YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Pediatric Human Subjects in Biomedical Research

100 FR17 (2013-1)

Please refer to the HIC website for application instructions and information required to complete this application. The Instructions are available at http://www.yale.edu/hrpp/forms-templates/biomedical.html Submit the original application and one (1) copy of all materials including relevant sections of the grant which funds this project (if applicable) to the HIC.	**HIC OFFICE USE ONLY**	
	DATE STAMPED-RECEIVED	PROTOCOL NUMBER

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: : The role of obstructive sleep apnea in the pathogenesis of hepatic steatosis in obese children and adolescents			
Principal Investigator: NICOLA SANTORO		**Yale Academic Appointment:** ASSOCIATE RESEARCH SCIENTIST	
Department: PEDIATRICS			
Campus Address: 330 CEDAR STREET, NEW HAVEN LMP3107			
Campus Phone: 2037376356	**Fax:**	**Pager:**	**E-mail:** nicola.santoro@yale.edu
Protocol Correspondent Name & Address (*if different than PI*)**:**			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone:	**Fax :**	**E-mail:**	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) ☐ **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Investigator Interests:

Does the principal investigator, or do any research personnel who are responsible for the design, conduct or reporting of this project or any of their family members (spouse or dependent child) have an incentive or interest, financial or otherwise, that may affect the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? Note: The Principal Investigator (Project Director), upon consideration of the individual's role and degree of independence in carrying out the work, will determine who is responsible for the design, conduct, or reporting of the research.

See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI

❑ Yes ✓ No

Do you or does anyone on the research team who is determined by you to be responsible for the design, conduct or reporting of this research have any patent (sole right to make, use or sell an invention) or copyright (exclusive rights to an original work) interests related to this research protocol?

❑ Yes ✓ No

If yes to either question above, list names of the investigator or responsible person:

The Yale University Principal Investigator, all Yale University co-investigators, and all Yale University individuals who are responsible for the design, conduct or reporting of research must have a current financial disclosure form on file with the University's Conflict of Interest Office. Yale New Haven Hospital personnel who are listed as con-investigators on a protocol with a Yale University Principal Investigator must also have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form:
http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

a. Internal Location[s] of the Study:

☒ Magnetic Resonance Research Center (MR-TAC)
☐ Yale Cancer Center/Smilow
☐ Yale Cancer Center/Clinical Trials Office
☐ Yale-New Haven Hospital
☐ Cancer Data Repository/Tumor Registry
☐ Yale University PET Center
☒ YCCI/Church Street Research Unit (CSRU)
☒ YCCI/Hospital Research Unit (HRU)
☐ YCCI/Keck Laboratories
☐ Yale-New Haven Hospital/Saint Raphael Campus

☐ Specify Other Yale Location:

b. External Location[s]:

☐ APT Foundation, Inc.
☐ Connecticut Mental Health Center
☐ Clinical Neuroscience Research Unit (CNRU)
☐ Other Locations, Specify:
☐ Haskins Laboratories
☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven
☐ International Research Site (Specify location(s)):

c. Additional Required Documents *(check all that apply)*: ☐ N/A

☒ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☒ *Pediatric Protocol Review Committee (PPRC) Approval Date:
☒ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
☐ *Radioactive Drug Research Committee (RDRC) Approval Date:
☐ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☒ Magnetic Resonance Research Center PRC (MRRC-PRC) Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
☐ Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at http://radiology.yale.edu/research/ClinTrials.aspx

Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.
We propose to recruit a multi-ethnic cohort of obese children and adolescents during two years. Together with data analysis the total duration of the study will be 2.5 years.

3. **Research Type/Phase: (Check all that apply)**

a. **Study Type**

☒ Single Center Study
☐ Multi-Center Study
Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No ☐
☐ Coordinating Center/Data Management
☐ Other:

b. **Study Phase** ☐ **N/A**

☒ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV
☐ Other (*Specify*)

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:

☒ Clinical Research: Patient-Oriented ☐ Clinical Research: Outcomes and

☐ Clinical Research: Epidemiologic and Behavioral
☐ Translational Research #1 ("Bench-to-Bedside")
☐ Translational Research #2 ("Bedside-to-Community")
Health Services
☐ Interdisciplinary Research
☐ Community-Based Research

5. Is this study a clinical trial? Yes ☒ No ☐

NOTE the current ICMJE (International Committee of Medical Journal Editors) definition of a clinical trial: "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes." Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events"

If yes, where is it registered?

Clinical Trials.gov registry ☒
Other (*Specify*)

*Registration of clinical trials **at their initiation** is required by the FDA, NIH and by the ICMJE.*

For more information on registering clinical trials, including whether your trial must be registered, see the YCCI webpage, http://ycci.yale.edu/researchers/ors/registerstudy.aspx or contact YCCI at 203.785.3482)

6. Does the Clinical Trials Agreement (CTA) require compliance with ICH GCP (E6)?

Yes ☐ No☐

7. Will this study have a billable service? A Billable Service is defined as a service or procedure that will be ordered, performed or result in charging in EPIC for individuals who are enrolled in a clinical research study, regardless if the charge is intended to be paid by the subject/their insurance or the research study.

Yes ☒ No☐

If you answered "yes", this study will need to be set up in OnCore Support.
Contact Thomas.debski@yale.edu.

8. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ___ No **X** *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? NO

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? NO

c. Will a novel approach using existing equipment be applied? NO

If you answered "no" to question 7a, or "yes" to question 7b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

SECTION III: FUNDING, RESEARCH TEAM AND TRAINING

1. **Funding Source:** Indicate all of the funding source(s) for this study. Check all boxes that apply.
   Provide information regarding the external funding source. This information should include identification of the agency/sponsor, the funding mechanism (grant or contract), and whether the award is pending or has been awarded. Provide the M/C# and Agency name (if grant-funded). If the funding source associated with a protocol is "pending" at the time of the protocol submission to the HIC (as is the case for most NIH submissions), the PI should note "Pending" in the appropriate section of the protocol application, provide the M/C# and Agency name (if grant-funded) and further note that University (departmental) funds support the research (until such time that an award is made).

