Study Title: Effects of High-Intensity Interval Training Exercise in Adolescents with Hepatosteatosis

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Background and Rationale

Non-Alcoholic Fatty Liver Disease (NAFLD) is characterized by fatty infiltration (steatosis ≥ 5% of liver volume) and is the most common chronic liver disease of childhood. The estimated prevalence of NAFLD is 29% to 38% in obese children[1]. NAFLD is rare before puberty, but can be diagnosed during puberty. Routine screening for NAFLD is recommended in at-risk groups and is done via measurement of serum alanine aminotransferase (ALT) level, but this test has a low specificity[1]. Using Magnetic Resonance Imaging (MRI), a reference diagnostic test, we demonstrated that 40% of obese children (10-17 years) who attended to our weight management clinic had NAFLD. Twice the sex-specific ALT level, a recommended cut-point for further evaluation[1], had only 30% specificity in our cohort. Further, ALT levels do not correlate with the stage of steatosis or fibrosis.[2] Recent findings suggest that the degree of hepatic fat content (i.e., Intrahepatic triglyceride [IHTG] percent) in patients with NAFLD plays an independent role in progression of disease to advanced characterized by inflammation (Non-Alcoholic Steatohepatitis [NASH]) and fibrosis[3,4]. This notion is supported by the finding that among adult NAFLD patients with no baseline fibrosis, those with a higher baseline IHTG (≥15.7%) developed fibrosis at a much higher rate (OR 6.67, 95% CI:1.01-44.1, $p<0.05$) after a 1.75 year follow up[5].

Despite the high prevalence and associated comorbidities, patients with NAFLD are usually asymptomatic, and routine screenings are unlikely to identify the majority of the patients. Also, there are no FDA-approved medical therapies to treat NAFLD, and the current standard of care is limited to controlling risk factors via lifestyle modifications, which is more effective in earlier and reversible stages of the disease process. Affected individuals usually have cardiometabolic disorders.[6,7] NAFLD is strongly associated with Insulin Resistance (IR), dyslipidemia, and hypertension, and is an independent risk factor for cardiovascular disease (CVD) morbidity and mortality[8,9]. In line with the literature, we found that all children with NAFLD in our cohort had IR (i.e., Homeostatic Model Assessment [HOMA]-IR ≥ 5) and lower cardiorespiratory fitness (as assessed by continuous treadmill graded protocol) and majority had markers of CVD (dyslipidemia or hypertension), but only 25% had elevated ALT (higher than twice the upper limit for sex). IR in the liver, muscle, and adipose tissue increases as IHTG increases[10]. Therefore, interventions aiming to decrease IR and improve cardiorespiratory risk factors will likely decrease IHTG, which may confer significant long-term health benefits.

Clinical and Public Health Significance: Given unfavorable outcomes of youth-onset NAFLD compared to adult-onset, and increasing societal burden of the disease in parallel to rising childhood and adult obesity, every effort should be made at an early stage to mitigate the long term negative consequences of untreated disease. Unfortunately, adherence to lifestyle modifications is a real challenge for most adolescents.

Scientific Premise: The mechanisms underlying NAFLD in adolescents with obesity have not been well-defined. Hepatocellular fat deposition is influenced by various factors, including circulating lipid levels, hepatic lipid uptake, *de novo* lipogenesis, hepatocellular fatty acid oxidation, and export of lipids from the liver[11]. Steatosis results from an imbalance between the forces/mechanisms favoring versus preventing intrahepatic fat deposition[12]. IR is pivotal in the development of steatosis and progression to NASH[1,11,13] According to the widely accepted adipose tissue (AT) expandability hypothesis[14], lipids begin to accumulate in liver and other organs when the storage capacity of the AT is exceeded as a result of IR. An important premise and the empirical foundation for the current proposal rests on the relationships between NAFLD, IR, and cardiorespiratory fitness. Reversal of IR is associated with a decrease in IHTG and improvement of liver histology. In line with this, anti-diabetic mediations improve biochemical and/or histological findings in NAFLD, either directly through regulating hepatic lipid metabolism, or indirectly via increasing hepatic and peripheral insulin sensitivity[15]. In addition to IR, obesity is

associated with lower cardiorespiratory fitness[16], hypertension, increased heart rate variability[17], atherogenic lipid profile[18], and lower adiponectin levels. Obese subjects with NASH have even lower levels of adiponectin levels compared to obese with simple steatosis[19]. Supervised exercise, in as little as 4 weeks[20,21], independently improves hepatic steatosis even in the absence of significant weight reduction or change in body composition, and increases adiponectin levels[20,22–25]. Additionally, exercise is thought to improve fibroblast growth factor-21 (FGF21) signaling. FGF21 has been identified as a potential target for obesity and NAFLD treatment due to its strong association with both conditions. A multitude of studies show that FGF21 decreases IR, stimulates peripheral glucose uptake and lipid oxidation, and decreases lipogenesis, at least in part through increased adiponectin, and these effects are augmented via exercise[26–29].

Strategies aiming to decrease IR and increase fitness and adiponectin will likely reduce IHTG. *Given the positive relationship between IR and steatosis and the inverse relationship between fitness, adiponectin, and steatosis, we propose that the exercise-induced reduction in IHTG will be more pronounced in those with a more significant decrease in IR and increase in fitness and adiponectin.* No previous pediatric studies have examined the longitudinal relationship between IR, fitness and adiponectin in response to exercise training to reduce IHTG in adolescents with NAFLD. The objectives of this study are to assess the cardiometabolic effects of a unique form of exercise strategy, high-intensity interval training (HIIT)[30] on IHTG in adolescents with NAFLD and to examine and compare the relationship between IHTG, fitness, IR, and adiponectin.

Significance to Fundamental Science: In addition to clinical and public health significance, this project will begin to address how HIIT intervention, improved fitness and insulin action regulate IHTG metabolism, and provide novel insights as to the role of these factors in hepatic lipid homeostasis.

Review of Relevant Literature:

Decreased cardiorespiratory fitness is associated with NAFLD: Reduced fitness level is associated with NAFLD status and progression[31]. Higher baseline fitness, independent of body composition, is a predictor for the effectiveness of interventions for reducing IHTG in NAFLD patients[32].

HIIT decreases IHTG: HIIT, defined as alternating cycles of short periods of intense aerobic and anaerobic exercise at maximum capacity, increases aerobic and anaerobic fitness[30]. HIIT is a well-tolerated and efficient intervention that improves glucose metabolism and markers of CVD in overweight and obese children[33–35]. HIIT was effective in decreasing hepatic steatosis in adults with NAFLD[24,31,36,37], but studies assessing the effect of HIIT on IHTG in obese adolescents with NAFLD are lacking. In a recent study involving mostly pre-pubertal children with NAFLD, Labayen et al.[38] showed HIIT could effectively decrease IHTG, even when IHTG is marginally elevated (mean 5.6% in the treatment group).

HIIT may directly regulate hepatic lipid metabolism in a weight independent manner: Mechanistically, HIIT stimulates hepatic lipid metabolism via hepatocyte transcription factor peroxisome proliferator-activated receptor alpha (PPAR-α)[39,40] and AMP-activated protein kinase (AMPK)[41,42]. AMPK inhibits the synthesis of fatty acids, cholesterol, and triglycerides, and activates fatty acid uptake and oxidation. PPAR-α regulates beta-oxidation, mitochondrial function, and hepatic lipid metabolism[31]. Furthermore, PPAR-α is the upstream regulator for hepatic FGF21 expression[43]. Also, HIIT is associated with increased hepatocellular mitochondrial number and function[40]. These findings support the direct role of exercise on liver metabolism.

HIIT improves cardiometabolic profile in obese individuals: HIIT increases maximal oxygen uptake, decreases blood pressure and IR in overweight or obese children[44] and adults[45], and

could be more effective in improving such risk factors compared to continuous, or low to moderate intensity interval training exercise[46]. A single bout of HIIT decreases insulin and glucose levels in healthy adolescents[47]. Obese adolescents with higher IR at baseline had the most decrease in IR following a total of 6 sessions of HIIT over 2 weeks.[48] A multitude of adult studies[49,50] show similar effects of HIIT on glucose level. HIIT decreases IR[51–53] and increases adiponectin[54,55] in obese adolescents.

