent Age*Group	0.91	0.001	0.033 0.268 0.022
19. Refuses to do physical activity	Parent Age Group Parent Age*Group	0.86	0.008	0.003 0.018 0.004
Total Score	Parent Age Group	0.50	0.043	0.879 0.014

Table B.5 Multivariate analysis of problem scale items with child age

Question	Problem Scale			
	Independent variable	r^2	p-Model	p-Independent variable
2. Eats too much food	Child Age Group	0.67	0.019	0.580 0.012
8. Argues about food (e.g. when you say No more)	Child Age Group	0.67	0.019	0.591 0.012
9. Demands extra helpings at meals	Child Age Group	0.38	0.184	0.343 0.078
17. Spends too much time playing video or computer games	Child Age Group Child Age * Group	0.84	0.007	0.013 0.117 0.020
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Child Age Group Child Age * Group	0.93	0.007	0.031 0.840 0.059
19. Refuses to do physical activity	Child Age Group Child Age * Group	0.91	0.001	0.002 0.032 0.003
Total Score	Child Age Group	0.41	0.153	0.768 0.066

Table B.6 Multivariate analysis of confidence scale items with parental BMI

Question	Confidence Scale			
	Independent variable	r^2	p-Model	p-Independent variable
1. Eats too quickly	Parent BMI Group Parent BMI*Group	0.54	0.082	0.949 0.036 0.065
2. Eats too much food	Parent BMI Group Parent BMI*Group	0.90	0.002	0.079 0.007 0.002
5. Yells about food	Parent BMI Group	0.60	0.015	0.022 0.665
8. Argues about food (e.g. when you say No more)	Parent BMI Group Parent BMI*Group	0.80	0.003	0.288 0.036 0.093
9. Demands extra helpings at meals	Parent BMI Group	0.45	0.064	0.426 0.300
17. Spends too much time playing video or computer games	Parent BMI Group Parent BMI*Group	0.74	0.009	0.960 0.018 0.048
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Parent BMI Group Parent BMI*Group	0.94	<0.001	0.765 0.005 0.003
19. Refuses to do physical activity	Parent BMI Group Parent BMI*Group	0.78	0.004	0.170 0.007 0.028
20. Complains about being unfit or feeling low in energy	Parent BMI Group Parent BMI*Group	0.63	0.037	0.315 0.028 0.071
Total Score	Parent BMI Group	0.53	0.032	0.437 0.185

Table B.7 Multivariate analysis of confidence scale items with child BMI

Question	Confidence Scale			
	Independent variable	r^2	p-Model	p-Independent variable
1. Eats too quickly	Child BMI Group	0.42	0.111	0.726 0.204
2. Eats too much food	Child BMI Group	0.69	0.008	0.473 0.270
5. Yells about food	Child BMI Group	0.17	0.467	0.540 0.315
8. Argues about food (e.g. when you say No more)	Child BMI Group Child BMI * Group	0.81	0.006	0.204 0.140 0.063
9. Demands extra helpings at meals	Child BMI Group Child BMI * Group	0.60	0.075	0.370 0.101 0.063
17. Spends too much	Child BMI	0.76	0.012	0.050

time playing video or computer games	Group Child BMI * Group			0.109 0.095
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Child BMI Group	0.89	0.001	0.063 0.139
19. Refuses to do physical activity	Child BMI Group Child BMI * Group	0.85	0.002	0.008 0.048 0.053
20. Complains about being unfit or feeling low in energy	Child BMI Group	0.23	0.340	0.948 0.543
Total Score	Child BMI Group	0.43	0.100	0.752 0.447

Table B.8 Multivariate analysis of confdence scale items with parental age

Question	**Confidence Scale**			
	Independent variable	**r^2**	**p-Model**	**p-Independent variable**
1. Eats too quickly	Parent Age Group	0.25	0.260	0.759 0.122
2. Eats too much food	Parent Age Group	0.68	0.005	0.473 0.002
5. Yells about food	Parent Age Group	0.43	0.074	0.134 0.053
8. Argues about food (e.g. when you say No more)	Parent Age Group	0.71	0.003	0.817 0.001
9. Demands extra helpings at meals	Parent Age Group	0.43	0.076	0.578 0.027
17. Spends too much time playing video or computer games	Parent Age Group Parent Age*Group	0.77	0.006	0.064 0.185 0.051
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Parent Age Group Parent Age*Group	0.91	0.001	0.033 0.268 0.022
19. Refuses to do physical activity	Parent Age Group Parent Age*Group	0.86	0.008	0.003 0.018 0.004
20. Complains about being unfit or feeling low in energy	Parent Age Group Parent Age*Group	0.67	0.022	0.037 0.070 0.032
Total Score	Parent Age Group	0.50	0.043	0.879 0.014

Table B.9 Multivariate analysis of confidence scale items with child age

Question	Confidence Scale			
	Independent variable	**r^2**	**p-Model**	**p-Independent variable**
1. Eats too quickly	Child Age Group	0.50	0.084	0.845 0.045
2. Eats too much food	Child Age Group	0.67	0.019	0.580 0.012
5. Yells about food	Child Age Group	0.29	0.301	0.257 0.173
8. Argues about food (e.g. when you say No more)	Child Age Group	0.67	0.019	0.591 0.012
9. Demands extra helpings at meals	Child Age Group	0.38	0.184	0.343 0.078
17. Spends too much time playing video or computer games	Child Age Group Child Age * Group	0.84	0.007	0.013 0.117 0.020
18. Complains about doing physical activity (e.g. This is boring, I'm too tired, My leg hurts)	Child Age Group Child Age * Group	0.93	0.007	0.031 0.840 0.059
19. Refuses to do physical activity	Child Age Group Child Age * Group	0.91	0.001	0.002 0.032 0.003
20. Complains about being unfit or feeling low in energy	Child Age Group Child Age * Group	0.65	0.077	0.047 0.126 0.056
Total Score	Child Age Group	0.41	0.153	0.768 0.066