PI	Title of Grant	Name of Funding Source	Funding	Funding Mechanism
Nicola Santoro	Gene/Nutrient Interaction in the pathogenesis of hepatic steatosis	American Heart Association	☐ Federal ☐ State ☒ Non Profit ☐ Industry ☐ Other For Profit ☐ Other	☒ Grant-M# ☐ Contract# ☐ Contract Pending ☐ Investigator/Department Initiated ☐ Sponsor Initiated ☐ Other, Specify:
			☐ Federal ☐ State ☐ Non Profit ☐ Industry ☐ Other For Profit ☐ Other	☐ Grant-M# ☐ Contract# ☐ Contract Pending ☐ Investigator/Department Initiated ☐ Sponsor Initiated ☐ Other, Specify:
			☐ Federal ☐ State ☐ Non Profit ☐ Industry ☐ Other For Profit ☐ Other	☐ Grant-M# ☐ Contract# ☐ Contract Pending ☐ Investigator/Department Initiated ☐ Sponsor Initiated ☐ Other, Specify:

IRB Review fees are charged for projects funded by Industry or Other For-Profit Sponsors. Provide the Name and Address of the Sponsor Representative to whom the invoice should be sent. ***Note: the PI's home department will be billed if this information is not provided.***

Send IRB Review Fee Invoice To:

Name:
Company:
Address:

2. **Research Team:** List all members of the research team. Indicate under the affiliation column whether the investigators or study personnel are part of the Yale faculty or staff, or part of the faculty or staff from a collaborating institution, or are not formally affiliated with any institution. **ALL members of the research team MUST complete Human Subject Protection Training (HSPT) and Health Insurance Portability and Accountability Act (HIPAA) Training before they may be listed on the protocol. See NOTE below.**

	Name	Affiliation: Yale/Other Institution (Identify)	NetID
Role: Principal Investigator	Nicola Santoro	Yale University	[redacted]
Role: Co- Principal Investigator	Craig Canapari	Yale University	
Role: Study Personnel	Sonia Caprio	Yale University	
Role: Study Personnel	Ramnet Gill	Yale University	
Role: Study Personnel	Melissa M Shaw	Yale University	
Role: Study Personnel	Bridget Pierpont	Yale University	
Role: Study Personnel	Elvira Duran	Yale University	

NOTE: The HIC will remove from the protocol any personnel who have not completed required training. A personnel protocol amendment will need to be submitted when training is completed.

SECTION IV:
PRINCIPAL INVESTIGATOR/FACULTY ADVISOR/ DEPARTMENT CHAIR AGREEMENT

As the **principal investigator** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- Subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- All members of the research team will be kept apprised of research goals.
- I will obtain approval for this research study and any subsequent revisions prior to my initiating the study or any change and I will obtain continuing approval of this study prior to the expiration date of any approval period.
- I will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set by the University and qualify to serve as the principal investigator of this project or have acquired the appropriate approval from the Dean's Office or Office of the Provost, or the Human Subject Protection Administrator at Yale-New Haven Hospital, or have a faculty advisor.
- I will identify a qualified successor should I cease my role as principal investigator and facilitate a smooth transfer of investigator responsibilities.

PI Name (PRINT) and Signature _____ Date _____

As the **faculty advisor** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- This project has scientific value and merit and that the student or trainee investigator has the necessary resources to complete the project and achieve the aims.
- I will train the student investigator in matters of appropriate research compliance, protection of human subjects and proper conduct of research.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- The student investigator will obtain approval for this research study and any subsequent revisions Prior to initiating the study or revision and will obtain continuing approval prior to the expiration of any approval period.
- The student investigator will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set forth by the University and qualify to serve as the faculty advisor of this project.

Advisor Name (PRINT) and Signature _____ Date _____

Department Chair's Assurance Statement

Do you know of any real or apparent institutional conflict of interest (e.g., Yale ownership of a sponsoring company, patents, licensure) associated with this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

I assure the HIC that the principal investigator and all members of the research team are qualified by education, training, licensure and/or experience to assume participation in the conduct of this research trial. I also assure that the principal investigator has departmental support and sufficient resources to conduct this trial appropriately.

_____ _____

Chair Name (PRINT) and Signature Date

_____

Department

YNHH Human Subjects Protection Administrator Assurance Statement

*Required when the study is conducted **solely** at YNHH **by YNHH health care providers**.*

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:

- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to notify the IRB if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and had the support of the hospital for this research project.

_____ _____

YNHH HSPA Name (PRINT) and Signature Date

For HIC Use Only

_____ _____

Date Approved **Human Investigation Committee Signature**

SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** Given the growing prevalence of pediatric NAFLD and its relationship with prediabetes and type 2 diabetes in youth, it is urgent to find new therapeutic approach to this disease. Recent studies have shown an effect of obstructive sleep apnea (OSA) on hepatic fat accumulation and liver injury (1, 2, 3). In this study we will test the hypothesis the administration of continuous positive pressure (CPAP) along will allow not only to reverse the symptoms due to OSA, but also to reduce the hepatic fat content.

   Background: NAFLD is emerging as one of the most common complications of childhood obesity. It is associated with and predicts the metabolic syndrome, independent of overall obesity (4). Recently, studies in obese adolescents have demonstrated that increased ALT levels are associated with deterioration in insulin sensitivity and glucose tolerance, as well as with increasing FFA and triglyceride levels (5). Further studies showed that the prevalence of metabolic syndrome and prediabetes increases with the increases in hepatic fat content in a cohort of obese adolescents (4). Moreover, we found that the fatty liver is associated with a pronounced dyslipidemic profile characterized by large VLDL, small dense LDL, and decreased large HDL concentrations (6). Fatty liver, independent of visceral and intramyocellular lipid content plays a central role in the impairment of liver, muscle and adipose insulin sensitivity in obese adolescents (7). The progressive impairment of insulin sensitivity and possibly secretion in patients with NAFLD is testified also by the higher prevalence among obese children and adolescents with fatty liver disease of prediabetes and type 2 diabetes (8). Thus, fatty liver disease may be the hepatic component of the metabolic syndrome. The synthesis of triglycerides in the liver is nutritionally regulated, and its formation from simple carbohydrates requires multiple metabolic pathways, including glycolysis and pyruvate oxidation to generate acetyl-CoA for fatty acid synthesis, NADPH generation to supply the reductive power, packaging of fatty acids into a glycerophosphate backbone, and finally, lipoprotein packaging to export triglycerides (9). Recent studies have shown an association between fatty liver and obstructive sleep apnea (OSA) (10), a condition that has been estimated to affect up to 27% of obese children (11). In particular, OSA has been associated with the ALT levels (7, 9) and with the degree of steatohepatitis (1). Furthermore, animal studies have shown that chronic intermittent hypoxia, which is the main characteristic of OSA, leads to liver damage probably by inducing the generation of oxygen derived compounds, which in turn cause the peroxidation of lipids and the activation of pro-inflammatory pathways (2, 3). In fact, the foregoing animal studies support the hypothesis that nighttime hypoxemia triggers pathways that may induce steatosis and oxidative stress, leading to more advanced liver disease in obese patients. Generated reactive oxygen species may further amplify liver injury by activating hypoxia-inducible factor 1, a transcriptional activator and master regulator of oxygen homeostasis during hypoxia, and by up-regulating nuclear factor κ light-chain enhancer of activated B cells, with subsequent downstream induction of inflammatory pathways (12,13,14). Thus, nocturnal hypoxemia may be an important factor in the progression from isolated steatosis. These data have been recently replicated in children and adolescents. In fact, it has been shown that oxygen saturation nadir during polysomnography is related to hepatic steatosis and liver injury, demonstrated by liver biopsy (15).