HIIT has a long-lasting positive effect on IHTG and cardiovascular risk factors: Tjønna et al.[56] demonstrated that 3 months of twice weekly HIIT in obese adolescents not only reduced CV risk factors immediately after the intervention, but also the effects were long-lasting. Specifically, fasting and 2-hour glucose, insulin, HOMA, blood pressure and $\dot{V}O_{2max}$ were all significantly different at 12-months post-intervention compared to baseline. Similar results, including reduction of IHTG, were observed in obese adults 12 months after the cessation of a one-year moderate to vigorous exercise intervention[57], but these results were not replicated in another adult study which could be due to small sample size (n=10)[58]. Pediatric studies assessing the long term effects of exercise interventions on NAFLD are lacking.

Objective/Specific Aims

Based on available literature and our pilot data (shown below), **we hypothesize that insulin resistance (IR) and decreased cardiac fitness are central to the NAFLD in adolescents, and the degree of steatosis is negatively correlated with lower fitness and higher IR**. **Thus, exercise-driven improvements in IHTG levels will be positively associated with the increase in fitness and the decrease in IR levels in adolescents with NAFLD.** We will test our hypotheses through the following specific aims:

Aim 1: Assess the change in IHTG, and CVD and IR markers in subjects with NAFLD after 4 weeks of HIIT, compared to a sex-matched control group also with NAFLD with no training.

1A. Quantitate the change in IHTG. Hypothesis: IHTG will decrease after HIIT, and the change will be greater than that seen in controls.

1B. Measure the changes in fitness and markers of CVD. Hypothesis: HIIT will significantly increase fitness, decrease heart rate variability and blood pressure, and improve atherogenic lipid profile. The changes will be greater than that seen in controls. Changes in fitness and IHTG will correlate.

1C. Estimate changes on IR. Hypothesis: HIIT will decrease IR especially in those with higher baseline IR, and the changes will be greater than that seen in controls. Changes in IR will correlate with changes in fitness and IHTG.

Aim 2: Determine the post-intervention effects of HIIT on IHTG, and CVD and IR markers 3 months after completion of the HIIT in adolescents with baseline NAFLD.

2A. Quantitate the change in IHTG percent. Hypothesis: HIIT will have a sustained positive effect on IHTG, and that IHTG 3-months post-HIIT will be significantly lower compared to baseline level.

2B. Estimate changes on IR and other CVD markers. Hypothesis: HIIT will have a sustained positive effect on IR, and that IR 3-months post-HIIT will be significantly lower compared to baseline level.

Pediatric NAFLD has serious cardio-metabolic health outcomes. Findings of this study will inform clinical providers in the use of HIIT as a modality to treat adolescent NAFLD and associated morbidities, and produce preliminary data for mechanistic studies to probe pathways implicated in the pathogenesis of NAFLD.

Preliminary Results

We have successfully recruited 59 obese children for a pilot study and characterized their metabolic profile, including IHTG (via MRI)[59]. Forty percent of children had NAFLD in this cohort. Children with NAFLD have decreased cardiorespiratory fitness: We assessed fitness using continuous treadmill graded protocol[60]. While most healthy individuals are expected to walk 9-12 minutes, the duration was only 4.5 ± 3 min (range 1-7 min) in our cohort (either due to exertion or target heart rate been achieved). Elevated blood pressure (BP) was more prevalent in NAFLD group: Sixty percent of children with NAFLD had elevated BP z-score (adjusted for age, height, sex) compared to 33% of children without NAFLD. Children with NAFLD have higher IR: While all subjects had elevated IR as assessed by HOMA-IR, children with NAFLD had even higher HOMA-IR levels. Also, adiponectin (as an indirect marker for IR) was lower , and FGF21 was higher (likely a compensatory response) in NAFLD group **(Table 1)**. IR and elevated FGF21 have an additive effect on IHTG: When all children were stratified based on their median HOMA-IR and FGF21 levels, those with HOMA-IR ≥5.67 and FGF21 ≥145.2 pg/mL had higher IHTG percent compared to other groups **(Figure 1)**. Association of FGF21 and IHTG was dependent on the magnitude of IR. HOMA-IR values of the last two groups were comparable (9.74 ± 3. 65 vs. 9.18 ± 4.56, p=0.71). Taken together, our results and published literature[11,61–63] suggest that IHTG relates to insulin, FGF21, and adiponectin actions collectively. Strategies aiming to decrease IR and improve FGF21 sensitivity while increasing adiponectin levels could reduce IHTG and treat NAFLD.

Table 1: Metabolic markers are impaired in NAFLD

NAFLD Status	NAFLD (+) (n=24)	NAFLD (-) (n=35)	P value
IHTG %	11 ± 5.4	2.8 ± 1.1	<0.001
BMI (kg/m²)	39 ± 6.8	36 ± 5.3	0.04
Body Fat (%)	45.5 ± 6.1	43.3 ± 6.3	0.2
HOMA-IR	8.1 ± 4.1	5.7 ± 4	0.02
ALT (IU/L)	38.3 ± 14.3	29.2 ± 12.2	0.01
Adiponectin (ng/mL)	6.6 ± 3.3	8.3 ± 3.7	0.07
FGF21 (pg/mL)	185 ± 123	140 ± 84	0.10

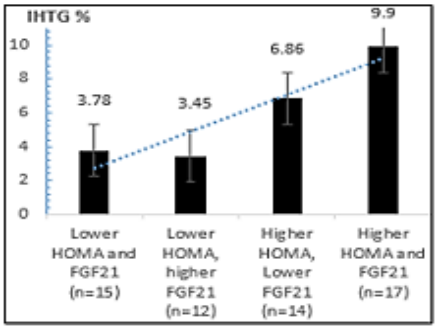

Figure 1: IHTG per median HOMA-IR and FGF21 in obese adolescents (n=58).

Study Design and Procedures

We propose a 4-week HIIT intervention in adolescents (ages 13 to 18, inclusive) with NAFLD. Up to 150 adolescents at-risk for NAFLD will be recruited to be able identify enough number of subjects with NAFLD (prevalence of NAFLD in our cohort was 40%; see data analysis section below). Our goal is to have 46 subjects with NAFLD to complete the study. Subjects will be randomized to either an exercise group or control group at a 5:1 ratio.

Because the objective of the study is to assess the effect of exercise in hepatic steatosis, we will use a multi-step approach to identify subjects with NAFLD and without disqualifiers (see

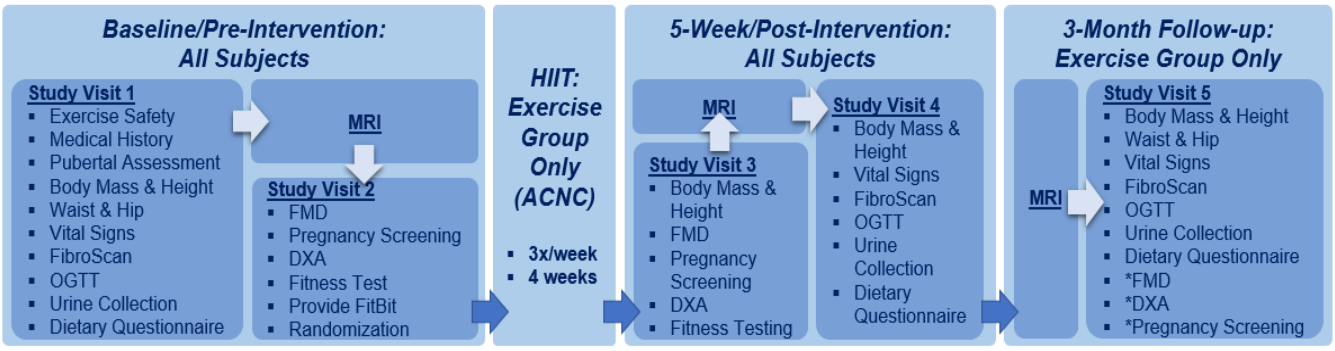

Figure 2. *Study Design. FMD, Flow-Mediated Dilation; DXA, Dual X-Ray Absorptiometry; OGTT, Oral Glucose Tolerance Test; MRI, Magnetic Resonance Imaging; ACNC, Arkansas Children's Nutrition Center; HIIT, High-Intensity Interval Training. *These assessments will only be completed if Visit 5 takes place at ACNC. Note: Physical Activity (PA) assessment via FitBit will also be conducted throughout the study.*

exclusion criteria). When available, electronic medical records will be reviewed to identify potential participants. Eligibility will be assessed using a screening form. Potential participants may be screened over the phone or in person. If determined eligible, potential participants will be invited to attend a study visit. Study visits will take place at the Arkansas Children's Nutrition Center (ACNC) and/or Arkansas Children's Hospital. Parents and/or participants will be instructed not to come to the study visit and other assessments if parent and/or participant is ill. The visit/assessments will be rescheduled if that is case to a time when parents and participants are well and asymptomatic. **Figure 2** summarizes the study visit schedule for the exercise and control groups as described in detail below.

