Study Title:

Treating PCOS with Semaglutide vs Active Lifestyle Intervention (TEAL Study)

Protocol Version Date: 11/4/2022

NCT03919929

Abstract

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COMIRB Protocol

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Project Title: Treating PCOS with Semaglutide vs Active Lifestyle Intervention (TEAL Study)

Version Date: 11/4/2022
Principal Investigator: Melanie Cree-Green

Financial Sponsor: National Institutes of Health (NIH)

Investigational Product name(s): Rybelsus® (semaglutide)

IND Sponsor listed with FDA: Melanie Cree-Green (IND# 146652)

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PROTOCOL SYNOPSYS	
Title:	Treating PCOS with Semaglutide vs Active Lifestyle Intervention (TEAL Study)
Sponsor:	National Institutes of Health
Phase:	Phase 3 Clinical Trial
Description of Sites/Facilities Enrolling Participants:	Children's Hospital Colorado
Description of Study Intervention:	Rybelsus (semaglutide) oral tablets vs dietary intervention
Study Duration:	60 months
Participant Duration:	5-6 months
Rationale:	In obese girls with polycystic ovary syndrome, testosterone and obesity combine to create unique pathology to increase metabolic disease including fatty liver and insulin resistance, which may be mediated by altered glucagon like peptide-1 activity. The investigators will treat girls with obesity and polycystic ovary syndrome for 4 months with a glucagon like peptide-1 receptor agonist compared to dietary intervention to primarily lower hepatic fat and secondarily improve whole body and adipose insulin sensitivity. Mechanisms of hepatic metabolism, including rates of de novo lipogenesis and relative mitochondrial flux will also be assessed. The objective of this clinical trial is to define the metabolic impact of 4-months of treatment with Rybelsus vs. diet therapy in girls with Polycystic Ovarian Syndrome (PCOS). Our previous research findings have shown that girls with PCOS have a higher degree of metabolic disease when compared to BMI matched controls. This includes an excess of non-alcoholic-fatty liver disease (NAFLD), dysglycemia, upregulated de-novo lipogenesis (DNL), hepatic, adipose and peripheral insulin resistance (IR) and decreased GLP-1 secretion. Our pilot data demonstrated that there were metabolic improvements with short-duration GLP-1 RA exenatide administration, with no side effects observed. The long-term consequences of NAFLD in girls with PCOS are of great clinical importance. This study will define the metabolic impact of a currently available medication on this common high-risk disease in youth thereby changing health outcomes.
Study Design:	This study is a randomized (2:1) semaglutide (Rybelsus®) vs dietary counseling. After completing a screening visit, participants will complete a baseline metabolic study and then be randomized to either semaglutide oral tablets or dietary counseling for a period of 4 months. There will be a check-in visit halfway through the study (mid-treatment visit), and then they will complete a final metabolic study at the completion of their 4 month intervention.
Primary Objective:	Aim 1: Quantitate change in IHTG in obese girls with PCOS after 4 months of GLP-1 RA therapy, compared to girls undergoing intensive dietary counseling and similar weight loss. Hypothesis: IHTG will decrease after GLP-1 RA therapy in PCOS girls and will be greater than that seen from dietary counseling alone. Methods: IHTG will be measured with MRI before and after interventions.
Secondary Objectives:	Exploratory Aim 2: Measure changes in substrate delivery and utilization after GLP-1 RA or intensive diet. A. Measure changes in rates of DNL and adipose IR, ie insulin suppression of FFA release Hypothesis: GLP-1 RA therapy will significantly improve adipose IR and decrease DNL, resulting in reduced IHTG. Changes will be greater than those seen from dietary

	counseling alone. Methods: Deuterated water will be utilized to measure DNL. Whole body and adipose insulin sensitivity will be modeled from FFA and glucose changes relative to insulin concentrations during oral sugar tolerance test (OSTT). B. Assess rates of hepatic TCA cycling and NAFLD severity Hypothesis: Excess cataplerotic TCA cycling provides substrate for DNL and contributes to hepatic damage via the production of reactive oxygen species. Methods: Oral U-$^{13}C_3$ glycerol will be consumed and serum glucose and VLDL undergo isotopomer analysis to determine relative TCA cycle flux. Hepatic ^{31}Phosphorus MR spectroscopy and MR elastography will be utilized to document NAFLD severity and ATP concentrations. xploratory Aim 3: Determine the extent to which diet or GLP-1 treatment alters neuronal response within, and functional connectivity between, homeostatic and non-homeostatic brain regions in adolescents with PCOS and obesity. Hypothesis: Connectivity between the homeostatic and non-homeostatic brain regions will be significantly greater after treatment with semaglutide compared to diet.
Number of Patients:	Up to 60, with the goal of completing 50
Enrollment Criteria:	Inclusion Criteria 1) Female 2) Ages 12-21 years 3) Sedentary- less than 2 hours of moderate (jogging, swimming etc) exercise a week. 4) BMI equal or greater than the 90th percentile for age and gender 5) PCOS per the most stringent NIH criteria adapted for adolescents (irregular menses >12 months post-menarche and clinical or biochemical hypertestosteronemia)[8,34] 6) Participants cannot be on hormonal contraception, so participants should remain abstinent or use reliable non-hormonal contraception (e.g. copper IUD) for the entire study period. For participants who receive semaglutide, they should avoid pregnancy for at least 2 months after stopping medication to avoid fetal exposure to the medication. Exclusion Criteria: 1) Diagnosed with or have a family history of medullary thyroid carcinoma (MTC) or Multiple Endocrine Neoplasia syndrome type 2 (MEN 2). Family history of medullary thyroid cancer or thyroid nodule palpated by endocrinologist at screening. 2) Use of medications known to affect insulin sensitivity: metformin (cannot have been used in the 3 months prior to screening), oral glucocorticoids within 10 days, atypical antipsychotics, immunosuppressant agents, HIV medications, hormonal contraception (cannot have been used in the 6 months prior to screening). Dermal patch or vaginal ring contraception methods. Weight loss medications or stimulants. Use of other products containing other GLP-1 agonists. 3) Currently pregnant or breastfeeding women. Development of pregnancy during the study period will necessitate withdrawal from the study. 4) Severe illness requiring hospitalization within 60 days. 5) Diabetes, defined as Hemoglobin A1C > 6.4% 6) BMI percentile less than the 90th percentile for age and sex. Weight >325 lbs. or <84 lbs. 7) Anemia, defined as Hemoglobin < 11 mg/dL

	8) Diagnosed major psychiatric or developmental disorder limiting informed consent. 9) Implanted metal devices that are not compatible with MRI 10) Use of blood pressure medications. 11) Known liver disease other than NAFLD or AST or ALT >100 IU/L. 12) Personal history of pancreatitis 13) Known renal disease of any severity or an eGFR at screening of <45ml/min/1.73m2 14) History of severe GI disease (e.g. gastroparesis) 15) History of gallstones 16) Untreated thyroid disease 17) History of hypersensitivity to semaglutide 18) Other causes of hyperandrogenism (example: tumor, CAH) or amenorrhea (untreated thyroid disease, tumor, primary ovarian failure, prolactinoma). 19) Active symptoms or undergoing treatment for anorexia nervosa or binging/purging disorder
Dose and Route of Administration:	Rybelsus® (semaglutide) is available in 3mg, 7mg, and 14mg oral tablets. Patients will be on the 3mg dose for the first 30 days, and then switch to the 7mg dose for the remainder of the study. Since our patient population will not have diabetes, we will not utilize the 14mg dose, as per packaging instructions, the 14mg dose is only used if additional glycemic control is needed.
Safety Assessments:	Patients will be instructed to contact study staff if they experience any adverse events. In addition, they will be assessed for adverse events when they come for their study visits and these will be graded based on the Common Terminology Criteria for Adverse Events (CTCAE) as described in the protocol.
Data Monitoring Committee:	A Data Monitoring Committee (DMC) will established by the investigator to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study.
Endpoints:	<u>Primary Endpoint:</u> 1. Change in Hepatic Fat Fraction Change from baseline in presence/severity of hepatic fat fraction will be measured with MRI, and calculated via the Dixon method as the proton density hepatic fat fraction, which ranges from 0-75%. [Time Frame: Baseline and 12 weeks] <u>Secondary Endpoints:</u> 2. Change in Rate of De Novo Lipogenesis Change from baseline of the rate of overnight de novo lipogenesis will be measured utilizing stable isotope methods with deuterated water, and expressed as the rate of newly synthesized lipids in the serum triglyceride fraction. [Time Frame: Baseline and 12 weeks] 3. Change in Whole Body Insulin Sensitivity Participants will undergo a 75 gram oral glucose tolerance test, and the change from baseline in whole body insulin sensitivity will be expressed as Si, calculated via the oral minimal model. [Time Frame: Baseline and 12 weeks] 4. Change in Adipose Insulin Sensitivity Change from baseline of adipose insulin sensitivity will be calculated as the percent suppression of free fatty acids, and the nadir of free fatty acids during the oral glucose tolerance test. [Time Frame: Baseline and 12 weeks] <u>Other Pre-specified Outcome Measures:</u> 5. Change in Relative Hepatic Mitochondrial Flux

	Change from baseline of relative hepatic mitochondrial flux will be assessed with an oral glycerol stable isotope tracer and subsequent blood draws while fasting. The outcome will be the relative proportion of hepaticly secreted glucose and glycerol that has undergone label rearrangement representative of excess mitochondrial metabolism - termed % indirect. [Time Frame: Baseline and 12 weeks] 6. Change in Functional Neuroimaging Measures Change from baseline of functional neuroimaging measures, specifically, the blood oxygen level-dependent (BOLD) signal in the hypothalamus, insula, anterior cingulate cortex, nucleus accumbens, amygdala, and superior and inferior frontal gyri, and connectivity between these brain regions. [Time Frame: Baseline and 12 weeks]
Statistical Analysis Plan and Rationale for Number of Patients	SA1 Statistical Plan Sample size justification. Sample size is based on the power to detect a difference between the standard diet + GLP-1 RA group and the intensive dietary counseling group in the primary outcome of change in IHTG. Estimates for power calculations were obtained from a recent randomized controlled trial of liraglutide in adult women with PCOS[68] and our baseline data on IHTG in obese girls with PCOS (Table 2). Assuming a standard deviation (SD) of 0.7% and a two-sided significance level of 0.05, a final sample size of 17 participants in the intensive diet group and 33 participants in the standard diet + GLP-1 RA group provides 90% power to detect a difference of -0.7% between group change. This difference is 50% smaller than the difference of -1.34% observed by Frøssing et al.[68] as their results are confounded by significant weight loss and therefore our study is powered conservatively. We anticipate a 1% decrease in hepatic fat. We estimate an approximately 5-10% drop out rate based on our group's experience from similar participant populations[9,10,111,116,118,182,183] and we will enroll up to 60 girls with the goal of 50 completers. Analysis plan. Data will be collected and managed in a REDCap database, a secure web-based application designed to support data capture for research studies, including validated data entry, audit trails, and automated export to statistical packages. Prior to the start of any formal analyses, variables will be examined for unusual values that need to be queried, patterns of missing values, and whether their distributions are non-Gaussian. Demographic and clinical characteristics will be summarized with descriptive statistics. Primary analyses will follow the "intent-to-treat" principle so that study hypotheses are tested under realistic conditions in which not all participants adhere to the intervention. This will include those who discontinue therapy whose data will be censored at the time of discontinuation, so that all data we have on the non-completers up until the time they leave the study will be included in this primary analysis. However, in order to fully evaluate the physiology and mechanism of action of semaglutide in this population, which is an important aim of this investigator-initiated study, we will also perform secondary analyses in participants who demonstrated at least 80% adherence (i.e., at least 80% medication taken in the GLP-1 arm and at least 80% of targeted weight loss achieved in the intensive lifestyle arm). For the primary outcome, we will compare the change in % IHTG with and without GLP-1 RA treatment using a t-test or Mann-Whitney U test. We expect the weight loss will be similar in the two groups; however, if there is a clinically significant difference between the groups, these comparisons will also be performed while adjusting for the amount of weight lost. Similarly, if there are differences in activity or obstructive sleep apnea severity, appropriate adjustments will be made. Similar analyses will be used for secondary outcomes (2 hour OGTT glucose, HOMA-IR, metabolomics markers, proportion of

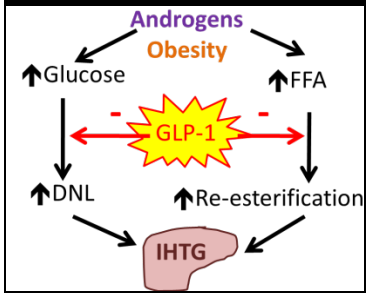
	direct/indirect glucose, EAA concentrations). Linear models will be used to identify the variables that are associated with change in IHTG, e.g., 2 hour OGTT glucose, HOMA-IR, metabolomics markers. SA2 Statistics Sample size justification. The primary outcomes for Aim 2 are (1) change in hepatic de novo lipogenesis (DNL) and (2) change in FFA nadir in the standard diet + GLP-1 group compared to the intensive diet group. Estimates of variability were obtained from the PI's pilot data (Figure 4b). We assumed a cross-sectional DNL SD of 3.36%, a pre-post correlation of 0.8, and a two-sided significance level of 0.05. With 50 participants, we will have 90% power to detect a between-group DNL difference of 2.1%, which is smaller than the difference of 2.6% reported by Armstrong et al. in adults[14]. Using the same assumptions and a cross-sectional SD of 23 nmol/L, we will have 80% power to detect a change of 22% in FFA nadir (Figure 4c), which is conservative given data in adults showing a decrease of 31% with liraglutide[67]. Analysis plan: Analyses for the primary outcomes of change in DNL and FFA nadir will be the same as Aim 1, as will the analyses for secondary measures of DNL (% suppression of triglyceride) and secondary outcomes (2-hour OSTT glucose, % TCA cycling, peripheral IR per OMM, metabolomics markers). From the study day 1 cross sectional data, we will use regression models to evaluate the relationships between markers of hepatic disease (IHTG and PDE/ATP) and DNL, excess substrate (FFA nadir, mean OSTT glucose) and measures of TCA anapleurotic cycling (% indirect glucose). Table 7 provides details on the power calculations for these models of primary interest, using estimates of variability obtained from the PI's pilot data. If the relationship between % TCA cycling and IHTG is significant, we will use mediation modeling to test if this effect is mediated by DNL. The distribution-of-the-product method will be used to construct 95% confidence intervals for the mediated effects, using the RMediation software package[184]. After investigating associations between variables in the cross-sectional data from study day 1, we will evaluate if baseline characteristics predict changes in the study primary outcomes, in order to further understand the pathophysiology and to identify patients benefit from particular interventions.

1. BACKGROUND AND SIGNIFICANCE

Polycystic ovary syndrome (PCOS) affects 6-10% of women, is defined by elevated testosterone and encompasses many co-morbidities including insulin resistance (IR), type 2 diabetes (T2D), nonalcoholic fatty liver disease (NAFLD) and decreased quality of life[1,2]. The testosterone excess in PCOS confers a unique additional risk for metabolic disease beyond obesity, with 3-4 fold higher rates of NAFLD and T2D relative to women with normal androgens[3-8]. In fact, our data in PCOS girls show a 50% prevalence of impaired glucose tolerance and hepatic steatosis (HS). Despite the high prevalence and gravity of co-morbidities seen in PCOS, widely effective therapeutic options are lacking. There is a *critical need* to understand the metabolic pathology underlying PCOS to inform new therapeutics. HS is one of the best predictors of future T2D and is already common in youth with PCOS[9,10]. Thus, our *long-term goal* is to understand hepatic substrate energetics as a gateway for prevention and treatment of metabolic and hormonal disease in obese youth with PCOS.

Based on our extensive preliminary data in adolescent girls[9-11], our *hypothesis* is that testosterone-induced alterations in hepatic energy metabolism, combined with excess substrate from poor diet and peripheral IR, are central to metabolic disease in PCOS (Figure 1). These effects are worsened by abnormalities in the incretin glucagon-like peptide (GLP-1), which are common in obesity[12-22]. PCOS girls in our studies have a high prevalence (50%) of NAFLD, defined as excess intrahepatic triglyceride (IHTG), relative to BMI-similar controls (15%)[9,10]. Obese PCOS youth in our cohort have increased rates of hepatic de novo lipogenesis (DNL) and increased free fatty acids (FFA) despite hyperinsulinemia. These PCOS girls also have significant muscle and hepatic IR relative to BMI-similar controls, which may provide substrates for DNL[9-11]. Androgens contribute to a unique disease pathology, increasing DNL gene expression and fatty acid synthesis rates[23,24]. Studies in adults with NAFLD have suggested that excess anapleurotic cycling of carbons through the TCA cycle provide excess acyl CoA for DNL and generate reactive oxygen species that contribute to hepatic fibrosis[25-28].

Fig 1: ↑ IHTG in PCOS is distinct from simple obesity and multi-factorial



Inadequate GLP-1 secretion/activity relate to HS in adults with diabetes, PCOS and NAFLD and animal studies have identified key roles for GLP-1 in preventing HS[12-22]. Our preliminary data demonstrate that GLP is similarly pathologic in PCOS girls. Limited studies show that GLP-1 receptor agonist (RA) therapy decreases IHTG in T2D adults and improves menstrual regularity in PCOS women[13,14,29-33]. Two doses of a short acting GLP-1 RA exenatide significantly improved glucose metabolism in our obese youth with PCOS regardless of HS status, showing the potential for clinical and metabolic improvements in PCOS youth.

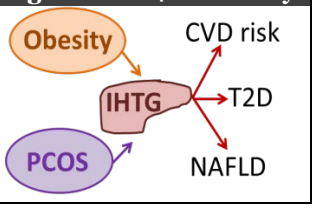
A.1 Significance

Obese girls with polycystic ovarian syndrome (PCOS) already have evidence of metabolic disease, are at high risk for metabolic decline as they age and therapeutic options are limited. New interventions focused during this vulnerable yet reversible stage are thus critical. PCOS affects 6-10% of U.S. women and 10-15% of obese women, with an estimated economic burden of $4 billion and is increasing in prevalence in parallel with the increase in obesity[1,2]. PCOS can first be diagnosed in adolescence and its phenotype includes high androgens, irregular/infrequent menses and dermatologic manifestations including facial hair, balding and severe acne[8,34]. Metabolic disease is more common in PCOS and worsens with obesity. Insulin resistance (IR) is nearly universal in PCOS and contributes to the earlier onset and rising incidence of non-alcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D) and cardiovascular disease (CVD)[3-8]. We found that >50% of obese girls with PCOS have hepatic steatosis (HS; ie early NAFLD) as well as severe IR and frequent dysglycemia, and CVD risk markers[9,10]. The primary therapy for PCOS with obesity is lifestyle modification, which can be very challenging for youth due to peer, family and socioeconomic issues. Unfortunately, current therapeutic options for PCOS and HS are inadequate. Approved medical therapies for PCOS include metformin, which only reduces PCOS severity in approximately half of patients and oral contraceptives which may actually worsen IR[8,34-41].