   Despite those evidences and the importance of non-alcoholic fatty liver disease (NAFLD) in the development of metabolic diseases, the information concerning the association between fatty liver and OSA in obese children and adolescents is quite sparse and in particular is unclear whether OSA itself can cause NAFLD or the two conditions just coexist as obesity complications. In this study we will test the hypothesis that **OSA is one of the determinants of hepatic fat accumulation.** To prove our hypothesis we will select a group of individuals with NAFLD and OSA, who will undergo a weight

maintenance diet and Continuous Positive Airway Pressure (CPAP) for 12 weeks. CPAP is FDA approved and represents the leading therapy for obstructive sleep apnea in children over age 7 and 40 lbs. To evaluate the effect of the CPAP on the intra hepatic fat accumulation we will evaluate hepatic fat content with MRI at baseline and after the intervention. The choice of the MRI to assess hepatic fat content is based on the evidence that this technique has been shown to be strongly related to the liver biopsy, which represents the gold standard for the assessment of fatty liver disease (16).

References:

1. Mirrakhimov AE, Polotsky VY. 2012 Obstructive sleep apnea and non-alcoholic Fatty liver disease: is the liver another target? Front Neurol 3:149.
2. Aron-Wisnewsky J, Minville C, Tordjman J, Lévy P, Bouillot JL, Basdevant A, Bedossa P, Clément K, Pépin JL. 2012 Chronic intermittent hypoxia is a major trigger for non-alcoholic fatty liver disease in morbid obese. J Hepatol 56: 225-33.
3. Savransky V, Nanayakkara A, Vivero A, Li J, Bevans S, Smith PL, Torbenson MS, Polotsky VY 2007 Chronic intermittent hypoxia predisposes to liver injury. Hepatology 45: 1007-13.
4. Cali AM, De Oliveira AM, Kim H, Chen S, Reyes-Mugica M, Escalera S, Dziura J, Taksali SE, Kursawe R, Shaw M, Savoye M, Pierpont B, Constable RT, Caprio S. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? Hepatology. 49: 1896-903; 2009.
5. Burgert TS, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA, Caprio S. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. J Clin Endocrinol Metab. 91: 4287-94; 2006.
6. Cali AM, Zern TL, Taksali SE, de Oliveira AM, Dufour S, Otvos JD, Caprio S. Intrahepatic fat accumulation and alterations in lipoprotein composition in obese adolescents: a perfect proatherogenic state. Diabetes Care. 30: 3093-8; 2007.
7. D'Adamo E, Cali AM, Weiss R, Santoro N, Pierpont B, Northrup V, Caprio S. The Central Role of Fatty Liver in the Pathogenesis of Insulin Resistance in Obese Adolescents. Diabetes Care 2010; 33: 1817-2.
8. Cali AM, De Oliveira AM, Kim H, Chen S, Reyes-Mugica M, Escalera S, Dziura J, Taksali SE, Kursawe R, Shaw M, Savoye M, Pierpont B, Constable RT, Caprio S. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? Hepatology. 2009; 49: 1896-903.
9. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest. 118: 829-38; 2008.
10. Tauman R, Gozal D. 2006 Obesity and obstructive sleep apnea in children. Paediatr Respir Rev 7: 247-59.
11. Kheirandish-Gozal L, Sans Capdevila O, Kheirandish E, Gozal D. 2008 Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. Chest 133: 92-9.
12. C. Culver, A. Sundqvist, S. Mudie, A. Melvin, D. Xirodimas, S. Rocha Mechanism of hypoxia-induced NF-kappaB. Mol Cell Biol, 2010; 30: 4901–4921
13. E.Y. Dimova, T. Kietzmann Hypoxia-inducible factors: post-translational crosstalk of signaling pathways Methods Mol Biol, 2010; 647: 215–236
14. G. He, M. Karin NF-kappaB and STAT3: key players in liver inflammation and cancer. Cell Res 2011; 21: 59–168

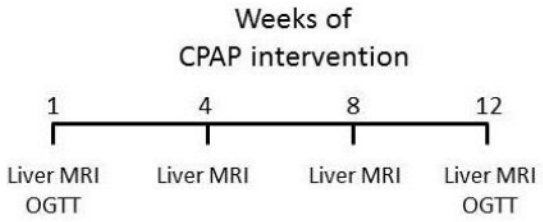
15. Sundaram SS, Sokol RJ, Capocelli KE, Pan Z, Sullivan JS, Robbins K, Halbower AC. Obstructive sleep apnea and hypoxemia are associated with advanced liver histology in pediatric nonalcoholic fatty liver disease. J Pediatr. 2014; 164: 699-706
16. T, Joshita S, Kodama R, Tanaka E, Uehara T, Sano K, Tanaka N.J Accurate and simple method for quantification of hepatic fat content using magnetic resonance imaging: a prospective study in biopsy-proven nonalcoholic fatty liver disease. Gastroenterol. 2010; 45:1263-71.

3. Research Plan: General Description

Obese children and adolescents between the ages of 9-21 diagnosed with obstructive sleep apnea (OSA) from a clinical sleep study and are at risk for fatty liver disease will undergo a screening abdominal MRI measurement of liver lipid content, and IV oral glucose tolerance test.

These at risk subjects will be identified by either elevated Alanine Aminotransferase (ALT) liver enzyme (≥35 U/L), positive family history of NAFLD, and/or known existence of disease. Those participants with hepatic fat content higher that 5.5% will be recruited.

Subjects diagnosed with OSA with a high hepatic fat content (greater than 5.5%) will receive the CPAP and after 4, 8 and 12 weeks intervention they will undergo additional MRIs and have liver function blood tests completed. Also, the patient will repeat an OGTT at 12 weeks of intervention. Along with the glucose testing, liver functions, lipid panel and hemoglobin A1C will be completed. All follow up testing will be billed to the research grant. We will repeat the MRI multiple times to assess the shortest timeframe to significantly reduce intra-hepatic fat content. See study design below



Study Schedule

Screening. The screening visit in the YCCI Research Unit will be scheduled and the PI or one of the members of the research team will explain the study in detail to each subject. Each potential subject and his/her parents will be given the consent to read and consider. If deciding to participate, then a medical history, physical exam, height, weight, vital signs will be performed to ensure that the individuals are healthy and it is safe for them to participate. A three day food record and stool sample will be obtained at this time. All subjects will be asked the medical history.

The physical exam will include:

- pulse rate and regularity at rest
- body composition as measured by Tanita scale
- blood pressure, supine, sitting and standing
- auscultation of the heart with specific attention to murmurs, gallops, clicks and rubs.

During the first visit the patients will undergo to the OGTT and abdominal MRI. Pubertal female subjects will be tested for pregnancy at screening and at each MRI. The subjects will be screened for metal implants and claustrophobia using the MRRC Safety Questionnaire.

