1. Protocol Title: Adolescent Bariatrics: Assessing Health Benefits and Risks
Short Title: Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS)

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2. Executive Summary

Bariatric surgery is currently the most effective method for achieving significant sustained weight reduction for those with severe obesity. Evidence from adult studies demonstrates strong treatment effects on weight and comorbid conditions, as documented by several high-quality randomized controlled trials and large observational studies(45). However, most of the quality evidence of effectiveness of surgery in adults has only short (1 year) to mid-term (3 year) follow-up, and very few adult data are available for the most commonly used bariatric procedure today, vertical sleeve gastrectomy (SG). For adolescents, outcome data for Roux-en-Y gastric bypass (RYGB) and SG remain exceedingly sparse, biased, and thus of limited value due to retrospective study designs, small sample sizes, non-standardized research methodology, missing data, poor collection of data on comorbidity change, and short duration of follow-up(54, 5). Due to the paucity of quality outcome studies and virtually no long term safety and efficacy data, the Teen-Longitudinal Assessment of Bariatric Surgery (Teen-LABS, or TL) study was designed specifically to fill this knowledge gap.

Teen-LABS is a longitudinal observational study of the safety and efficacy of bariatric surgery performed during adolescence. Patients who were considered eligible for inclusion in the current surgical study population were adolescents undergoing any weight loss surgical procedure at one of five participating clinical centers during the original 2007-2011 enrollment period. This approach was chosen to minimize bias and maximize the resemblance of the study population to real-life clinical populations undergoing modern-day bariatric procedures.

Between 2007 and 2012, TL recruited 242 adolescents undergoing RYGB (n=165), SG (n=67), and adjustable gastric band (n=14) procedures. The cohort is the largest and most thoroughly characterized sample of its kind and is uniquely positioned to answer questions about long term safety and efficacy of the interventions for the following reasons:

- Nearly the entire cohort has passed their 4 year post-operative anniversary
- Excellent long term retention - 97% of the cohort remain active participants
- Superior performance with annual visit completion at 90% overall
- Rigorous measures at in-person visits collecting scale weights and biochemical data
- Most of the cohort now have aged into adulthood, and are cooperative and enthusiastic about continuing study participation

While initial weight reduction occurs in an early postoperative window of time, the effects of the surgery, both beneficial and adverse, are ongoing. This has important implications for use of surgery in youth who will therefore be “exposed” to the effects of bariatric operations for many more decades to come, compared to middle-aged adults undergoing surgery. Continued follow-up of this cohort will answer important questions about the long-term outcome of the procedures being used today for weight loss, the durability of weight loss following these procedures and the effectiveness of weight loss surgery in reducing co-morbid disease. Long-term adverse effects of these procedures in adolescents are hypothesized and some may be entirely unknown.

In TL funding cycle 1 (2006-2011) and cycle 2 (2012-2016) study staff conducted baseline and longitudinal studies of the cohort (average length of follow-up is 6.9 years, ranging from 4.7 to 9.6 years). The TL study group (including ancillary studies) has published 33 manuscripts in peer-reviewed journals and has an additional 17 currently in preparation. In this current project period (since 2011) 28 manuscripts have been published. In the publications thus far, the study group has 1) described characteristics and challenges of the clinical programs that surgically treat severely obese adolescent at consortium sites, 2)

examined long-term effects of adolescent obesity, 3) investigated perioperative surgical safety, 4) assessed cardiometabolic risk factors, abnormal kidney function, fatty liver disease, musculoskeletal pain in severely obese adolescents, adherence to micronutrient recommendations, and the Impact of comorbid conditions on quality of life, Importantly, in 2016 the group published the first comprehensive three year outcome paper demonstrating weight loss, comorbidity outcomes, and complications.

The purpose of the cycle 3 funding (2016-2022) is to conduct long-term follow-up (8-15 years) of the cohort during which time the original aims of the study will be accomplished. These original aims including a broad assessment of outcomes of surgical treatment, including but not limited to physical, metabolic, and psychosocial health changes, analysis of nutritional and other risks over time, and collection of biospecimens (plasma, serum, urine, and genetic material). In addition, in cycle 3, we have exciting opportunities to address several additional aims, including 1) recruitment of additional participants who underwent sleeve gastrectomy to adequately power comparisons of benefits and risks of this newer procedure to gastric bypass, 2) address critical concerns about bone health in young adults who underwent bariatric surgery as adolescents, 3) obtain pilot data on post-operative cognitive function.

In summary, following the TL cohort into adulthood with high rates of cohort retention and data completeness presents us with a very unique opportunity to credibly examine durability of benefits and, due to the high quality pre- and postoperative phenotyping, to elucidate factors associated with beneficial outcomes. We are also presented with an unprecedented opportunity to rigorously test hypotheses related to long term safety of these procedures, and discover potential late adverse effects of the surgery on critical physiologic processes and body systems.

3. Purpose of the Study

3.A. Study history and context:

Severe obesity remains one of the most significant public health issues in pediatric medicine. Approximately 4.4 million children and adolescents in the U.S. are severely obese(51). The plateau in pediatric obesity prevalence(41) has not occurred for children with severe obesity (BMI ≥120% of the 95th percentile). In fact, the prevalence of severe pediatric obesity has increased(51). Severe pediatric obesity has numerous and serious health consequences (28), and the long-term prognosis is poor for the majority of these youth--approximately 90% will become severely obese adults(18). Most concerning is the fact that once severe pediatric obesity is present, virtually all non-operative treatment approaches--including intensive inpatient therapy(56) and pharmacologic means are ineffective for reversing the condition(13).

To facilitate and accelerate research in Bariatric Surgery, the National Institute of Diabetes & Digestive, and Kidney Diseases (NIDDK) established the Longitudinal Assessment of Bariatric Surgery consortium (LABS). The LABS research project focused only on adults undergoing bariatric surgery. However, an ancillary studies mechanism was also established to support other related research projects. Teen-LABS is one such related project. The primary goal of this observational Teen-LABS study is to characterize the safety and efficacy of bariatric surgery performed in adolescence. This study will provide critical scientific information to inform clinical decision-making regarding appropriate timing of bariatric surgery.

As described in the (now archived) original TL study protocol, data collection in TL was patterned after LABS to enable direct comparisons of adolescent and adult bariatric outcomes up to 2 years following surgery. These analyses are underway. The LABS study closed in June of 2016 after collection of 5 year data. TL will complete 5 year data collection in late 2017 to inform comparisons between cohorts as described below.

In the 2016-2022 cycle 3 period, TL will also obtain 10 year outcome data on the entire initial cohort of 242 adolescents. This revised protocol for the cycle 3 of TL funding will thus specify the long term clinical outcome data collection that represents key longitudinal data that will tell an important story over time since the measures have been collected from baseline and annually in the entire cohort. In addition, in cycle 3, new measures will address several new topics of interest. The aims and research plan in this protocol have been revised to synchronize directly with the plans that will be executed in cycle 3, and the reader is referred to the archived earlier version of the protocol for details regarding specific research goals and methods for the prior funding cycles.

3.B. Specific Aims:

Aim 1:
Assessment of mid-term (5 year) outcomes of adolescent compared to adult bariatric surgery, assessment of long-term (10 year) outcomes of adolescents who underwent bariatric surgery, and assessment of differences in outcome for RYGB and SG procedures performed in adolescence.

> Hypothesis 1.1: Teen-LABS participants will experience a higher comorbidity remission and lower comorbidity relapse rate at 5 years compared to adults enrolled in the LABS-2 study.

Hypothesis 1.2: Adolescents undergoing bariatric surgery will demonstrate durable improvements in BMI and cardiovascular status over long-term (10 year) follow-up.

Hypothesis 1.3: Compared to adolescents who underwent SG, those treated with RYGB will experience a greater reduction in BMI and greater cardiovascular risk factor reduction (increase in HDL cholesterol, decrease in triglycerides and blood pressure) over long-term follow-up.

Hypothesis 1.4: Compared to adolescents who underwent SG, those treated with RYGB will experience a greater rate of decline in folate, 25-OH vitamin D, and transferrin levels over long-term follow-up.

Hypothesis 1.5: Hospital admissions and additional surgical/ interventional procedures will continue to occur but technical (surgical) complications related to the original bariatric procedure will decline over time.

Hypothesis 1.6: Improvement in depressive symptoms and weight-related quality of life will be greater in RYGB compared to SG participants over long-term follow-up.

Aim 2:
To assess long term safety by measurement of bone mineral density in the surgical cohort, with comparisons to similarly obese non-surgical control participants Hypothesis 2.1: We hypothesize that bone mineral density and content will be lower in RYGB and SG participants compared to BMI-similar, non-surgical controls.

Exploratory Aim 3:
To evaluate cognitive functioning and behavioral constructs during the first decade following adolescent bariatric surgery. In particular, cognitive function, cognitive control, affect regulation, reward processing, risk taking behaviors (including problems with alcohol), and problematic eating behaviors will be assessed.

Exploratory Hypothesis: Impaired cognitive control, affect regulation dysfunction, and impaired reward processing will impact negatively on weight outcomes and increase the risk for problematic eating behaviors (e.g., loss of control eating) and alcohol problems. Interactions by procedure type will be assessed.