Frequent abbreviations used in this protocol: ALT= alanine aminotransferase; **BMI**=body mass index; **CVD**=cardiovascular disease; **DNL**=de novo lipogenesis; **D_20**=deuterated water; **FFA**=free fatty acids; **GLP-1**=glucagon-like peptide-1; **GLP-1 RA**=glucagon-like peptide-1 receptor agonist; **HOMA**=homeostasis model assessment; **HS**=hepatic steatosis; **IHTG**=intrahepatic triglyceride; **IR**=insulin resistance; **MRI**=magnetic resonance imaging; **MRS**=magnetic resonance spectroscopy **NAFLD**=non-alcoholic fatty liver disease; **NMR**=nuclear magnetic resonance; **OGTT**=oral glucose tolerance test; **OMM**=oral minimal model; **OSTT**=oral sugar tolerance test; **PCOS**=polycystic ovarian syndrome; **TCA**=tricarboxcylic acid cycle; **TG**=triglycerides; **T2D**= type 2 diabetes; **VAT**=visceral adipose tissue

Treating NAFLD in PCOS youth may confer significant long-term health benefits. Obesity and PCOS are synergistic for increasing the risk of NAFLD **(Figure 2).** Understanding and treating HS in girls with PCOS at the critical preclinical stages may be an important strategy to prevent future NAFLD, T2D and CVD as HS strongly and, independently predicts T2D and CVD development[42-44]. Elevated liver transaminases (a marker of NAFLD) predict dysglycemia and T2D onset[45]. Conversely, treatment of NAFLD with an effector of bile acid metabolism improves glycemia[46], arguing for a tight connection between glycemia and liver health. IR in adipose, muscle and hepatic tissue increases as the degree of HS increases[47]. HS can also progress to cirrhosis and liver failure[6,7,42]. Unfortunately, youth onset NAFLD is more severe at presentation and progresses more rapidly than adult disease[48,49].

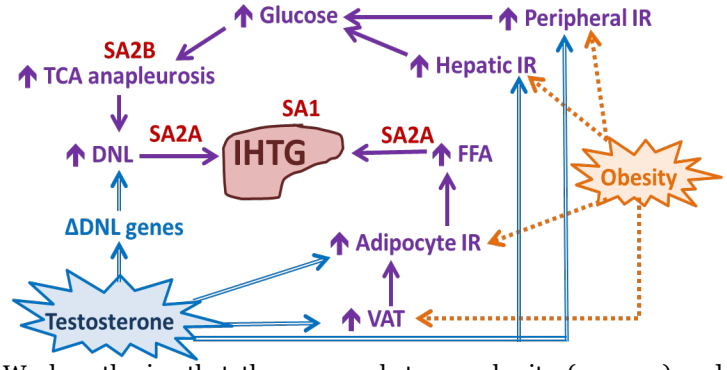
Fig 2. IHTG ↑morbidity



A.2 Scientific Premise

The unique mechanisms underlying HS in obese women with PCOS have not been well-studied, as the majority of mechanistic NAFLD studies have focused on men. Increased androgens in PCOS confer a pathologic metabolic milieu beyond that seen in obesity alone (**Figure 3**), and this process develops with disease onset or earlier, regardless of BMI[53-57]. Studies by our team in PCOS and others in adults with NAFLD indicate that upregulated hepatic de novo lipogenesis (DNL) and increased delivery of free fatty acids (FFA) to the liver are the two most important contributors to intrahepatic triglyceride (IHTG)[28,58-61]. NAFLD also relates to dysglycemia and IR[27,28,47,60,62,63]. Excess carbon availability (anaplerosis) to the TCA cycle leads to excess carbon availability for metabolic processes (cataplerosis) such as DNL and the production of reactive oxygen species which contribute to hepatic IR and progressive NAFLD[25,26]. Strategies to lower IHTG thus include decreasing DNL and FFA[60,64,65].

Fig 3. Androgens and obesity synergize to ↑ IHTG in PCOS



We hypothesize that the synergy between obesity (**orange**) and excess testosterone (**blue**) in PCOS (**purple**) has unique influences on IR, DNL and IHTG[50-52]. **Specific Aims in red.**

Glucagon-like peptide-1 receptor agonists (GLP-1 RA) are a promising choice to treat NAFLD in PCOS. GLP-1 RA lower serum glucose and thus substrates for DNL and increase insulin secretion, leading to greater suppression of FFA following a meal[13,18-20,66,67]. However, GLP-1 RA cause weight loss and it is unclear if the improvements in HS seen in adults with T2D or NAFLD are from direct GLP-1 activity or due to weight loss[13,66]. Limited GLP-1 trials in women with PCOS are promising[29-32,68]. We will use GLP-1 RA in PCOS to probe hepatic metabolism while also assessing if GLP-1 RA is a viable therapeutic option to improve metabolic disease in PCOS, beyond its weight loss affect.

A.3 Scientific Premise

Disinhibited eating behaviors (DEB) are associated with obesity and weight gain. Non-homeostatic brain systems promote DEB and are altered in obesity. Homeostatic brain systems control whole-body energy homeostasis, contribute to eating behavior and are altered in obesity. Despite the proven role of the hypothalamus in controlling eating behavior to maintain whole-body energy homeostasis, few studies in humans have focused on how the hypothalamus responds to weight-loss interventions. Limited data are available on functional connectivity between brain non-homeostatic and homeostatic systems and their coordinated role in DEB.

Semaglutide and other GLP-1 receptor agonists are thought to decrease appetite through direct interactions with the brain in the hypothalamus. Whereas this has been extensively studied in animals, there is extremely limited data in humans and almost none in adolescents. With the likely future marketing of semaglutide for obesity in youth, it is thus critical to start to understand how these medications function in the brain as opposed to dietary behavior weight loss.

The goal of this sub-aim is to better understand the function and interaction of both homeostatic and non-homeostatic systems in the context of DEB in obesity. Understanding communication between homeostatic and non-homeostatic brain regions and their relationship to DEB among adolescents with

PCOS and obesity undergoing weight-loss therapy will help to elucidate specific neural mechanisms that contribute to overeating, and ultimately to weight regulation.

2. HYPOTHESES AND SPECIFIC AIMS

Primary Specific Aim

Aim 1: Quantitate change in IHTG in obese girls with PCOS after 4 months of GLP-1 RA therapy, compared to girls undergoing intensive dietary counseling and similar weight loss.
Hypothesis: IHTG will decrease after GLP-1 RA therapy in PCOS girls and will be greater than that seen from dietary counseling alone. Methods: IHTG will be measured with MRI before and after interventions.

Secondary Specific Aim

Exploratory Aim 2: Measure changes in substrate delivery and utilization after GLP-1 RA or intensive diet.

A. Measure changes in rates of DNL and adipose IR, ie insulin suppression of FFA release

Hypothesis: GLP-1 RA therapy will significantly improve adipose IR and decrease DNL, resulting in reduced IHTG. Changes will be greater than those seen from dietary counseling alone. Methods: Deuterated water will be utilized to measure DNL. Whole body and adipose insulin sensitivity will be modeled from FFA and glucose changes relative to insulin concentrations during oral sugar tolerance test (OSTT).

B. Assess rates of hepatic TCA cycling and NAFLD severity

Hypothesis: Excess cataplerotic TCA cycling provides substrate for DNL and contributes to hepatic damage via the production of reactive oxygen species. Methods: Oral U-$^{13}C_3$ glycerol will be consumed and serum glucose and VLDL undergo isotopomer analysis to determine relative TCA cycle flux. Hepatic ^{31}Phosphorus MR spectroscopy and MR elastography will be utilized to document NAFLD severity and ATP concentrations.

Exploratory Aim 3: Determine the extent to which diet or GLP-1 treatment alters neuronal response within, and functional connectivity between, homeostatic and non-homeostatic brain regions in adolescents with PCOS and obesity.
Hypothesis: Connectivity between the homeostatic and non-homeostatic brain regions will be significantly greater after treatment with semaglutide compared to diet.

Overview of Clinical Studies

SA1 Review of Relevant Literature
Women with PCOS have a 2-3 fold increase in rates of HS and NAFLD. An estimated 50-70% of obese women with PCOS have HS, compared to 20-30% of similarly obese women without PCOS [4,69-75]. Increased IHTG in non-obese women with PCOS is also more common but not always found, likely due to varied definitions of PCOS[4,70,72,76,77]. In PCOS youth, only 2 published studies exist beyond ours and all three studies indicate increased rates of HS[9,57,78]. Many of these studies found associations between androgen concentrations and severity of HS, in addition to associations with PCOS status[4,69-72,79], suggesting a critical role of testosterone.

Decreases in HS are associated with improvements in metabolic disease. Both lifestyle interventions and pharmacotherapy have been utilized to acutely lower IHTG and decreases in IHTG are uniformly accompanied by improvements in insulin sensitivity and/or dysglycemia in animals and humans[46,80-85]. Stains induce mild improvements in NAFLD and decrease CVD risk more in those with existing NAFLD than those without, suggesting that the improvement in NALFD is additive to the direct lowering serum lipids[86].

Short-term dietary interventions can decrease HS but are difficult to implement long-term in a clinical setting. Diets with decreased calories and simple carbohydrates have been shown to decrease rates of HS across the lifespan[61,81,84,87-89]. Fructose is preferentially used by the liver for DNL and just 9 days of a low fructose diet reduced hepatic fat by 50% in youth with HS[90]. Unfortunately, this diet is difficult for most families to follow long-term and compliance with lifestyle recommendations is higher in a research setting than in the clinical setting[91, 84,92]. The American Association of Clinical Endocrinologists' guidelines for obesity state that reducing total caloric intake is

key for weight loss, with 4-10% weight loss recommended long-term to reduce NAFLD[93]. Pediatric NAFLD guidelines state that intensive weekly contact is needed to improve NAFLD in youth, which is beyond what most clinics and families can manage[94].

Effective pharmaceutical therapies to treat HS are lacking. Multiple pharmacologic strategies have been tested to treat NAFLD with no to moderate success[94-96]. Diabetes medications that lower glucose and thus DNL precursors such as metformin have had variable success[96-99]. Recent evidence indicates that SGLT-2 inhibitors, which increase glycosuria, decrease HS in adults with T2D, but may not be effective with the milder degree of dysglycemia seen in nondiabetic patients with PCOS[100]. Thiazolidinediones work via the peroxisome proliferator-activated receptor-γ and appear to decrease IHTG and glucose concentrations by increasing fat oxidation and redirecting TG storage to adipose tissue[85,101-105]. This class of medication is effective for treating metabolic and hormonal disease in women with PCOS, but the associated weight gain and possible serious side effects has reduced enthusiasm for thiazolidinedione use in youth[106,107]. Bile acid sequestrants alter bile acid metabolism and can lower IHTG and may do so through increasing incretins such as GLP-1[108]. Thus, there are no routinely recommended medications for HS in youth, but new classes of medications are being tested[93,94].

GLP-1 RA decrease HS in adults with NAFLD or T2D, although results are confounded by weight loss. GLP-1 RA therapy is currently used to improve glycaemia in T2D and to induce weight loss in obesity, and is a promising therapy for treating NAFLD[13]. GLP-1 is an incretin released in response to meals that increases insulin and decreases glucagon secretion[12-14, 15]. Individuals with obesity, NAFLD and T2D have both GLP-1 resistance and/or deficiency[21,22], and inadequate meal responses were reported in obese youth[109]. A recent secondary analysis of T2D patients receiving GLP-1 RA for glucose control demonstrated a decrease in IHTG and markers of NAFLD[66,110]. Further, decreases in HS induced by GLP-1 RA were related to improvements in glucose concentrations[66]. Studies in multiple animal models demonstrated the role of GLP-1 in the development and reversal of genetic or diet-induced IHTG accumulation[18-20].

Limited data indicates that GLP-1 treatment accompanied with weight loss decreases metabolic disease in women with PCOS. A small number of studies examined the role of GLP-1 RA in women with PCOS[29-32]. Obese women with PCOS had lower baseline and post-meal GLP-1 concentrations compared to obese controls. GLP-1 concentrations did not improve with oral contraceptives but did with metformin[29,30]. 6 months of GLP-1 RA reduced markers of liver fibrosis in obese PCOS women[31] and 3 months of GLP-1 RA therapy reduced visceral adipose tissue[32] or hepatic fat, although results are confounded by unequal weight loss between groups[68]. Improvements in ovulation rates, menstrual regularity and decreases in testosterone were also observed[33].

SA1 Preliminary Data

We have been increasing our knowledge of the physiology of HS, IR and dysglycemia in obese youth though 6 distinct studies, including 2 NIH-sponsored multi-center trials: RISE[111] and TODAY[112-114] (**Table 1**). The 4 local studies included stable isotope tracers, MR-measured IHTG to better understand pathophysiologic hepatic mechanisms[9,10,115-119]. Results from these studies that informed our proposed study are shown below. Our proposed team includes investigators from these trials, demonstrating our expertise in these type of trials in youth, as well as their tolerability by our adolescent participants and feasibility of the measures.

Table 1: Youth studies used for preliminary data

Study, N	Participant population	Team Role	Relevant methods
RESISTANT N=80	Lean and obese youth With or without T2D	Cree-Green Co-I Nadeau PI	Multi-tissue clamp-assessed IR ^{31}P MRS of muscle
AIRS N=73	Lean and obese girls With or without PCOS	Cree-Green PI Nadeau mentor	Multi-tissue clamp-assessed IR Markers of hepatic DNL 2 hour 75 gm OGTT
APPLE N=92	Obese girls With or without PCOS	Cree-Green PI Nadeau mentor	Multi-tissue OSTT-assessed IR Acetate tracer measured DNL 2 dose GLP-1 RA pilot trial
PLUM N=18	Obese girls With PCOS	Cree-Green PI Nadeau mentor	OSTT-assessed IR Isotopomer TCA cycle analysis ^{31}P MRS of liver
TODAY SITE N=45	Obese youth With T2D	Zeitler PI	Intensive lifestyle intervention Stop light diet counseling
RISE SITE N=34	Obese youth With T2D	Cree-Green Co-I Nadeau PI Zeitler Co-I	Standard lifestyle counseling Stop light diet counseling Multiple metabolic studies

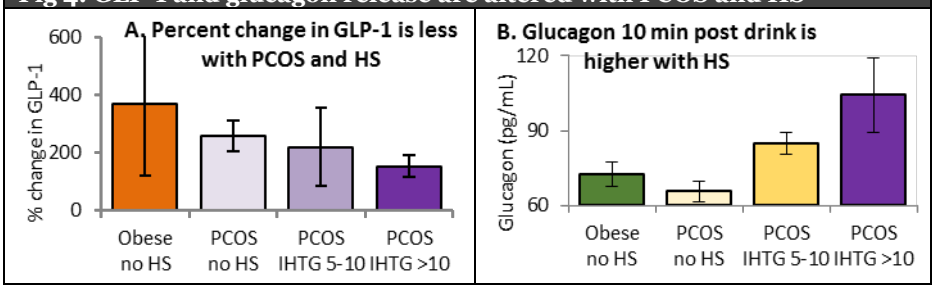
Obese girls with PCOS have excess IHTG and >50% have HS. Our data from more than 120 obese girls with PCOS, when compared to normal weight or similarly obese girls with and without T2D shows that PCOS girls have evidence of liver disease including 1) mildly increased fasting serum TG 2) liver transaminase (ALT) elevation severity similar to T2D girls 3) MRI-assessed IHTG even higher than in T2D girls **(Table 2)**. Most notably, ≈50% of obese girls with PCOS had clinically significant HS (defined as >5.5% liver fat), whereas only 15% of obese girls without PCOS had HS, and less severe abnormalities were also seen in normal-weight girls with PCOS[10]. These findings indicate that hepatic pathology occurs early in the course of PCOS. IHTG also strongly relates to elevations in free testosterone (r=0.437, p<0.001) and 75% of girls had HS if their free testosterone was >50% above the upper limit of normal.

Table 2: Obese PCOS girls have ↑IHTG, serum TG and markers of liver inflammation

Population	IHTG %	TG (mg/dL)	ALT (IU/L)
Lean Control	1.0±0.1	75±6	23±4
Obese Control	2.3±1.5	78±8	29±3
Obese PCOS	6.2±2.3	129±7	38±3
Obese T2D	4.5±1.8	207±7	40±2

GLP-1 and glucagon meal responses are abnormal in obese girls with PCOS. Serum GLP-1 and glucagon concentrations were assessed prior to and in response to an OSTT. Girls with PCOS had less GLP-1 release to an OSTT than obese controls, becoming progressively worse with greater degrees of HS **(Figure 4A)**. GLP-1 decreases glucagon and accordingly, glucagon concentrations 10 min into the OSTT were higher in girls with HS and PCOS **(Figure 4B)**.

Fig 4: GLP-1 and glucagon release are altered with PCOS and HS



Our current team can induce short-term, modest weight loss in obese youth utilizing the proposed methods. The PI, CO-I's and research study team have extensive research and clinical experience with lifestyle interventions in this and similar populations **(Table 3).** In the RISE study, in the first 3 months youth achieved a 1.9±0.25 kg weight loss utilizing the stop light diet as taught by our current staff. At completion of the PI's PCOS studies with identical inclusion criteria to that proposed (cross-sectional AIRS, APPLE and PLUM studies), girls with PCOS receive intensive lifestyle counseling utilizing the stop light diet and an individualized daily calorie target to induce weight loss based on metabolic cart determined basal metabolic rate and typical activity from the accelerometer. At clinical follow-up ~ 3 months later and with no additional coaching, the average weight loss is 1.8 kg. Based on the literature, our weight loss goals for the diet group are modest and similar to this last group of PCOS girls, with a goal of 2 kg. This goal will be tailored to match that seen in the GLP-1 RA group, but clearly there is room for further weight loss if needed, as we will be adding extensive follow-up and weight monitoring.

Table 3: Study team's success with short term weight loss in obese youth

Study	RISE	Clinical follow-up after PI's studies
Participants	Obese, both sexes T2D, pre-diabetes	Obese, girls PCOS
Weight loss (kg)	1.9±0.25	**1.8 ± 0.4**
Follow-up time	3 months	3.3 months

SA2 Review of Relevant Literature

Women with PCOS have IR in both adipose and peripheral IR in excess of that seen from obesity alone. Obese women with PCOS and HS have more IR than those without HS, indicating a link between HS and worsening IR[5]. IR in muscle, hepatic and adipose tissue is reported to be worse in PCOS adults than in adult women of similar BMI and thought to be related to increased androgens[53-56]. Similar findings are seen in PCOS girls[57].

Excess IHTG is secondary to excess DNL and substrate delivery from peripheral and adipose IR. Several studies in adults with NAFLD have linked increased IHTG to increases in DNL[28,60,63]. In obese adolescents and adults, increased IHTG was related to the degree of excess FFA from adipose IR[42,47,120]. Importantly, following weight loss or weight gain, DNL and FFA dynamically change in accordance with increases or decreases in IHTG[60,81]. NAFLD also relates to dysglycemia and peripheral IR[27,28,47,60,62]. Adipose IR and peripheral IR are tightly related in obese youth[62,121]. In obese women with PCOS, peripheral and adipose IR is closely related to increased IHTG[4,70,77,122] with similar results in two existing studies in youth[57,123].

Reductions in HS improve IR and clinical symptoms of PCOS. In youth, a combination of a thiazolidinedione, metformin and an androgen receptor blocker flutamide lowered IHTG and improved insulin sensitivity[76]. Further, change in IHTG was the best predictor of improved ovulation and lowered free testosterone[76]. However, flutamide is not available in the US and TZD's cause weight gain, precluding widespread use of this combination. A year of metformin reduced NAFLD and increased ovulation in overweight women with PCOS[124]. However, metformin does not consistently improve PCOS severity or NAFLD[34,125-127].

Hepatic ^{31}P MRS can be utilized to understand hepatic metabolism and document progressive NAFLD. In addition to the three ATP peaks and free phosphate (Pi), the liver contains phosphodiester peaks (PDE), thought to represent endoplasmic reticulum stress[128]. PDE's are higher in progressive NALFD[128-132] and this finding is consistent with findings in animals of increased hepatic stress and NAFLD[128,133,134]. ATP and Pi concentrations also relate to hepatic function[134]. MRS performed during an OSTT demonstrated increased mitochondrial flux thought to be secondary to excess FFA delivery to the liver[120].