Assessment of Glucose Tolerance Status

Oral Glucose Tolerance Test (OGTT) is completed at baseline and at the completion of 12 weeks of c-pap intervention. The study nurse will do a nursing assessment, including measuring the patient's height, weight, waist circumference, hip circumference, blood pressure, and pulse, along with evaluation of acanthosis nigricans and striae rubrae. The subject's percent body fat, fat mass and lean mass will also be measured using a Tanita scale. The nurse will obtain a family and medical history from the patient and/or the patient's parent/guardian. In addition, before starting the OGTT, the nurse will request a urine sample from the patient for analysis of microalbumin and creatinine, and to test for pregnancy. As these subjects will be followed by Yale's Obesity clinic and/or Respiratory, if the subject's doctor orders clinical laboratory tests, we will draw these while the subject's I.V. is in place. However, the subject's insurance company will be billed for these baseline tests.

The patient will receive 1.75 g/kg to a maximum of 75g of a sugar drink, orally. The patient will have one intravenous line. "Emla" or a local anesthetic (0.1cc buffered lidocaine) will be applied before the placement of the IV catheter. Blood will be drawn 10 times over three hours. Should abnormal glucose results be found, appropriate referrals will be made. We will draw approximately 60 cc of blood during this study. The blood will be analyzed for metabolic parameters, including glucose, lactate, insulin, proinsulin, c-peptide, IL-6, TNF-α, GLP-1, free fatty acids (FFAs), leptin, and adiponectin.

As part of the protocol, blood sample will be obtained for DNA extraction. A blood sample (16mL in two tubes) will be drawn at the baseline OGTT only. The DNA extraction will be stored frozen in laboratories that are locked when not in use and the information obtained will be kept on password-protected computers.

DNA will be extracted from whole blood with the use of a Flexigene Kit (Qiagen) and stored for future analyses. (See "Genetic Testing" section for further information)

Abdominal MRI. Magnetic Resonance Imaging (MRI) of the abdomen will be used to directly assess intra-abdominal fat deposition on a 1.5 Tesla magnet (Siemens). During the same session, we will acquire liver images, from which we will calculate the hepatic fat fraction and hepatic iron concentration. All patients will be screened for MRI safety before undergoing imaging of their abdomen. A localizing gradient echo sequence will be performed to allow accurate slice selection. Five true axial Fast Gradient Echo sections will be obtained with respiratory compensation through the abdomen, with the 3rd section at the L4/5 disc space. Saturation bands will be placed above and below the field of view in order to negate high signal in blood vessels. The largest field of view will be used to encompass the abdomen, although in larger patients this will be inadequate, and the sequence will need to be performed twice-once for the right side of the abdomen and once for the left. Each sequence should take approximately 6 minutes to acquire, and the entire scan should take no more than 45 minutes. The images will be analyzed for the amount of subcutaneous and visceral fat present. The hepatic fat fraction will be calculated using a modified Dixon technique. This will be performed using a single breath-hold scan of about 15 seconds to obtain a section through the liver. The standard Siemens body coil

will be used for transmit and receive and the imaging uses standard Siemens sequences. The FOV=400mm for all sequences.
These include:
1. a gradient echo localizer: 20 second scan time, TR = 24ms, TE = 6ms, 3 slices, 10mm thick. 256x128 matrix, flip = 30.
2. T1 FLASH sequence: 7 slices, 5mm thick, TE = 4.76ms, TR=100ms, 256x128 matrix. flip = 90.
3. Dixon sequence: T1 weighted flash, TE = 2.35, 4.76, TR=18ms, flip = 30, 256x128, slice thickness = 10mm, 1 slice.
4. Dixon true-Fisp sequences, (TE = 1.49, TR=2.98ms), (TE=2.7ms, TR=5.39ms), 256x128, 12 slices, thickness=10mm, flip=55degree.

The signal from an appropriate area of hepatic tissue will be used to calculate the fat fraction. Importantly, an excellent correlation was reported between the liver fat content measured by FAST-MRI and liver biopsy (r^2 = 0.853, $p<0.001$). The hepatic fat fraction will also be calculated using a three-point Dixon technique in combination with the iterative least-squares estimation method. As this method is implemented in a steady-state free precession sequence, it allows one to obtain multiple sections (typically 10-14 slices) through the liver within a single breath-hold scan of about 25-30 seconds. A scout image requiring a breath-hold of about 12 seconds will also be obtained. The hepatic iron concentration will be calculated using a T2 weighted and intermediate weighted gradient echo sequence obtained during two breath-hold sequences of about 26 seconds each. The signal from an appropriate area of hepatic tissue will be used to calculate the hepatic iron concentration. Subjects may be asked to repeat a scan if a sub-optimal image is obtained on the first attempt.

Evaluation of Dietary Intake: This will be done by a registered dietitian. Each subject will be instructed on how to keep a 3-day food diary at the time of assent/consent. The subject will return at baseline with their food recall and will also have a brief interview with the dietitian to determine their usual intake. The three-day food record will include two weekdays and one weekend day, while the brief interview with the subject and dietitian will serve as an opportunity to confirm portion sizes, brand names, and other details. Both the 3-day food diary and usual intake interview will be analyzed using the Nutrition Data System (NDS) for Research (version 4.02) (University of Minnesota). Macronutrient composition of diet, as well as types of fatty acids and carbohydrates will be included in the analysis.

Stool Specimen: The subject (or subject's parent/guardian) will be provided with a kit to collect stool. If the subject cannot provide the sample at the given time, they can complete it at home. Once collected, the container will remain at 4°C and be brought back to researchers within 12 hours. The bacterial DNA will be extracted and the bacterial gene coding for the 16S RNA subunit will be characterized by Next Generation sequencing to assess the bacterial diversity.

Intervention
Continuous Positive Airway Pressure (CPAP) is an FDA approved standard of care therapy for obstructive sleep apnea in children over age 7 and 40 lbs (1). CPAP is a non-invasive technique for providing single levels of air pressure from a flow generator, via a nose mask, through the nares. The purpose is to prevent the collapse of the oropharyngeal walls and the obstruction of airflow during sleep, which occurs in obstructive sleep apnea. Patients wear a face or nasal mask during sleep. The mask, connected to a pump, provides a positive flow of air into the nasal passages in order to keep the airway open. CPAP machines monitor compliance data, which is

recorded on a chip and transferred via a physical download or wireless transmission (2, 3). The intervention in this study would be the application of CPAP to obese children with persistent obstructive sleep apnea and fatty liver, with the independent variable being hours of CPAP use.

References.