Aim 4:
To obtain and store biospecimens (serum, plasma, and whole blood) from adolescents for research related to the aims of this study, and for future use by this consortium and ancillary studies for research into the pathophysiology of obesity and obesity related health problems.

3.C. Type of Study: Prospective observational cohort study

3.D. Sample Size

Originally, 242 adolescents were enrolled in TL during the first two funding cycles. During cycle 3 of funding (2016-2022) another wave of 38 individuals who underwent sleeve gastrectomy as adolescents will be recruited to enable meaningful comparison of key outcomes of this newer sleeve gastrectomy procedure with those who underwent the gastric bypass procedure. Finally, an additional 100 individuals who are similar demographically and have a similar BMI to TL participants but who have not undergone weight loss surgery will be recruited from the community to serve as controls for bone health studies.

3.E. Duration of Follow-up

- In the initial 5 year funding period for this project, the entire sample size was enrolled.
- The second 5 years of funding provided at least 3 years of follow up for the entire sample.
- The third 5 year cycle will permit 10 year follow-up of all of the original 242 participants (and up to 15 years follow-up for the earliest enrollees), and 8 to 10 year follow-up on n=38 additional sleeve gastrectomy participants that will be recruited in 2017-2018 described below in Aim 1c.
- Participants who enrolled in the earliest years of the study will also participate in cohort maintenance activities beyond their 10 years of complete study data collection. Continued retention measures (described below in section 5.G. and minimal self-report data collection will occur by way of telephone (or electronic) communication for participants who are 11-15 years postoperative during the 2016-2022 funding period.

3.F. Main Outcomes

- Clinical, metabolic, and psychosocial outcomes
- Bone mineral density and mineral content
- Key healthcare utilization events and death

3.G. Data Collection Schedule

- Pre-operative data is collected within 30 days prior to bariatric surgery.
- Follow-up assessments will occur immediately following hospital discharge from index operation, then at approximately 30 days, 6 months, and annually following date of bariatric surgery.
- Cohort maintenance (participant retention) activities will occur between annual visits

4. Significance of Study in Relation to Human Health

4.A. Overview. Obesity has become one of the leading health concerns among adults (39) and adolescents (40) in the United States. The traditional approach to weight loss consisting of diet, exercise, and medication generally achieves no more than a 5-10% reduction in body weight (65, 36, 4). Weight regain to or above baseline after such weight loss occurs in more than 90% of people undergoing non-operative therapy for both adult (63, 49) and adolescent (31, 47) age groups.

Extreme obesity has dramatically increased in prevalence over the past several decades, now affecting almost 5% of the US adult population and 4% of teenagers. Bariatric surgical procedures, which restrict stomach size or lead to decreased absorption of nutrients, are used to treat extreme or class 3 obesity (BMI ≥40 kg/m^2). These procedures often result in substantial weight loss and can have a dramatic effect on co-morbidities associated with obesity, such as improved control of blood sugar or even reversal of type 2 diabetes and obstructive sleep apnea (7). However, bariatric surgical procedures also carry substantial risks, including death.

Although an increasing number of people with extreme obesity and obesity-related complications are undergoing bariatric surgical procedures, there has been little systematic research to help determine the risks and benefits of bariatric surgery, or to provide guidance on appropriate patient selection. Of the several different types of bariatric procedures performed in the U.S., the Roux-en-Y gastric bypass (RYGB) (33, 22, 32) is the most commonly performed. The restrictive adjustable gastric band is increasing in use in the US and is the leading procedure performed outside of the U.S. (11, 15). The biliopancreatic diversion with or without the duodenal switch has also grown in use but is performed for adults by a smaller number of practitioners, and is generally not offered to adolescents.

Finally, the sleeve gastrectomy is also considered a surgical option for weight loss (38), and may be suitable for some adolescents.

Increases in bariatric procedure volume over the last decade has been truly remarkable with over 120,000 procedures performed in 2003 compared to less than 20,000 performed in 1993 (43, 24). Adolescent volumes have also increased recently. Over the years 1996-2003, Tsai estimated that over 3,000 bariatric procedures were done for adolescents, with 200 procedures per year between 1996 and 2000. From 2000 to 2003 however, a 3-fold increase in utilization of weight loss procedures (90% gastric bypass) occurred in adolescents (55). This growth may be related to the reported efficacy of these procedures, the availability of less-invasive laparoscopic procedures, increases in the pool of surgical candidates, increased media exposure of celebrity subjects who had successful bariatric procedures Johnson (24), and the identification of extreme obesity as a life-threatening disease (8, 16).

4.B. Teen-LABS study design and cohort characteristics

Teen-LABS is a prospective, longitudinal, observational study of the safety and efficacy of bariatric surgery performed during adolescence. Patients who were considered eligible for inclusion in the current surgical study population were adolescents undergoing any weight loss surgical procedure at one of five participating clinical centers during the original 2007-2011 enrollment period. This approach was chosen to minimize bias and maximize the resemblance of the study population to real-life clinical populations undergoing modern-day bariatric procedures.

Original Inclusion Criteria

- Individuals ≤19 years of age, and who are approved to undergo bariatric surgery by a Teen-LABS certified surgeon at one of the Teen-LABS clinical sites
- Primary caregivers of adolescent participants (for their weight, height, and demographic variables only).

Original Exclusion Criteria

- Informed assent/consent not obtained from the adolescent or, if subject < 18 years of age, informed consent not obtained from the adolescent's legally authorized representative
- Subject unable to communicate with local study staff

By February of 2012, all 242 TL participants had been enrolled and had undergone a primary bariatric operation as indicated in Table 1 below. Since the time of original enrollment, 232 are still active participants, as shown in the second column of Table 1.

Table 1: Teen-LABS participant characteristics at original and current cohorts

Characteristic	Original Cohort 242	Current Cohort† 232
Clinical Site, n (%)		
Cincinnati	94 (38.8%)	90 (38.8%)
Houston	59 (24.4%)	56 (24.1%)
Columbus	37 (15.3%)	36 (15.5%)
Pittsburgh	34 (14.1%)	32 (13.8%)
Birmingham	18 (7.4%)	18 (7.8%)
Surgical Procedure, n (%)		
Gastric Bypass	161 (66.5%)	153 (66.0%)
Adjustable Band	14 (5.8%)	14 (6.0%)
Sleeve Gastrectomy	67 (27.7%)	65 (28.0%)
Age at Surgery / Current Age (years)		
Median (min, max)	17.3 (13.3, 19.9)	23.1 (17.3, 27.7)
Sex, n (%)		
Male	59 (24.4%)	55 (23.7%)
Female	183 (75.6%)	177 (76.3%)
Race, n (%)		
White	174 (71.9%)	165 (71.1%)
Black	54 (22.3%)	54 (23.3%)
Other	14 (5.8%)	13 (5.6%)
Ethnicity, n (%)		
Non-Hispanic	225 (93.0%)	215 (92.7%)
Hispanic	17 (7.0%)	16 (6.9%)
Unknown	1 (0.4%)	1 (0.4%)

† Considering study withdrawal (n=6) and deaths (n=4)

Since the primary aim of TL has been to define efficacy and safety of bariatric surgery performed in adolescence, TL participants were extensively phenotyped at baseline and at each longitudinal follow-up visit (Table 2).

Table 2: Data collection	Baseline	6mo and annually
Baseline demographics	X	
Brief health status update, new contact information	X	x
Anthropometrics, % body fat	X	x
Blood pressure (in triplicate)	X	x
Central laboratory testing (see Table 4)	X	x
Medication use	X	x
Comorbidity Assessment	X	x
Healthcare utilization	X	x
Work/School history	X	x
Socioeconomic history	X	x
Behavioral assessment (comprehensive)	X	x
SF36 Health Survey	X	x
Beck Depression Inventory – II	X	x
Psychiatric and Emotional Survey	X	x
Impact of Weight on Quality of Life (IWQOL)	X	x
Gastrointestinal Symptoms Rating Scale	X	x
Urinary incontinence questionnaire	X	x
Berlin Sleep questionnaire	X	x
Excess Skin Survey		5yr only
International Physical Activity Questionnaire	X	x
400 Meter Walk Data Collection Form	X	x
Reproductive Health Pregnancy Questionnaire	X	x

Cohort maintenance, data quality, and study visit completion has been a clear focus of our group since enrollment began. Follow-up of TL participants as they transition into adulthood represents numerous visit completion challenges (e.g., competing demands for time, post-secondary education, complex lives outside of parental home). Standard cohort maintenance methodology collectively called the TL retention “tool-box” has been used with success. The elements of this toolbox include collection and cataloging of contact information (e.g., addresses, phone numbers, email addresses, social media usage) from the adolescent, caregiver, and a secondary contact person who is judged as likely to always know the whereabouts of the participant. Teen-LABS also utilizes frequent mailings (e.g., birthday cards, holiday cards, and quarterly TL newsletters). Adolescents also receive a “report card,” which provides individualized information on their post-surgical progress on key variables of interest (e.g., weight and body fat change over time). To inform participants of their blood pressure at the time of their visit, a letter will be provided for follow-up as needed.