Participants will be asked not to eat or drink anything (except water) after 10 pm the night prior to the study visits. Due to fasting, study visits will be scheduled in the morning. Study compensation will be provided via ClinCard and/or gift card(s). Lunch and/or snacks may be offered to participants and/or parents at study visits.

Baseline/Pre-Intervention: All Subjects

Study Visit 1 – This visit may last up to 4 hours. During this study visit, participants will be further assessed for eligibility. We will perform the following measurements upon participant consent, or parental consent and participant assent:

- Exercise Safety Assessment: Participants will be assessed for exercise safety using "The Physical Activity Readiness Questionnaire for Everyone" (2019 PAR-Q). Participants must be cleared for physical activity in order to continue in the study. If a participant is determined ineligible during the exercise safety assessment, study participation will be terminated before proceeding to the next measures.
- Medical History & Pubertal Assessment: A medical history questionnaire will be administered to further confirm eligibility. In addition, because the participant's pubertal development may influence the results from the imaging/blood samples, pubertal development must be taken into consideration. Subjects in the late stages of puberty will be recruited to minimize the confounding effect of puberty-associated changes in the body composition and insulin resistance on the test results. Confirming the pubertal status is essential because it is rare for a child to have NAFLD before puberty begins. Female participants will answer questions about their menstrual cycle. While males will be assessed by the study physician. The study physician will perform the pubertal assessment via Tanner

staging using Prader Orchidometer, if the subject does not demonstrate clear signs of late stages of puberty (e.g., facial hair growth, deepening of the voice, completed linear growth as assessed in the growth chart). A testicular size of 12-15 mL or larger corresponds to Tanner stage IV in males.[64] Pubertal assessment by self-report, particularly in males, is not a reliable measure of exact pubertal staging.[65] Late stage of puberty (i.e., Tanner stage IV or V) must be confirmed in order to continue in the study. If a participant is determined ineligible during the medical history and/or pubertal assessment, study participation will be terminated before proceeding to the next measures.

- Anthropometrics & Vital Signs: Body mass and height will be obtained using standardized techniques. In addition, we will also measure waist and hip circumferences using a tape measure. Blood pressure and heart rate will be assessed using standard techniques. If necessary, participants may be provided with a gown. Measures will be taken in duplicate, or triplicate if not within acceptable agreement.
- FibroScan: A FibroScan (FibroScan® 530 Compact, Echosens, Paris, France) will be performed to determine NAFLD status based on Controlled Attenuated Parameter (CAP) score. FibroScan**,** similar to an ultrasound scan, is a technique to assess liver health. FibroScan is a routinely used clinical tool in most centers to assess liver health. Outcomes of FibroScan are "Controlled Attenuated Parameter (CAP)" and "Transient Elastogram (TE)". CAP measurement strongly correlates with steatosis, while TE measures the stiffness of the liver as a surrogate marker of fibrosis, as described above. CAP values range from 100 to 400 dB/m, and higher numbers indicate more pronounced steatosis. TE values range from 2.5 kPa to 75 kPa, and higher numbers indicate more pronounced stiffness (fibrosis). Measurement via FibroScan is done when the subject is lying down on his/her back. The test involves a probe being passed over the right upper quadrant of the abdomen to measure the liver fat content and stiffness. The results are then immediately available for review once the scan is complete. No special preparation is required before the FibroScan. We will use the CAP cut-point of 241 dB/m as suggested by Shin et al. to determine baseline steatosis.[66] A subject with a CAP score of 241 dB/m or greater will be accepted as having steatosis. FibroScan test takes about 20 minutes to complete including the generation and interpretation of the results.

  In the rare instance that a valid scan is not feasible, the participant will be accepted as having NAFLD and will be allowed to proceed with the study. If upon initial Magnetic Resonance Imaging (MRI), the participant is found not to have NAFLD, then participation will be discontinued at that time.

Out of the participants whose eligibility is confirmed:

- Oral Glucose Tolerance Test (OGTT): Standardized 2-hour OGTT, as described by the American Diabetes Association, will be performed to assess the indices of insulin sensitivity and secretion. Venous blood sampling for the measurement of serum glucose, insulin, FGF21, adiponectin, and other analyses/metabolites of interest will be done at time 0 and 120 minutes by an experiences phlebotomist or physician via needle stick and/or intravenous line. Subjects will ingest a standard OGTT glucose drink (1.75 gram/kg, max 75 gram) within 5 minutes of blood sampling at time 0. HOMA-IR, QUICKI Index, and Matsuda Index (also known as insulin sensitivity index) will be calculated as previously described.[67,68] Up to a total of 40 mL of blood will be collected during OGTT. Glucose concentration will be measured using a standard calibrated point of care glucometer to determine the diabetes status instantly. Subjects with fasting glucose concentration ≥126 mg/dL **or** a 2-hour glucose concentration ≥200 mg/dL will be considered to have diabetes, an exclusion criterion. These subjects will be referred to the diabetes clinic for the management of their conditions and will

not continue in the study. Participants with no diabetes (those with fasting glucose <126 mg/dL **AND** 2-hour glucose <200 mg/dL) will continue in the study.

- Urine Collection: Urine will be collected to assess outcomes of interest, such as hydration status and to conduct metabolomics analyses.
- Dietary Questionnaire: The Block Food Frequency Questionnaire and/or Block Food Screeners will be used to assess dietary intake. The records will be analyzed using appropriate software to provide high quality, comprehensive, and complete nutrient data. Also, participants will be asked to follow their normal diet throughout the study enrollment.

Participants will be compensated with $50 at the end of the visit. Partial visits, due to ineligibility or any other reason, will be compensated with $25.

Study Visit 2 – This visit may last up to 3 hours to complete. During this visit, the following measurements will be performed:

- Flow-Mediated Dilation: Endothelial Function will be measured using ultrasound (GE Vivid 7 Ultrasound system with 5.0-13.0 mHz linear transducer, GE Healthcare, Chicago, IL, USA) to assess brachial artery flow-mediated dilation (FMD). This technology uses sound waves (ultrasound) to visualize the diameter of the brachial artery. A baseline image of the brachial artery will be recorded at rest. Three ECG electrodes will be placed on the torso of the subject, such that ultrasound measurements will be captured at identical times during the cardiac cycle (i.e. ECG-gating of ultrasound images). A blood pressure cuff will be placed around the lower arm (the opposite arm that was used for measuring blood pressure) and the cuff will be inflated to 50 mmHg above systolic pressure for 5 minutes, followed by rapid deflation of the cuff. The diameter of the brachial artery will be measured using edge detection software (Vascular Tools, Medical Imaging Applications, IA, USA) from ultrasound images captured during the R wave of the cardiac cycle (via ECG-gating) throughout the 5-minute recording protocol. The advantage of using ultrasound for FMD measurements is that this is a painless imaging test that uses non-invasive sound waves for imaging. There may be some discomfort from the blood pressure cuff since it will be applied tightly on the forearm. If the measurement is uncomfortable for the participant, and he/she requests to stop, then the procedure will be immediately aborted. FMD measurements will be done primarily by a registered clinical exercise physiologist or doctor with expertise in this procedure. However, other research staff trained and certified by Dr. Keshari Thakali (Director of the vascular physiology laboratory at ACNC) may conduct this measurement.
- Pregnancy Screening: A questionnaire will be used to assess pregnancy status in female participants prior to Dual X-Ray absorptiometry scan in order to ensure safety. Pregnancy testing via urine sampling will be conducted if parent/participant chooses this option.
- Dual X-Ray absorptiometry (DXA) scan: DXA scan is one of the best-standardized methods to assess the body composition (total body fat, lean body mass, visceral adipose tissue) of an individual. Body composition assessment is an essential part of the study due to the effects of whole-body adiposity and body composition on insulin resistance and NAFLD.
- Fitness Test: All subjects will undergo a cycle ergometer stress test to assess their fitness level and determine peak oxygen uptake (VO_2). This test will be conducted primarily by an ACSM registered clinical exercise physiologist (with M.Sc. in exercise physiology) with expertise in this procedure. Other research staff that have been certified by Dr. Elisabet Borsheim (Director of the Physical Activity Core at ACNC) may also conduct this measurement. Briefly, after evaluating the participants at rest and during a warm-up period, work load will be gradually increased until volitional exhaustion. The participants will be told that they are free to stop the test at any time if they do not want to continue the test, but will