1. Marcus, C. L., Brooks, L. J., Draper, K. A., Gozal, D., Halbower, A. C., Jones, J., et al. (2012). Diagnosis and Management of Childhood Obstructive Sleep Apnea Syndrome. *Pediatrics*, *130*(3), 576–584. doi:10.1542/peds.2012-1671.
2. Schwab, R. J., Badr, S. M., Epstein, L. J., Gay, P. C., Gozal, D., Kohler, M., et al. (2013, September 1
3. An official American Thoracic Society statement: continuous positive airway pressure adherence tracking systems. The optimal monitoring strategies and outcome measures in adults. *American Journal of Respiratory and Critical Care Medicine*. doi:10.1164/rccm.201307-1282ST

4. **Genetic Testing**

A. The DNA will be collected to assess whether common gene variants associated with fatty liver disease (*PNPLA3* rs738409, *GCKR* rs1260326 etc.) might also influence the relationship between NAFLD and OSA. Some of these variants and their relationship with fatty liver have been extensively investigated by our group (Santoro N et al. Hepatology 2010; Santoro N et al. Hepatology 2011, Santoro N et al. Diabetes Care 2013).

   i. We will collect the blood and extract the DNA from white blood cells.
   ii. the clinical information concerning the sleep study, the oral glucose tolerance test and the imaging studies will be collected.
   iii. the DNA will be identified with a number. We will keep one file containing the patients information, which will be accessible only for the PIs. Also, patients will be re-consented at the age of majority.

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects? Once the clinical data are collected, we plan to work on the unidentified samples.
C. Is widespread sharing of materials planned? NO
D. When and under what conditions will materials be stripped of all identifiers? The material will be stripped of identifiers at study end.
E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? Yes
   i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)? We will keep one file containing the patients' information, which will be accessible only for the PIs. If the patients will require their information to be withdrawn, the DNA will be discarded and all the clinical information deleted.

F. Describe the provisions for protection of participant privacy. The genetic material will be immediately unidentified, soon after being extracted. Describe the methods for the security of storage and sharing of materials. As long term storage of the genetic material is planned, it will be stored at-20C in the Yale Core Lab space.

5. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.
   Potential subjects will be recruited through the Yale Obesity Clinic or Yale Pediatric Respiratory. The PI or a member of the research team will describe the research study to the potential subject during clinic. The potential subject and parent/guardian will then be given the name and phone number of the research coordinator and asked to contact the coordinator if interested in the study. Alternatively, The PI or a member of the research team will ask permission from the potential subject and parent/guardian to have the research coordinator call them directly. The PI or a member of the research team may also inquire about whether the potential subject has eligible siblings.
   The following groups will be targeted for enrollment: 15 Obese children and adolescents between 9 and 21 years.

6. **Subject classification:** Check off all classifications of subjects that will be targeted for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☒ Children	☐ Healthy	☐ Fetal material, placenta, or dead fetus
☐ Non-English Speaking	☐ Prisoners	☐ Economically disadvantaged persons
☐ Decisionally Impaired	☐ Employees	☐ Pregnant women and/or fetuses
☐ Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects? ☐ Yes ☒ No (If yes, see HIC Application Instructions section VII #2 for further requirements)

7. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

Inclusion Criteria:

- Sleep Apnea as diagnosed by clinical sleep study (Apnea Hypopnea index greater than 1)
- Evidence of NAFLD as diagnosed by screening MRI (hepatic fat fraction ≥5.5%) Obese child/adolescent between 9-21 years old
- Compliance with using C-pap as instructed

Exclusion Criteria

- Medications or know disease known to alter glucose or insulin metabolism such as oral steroids, or certain psychiatric medications, such as Xeleca, Lithium and Paxil.
- Type 2 Diabetes Mellitus
- Medications for chronic anti-inflammatory effects
- Consumption of alcohol

8. How will **eligibility** be determined, and by whom?
   Eligible patients will have a diagnosis of OSA (Apnea hypopnea index higher than 1.5) and an hepatic fat content as assessed by the MRI higher than 5.5%. The eligibility will be evaluated by the Principal Investigators.

9. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research. We consider the risks of this study to be minimal. The specific risks are as follows:

- **Privacy Confidentiality.** Subjects may be concerned about privacy of personal health information and data collected and stored.

- **Indwelling Catheters.** Potential complications of indwelling catheters are hematomas and discomfort and rarely infection, thrombophlebitis and bleeding at the site. The incidence of this complication in our hands is less than 0.1%. Local hematomas are uncommon and on occasion there is short-lived local pain occurring at the site of venipuncture. Very rarely, subjects may also faint or become nauseated when indwelling catheters are placed. Subjects occasionally become nauseated, get a headache or feel shaky or lightheaded during or after the I.V. studies. Subjects may take an age-appropriate dose of Tylenol prn if a headache does occur. Any other symptoms will be addressed appropriately if they occur.
- **Amount of blood to be drawn.** A total of approximately 60cc of blood will be drawn during the OGTT. We will be using the Yale-New Haven Hospital (YNHH) standard of care guideline (blood sampling from a peripheral catheter) to re-infuse the blood saline discards. This method is used to prevent physiological anemia.
- **DNA Analysis.** The analysis of blood for genetic research raises special issues of confidentiality. Variation in some genes is known to be directly related to risk for certain illnesses. In some cases, knowledge of genetic information could have negative psychological consequences or could affect access to or retention of certain benefits or entitlements. For example, the information could potentially be used against an individual if it were revealed to insurance companies or potential employers. We will take precautions to ensure that confidentiality is maintained and that the genetic information is not unintentionally disclosed to inappropriate third parties. 16 cc of blood will be drawn for this analysis.
- **MRI.** Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.
  The patient will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens to the subject, the subject may ask to stop the study at any time and we will take the subject out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. The researcher will discuss this senstation to the subject and instruct to notify research staff if it happens..
  There are some risks with an MR study for certain people. If the subject has a pacemaker or some metal objects inside their body, the subject may not be in this study because the strong magnets in the MR scanner might harm the subject. Another risk is the possibility of metal objects being pulled into the magnet and hitting the subject. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. We also ask all people involved with the study to walk through a detector designed to detect metal objects. It is important to know that no metal can be brought into the magnet room at any time. Also, once the subject is in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

CPAP.

Continuous positive airway pressure is a commonly prescribed therapy for obstructive sleep apnea which is recommended for the treatment of obstructive sleep apnea in children and adults. In children it is recommended for children who have obstructive sleep apnea in spite of adenotonsillectomy or are not candidates for adentonsillectomy, usually due to the absence of significant enlargement of the tonsils and adenoids. It is comprised of a machine which applies pressure via a tube and mask to keep the upper airway from collapsing during sleep. The risks of CPAP use include: skin irritation and breakdown if the mask does not fit correctly; eye dryness if there is air leaking out of the mask into the eye during the night; air leaking out of the mouth if a nasal mask interface is used which may cause mouth dryness; fullness or nausea from swallowed air although this is uncommon in the range of pressures used commonly in clinical practice; and disrupted sleep from discomfort or noise from the machine during a period of habituation. In our study CPAP is provided as a part of clinical care in patients included in our study. To mitigate issues with skin irritation or mouth leak, the patients will have had an overnight sleep study to determine the optimal mask fit and pressure of therapy. At the time that the machine is provided by the home care company, mask fit is verified. Patients on CPAP therapy are followed up regularly in the Sleep Clinic until CPAP therapy is proceeding successfully. If issues with the mask develop, a new one is typically provided by the home care company.