Other retention strategies include a mid-year retention phone call, reimbursement for travel and lodging to participants living 150 or more miles from their TL site to attend their follow-up assessments, and home-based “field visits” for those participants unwilling/unable to return to a TL site. Home visits are conducted by Examination Management Services, Incorporated (EMSI; Dallas, TX). After appropriate training on the TL study protocol, and in collaboration

with each TL Site Coordinator, EMSI paramedical professionals complete TL annual assessments, including obtaining blood and biospecimens, as well as anthropometrics and blood pressure.

For participants unable to return to the TL Clinical Center or undergo a field visit, relationships with Quest Diagnostics® and The Little Clinic® have now been established to perform weight and blood pressure measurements and laboratory testing, greatly expanding capacity to avoid missed study visits. When TL participants can go to a Quest location, routine clinical laboratory testing can be conducted. This has helped with retention to meet our participants "where they are" and minimize the need for taking off work to attend a study visit.

When participants cannot be located using last known contact information, the Central Study Coordinator is also authorized to trace participants utilizing national information sources with this information then shared back to their original Clinical Center to facilitate scheduling study visits. Finally, the Central Retention Coordinator is responsible for executing a query of the National Death Index (National Center for Health Statistics) for any participant who cannot be located.

5. Research Plan for Cycle 3 of Teen-LABS

5.A. Overview.

Over time, surgery and weight loss have resulted in changes in the TL cohort. Currently, most TL participants are in their early twenties, a transition point in their lives (Table 3). Following the TL cohort into adulthood with exceptional rates of cohort retention and data completeness presents us with a very unique opportunity to credibly examine durability of benefits and, due to the high quality pre- and postoperative phenotyping, to elucidate factors associated with beneficial outcomes. We are also presented with an unprecedented opportunity to rigorously test hypotheses related to long term safety of these procedures, and discover potential late adverse effects of the surgery on critical physiologic processes and body systems, including bone health. This information will shape decisions about the timing of surgery and selection of procedures for many decades to come.

Table 3: Study timeline and participant aging

	1st period	2nd Funding Period					3rd Funding Period					
Year:	2007-2011	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Goal:	242 enrolled	Surgery complete	1 yr f/u	2yr f/u	3yr f/u	4yr f/u	5yr f/u	6 yr f/u	7yr f/u	8yr f/u	9yr f/u	10yr f/u
Avg age	17	18	19	20	21	22	23	24	25	26	27	28

5.B. Aim 1: a) Assessment of mid-term (5 year) outcomes of adolescent compared to adult bariatric surgery, b) assessment of long-term (10 year) outcomes of adolescent bariatric surgery, and c) assessment of differences in outcome for RYGB and SG procedures performed in adolescence.

5.B.1. Justification and research plans related to Aim 1a: Efficacy of adolescent RYGB compared to adult RYGB at 5 years. Originally, TL was designed to address questions about appropriate timing of the use of RYGB for treatment of severe obesity, comparing outcomes in adolescents to the adults enrolled in the LABS-2 study at 2 years postoperatively. These analyses are underway, and we have now obtained data from LABS-

2 and examined T2DM remission in TL adolescents (mean age=17) and adults (mean age=38) with a history of obesity at age 18 following RYGB treatment. Of the 23 adolescents and 120 adults with T2DM at baseline, median BMI decreased similarly by 30% and 32% respectively 2 years after surgery. T2DM remission (HbA1c<6.5% on no T2DM medications) was seen in 88% of adolescents and 69% of adults at 2 years (p=0.13). While not reaching significance, these trends suggest the validity of our original hypothesis: that the use of RYGB in adolescence will be associated with greater health benefits than the same treatment performed in adults. Additional analyses at 5 years follow-up will be critically important for determining durability of remission response not only for T2DM, but will also include other major comorbidities for which data collection methodology for the two studies was synchronized: hypertension, dyslipidemia, and abnormal renal function. In addition, published 5 year T2DM relapse rates for adults are as high as 50%(3); we hypothesize relapse frequency will be lower in adolescents. Since LABS-2 has now completed their long-term 5 year data collection, and all TL participants will complete 5 year follow-up by 2017, these long-term comparisons between adolescents and adults will be conducted in the extension period.

5.B.2. Research methods for Aim 1a:

Both LABS and TL have conducted clinical assessments and detailed interviews and questionnaires pre-operatively and at annual post-operative time points (Table 2) to assess risks of surgery, and changes in clinical, metabolic, and psychosocial characteristics following bariatric surgery. Detailed data about the surgical procedure and peri- and post-operative care is also collected to determine if components of the surgical procedure, and peri-operative and post-operative care as well as clinical/demographic patient characteristics are associated with post-operative risks and changes in patient status. Adult participants with history of obesity at age 18 years will be selected from LABS participants and their de-identified data from baseline to year 5 of follow up will be obtained from the LABS Data Coordinating Center. This will afford the opportunity for to understand broad ranging outcomes of bariatric surgery using duration of obesity as the moderating variable. Such an analysis will permit a realistic estimate of risks and benefits of use of bariatric surgery in adolescent years.

- TL data coordinating center (DCC) will obtain a de-identified dataset containing 5 year outcomes from LABS-2 participants.
- TL will compare and contrast anthropometric, clinical, and biochemical characteristics of the two cohorts, enabling a robust and longer term testing of our original hypotheses regarding the relative merits of bariatric treatment early (adolescents) or later (adults) in life.
- Biochemical characteristics will include the following measures (Table 4).

Table 4: Laboratory data collected in LABS and Teen-LABS

Laboratory Tests	Adult LABS	Adolescent TL
vitamin A	Teen-LABS*	Teen-LABS
parathyroid hormone (PTH)	Teen-LABS	Teen-LABS
25-OH vitamin D	Teen-LABS	Teen-LABS
Ferritin	Teen-LABS	Teen-LABS
Vitamin B1	Not drawn	Teen-LABS
vitamin B12	Teen-LABS	Teen-LABS
hs – CRP	LABS-2**	Teen-LABS
Glucose	Teen-LABS	Teen-LABS
Albumin (serum)	Teen-LABS	Teen-LABS
Insulin	LABS-2	Teen-LABS
Folate	Teen-LABS	Teen-LABS
Calcium	Teen-LABS	Teen-LABS
Lipids (TG, TC, HDL, LDL)	LABS-2	Teen-LABS
HbA1c	LABS-2	Teen-LABS
Creatinine	LABS-2	Teen-LABS
Cystatin C	LABS-2	Teen-LABS
Transferrin	Teen-LABS	Teen-LABS
Iron	Local Clinical***	Local Clinical
AST	Local Clinical	Local Clinical
ALT	Local Clinical	Local Clinical
Hemoglobin	Local Clinical	Local Clinical
total bilirubin	Local Clinical	Local Clinical
Alkaline phosphatase	Local Clinical	Local Clinical
Platelet	Local Clinical	Local Clinical
total lymphocyte count	Local Clinical	Local Clinical
urine albumin	LABS-2	Teen-LABS
urine creatinine	LABS-2	Teen-LABS
Complete Blood Count (CBC)*		Teen-LABS

* *Indicates that this assay was performed by the Teen-LABS study on saved serum from the adult LABS participants
** Indicates that this assay was performed by the LABS study on serum from the adult LABS participants
*** Indicates that these data were obtained clinically at the site where clinical care was delivered, and the variable was abstracted from chart review

5.B.3. Justification and research plans related to Aim 1b: Long-term (10 year) outcome of surgery for the original 242 TL participants.

The comprehensive, longitudinal phenotyping of participants already done in the study has allowed the examination of mid-term health and safety outcomes. These assessments have included extensive anthropometric, medical, surgical, and behavioral measures. Combining these longitudinal data collected in funding cycles 1 and 2 of the project with the long-term data from cycle 3 (which will include follow-up to year 10) will allow investigators to go well beyond descriptive reports and explore risk factors, associations, and postulate mechanisms of changes in weight and diseases in this important and understudied area. Importantly, the study is poised to also now document the long term durability of 1) anthropometric changes, 2) remission of comorbidities (including relapse of comorbid conditions), 3) quality of life, 4) depressive symptoms and 5) detect new onset (incident) nutritional deficiencies, pregnancies, abdominal re-operations, and death following adolescent bariatric surgery. An overarching aim of the TL study since inception has been the ability to collect and report adverse effects and event data. This safety assessment primarily takes the form of nutritional safety and unplanned healthcare utilization events. Nutrition assessment within TL is largely confined to micronutrient status. Bariatric surgery, by design, reduces