be encouraged to perform maximally. During the entire test, breath will be sampled and the composition will be measured using a metabolic cart (Medgraphics Ultima PFX® system, MGC Diagnostics Corporation, St. Paul, MN, USA). From this, the O_2 uptake and CO_2 excretion will be determined, and their respiratory exchange ratio (O_2 uptake/CO_2 expiration) will be assessed throughout the test. The participants will be required to wear an indirect calorimetry face mask for these measurements. The participants will be given ample time to become familiar with the equipment and face mask. A heart rate monitor (Zephyr Bluetooth Wireless Heart Rate Sensor, Medtronic, Boulder, CO) will be worn to measure the level of cardiovascular activity during the exercise test. Participants will be trained on how to assess their exertion levels, and ratings of self-perceived exertion will be assessed every two minutes using the children's OMNI scale.[69] This is a numerical scale from 0 to 10, with a score of 2 indicating "a little tired" and a score of 9 indicating "very, very tired," with associated pictures to represent perceived physical effort. Determination of peak VO_2 will take the following into consideration: a rating of perceived exertion ≥ 8 on the OMNI scale, and/or a peak heart rate ≥185 beats/min (or ≥85% of predicted peak heart rate), and/or a respiratory exchange ratio ≥ 1.1. The validity of the OMNI score will be evaluated by presence of visible signs of fatigue, including inability to maintain pedaling cadence.

- Randomization: Participants will be randomized to the control or exercise group. We will employ a randomized block design with sex as the blocking factor. Participants who are randomized to the control group will not undergo any exercise training. If randomized to the exercise group, participants will be offered to tour the exercise facility for familiarization with location and personnel.

Participants will be compensated with $25 at the end of the visit. A FitBit activity tracker will be provided for those who complete the study visit to account for differences in activity level at baseline and throughout the study.

Exercise Intervention (High-Intensity Interval Training (HIIT)): Exercise Group Only

HIIT Intervention – Exercise sessions will take place in the ACNC fitness facility. The session will begin with a 5-10 minute low-intensity warm-up, followed by ten 1-minute intervals using exercise equipment (such as elliptical machine and/or bike and/or treadmill) at a work rate that elicits 80-90% of the maximal heart rate determined during the VO_{2max} test. The participants will have two minutes to recover between each effort and will be encouraged to continue to turn the "pedals" over or walk slowly between high-intensity efforts. To finish the session, the participants will perform a 5-10 minute cool-down at a low intensity. Duration and/or intensity may be adjusted, if needed. Each participant will be asked to attend 3 weekly training sessions for 4 weeks. A minimum compliance rate of 50% will be required to remain in the study (at least 6 training sessions) by the end of the training period (4 weeks). Participants will be encouraged to do home exercises in between training sessions, but these will not be recorded other than via FitBit. All exercise sessions will be primarily directed and monitored by an exercise trainer who staffs the Core. However, an exercise physiologist may also conduct exercise training. The exercise trainer or exercise physiologist will monitor heart rate and OMNI score during sessions. Each HIIT exercise session is expected to last up to 1 hour. Compensation will be provided in increments of $10, $20, $30 and $40 at the end of the 3rd, 6th, 9th and 12th HIIT exercise sessions, respectively. Therefore, participants who attend all 12 HIIT sessions will receive a total of $100.

Participants can bring a friend or family member to workout with them if they would like. A separate release will be signed by the friend or family member in order to workout at the ACNC fitness facility.

5-Week/Post-Intervention: All Subjects

Study Visit 3 – This visit may last up to 3 hours and include: measurement of body mass and height, FMD, pregnancy screening (if female), DXA, and fitness test as described above. Participants who are in the control group will complete this visit 5 weeks ± 1.5 week after study visit 2. Participants will be reimbursed for their time with $25 at the end of the visit.

Study Visit 4 – This visit may last up to 4 hours and include: measurement of body mass and height, vital signs, OGTT, FibroScan, urine collection, and dietary questionnaire as described above. Participants will be reimbursed for their time with $50 at the end of the visit.

3-Month Follow-up: Exercise Group Only

Study Visit 5 – Participants from the exercise group will be asked to attend a follow-up visit 3 months ± 2 week after last HIIT session. This visit may last up to 4 hours and include: measurement of body mass and height, hip and waist circumference, vital signs, pregnancy screening (if female), DXA, FibroScan, FMD, OGTT, urine collection, and dietary questionnaire as described above. FMD, pregnancy screening (if female) and DXA will only be completed in the instance that this visit takes place at ACNC. Participants will be reimbursed for their time with $50 at the end of the visit.

Magnetic Resonance Imaging (MRI)

In addition to the study procedures described above, all participants will complete a MRI scan before the baseline/pre-intervention fitness test, and another MRI scan before the 5-week/post-intervention OGTT. Furthermore, participants in the exercise group will complete an additional MRI scan before the 3-month follow-up OGTT. The MRI scans will be done at the Arkansas Children's Hospital Radiology Department for the determination of hepatic fat content via magnetic resonance imaging. Each visit for completion of a MRI scan will last up to 1.5 hours. Participants will be reimbursed for their time with $50 after each visit.

Physical Activity Assessment

Furthermore, physical activity (PA) will be assessed throughout the study via FitBit device. The participant's activity information from his/her FitBit will be assessed and recorded. Multiple assessments may be made throughout study participation via checking the data through the website and/or mobile application. In the case of lack of adherence to FitBit wear (e.g., no data in an entire week, wearing FitBit less than half of the time, etc.), we may ask the participant to share physical activity information (e.g., step counts, activity minutes, etc.) from any other phone app that has been already installed on his/her phone. For instance, most, if not all, iPhone or Android devices have "Health" apps by default that automatically store physical activity data.

Any of the study measurements described above may be re-attempted during the study visit(s) if needed and if the participant is willing. In addition, subjects may be asked to return for a "repeat visit" in order to repeat and/or complete any study procedure(s). This will happen if the study procedure(s) is unsuccessful, incomplete, or cannot be completed due to unforeseeable reasons during the study visit. An additional $25 will be provided at the end of the repeat visit after re-attempt and/or completion.

Moreover, participants in the control group will be offered an opportunity to exercise at ACNC fitness facility after he/she completes the study. This will include one month of supervised exercise training (up to 12 sessions) within two months from when the participant completes this study. Participants can bring a friend or family member to workout with them if they would like. A separate release will be signed by the friend or family member in order to workout at the ACNC fitness facility.

Study Population

Recruitment of up to 150 participants will be conducted using IRB approved advertisements. Participants will be recruited from a variety of different settings, including select pediatric outpatient clinics with a high volume of at-risk patients for NAFLD (e.g., Weight Management Clinic, Gastroenterology Clinic, Adolescent Clinic, General Pediatric Clinic). Advertisements may be distributed in the form of flyers, postcards, and letters by direct mail, at information booths or events, or to various locations for posting or distributing, including but not limited to outpatient clinics, healthcare offices, health fairs, schools/universities, grocery stores, supermarkets, recreational centers, retail stores, websites (ACH, ACHRI, ACNC, UAMS, and others as applicable) and churches. Print and digital ads may appear in newspapers, magazines, social media, websites, and circulars. On-hold phone messages, text messages, and radio/television ads may also be used. In addition, research staff may contact parents who expressed an interest in our studies or who previously agreed to be contacted regarding future studies. Research staff may also contact parents/participants who have signed up for the AC research registry.