GLP-1 RA may alter intrahepatic energy metabolism. Mechanistic data suggest that all of the pathways related to increased IHTG as listed in figure 3 can be impacted by a GLP-1 RA. GLP-1 increases insulin and decreases glucagon secretion which lowers postprandial FFA and glucose,[12-15] potentially decreasing substrates for IHTG production[135]. Moreover, hepatocytes express GLP-1 receptors, thus GLP-1 can directly impact hepatic insulin signaling, leading to decreased gluconeogenesis and DNL through decreased carbohydrate uptake[16-18]. GLP-1 also suppresses genes directly involved in the formation of IHTG, particularly stearoyl-coA desaturase, a key enzyme in DNL [18-20]. Mechanistic studies on the role of GLP-1 RA in NAFLD are limited in humans. In healthy men, GLP-1 RA lowers post-prandial FFA, secondary to increased insulin[67]. GLP-1 RA decreased glucose release and DNL in adults with NAFLD**[14].** Thus, GLP-1 affects both drivers of increased IHTG: DNL and FFA delivery to the liver and is a target for reducing IHTG.

SA2 Preliminary Data

Obese girls with PCOS have significant multi-tissue IR and increased rates of dysglycemia. We examined the differential tissue-specific response to insulin in female youth with PCOS compared to Dr. Nadeau's studies of T2D, utilizing a 4 phase hyper-insulinemic euglycemic clamp with glucose and glycerol stable isotope tracers. By contrasting patterns of pathology between these disorders, we uncovered subtle differences that suggest unique mechanisms of metabolic change in PCOS. Obese girls with PCOS (N=38) had multi-tissue IR relative to similarly obese controls (N=19), but were not as IR as T2D girls (N=40)[136]. Whereas the concentration of insulin required to suppresses lipolysis assessed with glycerol Ra trended to be higher in PCOS compared to obese controls (p=0.06), FFA were higher during hyperinsulinemia and the amount of insulin required to suppress 50% of fasting FFA was significantly higher, indicating adipose IR **(Table 4).** The concentration of insulin required to suppress 50% of fasting glucose release was 2-fold higher in girls with PCOS (p=0.04), indicating hepatic IR and may be reflective of dysregulated gluconeogenesis. Muscle IR, measured as glucose rate of disappearance was much worse (p<0.001) in PCOS. Following a 75-gram oral glucose tolerance test (OGTT), nearly half of PCOS girls had impaired glucose tolerance compared to 20% of obese controls. We found that OGTT insulin concentrations were different when girls were divided into tertiles of muscle IR **(Table 5).** We thus modelled OGTT glucose and insulin with the oral minimal model (OMM)[137-139] and found that 2 hour OGTT OMM data correlated well with the clamp muscle IR (r=0.65, P<0.001). Further, the OMM was more precise when extended to 6 hours, when insulin returns to baseline post-OGTT in this population.

Table 4: Obese PCOS girls have adipose, liver and muscle IR

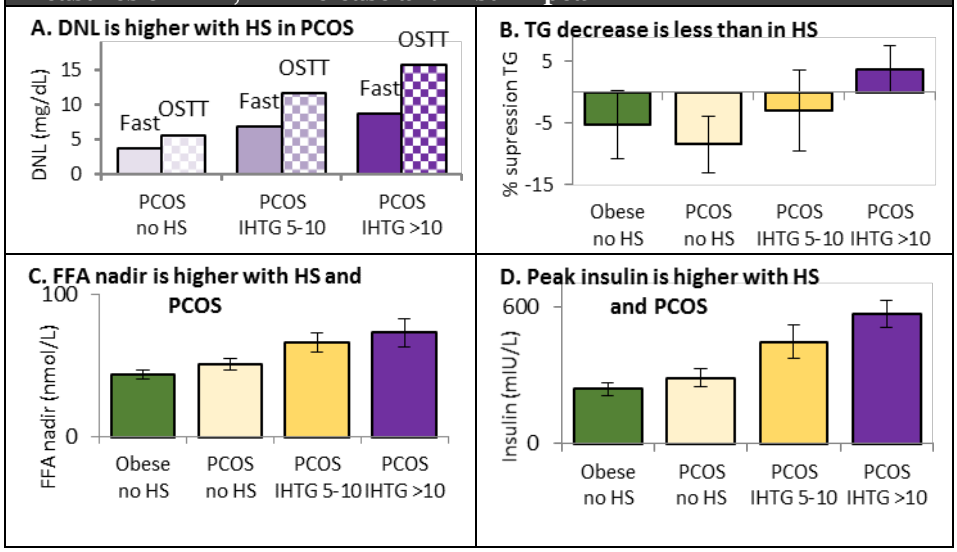
	PCOS	OB CONT
Adipose IR, insulin (mIU/L) to suppress 50% of fasting FFA	60±14	39±6
Hepatic IR, insulin (mIU/L) to suppress 50% of fasting EGP	100±25	50±15
Muscle IR, glucose rate of disappearance, mg•kgFFM•min	10±3	17±5

Table 5: OGTT insulin and OMM by muscle IR

Muscle IR (mg•kgFFM•min)	2 hr insulin (mIU/L)	2 hr OMM (Si)
<12	294±52	1.21±0.21
12-18	185 ± 48	2.6±0.38
>18	118 ± 19	5.6±0.51

HS in obese girls with PCOS relates to DNL, adipose IR and peripheral IR. Obese girls with PCOS undergoing clamps had higher serum concentrations of markers of DNL (i.e. long chain fatty acids with a double bond in the 7th carbon position[140]; N7 FFA; 29±13 nmol/g obese vs. 45±15 PCOS, p<0.01)[141] and the N7 FFA concentrations correlated with IHTG% (r=0.53, p=0.004)[9]. IHTG% also correlated with serum FFA nadir during the highest dose of the clamp (r=0.362, p=0.043) indicating a potential contribution of excess hepatic FFA delivery, secondary to adipose IR. Muscle IR correlated with IHTG (r=-0.374, p=0.021).

Fig 5. HS in PCOS relates to post-OSTT alterations in direct and indirect measures of DNL, FFA release and insulin peak

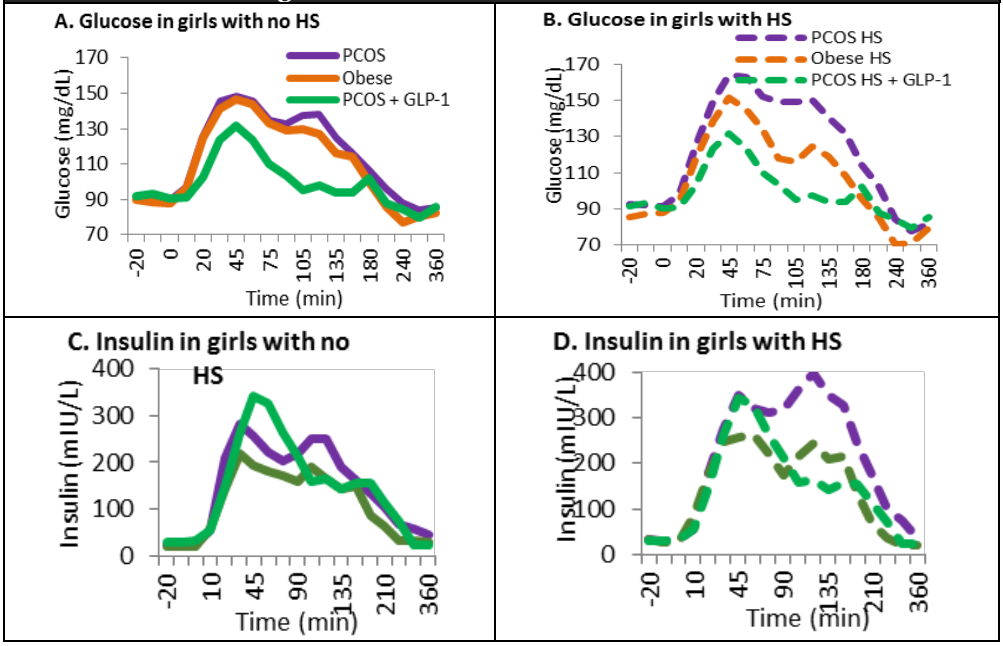


We next assessed the integrated glucose and fat response to an OSTT with 75 grams of glucose and 25 mg of fructose. With Co-I Dr. Parks and consultant Metabolomic Solutions, we are measuring the rate of hepatic DNL with an intravenous infusion of a 1,2-C_{13} acetate tracer in obese girls with PCOS fasting and following the OSTT, to better understand what happens in the post-prandial state. In girls with PCOS, fasting and post OSTT DNL increased with the degree of IHTG **(Figure 5A)**. In the entire cohort of 62 girls, serum TG decreased as a reflection of glucose stimulated DNL and TG secretion, in obese and PCOS without HS, whereas girls with PCOS and HS had a minimal change or increase in TG depending on the severity of HS **(Figure 5B).** Increases in serum TG following an OSTT have been shown to directly reflect changes in DNL[142]. The contribution of FFA to HS is again evident with this study design, as the OSTT nadir of FFA suppression **(Figure 5C)** was higher in both PCOS and increasing IHTG%, and OSTT FFA nadir correlated with IHTG% ($r=0.045$, $p<0.001$). Finally, peak insulin during the OSTT **(Figure 5D),** as a marker of overall IR, increased with PCOS and degree of HS. The FFA nadir appears to be related to IR, as the higher the FFA nadir, the higher the insulin ($r=0.504$, $p<0.0001$). *This combined data demonstrates that obese girls with PCOS have high rates of HS which relate to adipose IR (manifested as increased OSTT or clamp FFA), higher rates of DNL and whole body IR, when assessed with either a hyperinsulinemic euglycemic clamp or the simpler OSTT.*

In obese girls with PCOS, regardless of HS status, GLP-1 RA therapy lowers the OSTT glucose response, FFA nadir, TG release and glucagon, and increases first-phase insulin secretion. We gave a subgroup of 10 obese PCOS participants 2 doses of fast acting exenatide, 1 prior to dinner the night of admission and 1 thirty minutes prior to the OSTT the next day. As shown in **Figure 6A and B**, regardless of HS status, GLP-1 RA therapy decreased serum glucose concentrations significantly, below those seen in controls without PCOS. As expected, insulin secretion increased with GLP-1 RA (**Figure 6C and D**). Of note, the large second-phase insulin peak in

PCOS girls with HS (**Figure 6D**, **purple dashed line**) was completely mitigated with GLP-1 RA (**green dotted line**). GLP-1 RA also decreased fasting and post-prandial glucagon responses.

Fig 6. GLP-1 RA improves OSTT glucose via increased first phase insulin secretion in PCOS regardless of HS status

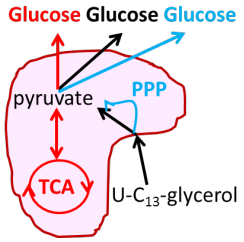


In terms of markers of DNL, post-OSTT TG suppressed 24±5% in PCOS girls without HS and 8±3% in girls with PCOS and HS which is greatly improved compared to without GLP-1 RA (8±5% and 3±3%, respectively **Figure 5B)**. The OSTT FFA nadir was also lower with GLP-1 RA in PCOS girls without HS 45±6 nmol/L, similar to controls in **Figure 5C**, and was also lower in girls with PCOS and HS at 74±4 nmol/L. *Thus, a short exposure of GLP-1 RA treatment induces multiple metabolic changes in obese PCOS girls regardless of HS status and prolonged therapy has the potential to cause even greater changes.*

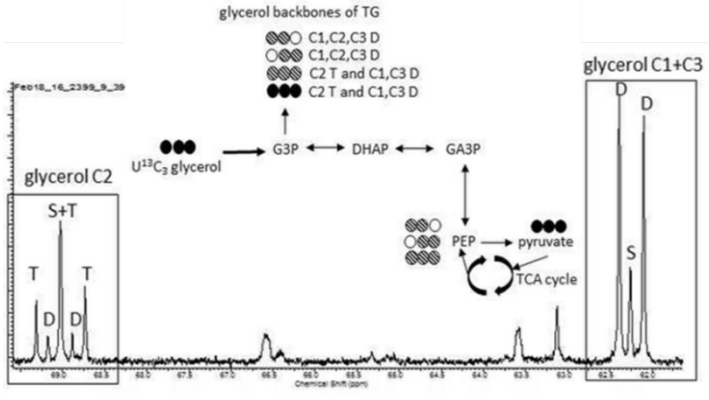
Excess hepatic TCA cycle activity may provide substrate for DNL and cause inflammation that promotes NAFLD progression. In adults with NAFLD, tracer-assessed rates of mitochondrial oxidation and anaplerotic/cataplerotic flux were 2-fold increased and related tightly to IHTG content[25]. Excess mitochondrial activity can produce reactive oxygen species and NAFLD severity per biopsy related to oxidative stress[26]. In animals, excess FFA provided the substrate for anaplerosis/cataplerosis and increased oxidative stress, and these processes could be prevented with fatty-acid oxidation knockout animals or metformin[26]. Our proposed project would confirm that similar metabolic processes are occurring in obese youth with PCOS.

Intrahepatic TCA cycle metabolism can be measured non-invasively with a U-C_{13} oral glycerol tracer in obese adolescent girls. Our team has demonstrated that TCA cycle anaplerosis/cataplerosis is up-regulated in NAFLD, yet the method is patient intensive and requires multiple isotopes[25]. Glycerol is nearly universally taken up by the liver, and when labeled with a carbon isotope can be used to trace hepatic metabolism. Co-I's Drs. Malloy and Jin first described this methodology in adult men[143,144]. The premise behind this methodology and sample spectra from our obese girls with PCOS are shown in **Figure 7**[143,144]. We successfully implemented this protocol in obese girls with

Fig 7. Carbon isotopomers to assess percent hepatic anaplerosis in obese PCOS girls



Glycerol-derived carbons can go to gluconeogenesis directly, via pentose phosphate pathway (PPP, blue) or the indirect pathway (red) where the carbons go via the TCA cycle.

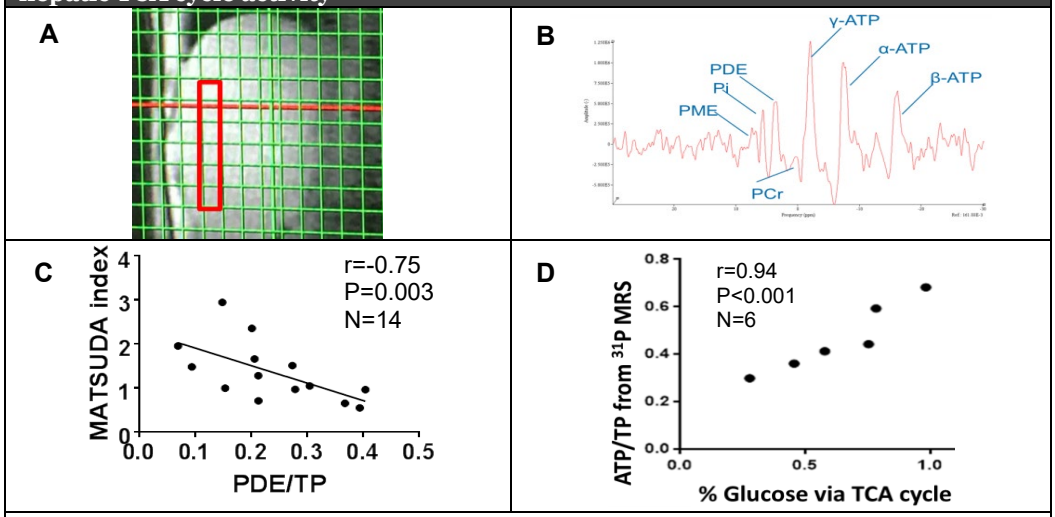


A similar process measures glycerol contribution to TG synthesis.

PCOS and confirmed that the isotopomer outcomes of interest for Aim 2B, including the percent of carbons originating from hepatic anapleurotic cycling in the TCA cycle and then contributing to triglyceride synthesis and gluconeogenesis, can be measured with this methodology in our patient population. Our preliminary data indicates that an increased indirect/direct ratio in glucose, indicative of higher proportions of cataplerosis, trends to be related to worse HOMA (R=-0.75, p=0.05 with 7 participants) and higher ALT. *This data demonstrates that this methodology has been optimized for our patient population and when combined with the clinical outcomes, rates of DNL and gluconeogenesis can yield powerful insights into underlying mechanisms of pathology.*

Hepatic ^{31}P-MRS demonstrates alterations in intrahepatic phosphate concentrations associated with IR and isotopomer-assessed TCA cycle activity. We implemented a ^{31}P-MRS protocol with consultant Dr. Befroy of Peak Analysts and Co-I Dr. Brown to measure fasting hepatic phosphate concentrations with a custom built 18 cm H/P dual tuned coil utilized on a large bore Siemens 3.0 Tesla dedicated research magnet, which is large enough to collect data in obese individuals. Data shown (**Figure 8**) are from 14 obese girls with and without PCOS who had an OSTT, of whom 6 also had the U-C_{13} oral glycerol drink with isotopomer analysis of serum.

Fig 8: Hepatic phosphate compounds are measuresurable with ^{31}P MRS in obese girls weighing up to 325 pounds and releate to measures of whole body IR and hepatic TCA cycle activity



A typical pre-spectra image of the liver with studied tissue outlined in red is in **9A**. A sample spectra is in **9B**. A low phosphocreatine (PCr) peak confirms capture of liver tissue without muscle in the area sampled. Outcome measures, include: ATP/total phosphate (ATP/TP)(marker of overall energy state) and PDE/ATP (normalized marker of oxidative stress). **9C** Those with a higher PDE/total phosphate ratio, ie greater oxidative stress have more severe IR with a lower Matsuda score. The ATP/TP ratio and TCA cataplerotic cycling, represented as glucose % TCA, correlate strongly (**9D**), validating ATP/TP as a non-invasive measure of hepatic TCA activity.

Deuterated water is well tolerated in youth and overnight dosing is adequate to measure DNL. The assessment of DNL with deuterated water method has been used successfully in obese youth by our Co-I Dr. Parks, and we thus are confident in our ability to perform this analysis[58] (NCT02960659). Previous protocols utilized a prolonged water loading protocol to increase the precursor enrichment[60]. Dr. Parks adapted the protocol to a more feasible multi-bolus overnight protocol with adequate precursor enrichment in obese individuals[28,58].

3. STUDY RATIONALE

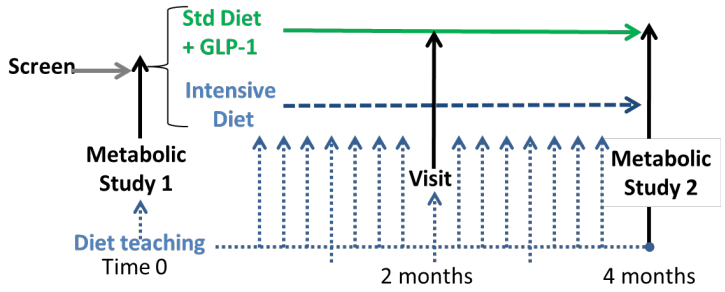
Here we propose a 4-month randomized study of GLP-1 RA semaglutide oral tablets vs. diet therapy in 50 drug-naive obese girls with PCOS. The *rationale* for this research is our observation of excess NAFLD, dysglycemia, upregulated DNL, hepatic, adipose and peripheral IR and decreased GLP-1 secretion in PCOS girls combined with pilot data demonstrating metabolic improvements with short-duration GLP-1 RA administration. The long-term consequences of NAFLD in girls with PCOS are of great clinical importance. This study will define the metabolic impact of a currently available medication on this common high-risk disease in youth thereby changing health outcomes. As such, this is a paradigm-changing approach to the management of this life-long disease. In addition, the innovative methodology employed will allow for non-invasive determination of specific mechanisms involved in metabolic disease in PCOS.

4. STUDY DESIGN

Overall Study Design

All aims will be accomplished from a cohort of 50 obese girls with PCOS. Girls will complete a 16-week randomized, open-label intervention study **(Figure 9)**. Treatments will consist of 1) a GLP-1 RA, semaglutide (Rybelsus®), with standard dietary counseling (n=33 girls) or 2) intensive nutritional counseling (n=17 girls). 16 semaglutide will be enrolled first, to determine target weight loss of the intervention group, then the remainder will be randomized 1:1. Metabolic studies will be performed prior to and after 4 months of intervention. A mid-point visit at 2 months to assess compliance, side effects, HbA1c, BMI and PCOS severity will occur.