nutritional intake, and anatomic features of RYGB and SG can predispose to specific micronutrient deficits with adverse effects on neurological, cardiovascular, epithelial, and hematological processes. Many problems will only manifest as true deficiency syndromes that cause disease many years after surgery. In light of the concerning trends in several micronutrients monitored in the TL study(23), we hypothesize that long term, adolescents who underwent RYGB will experience a greater rate of decline in folate, 25-OH vitamin D, and transferrin levels compared to those who underwent SG. To test this hypothesis, the study will continue to longitudinally monitor serum levels of micronutrients (Table 4). Analyses exploring predictors of deficiency will take into consideration important covariates including supplement adherence in each cohort, as well as use of contraception that may suppress menstruation which in turn may reduce iron loss in females. Healthcare utilization events linked to micronutrient deficiency (e.g., transfusions or infusion therapy), as well as pregnancy in females, which also affect micronutrient levels, will also be taken into account. Accomplishment of this aim should lead to evidence-based recommendations in the future so that patients may better avoid nutritional deficiencies. Collection and analysis of incident healthcare utilization events in the years following surgery are of considerable interest since they may represent late adverse effects of adolescent bariatric surgery. Objective and detailed adverse event data represents an enormous knowledge gap in the bariatric literature broadly. We believe that hospital admissions and additional surgical/intervention procedures will continue to occur in the surgical cohorts but technical (surgical) complications related to the original bariatric procedure will decline over time. We have demonstrated that our methodology for collection of such events and complications is robust(23). These efforts will continue going forward, including collection of important information about pregnancies and childbirth. These data will be of considerable interest to ancillary study investigators studying multigenerational effects of obesity or surgery. However, specifically relevant in the extension period will be the comparison of adverse events in RYGB and SG cohorts. RYGB and partial gastrectomy have been associated with excessive loss of muscle mass, metabolic bone disease and increased fracture risk, suicide, alcoholism, and accidental deaths(67, 61, 2). In addition, some patients experience such severe gastroesophageal reflux after SG that conversion to RYGB is needed, and one such conversion has already occurred in the TL cohort. Understanding more about such risks will inform families prospectively considering RYGB and SG in the future and will direct risk prevention, management, and minimization efforts.

5.B.4. Research methods for Aim 1b: To achieve our objectives, the approach we use for continuity of longitudinal, comprehensive data collection is of considerable importance and the core longitudinal data collection strategy from prior funding cycles (Table 2 above) will continue during cycle 3.

- Annual follow-up timed to coincide with anniversary of initial bariatric procedure.
- In the cycle 3 period, follow-up data will be collected using methodology which has been successful for us in the past.
- Full study visits in years 5, 6, 8, and 10 will be conducted either in-person at the participant's original clinical center, as an EMSI home research visit, or participant will be given the option of going to a local The Little Clinic or Quest for their visit. The main research assessments for these visits are shown in Table 5: comprehensive examination including physical measures, coordinator and clinician assessments, and a battery of self-report questionnaires including patient reported measures of QOL, depressive symptoms, weight management behaviors, and healthcare utilization will be obtained.
- Additional items will be queried as single items on a follow-up inventory annually in the 3rd cycle. The postoperative history or current state of the following conditions will be addressed:
    - Skeletal fractures

    - Treatment for specific nutritional deficiencies
    - Infusion therapy or blood transfusion for iron deficiency or anemia
    - Diagnosis of cancer, and if so, type, date of diagnosis, and treatment modality
- Blood and urine specimens sent to central reference laboratory and to central biospecimen repository for analysis and storage at full study visits (Table 6).
- In years 7, 9, and 11-15 full study visits will not be conducted. For those participants who pass through these time-points during the 3rd cycle, contact will be maintained and a brief health interview will be done by telephone. Interim contact as specified in retention plan between annual time-points will insure cohort maintenance, collect change of address, and health status updates.

Cohort maintenance and Retention: Appropriate assessment of safety and efficacy requires a high rate of participant retention during follow-up. During the initial study period, our retention has been strong, reflecting both our ability to generate appeal to participants for ongoing participation, as well as our developing a well-designed and responsive retention plan. We plan to continue our proven successful cohort maintenance and retention efforts (described in detail in section 5.G. below) in the cycle 3 period.

5.B.5. Justification and research plans related to Aim 1c: Assessment of efficacy and durability of sleeve gastrectomy compared to RYGB in TL participants.

Due to the substantial weight loss seen in adults after SG, and a number of potential advantages over RYGB and gastric banding, this operation has gained considerable popularity, surpassing the number of RYGB cases in some parts of the U.S. in 2012(46). All bariatric procedures have targeted the stomach but until recently, gastric resection has rarely been an element. SG is an irreversible procedure, removing 75-80% of the stomach leaving sleeve of residual stomach. As the procedure involves no intestinal diversion, it is simpler to perform, has less predictable micronutrient malabsorption, and reduces other risks such as marginal ulceration. Unlike RYGB, there are no potential anatomic areas for internal hernia and subsequent life-threatening bowel incarceration/strangulation, a definite consideration for an adolescent with another 60 to 70 years to live. There is a relative paucity of data about long-term outcomes, durability, or possible pathophysiologic and/or adverse effects of SG in adults and even less is known for adolescents. Preliminary evidence in adults suggests that this operation may have similar effects on weight loss and comorbidities(26, 50) with fewer nutritional concerns(53, 2, 19) compared to RYGB.

During the design of TL, the study was powered to examine outcomes of RYGB not SG. However, during enrollment, n=67 patients who underwent SG were enrolled in the study, providing an opportunity to examine some outcomes of this procedure when used in adolescence. Indeed, with the current sample of RYGB and SG cases, we have some intriguing preliminary 4yr data, including:

- Modestly greater rates of decline in BMI, triglycerides, blood pressure, and depressive symptoms in those who underwent RYGB as compared to SG.
- Modestly greater rates of increase in HDL cholesterol and quality of life in RYGB compared to SG.
- Trends for greater abnormality in PTH and vitamin A after RYGB compared to SG at 4 years.

Table 5: TL data collected (rows) at years (columns)*		5	6	7	8	9	10	11	12	13	14	15
	Surgeon/Coordinator Completed											
ANTH	Anthropometrics	x	X		x		x					
ANTH	Blood Pressure	x	x		x		x					
MWE	400 Meter Walk Eligibility Form	x	X		x		x					
MWF	400 Meter Walk Data Collection Form	x	X		x		x					
CAF	Comorbidity Assessment - Follow-up	x	X		x		x					
HC	Healthcare Utilization Form	x	X	x	x	x	x	x	x	x	x	x
CLAB FH	Central Lab - Fresh	x	X		x		x					
CLAB_FZ	Central Lab - Frozen	x	X		x		x					
BDI2S	Beck Depression Inventory Scoring & Action Plan	x	X		x		x					
SHORT	Short Form	x	X	x	x	x	x	x	x	x	x	x
AE	Adverse Events											
INF	Inactivation Form											
MRF	Mortality Report Form											
UPR	Unanticipated Problem Report											
ICT[#]	Informed Consent Tracking form											
	Patient Completed											
CI	Contact Information	x	X	x	x	x	x	x	x	x	x	x
CDI	Caregiver Demographic Information	x										
SWH	School and Work History	x	X		x		x					
BF	Behavior - Follow-up	x	X		x		x					
SF36	SF36 Health Survey	x	X		x		x					
BDI2	Beck Depression Inventory - II	x	X		x		x					
PETSF	Psychiatric and Emotional Test Survey - Follow-up	x	X		x		x					
IWQOL	Impact of Weight - (IWQOL -Lite)	x	X		x		x					
GSRS	Gastrointestinal Symptoms Rating Scale	x	X		x		x					
UIF	Urinary Incontinence - Follow-up	x	X		x		x					
BS	Berlin Sleep	x	X		x		x					
RHF	Reproductive Health - Follow-up	x	X		x		x					
MED	Medications	x	X		x		x					
IPAQ	International Physical Activity Questionnaire	x	X		x		x					
IPS	International Prevalence Study on Physical Activity	x	X		x		x					
FX	Fracture Survey	x	x		x		x					
ESS	Excess Skin Survey	x					x					
RHP	Reproductive Health Pregnancy Questionnaire	x	x		x		x					
UPPS-P	UPPS-P Behavior Scale						x					
SPSRQ	Sensitivity to Punishment and Sensitivity to Reward Questionnaire						x					
M-CQ	Monetary-Choice Questionnaire						x					
DERS	Difficulties in Emotion Regulation Scale						x					
ATQ-EC	Adult Temperament Questionnaire-Effortful Control						x					
SBQ	LABS SBQ						X^					

* **In years 7, 9, and 11-15, cohort maintenance and minimal data collection by annual phone call will be performed**

ICT forms will be completed and submitted whenever a new Informed Consent is obtained

^ **Or after, if not collected at year 10**

Table 5a: Summary of forms for bone component (DEXA scans)

	Surgery cases	Controls
Control subject screening		X
Surgery subject screening	X	
Consent		X
ANTH	X	X
Calcium & vitamin D intake	X	X
Fracture history	X	X
DXA performance	X	X
IPAC	X	X
Control subject medical & reproductive history		X
pQCT performance*	X	X

*Cincinnati only

Table 5b: Summary of Teleforms for newly enrolled VSG participants to be completed at study entry