AC electronic medical records may also be reviewed by study team to identify potential participants, and patients/parents may be contacted and informed about this study. In addition, staff, investigators, or other health care providers at Arkansas Children's may assist in identifying participants at clinic visits and ask if they would like to be contacted about research, or if they would be interested in answering some screening questions. If interested, contact information may be obtained directly from patient/parent or from AC electronic medical record or from healthcare provider, so that the study team member may follow up to inform parent/patient about the study and ask screening questions. Also, when applicable, patients/parents may be screened in person at the time of their visit.

In all recruitment instances, the research team member and/or health care provider will educate the parents and potential participants about the study and if interested determine potential eligibility. A copy of the consent and assent may be provided prior to the study visit for information. Eligibility will be assessed through the screening process prior to scheduling a study visit and will be reviewed/confirmed at the time of the visit. At the visit, participant's consent or parental consent and participant's assent will be obtained in person.

Study staff may also recruit patients at Arkansas Children's with MyChart accounts. Study staff may access the electronic medical records of AC patients and search for eligible participants. Once study staff determines that a patient may be eligible, staff will post the study information in their MyChart account under the available studies page. This page lists all studies the child may be eligible for that have chosen to recruit using MyChart. On this page, the parent or patient has the option to indicate if they are interested in the study or not. If they are interested, the MyChart system will notify study staff. Study staff will follow up with the interested patient (if 18) or patient's parent to provide more information about the study. This may be done by providing/mailing a letter/flyer to the parent and/or patient or by directly contacting parents or patients via phone or email to let them know about the research opportunity. If the parent or patient indicates an interest in the study, study staff will proceed to determine potential eligibility and schedule a visit where informed consent/assent would be obtained in person.

Participants may also be recruited among those who have previously participated in other studies (see below). Contact information of these subjects will be looked up from their AC electronic medical records. The research team may call and/or send IRB approved ads, texts, emails, and letters to inform participants about the new study.

- *Fibroblast Growth Factor-21: An Adjunct Biomarker for Early Detection of Non-Alcoholic Fatty Liver Disease in Children* (IRB Protocol: 206278): Eighteen subjects, between the ages of 10-17 years completed this study between November 2017 and January 2019, were found to have NAFLD via magnetic resonance imaging. Of these, participants for whom consent was obtained to be contacted for future studies and have their information or samples used for future research may be recruited.
- *Liver Assessment via FibroScan* (IRB protocol: 260576): Participants will be recruited based on their FibroScan results (i.e. CAP score of 241 dB/m or greater). Of these, participants for whom consent was obtained for contact for future research, use of information obtained over the course of this study in future research studies related to liver and/or metabolic health, and use of information obtained over the course of this study for screening purposes for other studies related to liver and/or metabolic health, may be recruited.

Information from the AC medical records regarding a participant's contact information, demographics, and health, including medical history, medical diagnoses, medicines, puberty, date of birth, anthropometrics, vital signs, blood and imaging results, and other test results and labs may be collected upon consent/assent. This information may be collected, retrospectively (i.e., collection of information already present in the medical record at the time of enrollment) and prospectively (i.e., collection of new information that may become available after study enrollment), from the time of study enrollment up to the time that data analysis for this study is complete and results have been published (up to 3 years from enrollment).

Inclusion Criteria for Initial Enrollment:

- Ages 13-18 years (inclusive) for both sexes
- Any ethnic/racial background
- English speaking competence
- Obesity (BMI≥ 95th percentile for age and sex)
- Low risk to participate in an exercise program during initial phone screening
- Late stages of puberty (i.e., Tanner stage IV or V)
    - Female participants should have attained menarche. By definition, onset of menstrual cycles corresponds to a minimum of Tanner stage IV breast development in females. A verbal confirmation of the attainment of menstrual cycles will be enough.
    - For male participants, a verbal confirmation of late puberty signs (e.g., facial hair growth, deepening of the voice, completed linear growth) will be enough for initial enrollment. However, pubertal assessment will be conducted by the study physician upon consent/assent to confirm late pubertal status (see above).

Inclusion Criteria to Continue in the Study:

- Cleared for physical activity
- Presence of NAFLD as determined by FibroScan CAP score or MRI (see above)
- Confirmed lack of diabetes as determined by OGTT (see above)
- Confirmed late stages of puberty (i.e., Tanner stage IV or V) (see above)
- Confirmed eligibility per medical history (see above)

Exclusion criteria:

- Pre-pubertal or early stages of puberty
- Pregnancy (verbal assessment)
- Confirmed lack of NAFLD in the past 6 months via biopsy, FibroScan or MRI
- Presence of an implantable medical device or metal objects in the body (a contraindication for FibroScan)

- High-risk to participate in an exercise program as determined by "The Physical Activity Readiness Questionnaire for Everyone" (see above)
- Unable to cooperate with study procedures and tests as determined by study PI, including genetic or physical conditions impacting mobility over the past year
- Having known chronic illnesses/disorders that may independently affect study outcome measures: type 1 diabetes mellitus, T2D, neurologic (e.g., epilepsy), developmental (developmental delay, autism spectrum disorder), endocrine (Cushing's, growth hormone deficiency), hepatic (other than NAFLD), autoimmune, cardiac and renal disorders
- Taking any of the following medications that can affect study outcome at the time of enrollment: insulin, metformin or any other anti-diabetics, antipsychotics, oral steroids, and anabolic drugs (growth hormone replacement therapy, testosterone, and oxandrolone). The participant will not be excluded from the study if he/she begins them during the study unless recommended otherwise by the primary physician.
- Adolescents who have a history of claustrophobia.
- Adolescents who need sedation in order to complete MRI.
- Adolescents determined ineligible by the PI or delegated staff.

Risks and Benefits

Subject Confidentiality: A risk to study participants is the potential for loss of confidentiality of study data. Measures to protect the confidentiality of study data will be implemented as described in the Data Handling and Recordkeeping section below.

Blood Sampling and OGTT**:** There is a small risk that participants will encounter bruising and/or infection after having blood taken, however, the use of well-established blood draw techniques, and antiseptic procedures will ensure minimal risk. Participants may also experience sickness or dizziness. Oral glucose tolerance test is routinely used in the clinics to screen for diabetes in at-risk individuals. There is no known risks of glucose drinks. If a subject is identified having diabetes via OGTT, appropriate referrals will be arranged and care will be provided without further delay.

FibroScan: There are no risks to having a FibroScan done. It is not invasive and is painless. It is similar to an ultrasound scan. Pregnancy is not a contraindication for FibroScan; however, pregnancy-associated physiological changes in liver structure may alter the results of FibroScan.

A TE score greater than 8.5 kPa was previously shown to be associated with advanced stages of fibrosis. If we identify any subject with a TE score of 8.5 kPa or greater, we will consult the results with Dr. Jonathan Dranoff, who is an experienced hepatologist at the UAMS GI clinic and the co-investigator of this study, for further guidance.

Dual-Energy X-ray Absorptiometry (DXA): This procedure will be used to determine body composition, and it exposes subjects to a very low dose of radiation (x-rays). The dose is only slightly greater than normal background radiation levels and is far less than that received during a standard chest x-ray (DEXA: < 0.1 mrem, Chest x-ray: 25 mrem). Otherwise, there are no known risks associated with the performance of this procedure. Because of the use of radiation, this procedure may pose risk to an unborn child.

Fitness Test and HIIT exercise: During the fitness test and HIIT exercise sessions, participants may experience discomfort associated with exercise which is normal. However, this and any other procedure can be aborted at any time at the request of the participant. The United States Centers for Disease Control and Prevention (CDC) and The American Heart Association (AHA), recommend that children be physically active for at least 60 min per day with moderate- or

vigorous-intensity in their aerobic activity.[70,71] These entities stress that vigorous-intensity aerobic activity should be included on at least 3 days per week. In the event of an adverse event or unanticipated problems, this will be reported to the study PI, the IRB, and the study sponsor in accordance with IRB Policy 10.2.