Figure 9: Overall study design



Participants will have 4 visits: A screening to ensure eligibility, 2 identical study visits before and after therapy and a mid-point check-in visit (black arrows). 16 GLP-1 will be completed to determine typical weight loss, then remaining girls will be randomized 1:1. During treatment, all participants will communicate with study staff weekly and have biweekly weights (**blue dashed arrows**). Extra visits will occur in diet participants not meeting weight loss goals.

Inclusion criteria	Exclusion criteria
Female	Diabetes (HbA1c ≥6.5%)
PCOS	Liver disease other than NAFLD
Age 12-21 years	Liver transaminases >100 IU/L
BMI >90 %ile for age/sex	Medications affecting IR
	Anemia (hemoglobin <11 mg/dL)
	Implanted MRI-incompatible metal

Participants: PCOS as defined as in our previous work by the most stringent NIH criteria, with adaptation for adolescents (oligomenorrhea >12 months post-menarche or primary amenorrhea after age 15 years and clinical/ biochemical hypertestosteronemia)[8,34,145]. NIH criteria were chosen as they identify women with a higher metabolic disease risk[34,146] than the Rotterdam PCOS criteria[77]. Rotterdam criteria also include ovarian ultrasound, which cannot be applied in teens as ovarian norms do not exist for youth[145,147,148]. Other inclusion/exclusion criteria are in **Figure 9** and include HbA1c to exclude diabetes, per guidelines, since non-controlled OGTT's have 20% discordance in youth[149]. Girls will be instructed not to change physical activity, as increased activity can independently improve HS[80,82].

Recruitment plan: Participants will be recruited from our adolescent obesity and PCOS specialty clinics. The PI has enrolled >120 obese girls with PCOS for research studies in the last 6 years and finished every study ahead of schedule. As the director of our growing multi-disciplinary PCOS clinic with a 7-state referral area, we do not anticipate a change in this pattern. If needed, we can add community gynecology and endocrinology clinics.

Incentives: Participants will be reimbursed for their time for each visit. To aid in retention, additional monetary incentives are given for completing the 2 month and final study visit, as in our previous studies[111,114].

Randomization: The first 16 participants who are enrolled in the study will automatically be randomized to GLP-1 RA (~50% of the group). These 16 participants must complete the 4-month study treatment and complete their final study visits. From these participants we will get their typical weight loss by measuring changes in weight from baseline to mid- and final-treatment. From this we will attempt to match the same amount of weight loss in the participants who are randomized to the lifestyle group. After the first 16 participants complete the study, the

remaining participants will be randomized 1:1 to intensive lifestyle or GLP-1 RA by the study team statistician, with the randomization schedule created prior to any enrollment.

SA1 Detailed Methodology

MRI for IHTG. No MRI studies utilize contrast. IHTG percent will be assessed with MRI using multiple hepatic slices through the entirety of the liver and a modification of the Dixon technique as in our previous studies[9,136,141] on a Siemens 3.0 Tesla research-dedicated magnet (Siemens Skyra large bore magnet with full research and multi-nuclear capabilities). The Dixon method has excellent correlation with IHTG from liver biopsy and measures fat from the entirety of the liver which is important as IHTG is not homogenously distributed. Whereas the PI and Co-I Dr. Brown have extensive experience with proton MR spectroscopy[150-156], MRI is preferable to proton MRS in obese participants because the signal depth of penetration can be reduced by abdominal obesity and only small area of the liver tissue is sampled. Visceral and subcutaneous fat will also be calculated from MRI as in our previous studies[9,136,141]. Our research MRI has in-MRI movie-viewing and thus is very tolerable to youth.

Dietary intervention: Stop Light Diet, data driven personalized daily calorie goals, biweekly measured weight, frequent dietician contact, and meal replacement therapy if needed. The stop light diet was first described for improving youth dietary choices over 20 years ago[157]. It was adapted for use in obese adolescents for the TODAY[113,114] study, and all of the materials are available free-of-charge online (https://portal.bsc.gwu.edu/web/today/materials). We implemented a similar strategy with reduced intensity of teaching in the RISE study[111]. Similar to the education that our team delivers with the PI's current cross-sectional studies, youth and their families will receive intensive dietary teaching by an experienced study dietician after randomization has been completed[158]. Additionally, participants will be oriented with the website Calorie King (www.calorieking.com) to assist them in assigning foods to the red (rarely), yellow (sometimes) or green (anytime) category. Individual targeted weight loss will be translated into daily calorie goals as determined from the measured basal metabolic rate plus the participants' activity factor from their 7-day accelerometer data and target weight loss. The dietician will receive bi-weekly weights and speak to the participants and their families in the diet arm weekly to encourage adherence, answer questions regarding food categories and offer general support. Participants will also be given the option of completing a 3-day photographic food diary prior to their scheduled in-person visits to help direct their diet therapy. Goals will be adjusted every 1-2 weeks, based on bi-weekly weight data. Intensification with in-person visits will be added if needed. At clinical discretion of the study RD, if weight loss trajectory is not meeting intervention goals, RD will add meal replacement shakes to intervention, ordered in participant's flavor preference.. All participants will receive diet counseling at the final study visit and a follow-up phone call as they transition back to clinical care.

Rybelsus® (semaglutide). We have selected semaglutide for many reasons including: ease of use, oral formulation, excellent tolerability **(Table 6),** less weight loss with equal glucose lowering effects and quantity of preliminary data available to-date in obese youth with the same class medication exenatide[159]. Exenatide was one of the first GLP-1 RA approved by the FDA, thus most studies in youth have used exenatide[160-166]. Oral semaglutide is the first subsequent GLP-1 RA to have a similar side-effect profile to that of exenatide. This combined with the oral formulation, which eliminates the possibility of injection site reactions, means that Rybelsus has the most favorable risk/benefit ratio of any available GLP-1 RA. Our team has extensive experience with encouraging adherence to daily oral study medication through coaching, parental involvement and additional incentives with typical adherence of 85-95%. Study staff will text the participant weekly reminders and encourage setting of daily reminders on participants phones, if applicable..

Table 6: GLP-1 RA effects per manufacturer

Drug & Weekly Dose	Nausea	Weight loss
Exenatide 2 mg	8%	1.4 kg (28 wk)
Dulaglutide 1.5 mg	21%	2.3 kg (26 wk)
Semaglutide 1 mg	20%	4.7 kg (30 wk)
Semaglutide (oral) 7 mg	11%	2.2 kg (26 wk)
Liraglutide 1.8 mg *daily	20%	3.3 kg (26 wk)

GLP-1 RA are overall well tolerated in youth and the primary side effects are nausea and hypoglycemia[160-166]. Co-I Dr. Zeitler is an investigator for a multi-center exenatide trial in youth with T2D (NCT01554618, NCT00658021) which is well underway. A trial of exenatide in obese youth with hypothalamic obesity secondary to brain tumor resection is also currently enrolling (NCT01484873). The medication has been well tolerated in both of these studies with no significant hypoglycemia or nausea leading to discontinuation at our local site or in the hypothalamic trial (personal communication with PI Dr. Ashley Shoemaker). There is a concern for increased risk of medullary thyroid cancer following long-term use of GLP-1 RA but recent safety results from a large multi-year study in adults (LEADER) showed that this risk is minimal if those with thyroid cancer risk factors are excluded [168]. Finally, there

was concern for pancreatic disease with GLP-1 RA, but again the recent pancreatic safety data from the LEADER study was also very reassuring[168].

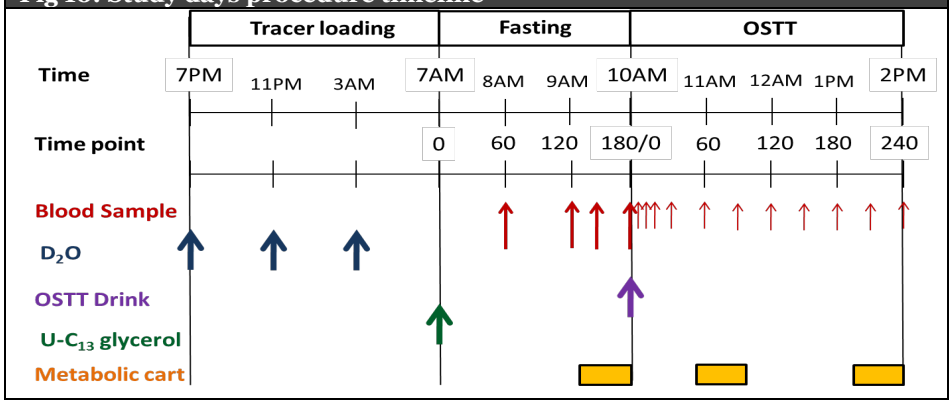
Physical Activity: Activity affects IR and HS. Habitual activity will be assessed objectively with a gold-standard waterproof thigh-worn accelerometer (ActivPal, PAL Technologies), sensitive to sedentary and light activities. This approach is ideal for the proposed sedentary population[169] to measure sedentary, upright, and ambulatory activity counts, intensity, energy expenditure and METs. The participant will be mailed an accelerometer to be worn for the 7 days prior to each study visit. We have utilized accelerometers extensively in youth and we are familiar with their use and have the necessary software and interpretation skills. Pre-intervention, this data will be utilized to customize calorie goals and post-study can be utilized to control for changes in activity, if needed.

Body Composition: DXA (Hologic, Waltham, MA) will be performed for fat mass and fat-free mass [170].

SA2 Detailed Methodology

Overall Metabolic Study Day All of these types of methodologies are currently in use by the PI and CO-I's in similarly obese youth and the study day plan is well tolerated in our patient population. Variations in diet, activity, menstrual phase and sleep affect metabolism and will be controlled and/or assessed prior to each study visit, as in our current protocols. Study visits will be performed in the follicular phase of the menstrual cycle where possible (most girls with PCOS are in a follicular-like non-ovulatory state), preceded by 3 days of no strenuous physical activity and a typical diet for the participant. A standardized afternoon snack and dinner will be provided the night prior to the metabolic study. Data on dietary intake (7-day food diary), habitual activity (3 day questionnaire and accelerometer for 7 days prior to study) and sleep quality (surveys and sleep watch for sleep apnea) are collected. Study day 1 will include an abdominal MRI, hepatic MR elastography (MRE) as measure of fibrosis, hepatic ^{31}P-MRS, brain rs-fMRI, and DXA. Participants will be admitted for an inpatient overnight monitored fast followed by an OSTT with isotope tracers on day 2, assessment of other contributors to HS and IR and PCOS severity. For the OSTT (approximate times shown in **Figure 10**) participants will receive deuterated water overnight to assess DNL, a fasting assessment of hepatic glycerol TCA flux with a U-C_{13} glycerol drink (**Figure 10**[143]), followed by an OSTT with 75g of glucose and 25g of fructose. Indirect calorimetry will be performed throughout the OSTT to measure the rate of fat oxidation. The study day is complete at 2:30 PM.

Fig 10: Study days procedure timeline



Variables known to be closely related to IHTG and IR will also be measured: lipid panel, HbA1c, inflammatory markers (C-reactive protein, AST, ALT, FGF-21; metabolic hormones (glucagon, GLP-1 and adiponectin), sex-steroids (DHEA-S, free and total testosterone, sex hormone binding globulin, progesterone, estradiol); and body composition (BMI, waist:hip ratio, fat and fat-free mass by DXA) and sleep apnea. Targeted metabolomic analysis to assess for serum markers of DNL and mitochondrial oxidation including fatty acids, acylcarnitines[171] and bile acids will be measured by the University of Colorado metabolomics core in fasting and 120-minute post-OSTT drink samples to assess changes with the OSTT.

Measures of hepatic DNL. Rates of hepatic DNL will be calculated based on tracer incorporation of deuterium from oral D_20 into VLDL-secreted palmitate with samples analyzed via GCMS by Co-I Dr. Parks, as previously described in youth[58,172,173]. Water will be given in the amount of 3 ml/kg fat free mass (per DXA), to reach a goal enrichment of 0.3%. We will utilize deuterated water rather than U-C_{13} acetate as in our current study to reduce participant burden, need for multiple IV's and blood volume. Further, the acetate tracer has a carbon label and cannot be combined in a single protocol with the U-C_{13} glycerol tracer needed for the isotopomer analysis. An alternative measure for DNL, the % change in TG secretion during the OSTT (TG_0-TG_{180}/TG_0 *100) will also be calculated.

Serum concentrations from fasting and end OSTT 16:0, 16:1 N7, 18:0, 18:1 N7 and 18:2 will be determined from metabolomics analysis as markers of DNL as in our preliminary data.

Measures of adipose and peripheral IR. Prior to and during the OSTT we will perform frequent sampling of glucose, insulin and FFA. Whole body insulin sensitivity (Si) will be calculated with a 4 hour OMM, utilizing SAM II software[137]. Adipose IR will be calculated as the FFA nadir as in our preliminary data.

Measures of proportion anaplerosis. Glucose and VLDL-TG are isolated from serum, as previously described[143]. Samples are then analyzed by Co-I's Drs. Malloy and Jin utilizing high resolution 13C NMR with a Varian Inova 14.1 T spectrometer (Agilent, Santa Clara, CA) equipped with a 3-mm broadband probe with the observe coil tuned to 13C (150 MHz)[143]. All NMR spectra are analyzed using ACD/Labs NMR spectral analysis program (Advanced Chemistry Development, Inc., Toronto, Canada). Endpoints include the relative proportion of carbon from TCA cycling contributing to TG synthesis and gluconeogenesis as shown in **Figure 7**. ([1,2-13C2] or [2,3-13C2]) glycerol in TAGs reflects the "indirect" contribution from [U-13C3] glycerol through the TCA cycle, representative of cataplerosis. Total gluconeogenesis from [U^{13}C] glycerol is measured as the sum of all glucose isotopomers with ^{13}C in excess. Because the incorporation of ^{13}C tracer in position 4,5,6 is not affected by other pathways (pentose phosphate pathway), the labelling of [4,5-$^{13}C_2$]- or [5,6-$^{13}C_2$] glucose indicates metabolism of [U-$^{13}C_3$] glycerol through the TCA cycle prior to gluconeogenesis. Therefore, indirect gluconeogenesis through the TCA cycle from glycerol is measured by [5,6 $^{13}C_2$]/ [4,5,6 $^{13}C_3$] glucose. All of the diet intervention group will get the glycerol tracer, and approximately half of the semaglutide group will get the glycerol tracer.

Hepatic ^{31}P-MRS. Imaging and MRS will be performed on a Siemens 3.0 Tesla MRI magnet. A custom ^{1}H/^{31}P abdominal coil will be used for imaging and MRS (Clinical MR Solutions, Brookfield, WI) as in our previous ^{31}P work[174]. This does not involve contrast agents. 256 individual ^{31}P MRS spectra are acquired with a 2.3 second echo time and a 2 second repetition time from the entire liver **(Figure 8A).** After minimal phasing corrections, the 6 continuous spectra in the superior/inferior direction with the largest phosphocreatine peak, representing intercostal muscle, are identified and then 6 hepatic spectra, 2-3 voxels medial of the muscle spectra with the largest ATP signal but no PCr signals are identified and summed (**Figure 8b**). Peak positions and areas of interest [phosphocreatine (PCr), inorganic free phosphate (Pi), ATP (3 peaks), PDE and PME] are determined by time domain fitting with jMRUi , utilizing AMARES (A Method of Accurate, Robust and Efficient Spectral fitting), a nonlinear least-square-fitting algorithm using our previously built prior knowledge files. We have utilized a similar method for muscle and liver ^{31}P analysis for the previous 9 years[10,115,118,175,176].

Indirect Calorimetry: Resting VO2 (ml/kg/min) and VCO2 (ml/kg/min) measurements will be collected prior to the OSTT and twice during the OSTT to determine changes in fat oxidation and metabolic flexibility.

SA3 Detailed Methodology

rs-fMRI Image Preprocessing. rs-fMRI preprocessing will be conducted in MATLAB using Statistical Parametric Mapping (SPM 12, Welcome Trust Centre for Neuroimaging, London, UK). Translational movement (i.e., x, y, z) and rotational movement (i.e., pitch, roll, yaw) will be quantified in SPM. All volumes will undergo framewise displacement quality assurance checks via implementation of the MRIQC Toolbox, such that volumes exceeding greater than 1mm or 0.3 degrees rotation will be excluded. All functional images will be slice-time corrected to the slice acquired halfway through the volume collection. Volumes will be realigned to the first volume, co-registered to the participant's anatomical T1, and spatially normalized to MNI152 template space. Normalized images will be smoothed using an 8-mm full-width half-maximum Gaussian kernel. Physiologic noise specifically, pulse and respiratory rate will be quantified and aligned with the BOLD time series data via the PhysIO Toolbox and removed through covariate adjustment in all models to maximize the temporal signal-to-noise ratio in the defined ROIs. Additionally, movement parameters will be used as covariates in all models.

5. OUTCOME MEASURES

Primary Outcome Measure

For the primary outcome, we will compare the change in % IHTG from baseline to end of treatment, with and without GLP-1 RA treatment.

The primary outcomes for Aim 2 are (1) change in hepatic de novo lipogenesis (DNL) and (2) change in FFA nadir in the standard diet + GLP-1 RA group compared to the intensive diet group.

Secondary Outcome Measure

Variables known to be closely related to IHTG and IR will also be measured: lipid panel, HbA1c, inflammatory markers (C-reactive protein, AST, ALT, GGT, FGF-23); metabolic hormones (glucagon, GLP-1 and adiponectin), sex-steroids (DHEA-S, free and total testosterone, sex hormone binding globulin, progesterone, estradiol); and body composition (BMI, waist:hip ratio, fat and fat-free mass by DXA) and sleep apnea. Targeted metabolomic analysis to assess for serum markers of DNL and mitochondrial oxidation including fatty acids, acylcarnitines, and bile acids. Hepatic mitochondrial function per MR spectroscopy and isotopomer analysis. Insulin sensitivity as assessed with the oral minimal model. Whole blood for DNA samples for future genetic analysis associated with hepatic steatosis. Stool samples will be collected to look at changes in microbiome pre-post treatment. Functional neuroimaging measures, specifically, the blood oxygen level-dependent (BOLD) signal in the hypothalamus, insula, anterior cingulate cortex, nucleus accumbens, amygdala, and superior and inferior frontal gyri, and connectivity between these brain regions.

6. PARTICIPANT SELECTION

Study Population

Description of Population to be Enrolled:

Study staff aims to complete 50 obese girls with PCOS (untreated). This is the number of participants needed to be completed statistically, thus more participants may be enrolled to allow for screen failures and dropouts. Total enrollment will be up to 60 participants.

Ethnic Categories	Gender		
	Females	Males	Total
Hispanic or Latino	20	0	**20**
Not Hispanic or Latino	30	0	**30**
Ethnic Categories: Total of All Participants	**50**	**0**	**50**
Racial Categories			
American Indian/Alaska Native	2	0	**2**
Asian	5	0	**5**
Native Hawaiian or Other Pacific Islander	5	0	**5**
Black or African American	15	0	**15**
White	23	0	**23**
Racial Categories: Total of All Participants *	**50**	**0**	**50**

Inclusion Criteria

1) Female
2) Ages 12-21 years
3) Sedentary- less than 2 hours of moderate (jogging, swimming etc) exercise a week.
4) BMI equal or greater than the 90th percentile for age and gender
5) PCOS per the most stringent NIH criteria adapted for adolescents (irregular menses >12 months post-menarche and clinical or biochemical hypertestosteronemia)[8,34]
6) Participants cannot be on hormonal contraception, so participants should remain abstinent or use reliable non-hormonal contraception (e.g. copper IUD) for the entire study period. For

participants who receive semaglutide, they should avoid pregnancy for at least 2 months after stopping medication to avoid fetal exposure to the medication.