EF	Enrollment Form
ANTH	Anthropometrics / Blood Pressure
MWE	400 Meter Walk Eligibility Form
MWF	400 Meter Walk Data Collection Form
CAF	Comorbidity Assessment - Follow-up
HC	Healthcare Utilization Form
CLAB FH	Central Lab – Fresh
CLAB_FZ	Central Lab – Frozen
NIH-DNA	NIDDK –shipment form
ICT	Informed Consent Tracking form
BDI2S	Beck Depression Inventory Scoring & Action Plan
SHORT	Short Form
AE	Adverse Events
INF	Inactivation Form
MRF	Mortality Report Form
UPR	Unanticipated Problem Report
	Patient Completed forms
CI	Contact Information
SWH	School and Work History
BF	Behavior - Follow-up
SF36	SF36 Health Survey
BDI2	Beck Depression Inventory – II
PETSF	Psychiatric and Emotional Test Survey - Follow-up
IWQOL	Impact of Weight - Kids (IWQOL -Lite yrs 6&8)
GSRS	Gastrointestinal Symptoms Rating Scale
UIF	Urinary Incontinence - Follow-up
BS	Berlin Sleep
RHF	Reproductive Health - Follow-up

MED	Medications
IPAQ	International Physical Activity Questionnaire
IPS	International Prevalence Study on Physical Activity
ESS	Excess Skin Survey
RHP	Reproductive Health Pregnancy Questionnaire

Table 6: Laboratory Testing	**Clinical Center and EMSI visits**	**Quest visits**
vitamin A	Central Lab	
parathyroid hormone (PTH)	Central Lab	
25-OH vitamin D	Central Lab	Quest
Ferritin	Central Lab	Quest
Vitamin B1	Central Lab	
vitamin B12	Central Lab	Quest
hs – CRP	Central Lab	
Glucose	Central Lab	Quest
Albumin (serum)	Central Lab	Quest
Insulin	Central Lab	
folate	Central Lab	Quest
Calcium	Central Lab	Quest
Lipids (TG, TC, HDL, LDL)	Central Lab	Quest
HbA1c	Central Lab	Quest
Creatinine	Central Lab	Quest
Cystatin C	Central Lab	
transferrin	Central Lab	
urine albumin	Central Lab	Quest
urine creatinine	Central Lab	Quest
Complete Blood Count (CBC)*	Central Lab	Quest
Iron	Local Clinical	
AST	Local Clinical	Quest
ALT	Local Clinical	Quest
Hemoglobin	Local Clinical	Quest
total bilirubin	Local Clinical	Quest
Alkaline phosphatase	Local Clinical	Quest
Platelet	Local Clinical	Quest
total lymphocyte count	Local Clinical	Quest

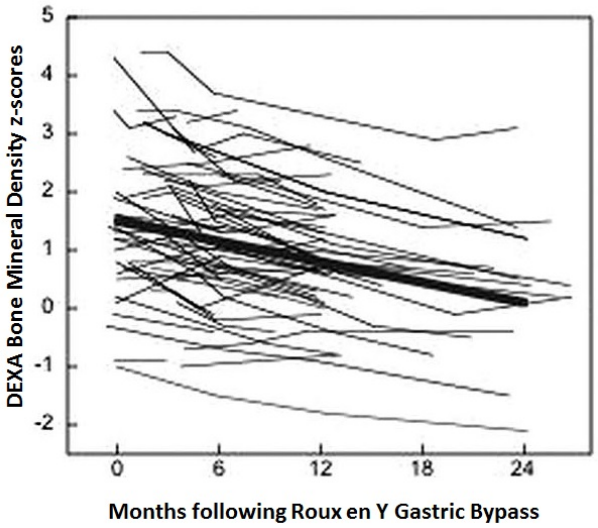
Our power analyses demonstrated that another 38 SG participants will be sufficient to formally test hypotheses comparing SG and RYGB subgroups (see statistical analysis plan, section 5.K.1). Consideration was given to new baseline recruitment of individuals undergoing operation in the extension period at study sites, but this approach was rejected as it would result in asynchronous groups within the cohort and an extended wait period before which late outcomes would be known for an additional 38 SG participants. To maximize the long-term (5-10 year) SG follow-up visits in the extension period, and to maintain uniformity with our consecutive enrollment methodology, we instead screened operative case logs from 2012-2013 at study sites. Sixty-four (n=30 in 2012 and n=34 in 2013) individuals who met original study inclusion/exclusion criteria were identified. These 64 have similar characteristics to SG participants already enrolled: mean age, 16.8 years; mean BMI 53.1 kg/m^2; 63% female.

5.B.6. Research methods for Aim 1c:
The original study cohort of 242 adolescent participants was recruited between 2007 and 2011. Enrollment of 38 additional SG participants will be achieved in 2017-2019 to permit adequately powered comparisons of important long term outcomes between SG and RYGB participants. Operative logs at Cincinnati Children's Hospital and Nationwide who performed SG in 2012 through 2015 will be screened to identify potential new SG participants. Cases identified will be sorted by date of SG surgery and these individuals will be consecutively recruited to participate. Recruitment phone calls and letters will be used, as well as recruitment using typical means by which the clinical team maintains contact with these individuals including routine clinic visits, as available. After enrollment, data that map to TL core baseline variables and early postoperative measures (weight/height, comorbidities, laboratory data, and surgical complications) at the time-points typically collected by TL (e.g., baseline, 30d, 6mo, and annual) will be abstracted from medical records. Major healthcare utilization events (operations, other procedures, or hospitalizations) during or following bariatric surgery will also be abstracted so that potential complication of surgery or of underlying medical conditions can be analyzed. An in-person research visit will then be conducted to collect a

complete annual visit research dataset, identical to the annual in-person full visits conducted for all other TL participants. In this manner, all 38 new enrollees will be eligible to contribute long-term outcomes data by 2018, and will be serially studied every year during this project period (2016-2022). In summary:

- Operative logs at the TL participating institutions will be screened for individuals who underwent SG surgery and meet the following criteria
    - ≤19 years of age at the time of primary SG bariatric operation in 2012-2015
    - Surgery performed by a Teen-LABS certified surgeon
    - No prior gastric or intestinal surgery prior to the index bariatric operation in 2012-2013
    - Able to communicate with study staff
- Those meeting criteria will be consecutively and sequentially recruited to build a cohort of n=33 using in-person at clinic visits, or phone calls and letters
- After informed written consent is obtained, data that map to TL baseline and early postoperative measures (only items from Table 5 above that can be collected retrospectively including weight/height, comorbidities, laboratory data, surgical complications) will be abstracted from medical records
- An in-person research visit will then be arranged to collect a complete annual visit dataset prospectively (Table 5).
- In this manner, all 38 new SG enrollees will be eligible to contribute long-term (≥5 year outcomes) data by 2018, well within the upcoming project period (2016-2021).

Figure 1: Whole body BMD



Total body DXA was used to measure BMD in adolescents undergoing RYGB in Cincinnati (from Kaulfers et.al. *Pediatrics*, 2011)

5.C. Aim 2: To assess long term safety by measurement of bone mineral density in the surgical cohort, with comparisons to similarly obese non-surgical control participants.

5.C.1. Justification and research plans related to Aim 2: Cycle 3 of TL will also provide an opportunity to address critical concerns about long term safety of bariatric surgery performed early in life in adolescents. During cycle 1 and 2, TL has collected data pertaining to nutritional safety (micronutrients assays) and important health events (pregnancies, childbirth, abdominal operations, and deaths) that bear on surgical safety. These areas of active, ongoing surveillance continue to be of high importance and will continue in the measures shown in Table 5 above. However, in cycle 3, the study will add measures of bone health to the long term safety data collected. Physiologic increases in bone mass and density (BMD) typically occur throughout childhood and into the 3rd decade of life. Bone mass and density are relatively stable in the 3rd to 5th decades of life and slowly decline thereafter. Weight is a strong determinant of BMD at all ages, and weight reduction is associated with reduced BMD. Bariatric surgery is associated with bone loss in the short term due, in part, to weight loss (20, 12, 62, 25, 42, 17, 35, 60, 10, 48, 9).

Previously, we examined bone mineral content and BMD in adolescents who underwent RYGB surgery(27). As shown in Figure 1, whole body dual energy xray absorptiometry (DXA) scans were acquired pre-operatively (BMD z scores 1.5-2) and BMD declined (normalized) over 24 months postoperatively (BMD Z score =0). We estimated that weight loss alone accounted for only 14% of the decrease in whole body bone mineral content (BMC) and

BMD, indicating that other factors may play a significant role. Others have reported significant declines in BMD (-9%) 12-36 months following bariatric surgery in adults at the hip, a weight-bearing site(20, 12, 25, 42, 17, 60, 10, 52, 66). Bone loss also appears to occur up to 48 months after surgery(62, 25, 42). For example, 4 year follow-up of RYGB (n=15), SG (n=2), and banding (n=5) in adults showed 10% loss of hip BMD at 4 years postoperatively; since weight loss had ceased at 1 year, this continued bone mineral density loss was not likely related to weight reduction alone. Similar loss of BMD was observed at the radius (non-weight bearing site), consistent with other reports(20, 25, 42). These findings suggest that other factors, possibly including nutritional deficiencies, poor quality dietary intake, and variable compliance with supplements, all likely play a role in BMD change postoperatively. In addition, since the dominant procedure today results in profound hypoghrelinemia (a hormone known to regulate body composition bone mass) there is additional concern that despite no malabsorptive component, SG could also result in accentuated adverse effects on bone density. Even small annual declines in BMD are important as they accumulate over decades and increase fracture risk later in life. This is particularly salient for adolescents who undergo bariatric surgery as they have life-long "exposure" to their altered anatomy.