Magnetic Resonance Imaging: MRI is a safe and painless test that uses a magnetic field and radio waves to produce detailed pictures of the liver and identify hepatic fat content. It does not give radiation. During the procedure, a participant needs to stay still as much as possible to prevent distortion of the images. Because the MRI scanner we use generates a strong magnetic field, people with metal implants (e.g., cochlear implants, cardiac pacemakers, certain prosthetic devices, etc.) are not eligible to participate in this study. Individuals with claustrophobia will be rejected as well because the risk of giving sedation outweighs the potential benefits from participating in this study. ACH Radiology Department is a very well-established site for imaging in children; therefore the risk is minimal.

Flow-Mediated Dilation: The risks are no more than what happens in everyday life, or in a routine physical examination where blood pressure is measured. The procedure may be uncomfortable, because the blood pressure cuff will stay inflated tightly on the participant's arm for 5 minutes, which is longer than in a typical visit to the doctor. There may be some discomfort, like the hand feeling cold and feeling like it is "asleep" from the blood pressure cuff since it will be applied tightly on the forearm.

Subjects may directly benefit from participating in this study. As stated above, exercise is one of the mainstays of the NAFLD treatment. Participants will receive supervised exercise training from an experienced exercise trainer, or exercise physiologist. The knowledge gained from the study will shed light on the effect of HIIT exercise on NAFLD outcomes in adolescents. Findings from this study may help designing individualized physical exercise regimen for the patients with NAFLD.

Data Handling and Recordkeeping

The principal investigator, co-investigators, and study staff will complete and maintain appropriate CITI training. All study subject material will be assigned a unique identifying code or number. The key to the code and participants' study data will be kept in locked file(s), and/or secure UAMS and/or Arkansas Children's maintained server(s) and/or database(s) (e.g., REDCap). All data and communications will be recorded in standardized paper and/or electronic case report forms. Data will be collected and managed using REDCap. The data integrity will be password protected. Only study investigators and staff will have access to the code and information that identifies the subject in this study.

The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. In addition, the PI and primary mentor (Jon Dranoff, MD) will review the data on a quarterly basis together. The information gained from this study that can be linked to a subject's identity will not be released to anyone outside of the study team other than the subject, subject's parent, and the subject's physician (if applicable). The results of this study may be published in scientific journals and/or presented at scientific meetings and conferences without identifiers. All procedures will be performed by appropriately trained and credentialed personnel supervised by the PI.

Coded samples and/or information may be shared for the purposes of this study (i.e. within the purview of this protocol) with local collaborators (e.g., Arkansas Children's Nutrition Center, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, Arkansas Children's Hospital) and/or non-local collaborators. At the conclusion of the study, the data will be stored to inform future proposals, grants, protocols, and procedures. The key to the code will

not be destroyed while information and/or samples are kept. If consented, information and/or samples may be used in future studies related to liver and/or metabolic health.

Specimen Handling and Storage

Samples will be stored in freezers in the Arkansas Children's Nutrition Center and/or Arkansas Children's Research Institute. The freezers are constantly monitored to ensure that the proper temperature is maintained. Samples will be identified using the subject's unique study acronym along with the date of sample collection and type of sample (e.g. EDTA plasma, serum). Remaining samples may be stored indefinitely or until used up for future analyses. If consented, samples may be used in future studies related liver and/or metabolic health. Participants and/or parents may contact the study's principal investigator, if they choose to withdraw consent to use samples and/or information in future research. In this case, a written letter addressed to the principal investigator expressing their desire to withdraw their consent would be required. If the information and/or samples have been shared or if publication of results has occurred, then we may not be able to remove information and/or samples.

Data Analysis

Exploratory data analysis will be conducted prior to formal statistical analysis. We will examine the distributions of all key variables to check for data errors and ensure that modeling assumptions are not violated. Numerical summaries including means, standard deviations, medians and histogram graphical techniques will be used for continuous variables and frequencies will be computed for categorical variables. For both aims, the primary outcome is IHTG level measured by both FibroScan and MRI, and secondary outcomes are fitness level, and various cardiometabolic disease markers (i.e.; insulin, glucose, adiponectin, etc.). The immediate (Aim 1) and sustained (Aim 2) effect of HIIT will be measured by comparing the changes in outcomes between groups using a linear mixed-effect framework with a subject-level random intercept. Changes in the outcomes are defined as difference between follow up visits (5-week and 3-month) versus the baseline. The independent variables will be group (intervention vs control), visit time (baseline, 5-week, 3-month) and their interaction term. This model specification can test the effect of intervention as well as difference between visits, while correctly account for the within-subject correlation due to repeated visits. Post-hoc contrasts of visit time between 5-week and baseline will be used to test the hypotheses under Aim 1, while contrasts between 3-month and 5-week will be used to test the hypotheses under Aim 2.

As an exploratory aim, diagnostic performance of FibroScan for the NAFLD adolescent will be determined by discriminating NAFLD status defined by FibroScan against that by MRI to calculate sensitivity, specificity, positive and negative predictive values. Additional agreement statistics such as concordance correlation coefficient and Bland-Altman plots will be provided for the continuous measurements of IHTG levels measured by both methods.

In our pilot data, the IHTG levels were 11.5±5.4 percent at baseline for NAFLD subjects (N=23). With a pre-defined clinically significant change of 20% relative decrease from baseline, we expect the NAFLD subjects' fat percent will be decreased to an average of 9.2±5.4 percent after HIIT intervention, which is an equivalent effect size of 0.43. In order to detect this effect size at 80% power, we will need 38 NAFLD participants in the intervention group, therefore 8 NAFLD subjects in the control group. Accounting for a 20% dropout rate and false-negative screening from FibroScan, we propose to enroll 58 subjects with NAFLD. Therefore, we need to screen no less than 150 subjects at-risk for NAFLD at baseline, to be able to identify 58 subjects with NAFLD (NAFLD prevalence was 40% in our pilot study, see preliminary data section above).

Ethical Considerations

This study will be conducted in accordance with all applicable government regulations and the University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each adult subjects or legal representative of minor subjects and the assent of minor subjects, using the IRB-approved consent and assent forms, will be obtained before subject is submitted to any study procedure. Any subject who turns 18 years old during his/her participation in the study will be re-consented to provide his/her own consent. All subjects for this study will be provided with consent/assent forms describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The consent form will also be available in Spanish for Hispanic parents who do not have English speaking competence. In this instance, in-person or remote interpreter services will be available during the consent process. However, for the remainder of the study, only remote interpreter services will be available. Participants may also intermediate communications with their Hispanic parents during participation in the study.

A copy of the consent/assent may be mailed or e-mailed to parent/participant for information prior to enrollment. The person obtaining consent/assent will thoroughly explain each element of the document and outline the risks, benefits, and requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. The consent form must be signed by the subject or legally authorized representative and the person obtaining the consent, and the assent form must be signed by the minor participant. A copy of the signed consent and assent forms will be given to the participant/parent, and the informed consent process will be documented in the research record. In addition, a signed copy of the consent form may be placed in the patients' medical record. Parents/participants may also be contacted (e.g., phone, text, email, letter) during their participation in the study and/or in preparation for study visits, including prior to enrollment. During the study, parents and/or participants may withdraw study consent verbally. In this case, the subject will no longer participate in the study and no further information, data, and samples will be collected. However, we may still keep and use any information, data, and samples that have been already collected from parent and/or participant.

During the consent/assent process, parents/participants will also be given the option to allow for contact for future research, and allow for use of information and samples obtained over the course of this study in future research studies related to liver and/or metabolic health. If agreed, information and specimens will be stored indefinitely (or until used up) for potential use in future research studies. De-identified samples and information may be shared for future research use (i.e. outside the purview of this protocol) with local researchers (e.g., Arkansas Children's Nutrition Center, Arkansas Children's Research Institute, University of Arkansas for Medical Sciences, Arkansas Children's Hospital) and/or non-local researchers. In this case, samples may only be shared with researchers who who do not express a plan to use them for genetic research. In addition, inquiring researchers will be informed that the stored samples do not have consent for future genetic research. Participants may choose to withdraw permission for future use of information and/or samples at any time they decide. A written letter addressed to the principal investigator expressing their desire to withdraw their consent would be required. If the information and/or samples have been shared or if publication of results has occurred, then we may not be able to remove information and/or samples. Consent for future contact and use of information and/or samples for future research will be separate from consent to participate in the

current study. Their decision will not affect their eligibility for this study. Participants may call the investigators or research staff to obtain more information if needed.