Exclusion Criteria

1) Diagnosed with or have a family history of medullary thyroid carcinoma (MTC) or Multiple Endocrine Neoplasia syndrome type 2 (MEN 2). Family history of medullary thyroid cancer or thyroid nodule palpated by endocrinologist at screening.
2) Use of medications known to affect insulin sensitivity: metformin (cannot have been used in the 3 months prior to screening), oral glucocorticoids within 10 days, atypical antipsychotics, immunosuppressant agents, HIV medications, hormonal contraception (cannot have been used in the 6 months prior to screening). Dermal patch or vaginal ring contraception methods. Weight loss medications or stimulants. Use of other products containing other GLP-1 agonists.
3) Currently pregnant or breastfeeding women. Development of pregnancy during the study period will necessitate withdrawal from the study.
4) Severe illness requiring hospitalization within 60 days.
5) Diabetes, defined as Hemoglobin A1C > 6.4%
6) BMI percentile less than the 90^{th} percentile for age and sex. Weight >325 lbs. or <84 lbs.
7) Anemia, defined as Hemoglobin < 11 mg/dL
8) Diagnosed major psychiatric or developmental disorder limiting informed consent.
9) Implanted metal devices that are not compatible with MRI
10) Use of blood pressure medications.
11) Known liver disease other than NAFLD or AST or ALT >100 IU/L.
12) Personal history of pancreatitis
13) Known renal disease of any severity or an eGFR at screening of <45ml/min/1.73m2
14) History of severe GI disease (e.g. gastroparesis)
15) History of gallstones
16) Untreated thyroid disease
17) History of hypersensitivity to semaglutide
18) Other causes of hyperandrogenism (example: tumor, CAH) or amenorrhea (untreated thyroid disease, tumor, primary ovarian failure, prolactinoma).
19) Active symptoms or undergoing treatment for anorexia nervosa or binging/purging disorder

Participant Recruitment Plan

Participants will be recruited from our adolescent obesity and PCOS specialty clinics. The PI has enrolled >120 obese girls with PCOS for research studies in the last 6 years and finished every study ahead of schedule. As the director of our growing multi-disciplinary PCOS clinic with a 7-state referral area, we do not anticipate a change in this pattern. If needed, we can add community gynecology and endocrinology clinics.

Informed Consent Plan

Appropriately qualified and informed personnel who have completed the COMIRB and HIPAA course requirements will fully explain the study protocol and consent form to the participant and guardian verbally in the language they understand. The explanation will be conducted in a quiet environment with adequate time given for the participant and guardian to review the study procedure before the commencement of the study. Asking the participant to explain the study in their own words will assess the participant's understanding. If non-English speaking participants are enrolled in the study, the investigators will adhere to Section 10C of the COMIRB Instructions for Clinical Investigators regarding the consent of these participants. The consent form will also be translated into Spanish. The qualified personnel mentioned above will then obtain written consent from the guardian and assent from the participant, co-signed on the consent form, or in participants who are 18 years or older, direct consent. The participant and guardian will be provided a copy of the consent form for better understanding and record purposes.

Special Consent/Assent Plan

Consent will be obtained from all participants in the study. Following explanation, all participants under 18 years old will co-sign the consent form in addition to the parents signing the consent form. All participants age 18 or older will sign the standard consent form.

Participant Compensation, Incentives and Rewards

Participants will be compensated with Target gift cards for completion of each study visit. The initial Visit 1 consisting of informed consent, and blood draw will result in a $50 gift card. Each of the imaging visits (Visits 2a and 4a) will be $50 each. Each of the overnight visits (Visits 2b and 4b) will be $150 each. Participants will be paid $50 for completing the mid-treatment visit (Visit 3). In an effort to maintain participant retention and completion of the study, the participants will be rewarded an additional $50 if they complete all study visits. Compensation for all completed visits can total up to $550, and participants will only be paid for the visits they complete.

Alternative Treatment

The alternative is for participants to not participate in the study.

Consideration of Specific Participant Categories

1. Inclusion of Women

All participants will be women, as PCOS only occurs in females.

2. Inclusion of Minorities

Every effort will be made to include a diverse participant distribution. PCOS affects Caucasians, Hispanics and African Americans equally.

3. Inclusion of Children

All participants will be between ages 12 and 21. Insulin sensitivity needs to be studied in the adolescent age group as available is scarce in this age group and it is critical to understand the pathophysiology of PCOS in its developing stages.

7. CONCURRENT MEDICATIONS

All participants should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies. Study medication should be taken at least 2 hours apart from any regular medications.

Allowed Medications and Treatments

Prior to starting any new medications, participants should contact the study team.

Individuals treated with semaglutide may have an improvement of PCOS, including improved rates of ovulation and therefore the possibility for pregnancy if sexually active. Non-hormonal methods of contraception including barrier methods (condoms), abstinence or seeking placement of a non-hormonal intrauterine device will be discussed at randomization, mid-study check-in and at the end of the study. Condoms will be offered to all participants at each of these time points.

Prohibited Medications and Treatments

The following medications are prohibited during the study and administration will be considered a protocol violation.

Medications known to affect insulin sensitivity: metformin (cannot have been used in the 3 months prior to screening), oral glucocorticoids within 10 days, atypical antipsychotics, immunosuppressant agents, HIV medications, hormonal contraception (cannot have been used in the 6 months prior to screening). Weight loss medications or stimulants. Use of other products containing other GLP-1 agonists. Dermal patch or vaginal ring contraception methods. Blood pressure medications

8. STUDY TREATMENTS

Method of Assigning Participants to Treatment Groups

Treatment 1: Rybelsus® (semaglutide, GLP-1 RA) (n=33 girls)

Treatment 2: Lifestyle Intervention: Intensive nutritional counseling (n=17 girls)

The first 16 participants who are enrolled in the study will automatically be randomized to GLP-1 RA (~50% of the group). These 16 participants must complete the 4-month study treatment and complete their final study visits. From these participants we will get their typical weight loss by measuring changes in weight from baseline to mid- and final-treatment. From this we will attempt to match the same amount of weight loss in the participants who are randomized to the lifestyle group. After the first 16 participants complete the study, the remaining participants will be randomized 1:1 to intensive lifestyle or GLP-1 RA by the study team statistician, with the randomization schedule created prior to any enrollment.

Investigational Drug Information

Drug Name: Semaglutide

Drug Trade Name: Rybelsus®

Other Names: GLP-1 RA

Dosage Form: oral tablets

Dosage Information: Once daily 3mg dose for 30 days, then once daily 7mg dose for remainder of study. If participants cannot tolerate 7 mg, the dose will be backed down to 3 mg for 1-2 more weeks, then another attempt made to increase to 7 mg. If they still cannot tolerate 7 mg, the duration of therapy will be completed with 3 mg.

Storage: Children's Hospital Colorado Investigational Drug Services

Drug Ordering and Drug Accountability: Children's Hospital Colorado Investigational Drug Services

Measures of Treatment Compliance

Participants on GLP-1 RA will be asked to keep a patient diary noting any adverse events. They will be asked to bring their patient diary to each study visit along with all used and unused study drug containers. Study staff will also send reminders to help with compliance.

Participants randomized to the intensive Lifestyle Intervention will be contacted by a Registered Dietician. Individual targeted weight loss will be translated into daily calorie goals as determined from the measured basal metabolic rate plus the participants' activity factor from their 7-day accelerometer data and target weight loss. The dietician will receive bi-weekly weights and speak to the participants and their families in the diet arm weekly to encourage adherence, answer questions regarding food categories and offer general support. Goals will be adjusted every 1-2 weeks, based on bi-weekly weight data. Intensification with in-person visits will be added if needed.

9. STUDY PROCEDURES AND GUIDELINES

A Schedule of Events representing the required testing procedures to be performed for the duration of the study is diagrammed in Appendix 1 below.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the participant or participant's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

Clinical Assessments:

Concomitant Medications

All concomitant medication and concurrent therapies will be reviewed at all visits. Reminders to take study medication 2 hours separate from regular medications will be made at all visits.

Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening Visit 1.

Medical History

Relevant medical history, including history of current disease, other pertinent history, and information regarding underlying diseases will be recorded at Screening Visit 1.

Physical Examination

A complete physical examination will be performed by either the investigator or a sub-investigator who is a physician at the Screening Visit 1. Qualified staff (MD, NP, and PA) may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

Adverse Events

Information regarding occurrence of adverse events will be captured throughout the study. Duration (start and stop dates), severity/grade, outcome, treatment and relation to study drug will be recorded.

Assessments for symptoms of hypoglycemia will be conducted in participants at baseline, 2 months and the end of the study. The family will be counseled on symptoms of hypoglycemia and counseled to contact the study staff immediately with any increase from baseline in symptoms of hypoglycemia. Symptoms include acute feelings of lightheadedness, shaking, nausea, sensations of hunger, heart racing and rarely vomiting. Treatment for this is eating a small carbohydrate snack.

Clinical Laboratory Measurements

Blood Chemistry Profile

See Appendix 2 for blood sample analysis during the Screening Visit 1 and during the Oral Sugar Tolerance Test (Visits 2b and 4b). A finger stick blood draw will also be done at Mid-Treatment Visit 3 to test HbA1c.

Pregnancy Test

A urine pregnancy test will be obtained from all participants at Screening Visit 1, Visit 2a, Visit 3, and Visit 4a.

Urinalysis

Urine will be collected and stored for future analysis of hormone and metabolic related markers.

EVALUATIONS BY VISIT (SEE APPENDIX 1 FOR REFERENCE)

Visit 1 (Screening)-Outpatient Pediatric CTRC at Children's Hospital Colorado

1. Review the study with the participant (participant's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Assign the participant a unique screening number.
3. Record demographics data.

4. Record medical history
5. Record concomitant medications.
6. Perform a physical examination, if one wasn't done clinically within 3-months of screening visit.
7. Perform and record vital signs.
8. Collect blood for clinical laboratory tests. See Appendix 2, for screening labs.
9. Perform urine pregnancy test
10. Schedule participant for Baseline Metabolic Study Visits 2a and 2b.
11. Pregnancy prevention counseling and offer condoms

Visit 2a (Imaging)-Leprino Building or Anschutz Health Sciences Building and UCD Brain Imaging Center

1. Perform urine pregnancy test
2. Perform DEXA scan
3. Perform MRI of abdomen and liver, P-MRS of the liver, and rs-fMRI of the brain.
4. Perform cardiovascular tests: EndoPAT and Dynapulse

Visit 2b (Overnight)-Pediatric CTRC at Children's Hospital Colorado or University Hospital

1. 7-days prior, mail out gut bacteria collection kit, accelerometer and sleep watch.
2. Perform abbreviated physical examination.
3. Perform and record vital signs.
5. Perform WatchPAT sleep study.
6. Give 3 doses of deuterated water oral tracer overnight.
7. Nurse will collect urine and place IV first thing in the morning.
8. Nurse will draw baseline labs and then participant will drink the Oral Glycerol Tracer (~7AM). This tracer will be given to all the diet intervention group and approximately half of the GLP-1 RA group. Samples for NMR analysis will be collected.
9. Measure basal metabolic rate and two additional metabolic carts during the Oral Sugar Tolerance Test (OSTT).
10. Participant will drink the glucose drink (~10AM) and complete the (OSTT) with frequent blood draws (See Appendix 2 for samples that will be drawn).
11. Administer participant questionnaires (see list in Appendix 3).
12. Participant will be randomized to GLP-1 RA or Intensive Lifestyle Intervention.
13. Baseline hypoglycemia assessment
14. Pregnancy prevention counseling and offer condoms
15. If randomized to Rybelsus, hypoglycemia and orthostatic hypotension symptom recognition teaching.

Visit 3 (Mid-Treatment Visit)-Outpatient Pediatric CTRC at Children's Hospital Colorado

1. Perform physical examination.
2. Perform urine pregnancy test.
3. Perform and record vital signs.
4. Perform venipuncture blood draw for HbA1c and complete metabolic panel.
5. Administer 3DPAR and FFQ Questionnaires.

6. If randomized to Rybelsus, hypoglycemia and orthostatic hypotension symptom recognition teaching.

Visit 4a (Imaging)-Leprino Building or Anschutz Health Sciences Building and UCD Brain Imaging Center

1. Perform urine pregnancy test
2. Perform DEXA scan
3. Perform MRI of abdomen and liver, P-MRS of the liver, and rs-fMRI of the brain.
4. Perform cardiovascular tests: EndoPAT and Dynapulse

Visit 4b (Overnight)-Pediatric CTRC at Children's Hospital Colorado or University Hospital

1. 7-days prior, mail out gut bacteria collection kit, accelerometer and sleep watch.
2. Perform abbreviated physical examination.
3. Perform and record vital signs.
5. Perform WatchPAT sleep study.
6. Give 3 doses of Deuterated Water oral tracer overnight.
7. Nurse will collect urine and place IV first thing in the morning.
8. Nurse will draw baseline labs and then participant will drink the Oral Glycerol Tracer (~7AM). This tracer will be given to all the diet intervention group and approximately half of the GLP-1 RA group. Samples for NMR analysis will be collected.
9. Perform basal metabolic rate and two more metabolic carts during the Oral Sugar Tolerance Test (OSTT).
10. Participant will drink the glucose drink (~10AM) and complete the (OSTT) with frequent blood draws (See Appendix 2 for samples that will be drawn).
11. Administer participant questionnaires (see list in Appendix 3).
12. Hypoglycemia Assessment
13. Pregnancy prevention counseling and offer condoms
14. Reminded to call study team in the next 4 weeks with new onset GI symptoms, worsening symptoms of hypoglycemia or postural hypotension.

Risk / Benefit Assessment

Please note: with the emergence of COVID-19 we must follow hospital policies for screening hospital patients and visitors. This may include testing for COVID-19. This may involve a nasal swab, and the risks of this are discomfort and potentially bleeding from nose. To limit participant time in our clinics, portions of study visits may be done virtually (phone or Telehealth).

1. Blood Sampling
Description: Blood will be drawn for Complete Blood Count (CBC), HbA1c, total and free testosterone, and sex hormone binding globulin. If participants have not had a full evaluation for oligomenorrhea, Prolactin, DHEAS, LH, FSH, TSH, total T4 or 17-OH progesterone may be drawn.
Risk: Minimal. Risk of pain, bruising at site of blood draw, excessive amount of blood.
Minimizing Risk: Certain studies at our institution draw over 7ml/kg in 6 weeks, or up to 7 ml/kg in a single draw, but include iron supplementation. Otherwise, the routine guidelines in our Pediatric CTRC are 2.5ml/kg for a single draw and no more than 5 ml/kg over a 4 week period. Our baseline visit will include 11.5 ml of blood (HbA1c, Hb, Cr, AST, ALT) and 25.5 ml of blood for PCOS patients (additional draw for T4, TSH, prolactin, LH, FSH, 17OH progesterone and DHEAS). The OSTT visit includes up to 300 ml of blood which will occur within 4 weeks of the

initial visit. Thus, our OSTT visit is within the NIH Clinical Center guidelines of 9 ml/kg in 6-8 weeks and within Children's Hospital Colorado's institutional guidelines of 5 ml/kg. In addition, by study design, participants are screened by our baseline CBC and excluded if anemic, further increasing the safety of the study regarding blood draws. We will use a minimum weight cutoff of 38 kg to remain below the most conservative pediatric CTRC blood drawing guidelines. This screening also helps to increase the safety of the blood draw. In addition, the blood planned to be frozen and held could also be omitted if needed to reduce blood volume for a particular participant. Finally, our CTRC has a system to track other studies participants might enroll in, and we ask during our consent process if the participant has been involved in any other studies in the past 6 weeks to avoid excessive blood drawing.
Justification: Screening laboratory measurements are necessary to assure that patients meet inclusion/exclusion criteria before any further study is completed. A CBC is necessary as a screening lab, to rule out anemia. A hemoglobin A1c can be used to rule out diabetes. Hormone levels of free and total testosterone, and sex hormone binding globulin are needed to categorize patients as having PCOS, and prolactin, DHEAS, LH, FSH, TSH, total T4 or 17-OH progesterone to rule out other causes of oligomenorrhea, if not done previously.

2. IV Risks
Description: One peripheral IV will be placed during the OSTT for drawing blood samples.
Risk: There is temporary discomfort when the needle goes in and 10% of the time there is a small amount of bleeding under the skin that may produce a bruise. Rarely, there is a risk of a blood clot forming or infection. We will use a low dose of a medication called heparin to try to prevent blood clotting.
Justification/Minimization: These studies involve sampling blood at multiple time points. Thus, an IV is needed, so as to avoid multiple needle sticks. These studies are focused on measured rates of change which necessitates the sampling of the same test over time. Proper sterile technique will be used with blood draws and IV placement to decrease the infection risk. EMLA cream will be used if participant desires to minimize pain of IV.

3. Oral Sugar Tolerance Test (OSTT):
Description: An OSTT will be performed with multiple blood draws. The purpose of the OSTT is to provide a controlled oral stimulus to effect changes in lipolysis and hepatic glucose release.
Risk: The participants rarely experience nausea within 15 min of consuming the drink, however, the amount of carbohydrate is very similar to a large soda, which is regularly consumed by this patient population.
Justification/Minimization: A standard oral challenge is needed to study lipogenesis, lipolysis and gluconeognesis in the fed state. We have chosen to start with a standard glucose and fructose load, to simplify the mathematical modeling. Dynamic carbohydrate metabolism in youth is made more relevant by the recently reported TODAY study, showing a decline in beta cell function in youth with newly diagnosed type 2 diabetes that was much more rapid than what has been reported in adults, and not prevented by metformin in the majority of the youth[177]. Our team of investigators, CTRC research nursing staff and physicians are well experienced with the OSTT blood draw procedure. A floor nurse located on the will be available during our inpatient visits and patients will be distracted by TV or other similar means during the OSTT, to minimize queasiness.

4. Stable Isotope Studies Glycerol:
Description: Oral stable isotope tracer of glycerol will be utilized to determine rates of intrahepatic substrate flux. These are substances normally present or produced in the body, and thus pose no more risk than typical glucose infusions. Measurements of these metabolic processes are only able to be made with the utilization of stable isotope tracers.
Risk: We are utilizing an isotope which already exists in all humans, but are simply increasing the percentage. We are only giving this medication orally. These are NOT radioactive substances.
Justification/Minimization: Pyrogen-free ^{2}C13 glycerol will be obtained from the manufacturer and delivered to CHCO IDS. The IDS pharmacist will deliver the tracer to the pediatric CTRC (inpatient unit or 3rd floor outpatient) once ordered by the physician.

5. Stable Isotope Studies Deuterated water:
Description: Oral stable isotope tracer of deuterated water will be utilized to determine rates of de novo lipogenesis and gluconeogenesis. These are substances normally present or produced in the body, and thus pose no more risk than typical water unless consumed in large amounts, to increase total body enrichment in excess of 20%[178]. Measurements of these metabolic processes are only able to be made with the utilization of stable isotope tracers.

Risk: We are utilizing an isotope which already exists in all humans, but are simply increasing the percentage. We are only giving this medication orally. These are NOT radioactive substances. The planned dose is 3 ml/kg as has been used previously in obese youth[179], with a goal enrichment of 0.3%, well below the 20% known to cause side effects. At the proposed dose, few participant experienced nausea, but it was extremely well tolerated (personal communication with PI's Dr. Santoro and Parks).
Justification/Minimization: Pyrogen-free deuterated water will be obtained from the manufacturer and delivered to CHCO IDS. The IDS pharmacist will deliver the tracer to the pediatric CTRC (inpatient unit or 3rd floor outpatient) once ordered by the physician.

6. Standard Diet
Description: A dinner and snack will be provided during the overnight visit. Daily calorie intake will be calculated using the St. Jeor Equation for females: ([10(weight in kg) + 6.25(height in m) – (5*18) – 161] X Activity Factor x 1.25). 1.25 x weight maintenance was chosen as this as most similar to our participants food consumption based on pilot participant's food frequency questionnaires and optimal for detection of hepatic glucose Ra [180]. The snack will be calculated to be 10% of the daily calorie intake and the dinner will be calculated to be 26% of the daily calorie intake.
Risk: None
Justification/Minimization: Variations in diet, activity and circadian rhythms affect metabolism [155].