In cycle 3, **the hypothesis that bone mineral density will be lower following both RYGB and SG procedures compared to BMI-similar controls will be tested.** Further, relationships between BMD and physiologic factors related to bone health will be examined. An early indicator of potential problems with skeletal mineral loss comes from measurements of vitamin D and PTH already collected in TL. Our preliminary data shows a worrisome trend of declining vitamin D (-8%) and increasing PTH levels (+30%) from baseline to 4 years after RYGB, suggestive of progressive alterations in calcium homeostasis. The trend in rising PTH was not observed for sleeve gastrectomy (SG) participants. These findings support the **hypothesis that BMD z-scores will be related to 25-OH vitamin D levels and reported intake of calcium, and inversely related to PTH levels.**

Non-surgical control cohort for DXA study

The TL surgical participants are well-known to the clinicians at study sites and have been followed by the TL study for many years. To accomplish the goal of characterization and interpretation of bone health in this surgical cohort, it is important to have comparison data from a group of n=100 individuals who did not undergo gastrointestinal surgery for surgical weight loss but who are of similar weight to the surgical cohort. Thus, a new cohort of control participants will be recruited in the cycle 3 period in Cincinnati, OH, Houston, TX, and Columbus OH. This group will undergo DXA examinations in an identical manner to the surgical cohort and will be carefully selected so that they are similar to the surgical cohort in terms of sex, race, and weight characteristics.

Control participants will be recruited using IRB-approved materials and a variety of mechanisms including print advertising; e-mail blasts, social and digital media; news/broadcasts; face-to-face marketing; and flyers at Cincinnati Children's, UC Health and in the community. Advertisements will provide phone and/or email contact information so potential participants can reach out to the study team with questions and get more information. Potential participants that learn about the research protocol through e-mail or advertisement may also have the option of completing an electronic eligibility screener. Individuals who are not eligible will be notified after completion of the screener. Individuals who are potentially eligible to participate can choose to provide their contact information so that research staff can contact them about participation.

Inclusion criteria for non-operative cohort:

- Individuals (n=100) who have a BMI in the range of the current (2017) postoperative BMI of the existing TL cohort (e.g., weight reduced)
- Demographically similar to existing TL cohort:
    - 75% female, 25% male
    - Age: 20-30 years
    - BMI: 25-60 kg/m^2
    - Race: 68% white, 32% non-white
- Reside in proximity to Houston, Cincinnati, or Columbus TL clinical centers

Exclusion criteria to reduce confounding in this cohort:

- Prior gastric resection, or prior intestinal surgery is an exclusion
- Medication Use: Current or historical use of bisphosphonate medications, use of >5mg/day of systemic prednisone or its equivalent for > 3 month duration at a time within the last year, use of anti-epileptic medication such as phenytoin or carbamazepine in the last year, or use of heparin therapy for >3 months in the last year
- History of chemotherapy or radiation treatment
- Immobility or wheelchair bound
- Currently (or within the last 6 months) pregnant or lactating. A urine pregnancy test will be obtained at the start of the study visit to confirm that participants are not pregnant.
- Depo-Provera contraceptive use in the three years or prior use for greater than 24 months in total
- Those with medical conditions that affect bone density at sites of interest (hip/radius) will be excluded. Specifically:
    - osteogenesis imperfecta,
    - hypogonadotropic hypogonadism,
    - panhypopituitarism,
    - primary ovarian failure,
    - syndromic forms of obesity (i.e. Prader-Willi Syndrome, Cohen Syndrome, Bardet-Biedl Syndrome),
    - known genetic forms of obesity (POM-C mutations, MC4R mutations),
    - Cushing Syndrome,
    - History of prior hip or spine fracture or ≥ 3 long bone fractures,
    - slipped capital femoral epiphysis,
    - primary hyperparathyroidism,
    - hyperthyroidism,
    - leukemia,
    - lymphoma.

5.C.2. Research methods for Aim 2:

Bone health data will be collected at a single study visit corresponding to an annual visit between 6-11 years after bariatric surgery. Dual energy-x-ray absorptiometry (DXA) will be used to measure BMC and aBMD once in each surgical participant and once in each of 100 individuals who have not undergone abdominal surgery but who are demographically and weight matched to TL participants during the 2016-2021 period (nonsurgical control participants). DXA scans of the total body, hip (femoral neck), lumbar spine and distal radius will be obtained to provide a comprehensive measure of bone mineral density throughout the skeleton. DXA scans will be acquired using the Hologic Discovery or Horizon and standard positioning as described by the manufacturer. To summarize the research plan for this aim:

- Cross-sectional design comparing bone density of RYGB and SG participants relative to

a BMI-similar, non-surgical control group

- TL participants at study sites with a Hologic DXA machine (CCHMC, Nationwide, and Baylor) will be invited to undergo bone density measurement by DXA at one study visit between 2016-2021.
- Control participants (n=100) who are weight stable and have a similar BMI to postoperative BMI of surgical group, and who are demographically similar will be recruited from community at each site in proportion to number of surgical participants at that site. Frequency matching approach rather than 1:1 matching, excluding individuals on medications or with medical conditions known to have strong effects on BMD (see exclusion criteria above). Control participants will be recruited throughout this funding cycle to provide consistency in timing of bone measurements with the surgery patients.
- This design is the most appropriate to determine if ***attained*** BMD and bone mineral content are appropriate or less than expected for attained post-surgical BMI and addresses the clinically relevant question of whether bariatric surgery has a long-term adverse impact on BMD by defining whether BMD is lower than expected for body weight.
- BMC and areal BMD measured at hip (femoral neck), lumbar spine, distal radius, whole body.
- Primary outcome measure is hip BMD; hip, spine and radius are often sites of osteoporotic fracture.
- Whole body scan provides lean and fat mass, helpful for interpreting BMD. It is important to know if there are differences in lean mass between the weight-matched control and surgical groups and whether this explains differences in observed BMD.
- All scans will be analyzed centrally at CCHMC using Apex 5 software. Differences in calibration among DXA machines will be accounted for by including 'site' in statistical models. Measurement error in BMD measurements due to obesity will be equally distributed across our BMI-similar groups, thus should not confound our comparisons. When CCHMC receives the scans for analysis, full birthdates are listed, thus PHI will be shared, and we have included this in the Informed Consent documents that will be signed by each participant.
- Radiation exposure associated with the DXA scans will be less than 90 micro Sieverts, which is comparable to approximately 11 days of back ground radiation.
- Additional information on dietary calcium and vitamin D intakes and fracture history will be collected by questionnaire on surgery cases and controls.

At the Cincinnati site only, surgery participants and controls will have bone measurements by peripheral QCT (pQCT) as well as DXA. We will obtain pQCT scans to better characterize effects of bariatric surgery on bone mineral density and cross-sectional dimensions. PQCT scans will be obtained on tibia at the distal 4% and 30% sites and on the radius at the 4% and 30% sites. These measurement sites were chosen to maximize the types of information to be obtained. The 4% site of the tibia and radius are within the distal metaphysis and are composed primarily of trabecular bone. The 30% site of the tibia and radius are located in the diaphysis are predominantly cortical bone. PQCT scans will be acquired using the Stratec XCT 2000 scanner with a voxel size of 0.4 mm and a speed of 25 mm/sec. The calibration stability of the pQCT is assessed daily by scanning a phantom and evaluating the vBMD results. Our long-term reproducibility of phantom scan measurements, expressed as a %CB, is ≥0.3% for total and cortical areas, and total, trabecular and cortical vBMD. Radiation exposure associated with these pQCT scans is minimal (<10 micro Sieverts).

5.D. Exploratory Aim 3: To evaluate cognitive functioning, behavioral constructs, psychopathology, and suicidal ideation/behaviors during the first decade following adolescent bariatric surgery.

5.D.1. Justification and research plans related to Exploratory Aim 3: Obesity is an established risk factor for adverse neurological outcomes in adults and elevated BMI is also associated with neurocognitive impairment in adolescents(1, 29), especially in those adolescents with severe obesity(37). The TL cohort will provide an opportunity to assess post-operative cognitive function. In the renewal period of TL, cognitive function will be measured to preliminarily characterize this important domain and gain insights into possible differences between RYGB and SG, research that has never been done in adults or adolescents.

Separately, impaired cognitive control, affect regulation dysfunction, and impaired reward processing may impact negatively on weight outcomes and increase the risk for problematic eating behaviors and alcohol problems(14). Affect dysregulation is associated with mood disturbance, eating disorders and alcohol use disorders(30, 21). Deficits in cognitive control may impair decision-making around alcohol use and eating behavior(6, 64). While preliminary, these data will be novel and prove useful for interpreting the long-term weight loss outcomes and for examining possible mediating and moderating variables(44).