Minimizing Risks: Describe the manner in which the above-mentioned risks will be minimized.

Privacy Confidentiality. Safeguards will be in place via password protected computers, encryptions of data when necessary, as well as secure file transfers.

Our subjects will be questioned and examined prior to the study. During the study, our subjects will be carefully monitored for any discomfort with IV lines. The discomfort during placement of the indwelling catheter will be minimized by offering the subject/parent the option of utilizing a topical anesthetic at the venipuncture site. The risk of subjects feeling faint or lightheaded will be minimized by having them remain supine during their indwelling catheter placement. If phlebitis occurs, it may be minimized through the application of heat and elevation of the arm. In addition, nurses who insert indwelling catheters have special training and experience in drawing blood from obese subjects.

For the MRI study, the subjects will be asked whether they become nervous while being in an enclosed space. Subjects will be monitored throughout the study; if the subject feels anxious during this study and wishes to end it, we will take the subject out of the magnet. Subjects will be asked to remove all metal before the scans.

For CPAP treatment, the subjects will receive mask fitting and habituation in the sleep laboratory as per routine clinical protocols. Subsequently, the home equipment will be provided by the home care company including follow up for equipment issues. Patients will follow up in Sleep Clinic within one month of CPAP, and then subsequently as per clinical routine.

DNA Analysis: To minimize the risk to the subject's confidentiality, all samples for DNA analysis will be labeled with a study identification number that cannot be linked to the subject's identity; however, a link to the subject's identity will be kept on a master list. This information will be stored on a password protected computer on a password protected data sheet and the specimen will be stored in a locked laboratory when not in use. No results from the DNA analysis will be returned to the subject/parent or any of the subject's health care practitioners and will not be placed in the subject's medical record. If the subject/parent wishes to withdraw

samples, all links to the subject's identifiers will be destroyed and the samples will be anonymized. Any data obtained from the sample up to the point of subject/parent withdrawal will continue to be used in the research. The sample and data will not be used or tested for purposes other than those authorized and the sample will be retained for as long as it is deemed useful for research purposes. The genotyping will be specific to not only fatty liver disease, but also to diabetes, obesity, and cardiovascular disease.

11. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study?
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study?
    c. Data and Safety Monitoring Plan
    For Data and Safety Monitoring Plan templates, see
    http://www.yale.edu/hrpp/forms-templates/biomedical.html

. The overall risk assessment is as follows: This study poses minimal risk to the subject.

b. This study poses minimal risk to the children participating in the study.

The principal investigator is responsible for monitoring the data and conducting safety reviews every six months. During the review process the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Either the principal investigator or the HIC have the authority to stop or modify the study.

This protocol presents minimal risks to the subjects and adverse events or other problems are not anticipated. In the unlikely event that such events occur, serious and unanticipated and related adverse events or unanticipated problems involving risks to subjects or others will be reported in writing within 48 hours to the HIC (using the appropriate HIC forms from the website) and the NIH. The investigator will apprise fellow investigators and study personnel of all adverse events that occur during the conduct of this research by e-mail or ad hoc meeting. The protocol's research monitors; the Yale Center for Clinical Investigation Research Subject Advocates (YCCI RSAs) and NIH will be informed of serious adverse events within 48 hours of the event becoming known to the principal investigator.

d. For multi-site studies for which the Yale PI serves as the lead investigator: N/A
    i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
    ii. What provisions are in place for management of interim results?
    iii. What will the multi-site process be for protocol modifications?

17. **Statistical Considerations:** Describe the statistical analyses that support the study design.
Statistical Considerations:
Statistical Analysis Plan

Power and Sample Size:
The hypothesis of our primary aim is that CPAP will decrease the intra hepatic fat accumulation measured with MRI between baseline after 4, 8 and 12 weeks of intervention in obese youth. A sample size of 15 produces a two-sided 95% confidence interval with a distance from the mean paired difference to the limits (i.e., precision or margin of error) that is equal to 0.55 when the estimated standard deviation of the paired differences is 1.

Statistical Methods:
For all analyses, a two-sided p-value of 5% will be used to test for statistical significance and will be performed using SAS v9.3 (SAS Institute, Cary, NC). We will calculate descriptive statistics (i.e., mean, SD, median, interquartile range, N and percentage) for all participants at baseline 4, 8 and 12 weeks of intervention. The difference in hepatic fat content between baseline and 4, 8 and 12 weeks will be tested using one-sample Wilcoxon signed rank-test. If necessary, we will also investigate whether or not the change (e.g., decrease) could be attributed to the differences in patients' demographic and other clinical characteristics, e.g., by the means of Wilcoxon rank sum test in patients from different groups stratified by some factor (e.g., male vs. female). This, however, pertains to hypothesis generation for a larger future study.

SECTION VI: RESEARCH INVOLVING DRUGS, BIOLOGICS, PLACEBOS AND DEVICES

A. DRUGS and BIOLOGICS N/A

1. **Identification of Drug or Biologic:** What is (are) the **name(s)** of the drug(s) or biologic(s) being used? **N/A**.

All protocols which utilize a drug or biologic **not** approved by, but regulated by, the FDA must provide the following information:

What is the Investigational New Drug (IND) **number** assigned by the FDA?

Who holds the IND?

All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: _____
Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step)
Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States. If there is no IND and an exemption is being sought, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1
The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☐ Yes ☐ No
ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. ☐ Yes ☐ No
iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product. ☐ Yes ☐ No
iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☐ Yes ☐ No
v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☐ Yes ☐ No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):
- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

3. **Source:** a) Identify the source of the drug or biologic to be used.

   b) Is the drug provided free of charge? ☐ Yes ☐ No
   If yes, by whom?

4. **Storage**, **Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.
   Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

   Check applicable Investigational Drug Service utilized:

   ☐ **YNHH IDS**
   ☐ **CMHC Pharmacy**
   ☐ **PET Center**
   ☐ **Other:**
   ☐ **Yale Cancer Center**
   ☐ **West Haven VA**
   ☐ **None**

   Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

5. **Use of Placebo:** ☐ **Not applicable to this research project**
   Provide a justification which addresses the following:
   a. Describe the safety and efficacy of other available therapies (if any).
   b. State the maximum total length of time a participant may receive placebo while on the study.
   c. Address the greatest potential harm that may come to a participant as a result of not receiving effective therapy (immediate or delayed onset.)
   d. Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Use of Controlled Substances:**
   Will this research project involve the use of controlled substances in human subjects?
   ☐ Yes ☐ No *See HIC Application Instructions to view controlled substance listings.*

   If yes, is the use of the controlled substance considered:
   ☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.
   ☐ Non Therapeutic: Note, the use of a controlled substance in a non therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. *Note, the use of a controlled substance in a non-therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See HIC Application Instructions for further information.*

7. **Continuation of Drug Therapy After Study Closure** ☐ **Not applicable to this project**

Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended? ☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

☐ No If no, explain why this is acceptable.