In addition, consent for release of data may be obtained from participants who have also taken part in *Fibroblast Growth Factor-21: An Adjunct Biomarker for Early Detection of Non-Alcoholic Fatty Liver Disease in Children* (IRB Protocol: 206278) and/or *Liver Assessment via FibroScan* (IRB protocol: 260576). If parent/participant agrees to release data, a copy of the signed consent for release of data will also be provided.

Dissemination of Data

Results of this study may be used for presentations, posters, publications, or intramural/extramural grant applications. The publications will not contain any identifiable information that could be linked to a participant. The study will be listed on clinicaltrials.gov in accordance with requirements.

References

1. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nu. *J Pediatr Gastroenterol Nutr*. 2017. doi:10.1097/MPG.0000000000001482
2. Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int*. 2013. doi:10.1111/liv.12226
3. Dongiovanni P, Stender S, Pietrelli A, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. *J Intern Med*. 2018. doi:10.1111/joim.12719
4. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *J Hepatol*. 2015. doi:10.1016/j.jhep.2014.11.034
5. Ajmera V, Park CC, Caussy C, et al. Magnetic Resonance Imaging Proton Density Fat Fraction Associates With Progression of Fibrosis in Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology*. 2018. doi:10.1053/j.gastro.2018.04.014
6. Khan RS, Bril F, Cusi K, Newsome PN. Modulation of Insulin Resistance in Nonalcoholic Fatty Liver Disease. *Hepatology*. 2019. doi:10.1002/hep.30429
7. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism*. 2016. doi:10.1016/j.metabol.2016.01.001
8. Lonardo A, Sookoian S, Pirola CJ, Targher G. Non-alcoholic fatty liver disease and risk of cardiovascular disease. *Metabolism*. 2016. doi:10.1016/j.metabol.2015.09.017
9. Kim D, Touros A, Kim WR. Nonalcoholic Fatty Liver Disease and Metabolic Syndrome. *Clin Liver Dis*. 2018. doi:10.1016/j.cld.2017.08.010
10. Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, Muscle, and Adipose Tissue Insulin Action Is Directly Related to Intrahepatic Triglyceride Content in Obese Subjects. *Gastroenterology*. 2008. doi:10.1053/j.gastro.2008.01.075
11. Kawano Y, Cohen DE. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J Gastroenterol*. 2013. doi:10.1007/s00535-013-0758-5
12. Day CP, James OFW. Steatohepatitis: A tale of two “Hits”? *Gastroenterology*. 1998. doi:10.1016/S0016-5085(98)70599-2
13. Grander C, Grabherr F, Moschen AR, Tilg H. Non-Alcoholic Fatty Liver Disease: Cause or Effect of Metabolic Syndrome. *Visc Med*. 2016. doi:10.1159/000448940
14. Azzu V, Vacca M, Virtue S, Allison M, Vidal-Puig A. Adipose Tissue-Liver Cross Talk in the Control of Whole-Body Metabolism: Implications in Nonalcoholic Fatty Liver Disease. *Gastroenterology*. 2020. doi:10.1053/j.gastro.2019.12.054
15. Cholankeril R, Patel V, Perumpail B, et al. Anti-Diabetic Medications for the Pharmacologic Management of NAFLD. *Diseases*. 2018. doi:10.3390/diseases6040093
16. Pojskic H, Eslami B. Relationship Between Obesity, Physical Activity, and Cardiorespiratory Fitness Levels in Children and Adolescents in Bosnia and Herzegovina: An Analysis of Gender Differences. *Front Physiol*. 2018. doi:10.3389/fphys.2018.01734
17. Vasconcellos F, Seabra A, Katzmarzyk PT, Kraemer-Aguiar LG, Bouskela E, Farinatti P. Physical activity in overweight and obese adolescents: Systematic review of the effects on physical fitness components and cardiovascular risk factors. *Sport Med*. 2014. doi:10.1007/s40279-014-0193-7
18. Bamba V, Rader DJ. Obesity and Atherogenic Dyslipidemia. *Gastroenterology*. 2007. doi:10.1053/j.gastro.2007.03.056
19. Loomba R, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology*. 2009. doi:10.1002/hep.23119
20. Johnson NA, Sachinwalla T, Walton DW, et al. Aerobic exercise training reduces hepatic and visceral lipids in

obese individuals without weight loss. *Hepatology*. 2009. doi:10.1002/hep.23129

21. Medrano M, Cadenas-Sanchez C, Álvarez-Bueno C, et al. Evidence-Based Exercise Recommendations to Reduce Hepatic Fat Content in Youth- a Systematic Review and Meta-Analysis. *Prog Cardiovasc Dis*. 2018. doi:10.1016/j.pcad.2018.01.013
22. Hallsworth K, Fattakhova G, Hollingsworth KG, et al. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. *Gut*. 2011. doi:10.1136/gut.2011.242073
23. Ok DP, Ko K, Bae JY. Exercise without dietary changes alleviates nonalcoholic fatty liver disease without weight loss benefits. *Lipids Health Dis*. 2018. doi:10.1186/s12944-018-0852-z
24. Keating SE, Hackett DA, George J, Johnson NA. Exercise and non-alcoholic fatty liver disease: A systematic review and meta-analysis. *J Hepatol*. 2012. doi:10.1016/j.jhep.2012.02.023
25. Bacchi E, Negri C, Targher G, et al. Both resistance training and aerobic training reduce hepatic fat content in type 2 diabetic subjects with nonalcoholic fatty liver disease (the RAED2 randomized trial). *Hepatology*. 2013. doi:10.1002/hep.26393
26. Hui X, Feng T, Liu Q, Gao Y, Xu A. The FGF21-adiponectin axis in controlling energy and vascular homeostasis. *J Mol Cell Biol*. 2016. doi:10.1093/jmcb/mjw013
27. Zhang Y, Lei T, Huang JF, et al. The link between fibroblast growth factor 21 and sterol regulatory element binding protein 1c during lipogenesis in hepatocytes. *Mol Cell Endocrinol*. 2011;342(1-2):41-47. doi:10.1016/j.mce.2011.05.003
28. Emanuelli B, Vienberg SG, Smyth G, et al. Interplay between FGF21 and insulin action in the liver regulates metabolism. *J Clin Invest*. 2014;124(2):515-527. doi:10.1172/JCI67353
29. Kharitonenkov A, Wroblewski VJ, Koester A, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. *Endocrinology*. 2007;148(2):774-781. doi:10.1210/en.2006-1168
30. Engel FA, Ackermann A, Chtourou H, Sperlich B. High-intensity interval training performed by young athletes: A systematic review and meta-analysis. *Front Physiol*. 2018. doi:10.3389/fphys.2018.01012
31. Van Der Windt DJ, Sud V, Zhang H, Tsung A, Huang H. The effects of physical exercise on fatty liver disease. *Gene Expr*. 2018. doi:10.3727/105221617X15124844266408
32. Kantartzis K, Thamer C, Peter A, et al. High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut*. 2009. doi:10.1136/gut.2008.151977
33. Cao M, Quan M, Zhuang J. Effect of high-intensity interval training versus moderate-intensity continuous training on cardiorespiratory fitness in children and adolescents: A meta-analysis. *Int J Environ Res Public Health*. 2019. doi:10.3390/ijerph16091533
34. Thivel D, Masurier J, Baquet G, et al. High-intensity interval training in overweight and obese children and adolescents: Systematic review and meta-analysis. *J Sports Med Phys Fitness*. 2019. doi:10.23736/S0022-4707.18.08075-1
35. Tjønna AE, Stølen TO, Bye A, et al. Aerobic interval training reduces cardiovascular risk factors more than a multitreatment approach in overweight adolescents. *Clin Sci*. 2009. doi:10.1042/CS20080249
36. Winn NC, Liu Y, Rector RS, Parks EJ, Ibdah JA, Kanaley JA. Energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity — A randomized trial. *Metabolism*. 2018. doi:10.1016/j.metabol.2017.08.012
37. Hallsworth K, Thoma C, Hollingsworth KG, et al. Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: A randomized controlled trial. *Clin Sci*. 2015. doi:10.1042/CS20150308
38. Labayen I, Medrano M, Arenaza L, et al. Effects of exercise in addition to a family-based lifestyle intervention program on hepatic fat in children with overweight. *Diabetes Care*. 2020. doi:10.2337/dc19-0351
39. Zhang S, Liu Y, Li Q, et al. Exercise improved rat metabolism by raising PPAR-α. *Int J Sports Med*. 2011. doi:10.1055/s-0031-1271755
40. Linden MA, Fletcher JA, Morris EM, et al. Treating NAFLD in OLETF rats with vigorous-intensity interval exercise training. *Med Sci Sports Exerc*. 2014. doi:10.1249/MSS.0000000000000430