5.D.2. Research methods for Exploratory Aim 3: To minimize burden and attrition risk to Teen-LABS, these measures will be administered through an online Assessment Center portal. This approach is commonly-used in neuropsychological studies, and our consultant group has utilized it in numerous studies examining cognitive outcomes in severely obese persons, including those that pursue bariatric surgery. The platform for this data collection is WebNeuro, an online test site that provides a rapid and valid assessment of memory and other cognitive abilities. Test administration takes approximately 30 minutes and requires use of a keyboard and a mouse. Participants are provided with information for test completion through email and data is coordinated through an online dashboard system by study investigators. Data can also be extracted through this dashboard system. Data includes raw test scores (i.e. how many seconds it took to complete a given task) and standardized performance (i.e. how much faster or slower than peers did task completion take). Safety procedures for cognitive testing include de-identification of test data, secure storage, and review of test results by a licensed clinical neuropsychologist. This testing will be offered to all TL surgical participants around the time of their 10 year visit and to all non-operative control participants around the time of their single study visit.

Assessment of neurocognitive status. WebNeuro is comprised of 12 reliable and valid subtests that are known to be sensitive to the effects of obesity and bariatric surgery on the brain. After reading the instructions and completing practice items to ensure comprehension, participants are then asked to complete the subtest. Data is automatically stored after completion of each subtest. The following 12 constructs will be tested during this session:

- Finger tapping. This sensitive measure of psychomotor speed asks individuals to press the space bar as quickly as possible with their dominant hand throughout a pre-determined time period.
- Choice reaction time. This subtest is a reliable and valid measure of complex attention and speed of information speed. Individuals are asked to attend to the screen and respond to stimuli as quickly and accurately as possible.
- Memory Recognition - Verbal List-learning, immediate forced choice recall, and delayed forced choice recall. For this subtest, individuals are asked to learn, recall,

and recognize a list of 20 words. A filled delay interval of approximately 10 minutes allows for valid assessment of verbal memory consolidation, permitting examination of hippocampal function.

- Emotion Recognition (Explicit recognition). This brief subtest asks participants to attend to a series of faces presented on the screen and identify the pictured emotion (e.g. happy, sad, neutral). Following a filled delay, individuals are asked to recognize the faces, providing a challenging test of nonverbal memory.
- Forward digit span. This widely-used scale of verbal auditory attention asks individuals to attend to number strings of increasing length and correctly recall them.
- Verbal Interference. Similar to the Stroop Color Word Test, this measure provides a measure of cognitive inhibition and speed of information processing. During part 1, individuals are asked to read a series of color words as quickly as possible. During part 2, they are asked to name the color the word is printed in rather than read the word itself (i.e. word "red" in blue font).
- Switching of attention. This measure of set shifting and complex attention requires participants to connect a series of alternating numbers and letters. Similar to the Trail Making Test B, it provides a highly sensitive index of cognitive dysfunction.
- Go/No-Go. This measure asks individuals to quickly respond to target stimuli and to suppress this response to non-targets, providing a sensitive index of cognitive inhibition and response time.
- Sustained Attention (1-back task). This brief scale of cognitive vigilance and complex attention asks individuals to attend to a number series and identify whether the number on the screen is the same as the previous number.
- Executive maze. Similar to the Austin Maze task, participants are asked to find through way through a blind maze. In so doing, this challenging task provides a comprehensive measure of executive function, particularly perseverative tendencies.

Assessment of cognitive control, affect regulation, reward processing. The measures outlined below will be assessed during the 3rd funding cycle of the study. For surgical participants, the measures will be conducted within 3 months of the 10 year postoperative study visit. The self-report measures and computer tasks will administered via the web using software from Millisecond Software using the Inquisit 4 program.

- Difficulties in Emotion Regulation Scale (DERS; As described by Gratz & Roemer in 2004). The DERS is a 36-item self-report questionnaire that assesses six domains of emotion dysregulation (i.e., non-acceptance of negative emotions, inability to engage in goal-directed behavior when distressed, difficulties controlling impulsive behavior when distressed, limited access to effective emotion regulation strategies, lack of emotional awareness, lack of emotional clarity) and has been found to have acceptable internal consistency as well as adequate test-retest reliability and construct validity (Gratz & Roemer, 2004).
- UPPS-P Impulsive Behavior Scale (UPPS-P; Cyders & Smith, 2007). This is a 59-item self-report measure assessing five dimensions of impulsivity. Only the negative urgency and positive urgency subscales will be administered. The measure has demonstrated good internal consistency and validity (Cyders & Smith, 2007).
- Effortful Control Scale of the Adult Temperament Questionnaire (ATQ-ECS; Evans & Rothbart, 2007). This 19-item self-report measure assesses several components of dispositional effortful control, including attentional control (i.e., ability to voluntarily focus or shift attention), inhibitory control (i.e., ability to inhibit behavior), and activation control (i.e., ability to activate behavior as needed). The measure has evidenced good reliability and validity (Evans & Rothbart, 2007).

- Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ; Torrubia et al., 2001). This is a 44-item self-report measure that is based on Gray's behavioral inhibition and activation motivational systems conceptualization, and assesses sensitivity to punishment and reward. The measure has demonstrated adequate internal consistency, test-retest reliability, and construct validity. Only the Sensitivity to Reward subscale of the measure will be administered.
- Delay Discounting Task (Kirby et al., 2000; Kishhinevsky et al., 2012). Delay discounting tasks require participants to choose between receiving a larger amount of money after a delay versus receiving a smaller amount immediately. The size of the smaller immediate reward is adjusted based on previous responses, narrowing the range of choices until an indifference point (i.e., amount at which the smaller more immediate reinforcer and larger delayed reinforcer are judged as being of equal value) is determined for each delay interval.

Assessment of suicidal history, ideation, and related behaviors. The TL study has assessed suicidal ideation through one question on the Beck Depression Inventory during the first two grant cycles. For cycle 3, one questionnaire will be added to the study, termed the Suicidal Behaviors Questionnaire (SBQ). This one page instrument collects history of suicidal ideation and plans prior to surgery and after surgery. It also captures seriousness of the ideation, frequency of ideation, whether the individual has shared this ideation with others, whether the ideation has occurred in the past month, and how likely it is that the individual will make future attempts. Both of the questionnaires (BDI-II and SBQ) regarding suicide, will be completed by participants during in-person clinical visits and the safety action plan if necessary to be implemented indicated by a positive score has been approved by the Data Safety Monitoring Board of the Teen-LABS Continuation study.

5.E. General Clinical Research Methods

5.E.1. Informed Consent. All active TL participants are eligible for participation in additional research measures during cycle 3 of funding. In addition, new SG and control research participants being recruited will be eligible for all or select components of the research plan. Thus, new informed consent documents will be used and written consent obtained from all participants, former and new, according to the guidelines established by the Institutional Review Board at CCHMC and each specific site. All study procedures will be explained and performed using English language and participants must comprehend the English language to participate. The informed consent form is a means of providing information about the study to a prospective participant and allows for an informed decision about participation in the study. All participants must read, sign, and date a consent form before entering the study or undergoing any study-specific procedures.

The informed consent form will be revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the study.

A copy of the informed consent document will be given to a prospective participant for review. A Teen-LABS investigator or study coordinator will review the consent and answer questions. The prospective participant will be told that participation in the study is voluntary and that he or she may withdraw from the study at any time, for any reason. There will be specific consent/permission forms to address the special circumstances of this study:

- Informed Consent for use in re-consenting participants who had been engaged in the TL study during prior funding cycles

- Informed Consent for use in the n=38 new SG participants who underwent operation in 2012 - 2015 for whom retrospective chart abstraction and prospective study visits will be conducted
- Informed Consent for participants who have not undergone surgery who are new to the study and are enrolling as matched controls

5.E.2. Study Withdrawal

Participants who wish to discontinue participation in the research may withdraw his or her consent for this voluntary study at any time. A participant can terminate ongoing participation in the study by written request, at which time there will be no further study contact with the participant. Previously collected data and specimens will only be deleted or destroyed upon written request. Withdrawal will be reported to the IRB in annual progress reports.

5.E.3. Enrollment. Once an individual has been identified as eligible for the study, a Teen-LABS co-investigator or staff member will outline the study objectives. For individuals who are eligible and indicate a willingness to participate, the study coordinator will review and document inclusion and exclusion criteria, and then will discuss in detail the nature of the study and what is expected. All potential new participants and all existing TL cohort members will be ≥18 years of age in cycle 3. All previous participants in TL will be required to provide new written informed consent based on the new study objectives (including the DXA bone health component). Study objectives, procedures, benefits and risks will carefully discussed with the participant. Consent will be requested not only for study participation, but also for access to all medical records, submission of biospecimens, for future contact for participation in any additional research studies, and to have their contact information and Protected Health Information (PHI) sent to the Central Retention Office at University of Colorado, Denver (UCD) to be stored in a central database. If there are any questions or concerns about eligibility, these will be directed to the site investigator or Principal Investigator of Teen-LABS. Signed consent forms will be placed into the participant's research binder and a copy placed into the medical record according to each site's regulatory requirements.