7. Magnetic Resonance Imaging, Magnetic Resonance Spectroscopy, and resting state – functional Magnetic Resonance Imaging (MRI, MRS, rs-fMRI)
Description: The MRI will usually be obtained the day of admission to CHC), at the UCD Brain Imaging Center on the Fitzsimmons campus. A trained research radiographer who is supervised by Dr. Mark Brown, of UCD radiology, will perform an abdominal MRI to obtain hepatic, visceral and subcutaneous fat on a 3.0 T whole–body MRI scanner (Siemens MAGNETROM, Malvern, PA). Participants will lie supine while these measurements are obtained, need to hold reasonably still during the scan and cannot weigh >325 lbs. A second sequence to measure the amount of fibrosis (if any present) in the liver will be performed. A specialized phosphorus coil will be utilized to measure the concentration of ^{31}P via MRS. For the fMRI, participant's head will be stabilized within the head coil using foam padding. Participants will be instructed to keep eyes open with relaxed fixation on a projected bright crosshair on a dark background. Anatomical scans will be collected using a T1- weighted, 3D Magnetization Prepared Rapid Gradient Echo sequence and will take ~5 mins to complete. Blood Oxygen Level Dependent (BOLD) resting-state functional MRI (rs-fMRI) data will be collected using a multiband gradient echo planar imaging sequence with a 32-channel head coil. The rs-fMRI sequence will take ~20 min to complete. Pulse and respiratory rate will be recorded during all scanning procedures via a respiratory belt and pulse sensor.
Risks: Minimal. Participants may develop claustrophobia in the magnet.
Minimizing Risk: The participant is provided with audio protection and optional television to help increase comfort. Some participants might feel claustrophobic while having an MRI and the scan will be stopped if it cannot be tolerated. In addition, any participants with implanted metal that is not cleared by the MRI technician may not be able to have the MRI due to the type of magnet involved. There is a very small risk that structural brain abnormality is identified during the rs-fMRI scan. If the participant is determined to have a structural abnormality, the participant and their primary care provider will be notified, and the participant will be encouraged to follow-up with them. This notification may cause the participant psychological distress.
Justification/Minimization: MRI is a non-invasive and non-radiation method to assess body fat, and mitochondrial function. The risks are minimized by assuring patient comfort prior to starting the scan, placing eye goggle that plays movies on the participants. Further, per standard protocol, no patient will be placed into the scanner if they do not meet the rigorous safety standards for the MRI, including the absence of non-compatible implanted metal.
8. Body Composition
Description: Body composition will be measured using the DEXA technique and will be used to derive fat-free mass and % body fat. This technique relies on the absorption of dual electron wavelengths for the assessment of body fat, lean tissue, and bone mineral density. During the procedure, the participant will be supine on the measurement table, and the arm of the machine will slowly pass over their body.
Risk: Minimal. Radiation exposure
Justification/Minimization: Body composition is best assed via DEXA, and the amount of muscle mass is needed to standardize the OSTT results, since body weight can vary greatly. This procedure will deliver the radiation exposure that is 2 times the level of background radiation in Colorado. Participants will be tested for pregnancy immediately prior to DEXA, to ensure that they are not pregnant.

9. EndoPAT and Dynapulse
Description: The Dynapulse Pathway and the EndoPAT system are noninvasive portable systems that measure brachial artery distensibility and endothelial function, utilizing a standard sphygmomanometer cuff inflated in the same fashion as a sphygmomanometer to obtain blood pressure. The instrument derives brachial artery distensibility using the technique of pulse waveform analysis of arterial pressure signals obtained from the sphygmomanometer. Measurement of heart rate variability for autonomic tone will be performed using the EndoPAT
Risk: This procedure may lead to mild discomfort due to the blood pressure cuff being inflated.
Justification/Minimization: Endothelial function is a novel measure in PCOS and will aid in the determination of cardiovascular dysfunction with this population.

10. Body fat distribution
Description: Height, weight, waist circumference, and hip circumference will be measured. Body fat distribution will be determined using the waist-to-hip ratio where the waist circumference is measured 1/2 the distance from the xiphoid process to the navel and the hip circumference is measured at the level of the greater trochanter.
Risk: None
Justification/Minimization: IR has been associated with central obesity, as has hyperandrogenism. Whereas we are measuring central obesity with MRI, it is important to see if this simple non-invasive measure matches the MRI results, as it is a much simpler measure to follow clinically.

11. Accelerometer and Sleep Watch:
Description: Participant will be provided an accelerometer and a sleep watch (GT3X BT by Actigraph and ActiWatch by Philips Respironics) to be worn for seven days to measure level of habitual physical activity, which affects insulin sensitivity, and sleep patterns. The accelerometer and sleep watch will be worn on the participant's wrist. The wrist position has been validated to hip position actigraphy in this population.
Risk: There is no risk involved with the accelerometer.
Justification/Minimization: Accelerometers are effective tools for the objective measurement of physical activity [181] because they have the ability to continuously record physical activity data and such data can be used to estimate METs of activity. They provide more detailed information than pedometers, which only measure walking steps, and help get around the recall bias of questionnaires. We are currently using the GT3X BT Actigraph in adolescents in our other diabetes studies; therefore, we are familiar with their use in this population and have the necessary computer software and interpretation skills. The Actiwatch is being used as a tool for objective measurement of sleep patterns. The Actiwatch is fitted with a LED monitor that records multiple spectrums of light to better assess sleep patterns in this population.

12. Metabolic Cart:
Description: The metabolic cart measures the amount of air that the participant breathes in and out. The machine attaches to the participant's mouth through a tube, or a plastic bubble that is placed over the participant's head. There is the potential for experiencing claustrophobia from having the plastic bubble over the participant's head. A metabolic cart will be utilized multiple times during the OSTT study day to measure rates of oxygen consumption and carbon dioxide release. These rates can be utilized to calculate rates of carbohydrate and fat oxidation and resting energy expenditure.
Risk: Minimal risk of claustrophobia.
Justification/Minimization: These studies are well tolerated by youth, and involve placing a clear plastic hood over the participant's head for approximately 20 minutes. The data collected from the baseline study is also very useful for assisting obese participants in determining their true caloric needs, and useful in setting dietary goals for weight loss. This piece of information is thus utilized in post-study nutritional counseling.

13. Food Frequency Questionnaire (SEARCH FFQ)
Description: Customary macronutrient pattern will be ascertained by diet interview at the time of admission using a SEARCH FFQ, modified to incorporate common food choices among ethnically and regionally diverse youth aged 10-19 participating in another large childhood diabetes study, SEARCH (48). The instrument is self-administered with staff support to provide instructions, answer questions, and to review the form after completion, and captures the last week of dietary intake.
Risk: None

Justification/Minimization: Several of the measurements being assed are affected by prior nutritional intake. Furthermore, participants will receive dietary counseling at the end of the study, and by knowing what their previous dietary pattern is, suggestions for improvement can be tailored to their specific dietary habits.

14. 3DPAR Questionnaire
Description: A questionnaire (3DPAR) recalling the physical activity levels of the three previous days will be completed at Visit 2b, Visit 3 and Visit 4b.
Risk: None
Justification/Minimization: Physical activity can directly affect insulin sensitivity, our primary outcome measure. The 3DPar is a well validated measure to asses 3 days of physical activity in youth, and includes a variety of youth centric activities.

15. Strengths and Difficulties Questionnaire:
Description: This is a survey which identifies areas in a youth's life that they believe they are strong or weak in dealing with, as a measure of coping skills. Low coping skills have been associated with the development of depression.
Risk: None
Justification/Minimization: This survey can help identify youth at risk for depression or anxiety, and identify poor coping skills. It does not directly assess depression or suicidality.

16. WatchPAT and Questionnaires to assess for Obstructive Sleep Apnea
Description: The WatchPAT is a noninvasive portable system that measures the oxygen saturation and apnea hypopnea index. Three surveys querying signs and symptoms of obstructive sleep apnea.
Risk: No risk associated with the questionnaires and the WathchPAT, other than a mild discomfort from having to wear the watch and cuff around finger during sleep. It is possible that we will discover that the participant has obstructive sleep apnea, and will need to be referred for further clinical care.
Justification: Obstructive sleep apnea is associated with obesity, and can worsen both fatty liver and insulin resistance. Thus the presence of OSA must be accounted for when measuring either of these outcomes. These surveys selected are currently being utilized by the NIH multiple center study in obese youth at risk for diabetes, and are well validated in youth from multi-ethnic populations. If OSA is suspected during the course of the screen, the participants will be referred for further evaluation and treatment. The WatchPAT is an FDA approved device that can be used specifically for oxygen saturation and apnea hypopnea index and is approved in children as young as 12 years of age, within the age range of our study population.

17: Questionnaires on Motivation for Weight Loss, Binge Eating, and Social Support

Description: Four surveys asking about the participant's motivation to lose weight, indicators of uncontrollable eating habits, and the participant's social support from family, friends, and others (ie. Teachers, classmates) in achieving set goals.
Risk: None
Justification: Assistance in developing a participant-specific weight loss plan and targeting goals.

18: Gut Bacteria Collection:
Description: A week prior to visit three, participants will be provided with stool collection swabs to collect a small sample of stool from the toilet paper they use after having a bowel movement.
Risk: Although the risk is minimal, participants may feel uncomfortable taking a sample of stool from the toilet paper following a bowel movement. All participants will be instructed to follow proper bathroom etiquette as fecal matter can transmit diseases.
Justification: Studies in obese individuals with type 2 diabetes have alterations in the gut microbiota that may be related to NAFLD.

19: Rybelsus® *semaglutide):*
Description: Rybelsus® (semaglutide) is a GLP-1 receptor agonist. This medication comes in the form of daily oral tablets available in 3mg, and 7mg.*Risk:* 1) The most common side effects with Rybelsus® (semaglutide) may include nausea, abdominal pain, diarrhea, decreased appetite, vomiting, and constipation.. 2) Some patient can develop low blood sugars. Symptoms include acute feelings of lightheadedness, shaking, nausea, sensations of hunger, heart

racing and rarely vomiting. Treatment for this is eating a small carbohydrate snack. The family/participant should let the study team know if these symptoms develop. 3) From animal studies, there is a concern for increased risk of medullary thyroid cancer following long-term use of GLP-1 RA, but this has not been duplicated in human studies. 4) Inflammation of the pancreas and gallstones have rarely been associated with this class of medications. Symptoms include serve central abdominal pain that may radiate to the back, nausea and vomiting and may be worse after meals, particularly fatty meals. These symptoms will be reviewed with the family/participant and the family/participant should let the study team know if these symptoms develop. 5) It is very important not to get pregnant while taking this medication.
Justification: This class of medication is utilized for weight loss in normoglycemic patients and it is not known to cause hypoglycemia in patients without diabetes. Multiple clinical trials of use of medications of this class in pediatric patients have been published, showing good safety and efficacy data similar to that found in adults. Of this class of medications Rybelsus is the first oral GLP-1 RA therapy.. Finally, our pediatric providers are experienced in prescribing this medication in youth. From February 2017- February 2019, 65 unique pediatric patients were prescribed a GLP-1 receptor agonist by our pediatric endocrinology type 2 diabetes providers (Zeitler, Kelsey, Nadeau, Green, Williams) at the Anschutz Medical Campus.. Medullary thyroid cancer is extremely rare in humans and this risk is negligible with short-term use (as proposed by this study) and those with thyroid cancer risk factors are excluded. Patients will receive extensive education on how to properly take this medication and they will be encouraged to contact study staff if they experience any adverse events. The family should notify the study team for significant GI side effects and our team will also be routinely monitoring for this via phone contact. If a participant has emesis for > 24 hours or nausea limiting the ability to drink fluids, we will bring them in for hydration assessment that will include orthostatic blood pressures and complete metabolic panel (sodium, potassium, and renal function). Additionally, if a patient presents with symptoms consistent with pancreatitis during the study or follow-up period, we will bring them in for an assessment (physical exam, vital signs, blood draw), which will can include an amylase, lipase, and complete metabolic panel, along with consultation with gastroenterology.

20. Violation of Privacy and Loss of Confidentiality
Description: These are both risks to which research participants are exposed. The possibility of these risks increases when protected health information is collected. Every effort will be made to decrease this risk by limiting access to protected health information, storing this information in a password protected database, and identifying participants only by a unique identifier that is kept in a separate location in a locked container, traceable only by study personnel. All of the tests involve the risk of identifying asymptomatic abnormalities. The study may include risks that are unknown at this time.
Justification/Minimization: Every effort will be made to decrease the risk of loss of confidentiality by limiting access to protected health information, storing this information in a password protected database, and de-identifying study specimens.

11. ADVERSE EVENTS

a) Definitions of Adverse Events

According to the International Conference on Harmonization (ICH) guidelines (Federal Register. 1997;62(90):25691-25709) and 21 CFR 312.32, IND Safety Reports, and ICH E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:

An adverse event is any untoward medical occurrence in a participant or clinical investigational participant administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any untoward medical occurrence regardless of relationship to the medicinal (investigational) product.

Adverse events will be recorded from the time of informed consent.

b) Documentation and Monitoring Adverse Events

All baseline conditions and adverse events encountered during the treatment following participant consent through the last day of treatment will be recorded in the patient's medical records. Serious adverse events that are considered related (i.e., determined to be possibly, probably, or definitely related) to the investigational product by the treating physician will be documented on the patient's medical record.

c) Assessment of Adverse Events

For each adverse event, the start and resolution dates, severity, seriousness (i.e., whether the event meets the definition of an SAE, relationship of the event to the study drug, action taken regarding study drug, and outcome of the event will be documented on the patient's medical record.

(1) Severity

Common Toxicity Criteria for Adverse Events (CTCAE) Version 4.03 will be used to assess and grade adverse event severity, including laboratory abnormalities judged to be clinically significant. If the event is not covered in the CTCAE, the guidelines shown in the following table will be used to grade severity.

Adverse Event Severity Grading Scale:

Severity	Grade	Description
Mild	1	The event is of little concern to the participant and/or of no clinical significance. The event is not expected to have any effect on the participant's health or well being
Moderate	2	The participant has enough discomfort to interfere with or necessitate change in usual activities. The event is of some concern to the participant's health or well being. The event may require medical intervention.
Severe	3	The participant is incapacitated and unable to work or participate in many or all usual activities. The event is of definite concern to the participant and/or poses substantial risk to the participant's health or well being. The event is likely to require medical intervention and/or close follow-up
Life-Threatening	4	The participant is at risk of death due to the adverse event as it occurred. This does not refer to an event that, hypothetically, might have caused death if it were more severe.
Death	5	Death related to adverse event.

(2) Relationship to the investigational product:

The relationship of an adverse event to study drug will be assessed using the guidelines presented in the table below.

Adverse Event Relationships to Investigational Product:

Relationship to Drug	The Adverse Event:
Related	Exhibits previously known toxicity of agent; **or** Follows a reasonable temporal sequence from administration of the drug; Follows a known or expected response pattern to the suspected drug; Is confirmed by stopping or reducing the dosage of the drug; **and** Is not explained by any other reasonable hypothesis

Probably Related	Follows a reasonable temporal sequence from the time of study drug administration; **and/or** Follows a known response pattern to the study drug; **and** Was unlikely to have been produced by other factors such as the participant's clinical state, therapeutic intervention, or concomitant therapy
Possibly Related	Follows a reasonable temporal sequence from the time of study drug administration; **and/or** Follows a known response pattern to the study drug; **but** Could have been produced by other factors such as the participant's clinical state, therapeutic intervention, or concomitant therapy.
Unlikely Related	Does not follow a reasonable temporal sequence from the time of study drug administration; **and** Was likely produced by other factors such as the participant's clinical state, therapeutic intervention, **or** concomitant therapy, but for which relationship cannot be definitely ruled out.
Not Related	The adverse event can be determined with certainty to have no relationship to the study drug

(3) Adverse Event Outcome

Each adverse event will be characterized according to the outcomes described in the following table:

Adverse Event Outcomes:

Outcome	Description
Recovered/resolved	The participant has fully recovered from the event with no observable residual effects.
Recovering/resolving	The effects of the event are improving, or events have stabilized (are constant and not expected to improve or worsen) but have not returned to baseline
Not recovered/not resolved	The effects of the event are still present and changing. The event is not considered stabilized or resolved.
Recovered/resolved with sequelae	The participant has fully recovered from the event with some observable residual effects.
Fatal	The event was the primary cause of death (may or may not be the immediate cause of death).
Unknown	The event outcome is unknown.

Note: Death is an outcome of an event and not an event per se. Sudden death or death due to unexplainable cause(s) will be reported, but follow-up will be pursued until cause of death is determined.

(4) Action Taken with Investigational Product

Action taken with investigational product in relation to each adverse event will be characterized as follows:

Action taken with investigational product:

- None
- Drug withdrawn
- Drug interrupted
- Not applicable
- Other (specify in medical record)

12. SERIOUS ADVERSE EVENTS

Any adverse event or abnormal laboratory test value that is serious (see definition below) and occurs after administration of the investigational product will be documented by the Sponsor-Investigator within 24 hours of discovery of the event.

(a) Definitions

An adverse event will be classified as a serious adverse event (SAE) if it meets one of the following criteria:

Classification of Serious Adverse Events:

Classification	Description
Fatal	Adverse event resulted in death
Life Threatening	The adverse events placed the patient at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization	The AE required or prolonged an existing inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before the signing of informed consent in the study or routine check-ups are not serious adverse events by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization. Hospitalizations or prolonged hospitalizations for scheduled therapy of the underlying cancer or study target disease need not be captured as SAEs.
Disabling/incapacitating	Resulted in a substantial and permanent disruption of the patient's ability to carry out activities of daily living
Congenital anomaly or birth defect	An adverse outcome in a child or fetus of a patient exposed to the molecule or study treatment regimen before conception or during pregnancy.
Medically significant	The adverse event did not meet any of the above criteria, but could have jeopardized the patient and might have required medical or surgical intervention to prevent one of the outcomes listed above.

Death due to progressive disease (PD) will not be considered an SAE. However, if a patient requires hospitalization due to an AE related to PD, the specific sign or symptom leading to hospitalization will be reported as an SAE. If death due to PD occurs outside of hospitalization and is considered to be due solely to the patient's malignancy, no SAE report is required. The death will be recorded as part of the tumor response and patient disposition.

Serious adverse events that are considered related (i.e., determined to be possibly, probably, or definitely related) to the investigational product by the Sponsor-Investigator will be followed until the event resolves or stabilizes. Any SAE that occurs after treatment completion, and is considered by the Sponsor-Investigator to be related to the investigational product, will be documented and reported as appropriate.

(b) Reporting Serious Adverse Events to Regulatory Agencies

Events meeting the following criteria will be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report:

The Sponsor-Investigator will notify the appropriate review division at the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Sponsor-Investigator to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the investigational

product's Investigator Brochure. Such reports are to be telephoned or faxed to the appropriate review division at FDA and to the manufacturer of the investigational product within 7 calendar days of first learning of the event.

15 Calendar Day Written Report

The Sponsor-Investigator will notify the appropriate review division at the FDA, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the investigational product's investigator brochure.

Written IND Safety reports will include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the Sponsor-Investigator with the IND concerning similar events will be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with Analysis of Similar Events will be submitted to the appropriate review division at the FDA, and the manufacturer of the investigational product within 15 calendar days of first learning of the event, using FDA Form 3500A.

Written IND Safety Reports will be submitted to the IRB(s) of record per IRB Guidelines.

13. IND Annual Reports

The Sponsor-Investigator will provide annual reports to the appropriate review division at the FDA within 60 days of the IND's anniversary date, until the IND is withdrawn or terminated.