B. DEVICES

1. Are there any investigational devices used or investigational procedures performed in a YNHH Operating Room? Yes ☐ No ☒ *If Yes, please be aware of the following requirements*:

   a. A YNHH OR New Product/Trial Request Form must be completed. Please contact the OR Materials Manager, Chris Baillargeon, at 203-688-8912 for more information on this requirement;

   b. Your request must be reviewed and approved by the Operating Room New Technology Committee before patients may be scheduled; and

   c. The notice of approval from the OR New Technology Committee must be submitted to the HIC for the protocol file.

   Please contact Gina D'Agostino, gina.d'agostino@ynhh.org or 203-688-5052, to initiate the process.

2. **What is the name of the device to be studied in this protocol?** Continuous positive airway pressure (**CPAP**).

   Has this device been FDA approved? ☒ Yes ☐ No
   If yes, state for what indication.

3. **Background Information:** Provide a description of previous human use, known risks, and any other factors that might influence risks. If this is the first time this device is being used in humans, include relevant data on animal models.

   Continuous positive airway pressure is a commonly prescribed therapy for obstructive sleep apnea which is recommended for the treatment of obstructive sleep apnea in children and adults. In children it is recommended for children who have obstructive sleep apnea in spite of adenotonsillectomy or are not candidates for adentonsillectomy, usually due to the absence of significant enlargement of the tonsils and adenoids. It is comprised of a machine which applies pressure via a tube and mask to keep the upper airway from collapsing during sleep. The risks of CPAP use include: skin irritation and breakdown if the mask does not fit correctly; eye dryness if there is air leaking out of the mask into the eye during the night; air leaking out of the mouth if a nasal mask interface is used which may cause mouth dryness; fullness or nausea from swallowed air although this is uncommon in the range of pressures used commonly in clinical practice; and disrupted sleep from discomfort or noise from the machine during a period of habituation. In our study CPAP is provided as a part of clinical care in patients included in our study. To mitigate

issues with skin irritation or mouth leak, the patients will have had an overnight sleep study to determine the optimal mask fit and pressure of therapy. At the time that the machine is provided by the home care company, mask fit is verified. Patients on CPAP therapy are followed up regularly in the Sleep Clinic until CPAP therapy is proceeding successfully. If issues with the mask develop, a new one is typically provided by the home care company.

4. **Source:**

a) Identify the source of the device to be used. Continuous Positive Airway Pressure (CPAP) provided by home care companies.

b) Is the drug provided free of charge? ☐ Yes ☒ No
If yes, by whom? CPAP devices and associated supplies will be provided by home care companies as part of standard of care.

5. **What is the PI's assessment of risk level (significant or non-significant) associated with the use of the device?**

☐ **Significant Risk (SR) Device Study:** A study of a device that presents a potential for serious risk to the health, safety, or welfare of a participant and 1) is intended as an implant; 2) is used in supporting or sustaining human life; or otherwise prevents impairment of human health; 3) is of substantial importance in diagnosing, curing, mitigating or treating disease, or otherwise prevents impairment of human health; or 4) otherwise presents a potential for serious risk to the health, safety, or welfare of a participant.

Significant Risk Devices require an Investigational Device Exemption (IDE) issued by the FDA.

a. What is the **IDE number** assigned by the FDA?

b. Did the FDA approve this IDE as **Category A** (experimental/investigational) or as **Category B** (non-experimental/investigational)?

c. Who holds the IDE?

☒ **Non-Significant Risk (NSR) Device Study:** A study of a device that does not meet the definition for a significant risk device and does not present a potential for serious risk to the health, safety, or welfare of participants. Note that if the HIC concurs with this determination, an IDE is not required.

6. **Abbreviated or Exempt IDE:** There are abbreviated requirements for an IDE and there also are exemptions to the requirement for an IDE. *See the criteria in the HIC Application Instructions,* Section VI.B.4 at **http://www.yale.edu/hrpp/forms-templates/biomedical.html** *to determine if these pertain to this study.*

☐ **Abbreviated IDE or Exempt IDE** – *If criteria set forth in the HIC Application Instructions are met, copy and paste the completed relevant section from the Instructions into this application.*

7. Investigational device accountability

State how the PI, or named designee, ensures that an investigational device is used only in accordance with the research protocol approved by the HIC, and maintains control of the investigational device as follows:

a. Distributes the investigational device to subjects enrolled in the IRB-approved protocol: The CPAP represents a standard of care intervention for OSA. The device is provided by the healthcare companies and Dr Canapari will explain to the subjects how to use the device and monitor the compliance of the patients to the intervention.

SECTION VII: RECRUITMENT/CONSENT/ ASSENT

1. Targeted Enrollment: Give the number of subjects: 15

a. targeted for enrollment at Yale for this protocol: children and adolescents between 9 and 21 years of age, affected by OSA and fatty liver disease.

b. If this is a multi-site study, give the total number of subjects targeted across all sites N/A

2. Indicate recruitment methods below. Attach copies of any recruitment materials that will be used.

- ☐ Flyers
- ☐ Posters
- ☐ Letter
- ☐ Medical Record Review
- ☐ Departmental/Center Newsletters
- ☐ YCCI Recruitment Database
- ☐ Other (describe):
- ☐ Internet/Web Postings
- ☐ Mass E-mail Solicitation
- ☐ Departmental/Center Website
- ☐ Departmental/Center Research Boards
- ☐ Web-Based Clinical Trial Registries
- ☐ Clinicaltrials.gov Registry (do not send materials to HIC)
- ☐ Radio
- ☐ Telephone
- ☐ Television
- ☐ Newspaper

3. Recruitment Procedures:

Potential subjects will be recruited through the Yale Obesity Clinic or Yale Pediatric Respiratory. The PI or a member of the research team will describe the research study to the potential subject during clinic. The potential subject and parent/guardian will then be given the name and phone number of the research coordinator and asked to contact the coordinator if interested in the study. Alternatively, The PI or a member of the research team will ask permission from the potential subject and parent/guardian to have the research coordinator call them directly. The PI or a member of the research team may also inquire about whether the potential subject has eligible siblings.

4. Screening Procedures:

A. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☐ Yes ☒ No

B. If yes, identify any health information and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:

HIPAA identifiers:

☐ Names

☐ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.

☐ Telephone numbers

☐ Fax numbers

☐ E-mail addresses

☐ Social Security numbers

☐ Medical record numbers

☐ Health plan beneficiary numbers

☐ Account numbers

☐ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older

☐ Certificate/license numbers

☐ Vehicle identifiers and serial numbers, including license plate numbers

☐ Device identifiers and serial numbers

☐ Web Universal Resource Locators (URLs)

☐ Internet Protocol (IP) address numbers

☐ Biometric identifiers, including finger and voice prints

☐ Full face photographic images and any comparable images

☐ Any other unique identifying numbers, characteristics, or codes

5. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐ Yes, all subjects

☒ Yes, some of the subjects

☐ No

Dr. Canapari and Dr. Caprio will have a clinical relationship with some, if not all of the subjects, in Yale Respiratory clinic.