41. Smith BK, Marcinko K, Desjardins EM, Lally JS, Ford RJ, Steinberg GR. Treatment of nonalcoholic fatty liver disease: Role of AMPK. *Am J Physiol - Endocrinol Metab*. 2016. doi:10.1152/ajpendo.00225.2016

42. Marcinko K, Sikkema SR, Samaan MC, Kemp BE, Fullerton MD, Steinberg GR. High intensity interval training improves liver and adipose tissue insulin sensitivity. *Mol Metab*. 2015. doi:10.1016/j.molmet.2015.09.006

43. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic Fibroblast Growth Factor 21 Is Regulated by PPARalpha and Is a Key Mediator of Hepatic Lipid Metabolism in Ketotic States. *Cell Metab*. 2007;5(6):426-437. doi:10.1016/j.cmet.2007.05.002

44. Cvetković N, Stojanović E, Stojiljković N, Nikolić D, Scanlan AT, Milanović Z. Exercise training in overweight and obese children: Recreational football and high-intensity interval training provide similar benefits to physical fitness. *Scand J Med Sci Sport*. 2018. doi:10.1111/sms.13241

45. Batacan RB, Duncan MJ, Dalbo VJ, Tucker PS, Fenning AS. Effects of high-intensity interval training on cardiometabolic health: A systematic review and meta-analysis of intervention studies. *Br J Sports Med*. 2017. doi:10.1136/bjsports-2015-095841

46. García-Hermoso A, Cerrillo-Urbina AJ, Herrera-Valenzuela T, Cristi-Montero C, Saavedra JM, Martínez-Vizcaíno V. Is high-intensity interval training more effective on improving cardiometabolic risk and aerobic capacity than other forms of exercise in overweight and obese youth? A meta-analysis. *Obes Rev*. 2016. doi:10.1111/obr.12395

47. Cockcroft EJ, Williams CA, Tomlinson OW, et al. High intensity interval exercise is an effective alternative to moderate intensity exercise for improving glucose tolerance and insulin sensitivity in adolescent boys. *J Sci Med Sport*. 2015. doi:10.1016/j.jsams.2014.10.001

48. Cockcroft EJ, Bond B, Williams CA, et al. The effects of two weeks high-intensity interval training on fasting glucose, glucose tolerance and insulin resistance in adolescent boys: A pilot study. *BMC Sports Sci Med Rehabil*. 2019. doi:10.1186/s13102-019-0141-9

49. Jelleyman C, Yates T, O'Donovan G, et al. The effects of high-intensity interval training on glucose regulation and insulin resistance: A meta-analysis. *Obes Rev*. 2015. doi:10.1111/obr.12317

50. Ramos JS, Dalleck LC, Borrani F, et al. The effect of different volumes of high-intensity interval training on proinsulin in participants with the metabolic syndrome: a randomised trial. *Diabetologia*. 2016. doi:10.1007/s00125-016-4064-7

51. Burgomaster KA, Hughes SC, Heigenhauser GJF, Bradwell SN, Gibala MJ. Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J Appl Physiol*. 2005. doi:10.1152/japplphysiol.01095.2004

52. Helgerud J, Høydal K, Wang E, et al. Aerobic high-intensity intervals improve V̇O2max more than moderate training. *Med Sci Sports Exerc*. 2007. doi:10.1249/mss.0b013e3180304570

53. Talanian JL, Galloway SDR, Heigenhauser GJF, Bonen A, Spriet LL. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during exercise in women. *J Appl Physiol*. 2007. doi:10.1152/japplphysiol.01098.2006

54. Racil G, Ben Ounis O, Hammouda O, et al. Effects of high vs. Moderate exercise intensity during interval training on lipids and adiponectin levels in obese young females. *Eur J Appl Physiol*. 2013. doi:10.1007/s00421-013-2689-5

55. Balagopal P, George D, Yarandi H, Funanage V, Bayne E. Reversal of obesity-related hypoadiponectinemia by lifestyle intervention: A controlled, randomized study in obese adolescents. *J Clin Endocrinol Metab*. 2005. doi:10.1210/jc.2004-2427

56. Tjønna AE, Stølen TO, Bye A, et al. Aerobic interval training reduces cardiovascular risk factors more than a multitreatment approach in overweight adolescents. *Clin Sci*. 2009. doi:10.1042/CS20080249

57. Zhang HJ, Pan LL, Ma ZM, et al. Long-term effect of exercise on improving fatty liver and cardiovascular risk factors in obese adults: A 1-year follow-up study. *Diabetes, Obes Metab*. 2017. doi:10.1111/dom.12809

58. Pugh CJA, Sprung VS, Jones H, et al. Exercise-induced improvements in liver fat and endothelial function are not sustained 12 months following cessation of exercise supervision in nonalcoholic fatty liver disease. *Int J Obes*. 2016. doi:10.1038/ijo.2016.123

59. Tas E, Bai S, Ou X, et al. Fibroblast Growth Factor-21 to Adiponectin Ratio: A Potential Biomarker to Monitor

Liver Fat in Children With Obesity. *Front Endocrinol (Lausanne)*. 2020;11(September):654. doi:10.3389/fendo.2020.00654

60. Will PM, Walter JD. Exercise testing: Improving performance with a ramped Bruce protocol. *Am Heart J*. 1999. doi:10.1016/S0002-8703(99)70067-0

61. Giannini C, Feldstein AE, Santoro N, et al. Circulating levels of FGF-21 in obese youth: Associations with liver fat content and markers of liver damage. *J Clin Endocrinol Metab*. 2013;98(7):2993-3000. doi:10.1210/jc.2013-1250

62. Pagano C, Soardo G, Esposito W, et al. Plasma adiponectin is decreased in nonalcoholic fatty liver disease. *Eur J Endocrinol*. 2005. doi:10.1530/eje.1.01821

63. Yan H, Xia M, Chang X, et al. Circulating fibroblast growth factor 21 levels are closely associated with hepatic fat content: A cross-sectional study. *PLoS One*. 2011;6(9). doi:10.1371/journal.pone.0024895

64. Koskenniemi JJ, Virtanen HE, Toppari J. Testicular growth and development in puberty. *Curr Opin Endocrinol Diabetes Obes*. 2017. doi:10.1097/MED.0000000000000339

65. Rasmussen AR, Wohlfahrt-Veje C, De Renzy-Martin KT, et al. Validity of self-assessment of pubertal maturation. *Pediatrics*. 2015. doi:10.1542/peds.2014-0793

66. Shin J, Kim MJ, Shin HJ, et al. Quick assessment with controlled attenuation parameter for hepatic steatosis in children based on MRI-PDFF as the gold standard. *BMC Pediatr*. 2019. doi:10.1186/s12887-019-1485-8

67. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: Comparison with the euglycemic insulin clamp. *Diabetes Care*. 1999. doi:10.2337/diacare.22.9.1462

68. George L, Bacha F, Lee SJ, Tfayli H, Andreatta E, Arslanian S. Surrogate estimates of insulin sensitivity in obese youth along the spectrum of glucose tolerance from normal to prediabetes to diabetes. *J Clin Endocrinol Metab*. 2011. doi:10.1210/jc.2010-2813

69. Utter AC, Robertson RJ, Nieman DC, Kang J. Children's OMNI scale of perceived exertion: Walking/running evaluation. *Med Sci Sports Exerc*. 2002. doi:10.1097/00005768-200201000-00021

70. 2018 Physical Activity Guidelines Advisory Committee. 2018 Physical Activity Guidelines Advisory Committee Scientific Report. *US Dep Heal Hum Serv*. 2018. doi:10.1115/1.802878.ch1

71. Piercy KL, Troiano RP, Ballard RM, et al. The physical activity guidelines for Americans. *JAMA - J Am Med Assoc*. 2018. doi:10.1001/jama.2018.14854