14. DISCONTINUATION AND REPLACEMENT OF PARTICPANTS

Early Discontinuation of Study Drug

A participant may be discontinued from study treatment at any time if the participant, or the investigator, feels that it is not in the participant's best interest to continue.

The following is a list of possible reasons for study treatment discontinuation:

- Participant withdrawal of consent (or assent)
- Participant is not compliant with study procedures
- Adverse event that in the opinion of the investigator would be in the best interest of the participant to discontinue study treatment– classified as a CTCAE grade 3 attributable to the medication or a CTCAE regardless of attribution to the drug.
- Protocol violation requiring discontinuation of study treatment
- Lost to follow-up
- Positive pregnancy test
- If a patient has recurrent emesis at the lowest dose (3 mg) of semaglutide they will be removed from the trial or if they are so nauseated as to be unable to maintain adequate hydration, they will be removed from the trial.
- If participants cannot tolerate the study medication due to side effects, participants will be instructed to contact the study team immediately and they may be brought in to an additional visit, which may include a blood draw. Study team will evaluate their symptoms and determine if symptoms are severe enough for them to withdraw from the study, or if they are on the 7 mg dose, back down to 3 mg for at least a week, before reattempting to increase to 7 mg.

- We will monitor liver enzymes following the criteria listed below:
    - If the ALT at the second visit increases by > 40% and becomes > 100 IU/mL, and the participant is randomized to active drug, we will not start the drug immediately, but will repeat the CMP in 2-4 weeks. If the value remains >40% and > 100 IU/ml of baseline, the participant will be removed from the study.
    - Once on therapy, if there is an increase in ALT > 5x baseline and > 100 IU/ml at any visit, ALT will be repeated within 3-7 days and if the elevation is persistent, medication will be stopped for one week, and ALT repeated after 7-10 days of discontinuation. If ALT is no longer elevated medication will be restarted, with another ALT 7-10 days following restart of medication will be performed. If ALT is not elevated, trial will continue, if ALT returned to > 5x baseline and > 100 IU/ml, participants will be removed from trial. If removed from the trial, a repeat safety CMP will be performed 3-7 days after stopping drug.
    - Once on therapy, if participants develop persistent right upper quadrant pain or signs of jaundice, they will be brought in for assessment, including vital signs, physical exam and complete metabolic panel. The above guidance on point 2 will apply to laboratory values obtained.
    - It will be recommended that any participant who is removed from the trial have a follow-up appointment with hepatology, but this will not be part of the study.

Withdrawal of Participants from the Study

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for participant withdrawals. The reason for the participant's withdrawal from the study will be specified in the participant's source documents. Refer to the protocol for early termination procedures.

A participant may be withdrawn from the study at any time if the participant, the investigator feels that it is not in the participant's best interest to continue.

Replacement of Participants

Participants who withdraw or are withdrawn from the study will be replaced.

15. CRITERIA FOR STOPPING THE TRIAL

- Three patients develop the same Grade 3 Common Terminology Criteria for Adverse Events (CTCAE) attribution to the study drug OR
- Two patients develop any Grade 4 CTCAE attribution to study drug OR
- One patient develops a grade 5 CTCAE attribution to study drug

16. PROTOCOL VIOLATIONS

A protocol violation occurs when the participant or investigator fails to adhere to significant protocol requirements affecting the inclusion, exclusion, participant safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

Failure to meet inclusion/exclusion criteria

Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The investigator will determine if a protocol violation will result in withdrawal of a participant.

When a protocol violation occurs, it will be discussed with the investigator and a Unanticipated Problem form will be completed, and submitted to COMIRB, detailing the violation. This form will be signed by the Investigator. A copy of the form will be filed in the site's regulatory binder and in the COMIRB's files.

17. DATA SAFETY MONITORING

The PI, Dr. Melanie Green, will establish a Data Monitoring Committee (DMC) to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study. The committee will consist of members from outside institutions who are experts in the field: Tania Burgert, pediatric endocrinologist at Mercy Children's in Kansas City, Missouri; Ashley Shomaker, pediatric endocrinologist at Vanderbilt; and Lisa Chow, adult endocrinologist at the University of Minnesota Rochester, MN, and a pediatric gastroenterologist.. The DMC will talk for the purpose of monitoring study conduct and assessing patient safety. The conference calls will be held after the first three participants are complete, then approximately every 6 months, or with the occurrence of any acute adverse event.

The principal investigator and will meet with the research coordinator to monitor the protocol and the safety of the research participants. The PI will review all laboratory data and report any abnormal values to the patient and guardian and instruct the participant to follow-up with their PCP. If an abnormal result from a research procedure exists, the PI will notify the family and their PCP and refer the participant to the appropriate clinic for further evaluation. The PI may also share research results in a reasonable and prudent manner with appropriate medical professionals if the participant was seriously injured as a result of a procedure or if follow-up of the result of the procedure is in the best interest of the participant's health as determined by a medical professional. If immediate medical follow-up of participant required, the PI will share the research results via EPIC when clinically relevant. The PI will report adverse events, and any decision to suspend or halt the protocol to CTRC and COMIRB immediately. The PI will also prepare a written report for the yearly continuing review required by COMIRB and the CTRC. The PI will also submit relevant reports to the FDA as required per FDA regulations.

No protected health information will be collected until the appropriate HIPAA forms are completed. The protected health information that will be collected will include: Name and phone number, demographic information (DOB, sex, ethnicity, address, etc.), diagnosis (es), history and physical, laboratory or tissue studies, radiology studies, procedure results, survey/questionnaire results, research visit records, and portions of previous Medical Records that are relevant to this study. This information will be accessible only by the study investigators, Federal agencies overseeing human subject research, the Colorado Multiple Institutional Review Board, regulatory officials from the institution where the research is being conducted to monitor safety and compliance with policies.

18. STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

SA1 Statistical Plan

Sample size justification. Sample size is based on the power to detect a difference between the standard diet + GLP-1 RA group and the intensive dietary counseling group in the primary outcome of change in IHTG. Estimates for power calculations were obtained from a recent randomized controlled trial of liraglutide in adult women with PCOS[68] and our baseline data on IHTG in obese girls with PCOS (Table 2). Assuming a standard deviation (SD) of 0.7% and a two-sided significance level of 0.05, a final sample size of 17 participants in the intensive diet group and 33 participants in the standard diet + GLP-1 RA group provides 90% power to detect a difference of -0.7% between group change. This difference is 50% smaller than the difference of -1.34% observed by Frøssing et al.[68] as their results are confounded by significant weight loss and therefore our study is powered conservatively. We anticipate a 1% decrease in hepatic fat. We estimate an approximately 5-10% drop out rate based on our group's experience from similar participant populations[9,10,111,116,118,182,183] and we will enroll up to 60 girls with the goal of 50

completers.

Analysis plan. Data will be collected and managed in a REDCap database, a secure web-based application designed to support data capture for research studies, including validated data entry, audit trails, and automated export to statistical packages. Prior to the start of any formal analyses, variables will be examined for unusual values that need to be queried, patterns of missing values, and whether their distributions are non-Gaussian. Demographic and clinical characteristics will be summarized with descriptive statistics. Primary analyses will follow the "intent-to-treat" principle so that study hypotheses are tested under realistic conditions in which not all participants adhere to the intervention. This will include those who discontinue therapy whose data will be censored at the time of discontinuation, so that all data we have on the non-completers up until the time they leave the study will be included in this primary analysis. However, in order to fully evaluate the physiology and mechanism of action of semaglutide in this population, which is an important aim of this investigator-initiated study, we will also perform secondary analyses in participants who demonstrated at least 80% adherence (i.e., at least 80% medication taken in the GLP-1 arm and at least 80% of targeted weight loss achieved in the intensive lifestyle arm). For the primary outcome, we will compare the change in % IHTG with and without GLP-1 RA treatment using a t-test or Mann-Whitney U test. We expect the weight loss will be similar in the two groups; however, if there is a clinically significant difference between the groups, these comparisons will also be performed while adjusting for the amount of weight lost. Similarly, if there are differences in activity or obstructive sleep apnea severity, appropriate adjustments will be made. Similar analyses will be used for secondary outcomes (2 hour OGTT glucose, HOMA-IR, metabolomics markers, proportion of direct/indirect glucose, EAA concentrations). Linear models will be used to identify the variables that are associated with change in IHTG, e.g., 2 hour OGTT glucose, HOMA-IR, metabolomics markers.

SA2 Statistics

Sample size justification. The primary outcomes for Aim 2 are (1) change in hepatic de novo lipogenesis (DNL) and (2) change in FFA nadir in the standard diet + GLP-1 group compared to the intensive diet group. Estimates of variability were obtained from the PI's pilot data (**Figure 4b**). We assumed a cross-sectional DNL SD of 3.36%, a pre-post correlation of 0.8, and a two-sided significance level of 0.05. With 50 participants, we will have 90% power to detect a between-group DNL difference of 2.1%, which is smaller than the difference of 2.6% reported by Armstrong et al. in adults[14]. Using the same assumptions and a cross-sectional SD of 23 nmol/L, we will have 80% power to detect a change of 22% in FFA nadir (**Figure 4c**), which is conservative given data in adults showing a decrease of 31% with liraglutide[67].

Analysis plan: Analyses for the primary outcomes of change in DNL and FFA nadir will be the same as Aim 1, as will the analyses for secondary measures of DNL (% suppression of triglyceride) and secondary outcomes (2-hour OSTT glucose, % TCA cycling, peripheral IR per OMM, metabolomics markers). From the study day 1 cross sectional data, we will use regression models to evaluate the relationships between markers of hepatic disease (IHTG and PDE/ATP) and DNL, excess substrate (FFA nadir, mean OSTT glucose) and measures of TCA anapleurotic cycling (% indirect glucose). **Table 7** provides details on the power calculations for these models of primary interest, using estimates of variability obtained from the PI's pilot data. If the relationship between % TCA cycling and IHTG is significant, we will use mediation modeling to test if this effect is mediated by DNL. The distribution-of-the-product method will be used to construct 95% confidence intervals for the mediated effects, using the RMediation software package[184]. After investigating associations between variables in the cross-sectional data from study day 1, we will evaluate if baseline characteristics predict changes in the study primary outcomes, in order to further understand the pathophysiology and to identify patients benefit from particular interventions.

Table 7: Power calculations for regression models

Outcome (SD)	Predictor (SD)	Slope detected with 80% power
IHTG (6%)	DNL (3.4%)	0.66
IHTG (6%)	FFA nadir (23 nmol/L)	1.44
IHTG (6%)	Mean OSTT glucose (23 mg/dL)	0.10
IHTG (6%)	% indirect glucose (5%)	0.45
PDE/ATP (0.12)	% indirect glucose (5%)	0.01

Summarize Knowledge to be Gained:

SA1 Expected Results

We anticipate that after GLP-1 RA, IHTG will decrease by at least 0.7% compared to the diet intervention. With the mean of 6.2±2.3% IHTG in PCOS girls, this would change the clinical classification of HS in many girls. These findings would substantiate the role of GLP-1 RA as a treatment to reduce HS in obese girls with PCOS and potentially mitigate long-terms health effects associated with HS. Alternatively, if both treatment groups had a reduction in IHTG, data could be utilized to best predict a favorable response to diet or GLP-1 RA.

SA2 Expected Results
We expect that DNL and FFA will relate to IHTG at baseline and that changes in IHTG will relate to changes in DNL and FFA suppression. Further DNL will relate to glucose excursions, rates of hepatic anaplerosis and ATP concentrations measured with ^{31}P MRS. These findings will provide mechanistic data relating to IHTG in PCOS and will contribute to the overall understanding of the unique pathology seen in PCOS. This information may lead to the development of alternate approaches to treat HS in PCOS or even NAFLD in simple obesity.

Confirmation of our anticipated outcomes would provide critical evidence to pursue larger clinical trials with a GLP-1 RA. Longer trials of GLP-1 in adult women with PCOS showed an increase in ovulatory cycles and decrease in testosterone[185]. Future trials could include a longer duration to better assess changes in clinical severity in a larger adolescent cohort. If the DNL contribution to IHTG is significant in this population, future trials could examine the efficacy of glucose lowering SGLT-2 medication in PCOS girls with dysglycemia.

SA3 Expected Results
We expect that connectivity between the homeostatic and non-homeostatic brain regions will be significantly greater after treatment with semaglutide compared to diet. Neurobiologically, eating behavior is driven by both homeostatic and non-homeostatic regions in the brain. Understanding the communication between homeostatic and non-homeostatic brain regions and their relationship to weight-loss interventions among adolescents with obesity and PCOS will help to elucidate specific neural mechanisms that contribute to overeating, and ultimately to weight regulation.

Potential Scientific Problems:

SA1 Limitations/Alternate Approaches
1) As with any clinical trial, recruitment and retention can be challenging. We have successfully recruited over 120 obese girls with untreated PCOS using identical enrollment criteria, and thus our recruitment goals are in line with our previous work. We have never needed to recruit in the community or other endocrinology practices but can if needed. Our overall lab has been successful with 3-month, 21-month and 3-year interventional protocols and we have designed the study with retention techniques that were successful in similarly obese adolescent populations. The PI is the only investigator recruiting from this patient population at UC Denver, and the majority of recruitment for our existing funding would be complete by the time the proposed project would start, thus we will not lose potential participants to other studies. **2)** Adherence to treatment is also a challenge, and we will use texting and emails as preferred contacts in youth to send supplemental reminders. **3)** Major changes to diet and exercise could alter results, thus we will request participants in the GLP-1 RA group to not to initiate major diet or exercise changes during the study. Those in the diet group will be asked to not change activity during the study period. As acute exercise effects measurements of IR, we will request no exercise in the 72 hours prior to each admission. **4)** Among the three current sets of guidelines for care of adolescents with PCOS, the consistent initial recommendation is for lifestyle intervention[8,34,126]. Thus, those girls in the dietary arm are receiving standard of care, whereas those in the medication arm will be receiving a medication with a clear potential for benefit. **5)** We acknowledge the fact that inducing weight loss in adolescents with diet is challenging and not sustainable in most youth long-term. However, we are experienced and have been successful in the short-term with our planned approach in an identical patient cohort and will be utilizing several additional strategies. We will also perform statistical analysis accounting for differences in weight loss, should they not be equal between the groups. Although this is not ideal, this will be achieved by correcting the analyses for differences in weight loss. **6)** There are several other long-acting GLP-1 RA available that may have a similar glucose lowering effect, but a greater effect on weight loss and higher side effects and lower tolerability[159]. We choose to use Rybelsus (semaglutide)due to a)being the first GLP-1 RA therapy available in oral tablets instead of injection b) our preliminary data in youth with PCOS c) a small degree of weight loss which is realistic to obtain in a dietary intervention group.

SA 2 Limitations/Alternate Approaches
1) The reproducibility of glucose measurement from an OSTT in any individual is only 75%, however, this is when the effects of exercise, diet, sleep and menstrual cycle are not controlled[149]. An alternative would be to provide the diet for participants for 3 days prior to studies, although this would then be a measure of the diet itself and not the participant's home intake. As the oligomenorrhea in PCOS best approximates the follicular phase in terms of estrogen and progesterone concentrations, we will attempt to perform studies in the follicular phase and confirm this

with a serum progesterone concentration (<2 ng/mL is consistent with the follicular phase). The OSTT results will remain valid if serum progesterone concentration is >2ng/mL on the day of the OSTT, but this will be disclosed in publications. We will record the presence or severity of sleep apnea and can adjust for typical sleep duration if needed. **2)** An alternate approach for assessing insulin sensitivity would be a hyperinsulinemic euglycemic clamp, for which we have extensive experience in pediatrics[9,10,150,153]. We have elected not to use this approach as IHTG is formed primarily in the fed state and a clamp bypasses the effects of GLP-1. We have also validated that the 4 hour OMM approximates clamp results well in our patient population. **3)** Certain genotypes have been associated with increased rates of NAFLD and DNL[58,186,187]. As we are calculating the change per individual, this affect should be minimized. Further, cost-efficient yet comprehensive panels for multiple relevant genes are not yet available. At the time of initial consent, we will seek permission for future genetic testing and save blood samples for such testing, should we deem this data necessary for interpretation of results and testing panels for multiple genes become more available.

SA3 Limitations/Alternate Approahces
1) One limitation is that we may not see any change with treatment. In addition, we will not have enough participants for a fully powered study, but this is a pilot study to inform potential future studies. There is no alternative approach. However, even if no change is observed with treatment, it would still be an important finding.

Scientific Rigor: Plan for robust and unbiased results
Additional OSTT studies in this patient population will replicate and validate our preliminary findings, providing redundancy. Modulating hepatic metabolism via nutritional and pharmacologic means will help with establishing cause and effect for excess DNL and IHTG. We are appropriately powered to answer our hypotheses and are using robust statistical analyses to test our hypotheses in an unbiased manner. The protocol includes a control group and participants are randomized to groups once pilot data is collected.

Consideration of relevant biological variables
PCOS occurs in women only, and the metabolic consequences have been understudied, especially relative to investigations of NAFLD in men. However, NAFLD appears to occur differently in Blacks compared to Hispanics and Whites[94,188]. While we are not fully powered to examine these differences, our baseline data can be utilized to explore racial/ethnic differences and generate preliminary data for future applications

19. DATA COLLECTION, RETENTION AND MONITORING

Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant treated with the study drug.

The Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator.

Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis.

Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

Availability and Retention of Investigational Records

The Investigator must make study data accessible to authorized representatives of the Sponsor (or designee), COMIRB and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each participant must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that participant. The Investigator must ensure the reliability and availability of source documents from which the information was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of two years after centers have been notified that the IND has been discontinued.

20. ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only, with the exception of HIPPA protected hospital and CTRC CORE laboratories which require patient identifiers. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996).

Protocol amendments cannot be implemented without prior written COMIRB, except where it may be necessary to eliminate an immediate hazard to a research participant. In such case, the deviation will be reported to COMIRB as soon as possible.

The protocol and consent form will be reviewed and approved by COMIRB prior to study initiation.

APPENDIX 1. SCHEDULE OF STUDY VISITS

Study Calendar	Visit 1 (Screening)	BASELINE METABOLIC STUDY		Visit 3 (Mid-Treatment)	POST-TREATMENT METABOLIC STUDY	
		Visit 2a (Imaging)	Visit 2b (Overnight)		Visit 4a (Imaging)	Visit 4b (Overnight)
Consent & Eligibility Assessment	X					
History & Physical	X			X		
Intravenous Blood Draw	X		X	X		X
Urine Pregnancy Test	X	X		X	X	
Gut Bacteria Collection			X			X
Accelerometer and sleep watch			7-days prior			7-days prior
DEXA Scan		X			X	
MRI of abdomen and liver		X			X	
P-MRS of Liver & rs-fMRI of Brain		X			X	
EndoPAT and Dynapulse		X			X	
WatchPAT sleep study			X			X
Oral Glycerol and deuterated water tracers			X			X
Oral Sugar Tolerance Test (OSTT)			X			X
Metabolic Cart			X			X
Questionnaires (Refer to appendix 3)			X	X		X
Randomization- GLP-1 (Rybelsus) vs Lifestyle intervention			X			
Hypoglycemia Questionnaire			X	X		X
Pregnancy prevention counseling/condoms	X		X	X		X
Total Time of Visit (approximately)	2 Hours	4.5 Hours	21 Hours	1.5 Hour	4.5 Hours	21 Hours

Timeline: The time between the initial screening visit and completion of the study will be less than 6 months. After confirmation of eligibility participants will complete the Baseline Metabolic Study (Visit 2a and 2b) and they will be randomized to GLP-1 (Rybelsus) or Lifestyle. Visit 3 will be completed approximately 2-months post baseline metabolic study. The Post-Treatment Metabolic Study (Visit 4a and 4b) will be completed after 4-months of GLP-1 or Lifestyle Intervention.