6. Request for waiver of HIPAA authorization (N/A): (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one: For entire study: _____ For recruitment purposes only: __X____

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data; Properly assessing a subject's eligibility cannot be done without asking PHI.
ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data; We are not requesting a waiver of signed authorization.

By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:
   ☒ Compound Consent and Authorization form
   ☐ HIPAA Research Authorization Form

8. **Consent Personnel:** List the names of all members of the research team who will be obtaining consent/assent.
   Craig Canapari, M.D
   Nicola Santoro, M.D, PhD
   Ramnet Gill, M.D.
   Bridget Pierpont, M.A.
   Melissa Shaw, B.S.
   Elvira Duran, B.A.

9. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

The study will be explained at Pediatric Obesity Clinic or Pediatric Respiratory visit or may be recruited by phone if they have given permission to be contacted from previous studies. An authorized staff will explain the study and obtain consent if the child and parent are interested. If after explaining the study, the family would like to give the decision additional thought, we will follow up via phone (and answer additional questions, if necessary) and make a later appointment for consent if the family decides to enroll.

10. **Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

The researcher obtaining consent will ask the subject a series of questions to ensure that they have understood the main procedures involved in the study, along with the risks and commitment**.** Only after this they will be asked to give informed consent to participate.

11. **Documentation of Consent/Assent:** Specify the documents that will be used during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.

Compound Authorization and Consent Form for subjects older than 18 years and a Compound Authorization and Parental Permission Form for subjects younger than 18 years. Two different assent forms will be developed, one for children between 9 and 12 and a second one for children between 13 and 17 years. The researcher obtaining consent will ask the subject a series of questions to ensure that they have understood the main procedures involved in the study, along with the risks and commitment**.** Only after this they will be asked to give informed consent to participate.

12. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. Translated copies of all consent materials must be submitted for approval prior to use.

All study participants must be English-speaking.

13. **Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study.** If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☒ **Not Requesting a consent waiver**
☐ **Requesting a waiver of signed consent**
☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6)**

☒ **Requesting a waiver of signed consent for <u>Recruitment/Screening</u> only**

If requesting a waiver of signed consent, please address the following:

a. Would the signed consent form be the only record linking the subject and the research?
☒ Yes ☐ No

b. Does a breach of confidentiality constitute the principal risk to subjects?
☒ Yes ☐ No

OR

c. Does the research activity pose greater than minimal risk?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity.
☒ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

☐ **Requesting a waiver of signed consent for the <u>Entire Study</u>** (Note that an information sheet may be required.)

If requesting a waiver of signed consent, please address the following:

a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No

b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research pose greater than minimal risk? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

B. **Full waiver** of consent: (No consent from subjects will be obtained.)

☐ **Requesting a waiver of consent for <u>Recruitment/Screening</u> only**

a. Does the research activity pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☐ No

b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No

c. Why would the research be impracticable to conduct without the waiver?

d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

☐ **Requesting a full waiver of consent for the <u>Entire Study</u> (Note: If PHI is collected, information here must match Section VII, question 6.)**

If requesting a full waiver of consent, please address the following:

a. Does the research pose greater than minimal risk to subjects? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No

c. Why would the research be impracticable to conduct without the waiver?

d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

b. How will the research data be collected, recorded and stored?

c. How will the digital data be stored? ☐ CD ☐ DVD ☐ Flash Drive ☐ Portable Hard Drive ☐ Secured Server ☐ Laptop Computer ☒ Desktop Computer ☐ Other

d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?

All personal information will be handled in confidence and in accordance with local data protection laws. All research materials will be held in locked cabinets and stored on password-protected computers. Only study personnel will have access to the database to record results as they accrue. While being analyzed samples will be stored in locked areas in the secured laboratories of the investigator. When the results of the research are published or discussed in conferences, no information will be included that would reveal a subject's identity unless specific consent for this is obtained. The subjects will be informed of those parties who may have access to their data as listed on the Research Authorization form.

Do all portable devices contain encryption software? ☒ Yes ☐ No

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

With the permission of the subject, extra blood samples and information collected during this research study may be stored indefinitely and used by our research group for future studies on the pathophysiology of diabetes, obesity, hepatic disease, and cardiovascular disease. These extra blood samples will not be used for genetic testing. Any information derived from the additional studies will be kept confidential. The subject may at any time request that the blood samples be destroyed. When the results of the research are published or discussed in conferences, no information will be included that would reveal the subject's identity unless the subject's specific consent for this activity is obtained. Study results will continue to be stored on password-protected computers. Data analyses will be performed and the information may be used in papers or presentations by the research team, but no identifiable subject data will be revealed. Representatives from the Yale Human Investigation Committee and the National Institutes of Health may inspect study records during internal auditing procedures. However, these individuals are required to keep all information confidential.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data) The P.I. and research staff are the only ones that have access to the protected health information

g. If appropriate, has a Certificate of Confidentiality been obtained? N/A

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g. HIV testing – reporting of communicable diseases; parent interview - incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported. NO

SECTION IX: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

The study offers the prospect of direct benefit to the subjects. The OGTT will allow us to check the glucose tolerance status of the subjects. Should they are IGT or diabetic, appropriate referral

will take place. Furthermore, we will determine the lipid profiles, insulin sensitivity, distribution of abdominal fat, and percent body fat of each subject. In addition, a radiologist will review each abdominal MRI image and will inform us if any abnormalities are seen. We, in turn, will inform the subject and take appropriate action. Furthermore, the CPAP represents the standard of care for the treatment of the OSA.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?
   Patients approached about the study are always offered continuing care and follow-up at the Obesity Clinic/Sleep Clinic regardless of their decision to participate in the study.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
   To help offset the cost of the subjects' participation in the study; $50 for the OGTT done as part of this study. They will also receive $60 for the abdominal MRI. The total compensation a subject could potentially earn is $340. If subjects park in the Howard Avenue Garage, we can validate their parking.

3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

There will be no cost to participants.

4. **In Case of Injury:**
   a. Will medical treatment be available if research-related injury occurs?
   b. Where and from whom may treatment be obtained?
   c. Are there any limits to the treatment being provided?
   d. Who will pay for this treatment?
   e. How will the medical treatment be accessed by subjects?

Though unlikely, if injury should occur during any of the procedures, the overseeing physician and the study nurse will attend to the immediate needs of the patient. Should any acute care visit (i.e. emergency room or outpatient clinic), hospital admission or chronic care be necessary, the subject's medical insurance will be responsible for covering any incurred charges. All subjects will be provided with the phone number for the doctor and study personnel and an emergency number.