APPENDIX 2. SCREENING AND ORAL SUGAR TOLERANCE TEST (OSTT) LABS

Screening Labs	Purpose
HbA1C	Rule out type 2 diabetes, if > 6.4% participant to be excluded
ALT, AST, total bilirubin, alkaline phosphotase (Part of complete metabolic panel)	Ensure no severe liver disease, if >100 IU/L participant to be excluded normal participant is excluded
Hemoglobin & Hematocrit (part of CBC)	If participant is Anemic, they will be excluded
Sodium, Potassium, Creatinine and BUN (Part of complete metabolic panel)	To check for undetected renal dysfunction
Calcium and Albumin (Part of complete metabolic panel)	To screen for occult hypercalcemia potentially associated with MEN
Testosterone	Test for hyperandrogenism – required to meet NIH criteria for PCOS
1 sample for genetic analysis	Measure for common polymorphisms associated with hepatic steatosis[179,189]
Optional Screening PCOS labs PCOS status must be confirmed prior to enrollment. Referring physicians often do not perform the entire recommend work-up for oligomenorrhea (per 2013 Endocrine Society Clinical Guidelines for PCOS). The values are typically expected in PCOS publications	
TSH, total T4	Ensure no hypo or hyperthyroidism causing amenorrhea
LH, FSH	Rule out primary ovarian failure
17-hydroxyprogesterone	Rule out late onset congenital adrenal hyperplasia
DHEAS	Rule out adrenal tumor
Prolactin	Rule out prolactin secreting brain tumor
Mid-treatment	**Purpose**
Complete Metabolic Panel	This will include measures of transaminases, renal function and hypercalcemia.
HbA1c	
OSTT Labs	**Purpose**
Lipids and glycerol samples for tracer analysis	Determination of hepatic metabolism flux
Glucose	Determination of IR
Insulin	Determination IR
FFA	Measure of lipolysis
Glycerol	Measure of lipolysis
Glucagon	Gut hormone known to influence hepatic IR
GLP-1	Gut hormone known to influence hepatic IR
CRP	Marker of inflammation, know to effect IR
Leptin	Gut hormone known to influence hepatic IR
Adiponectin	Adipokine thought to influence adipose IR
Estradiol	Known to effect IR and HDNL
Progesterone	Demonstrate the participant is in the follicular phase of cycle, required for publication
Complete Metabolic Panel	This will include measures of transaminases, renal function and

	hypercalcemia.
Metabolomics	Correlation with hepatic metabolism flux
Stored blood	Future markers of glucose and fat metabolism, CVD or hormones related to PCOS
Apolipoprotein B	Measure of triglyceride secretion

APPENDIX 3. QUESTIONNAIRES BEING ADMINISTERED

Questionnaire	Description	Study Validity
Adolescent Sleep Hygiene Scale	Measurement of sleep patterns/habits	Cronbach's alpha ranges from .46-.74; total scale alpha = .80 LeBourgeois et al. 2005. *The Relationship Between Reported Sleep Quality and Sleep Hygiene in Italian and American Adolescents*
Center for Epidemiological Studies Depression (CES-D)	Measuring for depression	See Table 3 below, adapted from: Stockings et al. 2015. *Symptom screening scales for detecting major depressive disorder in children and adolescents: A systematic review and meta-analysis of reliability validity and diagnostic utility*
Cleveland Adolescent Sleepiness Questionnaire	Measurement of sleepiness during a typical week	alpha = 0.89 Spilsbury et al. 2007. *The Cleveland Adolescent Sleepiness Questionnaire: A New Measure to Assess Excessive Daytime Sleepiness in Adolescents*
SDQ: Strengths and Difficulties Questionnaire	Behavioral screening questionnaire	The internal reliability of the various self report scales was assessed using Cronbach's alpha coefficient. This was 0.82 for the total difficulties, 0.75 for emotional symptoms, 0.72 for conduct problems, 0.69 for hyperactivity, 0.65 for prosocial behaviour, and 0.61 for peer problems. Goodman et al. 2003. *The Strengths and Difficulties Questionnaire: a pilot study on the validity of the self-report version*
Sleep Disturbances Scale for Children	Gain understanding of sleep-wake rhythm and any problems in sleep behavior	Internal consistency ranged from .71-.79l test-retest reliability r = .71 Bruni et al. 1996. *The Sleep Disturbance Scale for Children (SDSC)Construct ion and validation of an instrument to evaluate sleep disturbances in childhood and adolescence*
3DPAR: Activities Scale	Measuring activity in the 3 days previous as a typical activity score	Interrater and test-retest reliability was 0.99 and 0.98, respectively ($P < 0.01$). The correlation between relative energy expenditure from the PDPAR (kcal.kg-1.l.d-1) and pedometer and Caltrac counts was 0.88 ($P < 0.01$) and 0.77 ($P < 0.01$), respectively. The correlation between percentage heart rate range (HRmax-HR-rest) and mean energy expenditure from the PDPAR was 0.53 ($P < 0.01$). The correlation between 1-min heart rates > 50% HRR sustained for 20 min and the number of 30-min blocks with a relative energy expenditure of at least four metabolic equivalent tasks (MET) was 0.63 ($P < 0.01$). The PDPAR provides valid and reliable estimates of physical activity and also accurately identifies bouts of moderate to vigorous activity. Weston et al. 1997 *Validation of an instrument for measurement of physical activity in youth*

Questionnaire	Description	Study Validity
Food Frequency Questionnaire	Measuring typical food intake over previous seven days.	The mean correlations, adjusted for measurement error, of food groups and nutrients between the FFQ and true usual intake were 0·41 and 0·38, respectively, with 57 % of food groups and 70 % of nutrients exhibiting correlations >0·35. Correlations were high for low-fat dairy (0·80), sugar-sweetened beverages (0·54), cholesterol (0·59) and saturated fat (0·51), while correlations were poor for high-fibre bread and cereal (0·16) and folate (0·11). Reliability of FFQ intake based on two FFQ administrations was also reasonable, with 54 % of Pearson correlation coefficients ≥0·5. Reliability was high for low-fat dairy (0·7), vegetables (0·6), carbohydrates, fibre, folate and vitamin C (all 0·5), but less than desirable for low-fat poultry and high-fibre bread, cereal, rice and pasta (0·2-0·3). Liese et al. 2015 *Relative validity and reliability of an FFQ in youth with type 1 diabetes* First described in 2006: Mayer-Davis et al. 2006. *Search FFQ Dietary Intake among Youth with Diabetes: The SEARCH for Diabetes in Youth Study*
Optional 7 Day Food Log	Optional record of 7-day diet to aid in food recall. Added per participant request to help fill out food frequency questionnaire.	N/A
Actigraphy Daily Sleep Diary	Recording bedtime/wake time during actigraphy. Needed to corroborate watch collected data	N/A
The Reward Based Eating Drive (RED) Scale	To assess the changes in reward eating behaviors	Epel, et al. (2014) The Reward-Based Eating Drive Scale: A Self-Report Index of Reward-Based Eating
Youth Quality of Life Instrument-Short Form (YQOL-SF)	Self-report measure of quality of life in youth	Validated in Adolescent quality of life, Part II:initial validation of a new instrument. *(Patrick, et al 2002)* Used in a diabetes prevention community in Latinx teens (Soltero et al. 2018) that used/reported on the short-form of the original measure
Treatment Self-Regulation Questionnaire (TSRQ	To capture the motivation for engaging in weight loss treatment	Mokhtari et al. (2017) Motivation and perceived competence for healthy eating and exercise among overweight/obese adolescents in comparison to normal weight adolescents
Three-Factor Eating Questionnaire	Measures of eating restraint, disinhibition, and hunger	Bryant et al. (2018) Development and validation of the Child Three-Factor Eating Questionnaire (CTFEQr17)

Child and Adolescent Social Support Scale for Healthy Behaviors	Survey designed to capture the role of family involvement in dietary habits	Cullum et al. (2015) A Review of the Child and Adolescent Social Support Scale for Healthy Behaviors
Adolescent Binge Eating Scale	Assessment of behaviors related to binge eating	Chamay-Weber et al. (2017) Screening Obese Adolescents for Binge Eating Disorder in Primary Care: The Adolescent Binge Eating Scale

Table 3
Validation evidence for the Center for Epidemiologic Studies Depression Scale (CES-D) in child and adolescent samples.

Source	*N*	Age and Gender (% m, % f)	Sample (location)	Scale name (no. of items)	Reliability	Criterion	Cutoff	Sensitivity	Specificity	PPV	NPV	AUC
Clinical												
Logsdon and Myers (2010)	59	13–18 (0, 100)	Adolescent mothers at 4–6 weeks postpartum (USA)	CES-D (20)	α=0.84	K-SADS-PL	16	0.7	0.52	0.25	0.12	0.62
Aebi et al. (2009)	140	Mean: 15.5 (33, 67)	Adolescents diagnosed with major depressive disorders (Switzerland)	CES-D (20)	α=0.83	Clinical interview	21	0.86	0.86	–	–	0.94
Non-clinical												
Betancourt et al. (2012)	367	10–17 (33, 67)	Children and adolescents (Rwanda)	CES-DC (20)	α=0.86	MINI-KID	≥ 30	0.82	0.72	–	–	0.83
Cuijpers et al. (2008)	1392	14–16 (52, 48)	Adolescents (Netherlands)	CES-D (20)	α=0.93	MINI	22	0.9	0.74	–	–	0.90
Thrane et al. (2004)	213	9–16 (54, 46)	Adolescents from three American Indian reservations (USA)	CES-D (20)	α=0.80	–	–	–	–	–	–	–
Yang et al. (2004)	2440	12–16 (52, 48)	Adolescents (Taiwan)	CES-D (20)	α=0.9	K-SADS-E	90th % tile	0.41	0.9	–	–	0.9
Prescott et al. (1998)	556	Mean: 16.8	Adolescent students from grades 9–12 (USA)	CES-D (20)	–	DISC	16	0.79	0.74	0.24	0.96	0.74
Garrison et al. (1991)	1231	12–14 (100, 0)	Child and adolescent boys from school sample (USA)	CES-D (20)	α=0.81	K-SADS-P	12	0.85	0.49	0.16	0.98	0.61
Garrison et al. (1991)	1234	12–14 (0, 100)	Child and adolescent girls from a school sample (USA)	CES-D (20)	α=0.86	K-SADS-P	22	0.83	0.77	0.32	0.98	0.77
Roberts et al. (1991)	1710	14–18 (47, 53)	Adolescents of nine senior high schools (USA)	CES-D (20)	α=0.89	K-SADS	24	0.84	0.75	0.08	0.99	–
Roberts et al. (1991)	804	14–18 (100,0)	Adolescent male sample of nine senior high schools (Roberts et al., 1991; USA)	CES-D (20)	–	K-SADS	22	–	–	–	–	0.87
Roberts et al. (1991)	906	14–18 (0,100)	Adolescent female enrolment of nine senior high schools (Roberts et al., 1991; USA)	CES-D (20)	–	K-SADS	24	–	–	–	–	0.83
Fendrich et al. (1990)	220	12–18	Children and adolescents at risk for depression according to their parents' diagnosis (USA)	CES-DC (20)	α=0.89	K-SADS-E	≥ 16	0.71	0.62	0.15	0.96	–

Note: *N*=Number of participants in the study sample. PPV=Positive predictive value. NPV=Negative predictive value. AUC=Area under the curve analysis. CES-D=Center for Epidemiologic Studies Depression Scale. CES-DC=Center for Epidemiologic Studies Depression Scale Child Version. α=Cronbach's alpha reliability co-efficient. K-SADS-PL: The Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime version. MINI-KID: The Mini-International Neuropsychiatric Interview. MINI: The Mini-International Neuropsychiatric Interview for children. K-SADS: The Schedule for Affective Disorders and Schizophrenia for School-Age Children. K-SADS-E: The Schedule for Affective Disorders and Schizophrenia for School-Age Children – Epidemiological version. DISC: The National Institute of Mental Health Diagnostic Interview Schedule for Children. K-SADS-P: The Schedule for Affective Disorders and Schizophrenia for School-Age Children Present Episode version.

APPENDIX 4. WATCHPAT SLEEP RESULTS FOLLOW-UP GUIDELINES

We anticipate that approximately 30-40% of our participants will have an abnormal apnea hyponea index (AHI), requiring some type of follow-up. We have worked with Drs. Ann Halbower, Stephen Hawkins and Ben Hughes, our primary pediatric sleep pulmonologists to develop a post-study follow-up algorithm. Of note, Dr. Hallbower is currently working with Kaiser to verify the accuracy of the results from the WatchPAT device as compared to inpatient polysomnograms in children younger than 12, and is very familiar with this device, it's output and limitations. Our youth fall into a grey zone in terms of what is an abnormal sleep study, as pediatric criteria are defined for less than 12, and adult for 18 or older. The international accepted clinical criteria are listed below, as well as the American Academy of Sleep Medicine's recommendations of how to handle age 12-17:

Pediatrics:

Mild OSA	Moderate OSA	Severe OSA
1 to 4.9	5 to 9.9	>10

Adults

Mild OSA	Moderate OSA	Severe OSA
5 to 14.9 + symptoms	15-30	>30

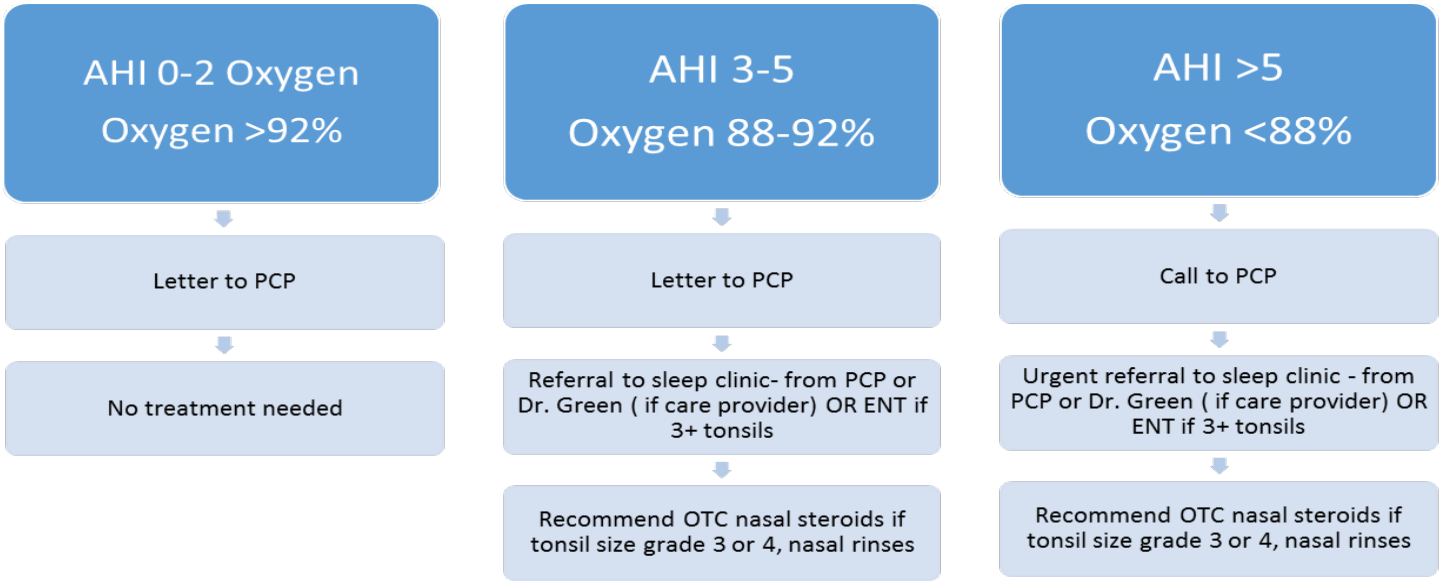
A. Ages for Which Pediatric Respiratory Scoring Rules Apply

1. Criteria for respiratory events during sleep for infants and children can be used for children <18 years, but an individual sleep specialist can choose to score children ≥13 years using adult criteria.[N1] RECOMMENDED

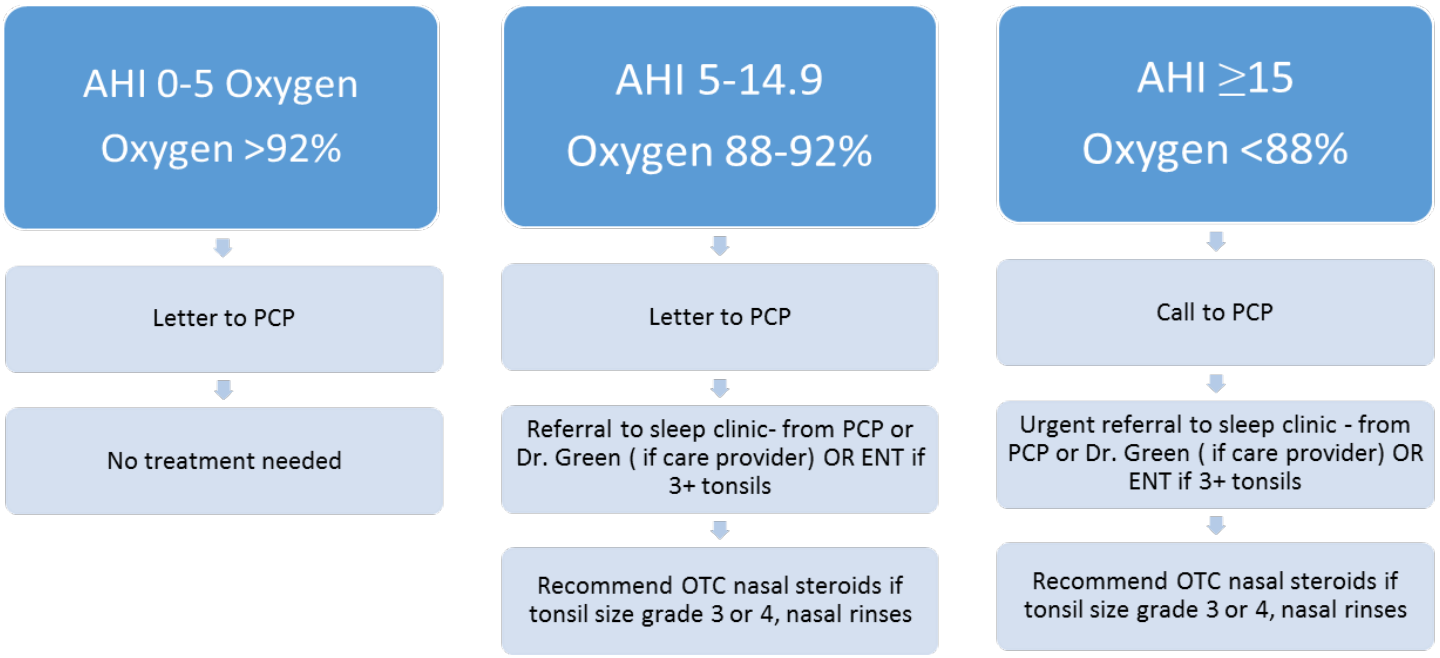
Note 1. Several studies suggest that the apnea hypopnea index (AHI) will be higher in adolescent patients when using pediatric compared to the adult rules presented in the 2007 version of the AASM scoring manual. As adult hypopnea rule 1A and pediatric hypopnea rules are similar, there may now be less difference in the AHI when using adult versus pediatric rules.

Since the recommendations are not concrete for the 12-17 year old range, we sought to follow what is being done in clinical practice at Children's Hospital Colorado sleep clinics, were a patient to have an inpatient polysomnogram. Sleep studies are read within 5 business days, and we will adhere to this same turnaround timeline. In terms of interpretation, currently, the pediatric guidelines are being applied for the 12-17 year old age group but 2 is considered normal in this age, and thus our post-study for the 12-17 year olds will follow this, and are shown below. Approximately half of the participants have a patient relationship with the PI Dr. Green, and thus she can order F/U evaluation if needed, and if not, Dr. Green will request the follow-up be arranged by the primary care provider. We have 2 algorithms by age.

For participants 12-17 years of age:



For participants ≥18 years of age:



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