Non-enrollment: A preoperative enrollment form (data collection form "POEF") will be completed whenever possible on participants who are eligible for participation in Teen-LABS. For those who are eligible but decline participation, attempts will be made to determine why eligible participants refused to participate. At those sites where IRB permits, the adolescent who declines participation in Teen-LABS will be asked to consent to having age, gender, race, ethnicity, height and weight recorded and stored in a de-identified fashion. The sole purpose of collecting this minimal, entirely anonymous data on non-participants will be collected to insure generalizability of study results procedures. This information from participants who decline to participate in the study will be compared to those who do consent to ascertain the generalizability of Teen-LABS results and to minimize selection bias.

5.F. Study Procedures, Visits, and Database Contents

5.F.1. General.

Permission will be requested to contact the alternative contacts should the patient become unreachable during the study period; permission will also be sought to use the subject's social security number to locate him or her in the event that the patient cannot be reached through any other contact information. The information will not be shared with anyone outside the study team.

Data collection forms and questionnaires listed in Table 5 above will be obtained by each clinical site from a password protected study website administered by the Teen-LABS Data

Coordinating Center (DCC). The Teen-LABS pre-operative evaluation, including relevant updated data collection, will be completed through patient assessments, including chart review, self-administered forms and interview with the patient as necessary and data recorded on data collection forms to be transmitted by email or facsimile to the DCC. Laboratory values, medication and co-morbidity information will be updated within 30 days of surgery. Weight and other measures will be obtained again within 30 days of surgery via the anthropometrics form. The Teen-LABS operative form will be used to collect operative details. The research coordinator also will review participants' medical records to determine if a reportable outcome event occurred.

A summary of the Teen-LABS data elements collected **from the surgical cohorts** at preoperative, 30 day, and 6 months, and annual follow-up time periods is bulleted below. These bulleted lists are descriptive in nature; the actual case report forms collect related information in a detailed fashion. In addition, Table 5 names the actual case report forms and indicates time of administration.

5.F.2. Baseline evaluation data elements from surgical cohort. These elements were collected in the original cohort of 242 participants within 30 days prior to surgery and will be abstracted from charts for the n=38 new SG participants being enrolled in cycle 3.

- Date of consent to participate in Teen-LABS
- Month and year of birth, gender, height, weight, race, ethnicity
- Anthropometrics and vital signs
- Nutritional supplements prescribed for postoperative period
- Fitness (400 meter corridor walk)*
- Most recent laboratory values and medications (within 90 days prior to operation)
- Comorbidities (T2DM, ischemic heart disease, hypertension, congestive heart failure, sleep apnea, history of deep vein thrombosis/pulmonary embolus, asthma, functional status, pulmonary hypertension, urinary incontinence, sleep hygiene)
- Psychosocial assessments*
- Smoking history, other conditions possibly affecting outcomes*
- Preoperative weight loss program characteristics*
- Weight history
- Polysomnography (sleep study) data files for those with OSAS diagnosed at baseline*

* indicates that this will only be collected if it was documented previously for the new SG enrollees and is available for abstraction

5.F.3. Operative data elements from surgical cohort

- Date of surgery
- Operative times
- Procedure(s) performed
- Whether a resident or trainee was present
- Method of surgical procedure
- Whether concurrent procedures were performed
- Test of anastomosis
- Deep vein thrombosis prophylaxis
- Intra-operative fluids administered
- Anesthesia risk-derived classification
- Whether post-operative anticoagulation was ordered
- Adverse intra-operative events
- Lowest reported or known body temperature

5.F.4. Full study visits at six and twelve months and annually in surgical cohort. The data in the bulleted list below will be collected within a designated window of time around the 6 month, 12 month, and annual anniversary of surgery for each surgical participant. The desired window for the 6 month timepoint will be between 5-7 months, and the window around the annual timepoints will be 60 days around the surgery anniversary date. Current funding estimates predict that the year 10 anniversary visit will be the last full study visit for each participant. Exceptions to this will be made for participants who miss(ed) his or her 10th year visit. . **Partial visits** will be conducted on odd years beginning in each participant's 7th postoperative year and extending until the close of active data collection activities in 2021. Milestone visit data collection will include:

- Anthropometrics (Anthropometrics Form)
- Nutritional supplements prescribed for postoperative period
- 400 meter corridor walk
    - The walk only conducted up to a participant's 5th annual visit year and not again until the 10th annual visit during 3rd grant cycle 2016-2022
- Most recent vital signs, laboratory values, medications (within 90 days of visit date)
- Comorbidity reassessment
- Psychosocial self-assessment forms
- Smoking status, other conditions possibly affecting outcomes
- Healthcare utilization
- Polysomnography (sleep study) data files (for those with OSAS diagnosed at baseline, data files for the 12 month follow-up sleep study, if done clinically, will be collected for research analysis)
- A **partial annual visit** (odd years beginning year 7) will consist of a telephone interview to complete interview-based questionnaires (e.g., anthropometrics, brief comorbidity status healthcare utilization, and retention surveys). No laboratory testing will be done by the study at these timepoints. The time estimated for this telephone interview will be <30 minutes.

400 meter corridor walk. After a brief medical screening, eligible participants will be asked to complete a 400 meter corridor walk at their usual walking pace at baseline, 6 months, and at annual study visits. During the 2016-2022 grant cycle, this test will be prospectively done at anniversary visits through year 5 and not again until year 10. **This is not done for home visits.** Details are outlined in the manual of procedures.

Laboratory studies: Blood (70mL) and urine (3.5mL) will be obtained for research purposes from adolescent participants who are undergoing bariatric surgery at baseline and at 6, 12 months and annually postoperatively. The maximal volume of 70 mL of blood drawn at each time point, predictably represents <2% of a individual with a body mass of 68 kg, far below that which would be considered excessive. A portion of the adolescent's plasma, serum, and urine that is not used immediately by Teen-LABS will be banked at the NIDDK Biospecimen Repository for future investigations. Serum, plasma, and urine will be aliquoted into approximately 30 separate cryovials (10 serum and 10 plasma and 10 urine) in volumes of 0.5 mL for storage at the NIDDK repository for future research. A portion of the adolescent specimens will be used by Teen-LABS investigators during the course of this study. The analyses to be performed using these specimens are identified in Table 6.

Also, one 10mL tube of whole blood (this volume included in above 70mL total) will be shipped to the NIDDK Genetics Repository for DNA extraction and storage. Participants in Teen-LABS agree to serum, plasma, and urine collection, but may opt out of DNA collection.

5.G. Cohort retention plan with remote/home visit plan and outpatient testing.

5.G.1. Contact information

Retention of participants and proactive efforts to ensure successful accomplishment of each study visit are a high priority for the study group. Detailed contact information for the participant, the parents or guardians, and two close relatives not living with the patient will be collected to aid in tracking participants in the event of missed visits.

The following specific information will be collected and maintained at the participant's study site:

- Social security number of the participant
- Contact information (i.e. address, phone number, email) of the participant/parents
- Contact information (i.e. address, phone number, email) of one more contacts (close friends or family) not living with the participant
- Social networking site identity (e.g., Facebook name)

If a participant expresses concern and or refuses to provide this information they will not be excluded from the research.

Once collected, this contact information will be stored in a separate secure database separate from the participant's research-related information and will not be shared with additional participating sites, external research coordinating centers or as a part of the main study database.

Consent will be obtained to have personally identifiable information and contact information stored centrally at UCD. The information will be stored in a central database for retention purposes and will not be shared with anyone outside the study team. In addition, permission will be obtained from the patient to obtain health and anthropometric information from their personal physician(s) in the event that information needs to be gathered from an office visit to their personal physician(s). By consenting to the study, participants agree to be contacted by investigators for participation in any additional research studies approved by the ancillary study committee.

To assure cohort retention, a "SHORT form" data capture instrument will be utilized to collect current contact information and important health related issues at the time of scheduling each milestone visit.

Detailed information will be ascertained from the participant or family member regarding the names, addresses, and phone numbers of people who might know where the participant is living in case the participant is no longer reachable by study staff in the future.

During study visits, participants will be provided with coordinators' business cards with reminders to call the toll-free number if they move or change phone numbers.

Participants will also be asked to provide a picture to be stored in the central database registry at UCD. This will provide us with an updated picture to the one taken prior to surgery and also serve as an additional means to confirm a participant's identity and is invaluable when searching for them on social network sites (i.e. Facebook).

5.G.2. Communication with study participants

To maximize our contact efforts various communication means (i.e. email, mailings, phone calls, social networks, and text messaging, etc.) will be utilized to update contact information, maintain contact, schedule appointments, remind participants of appointments, and for general study correspondence. A Teen-LABS study cell phone will be used for text

messaging by study staff members. Considerable efforts will be made to continually build rapport, stress the confidentiality of the data, and explain the importance of the research and how it could have a positive impact on other adolescents.

A Study Newsletter will be created and used to maintain contact with participants and maintain enthusiasm for the study. The Study Newsletter will be distributed to study participants and their parents/guardians several times per year. Various methods may be utilized to distribute the Study Newsletter (i.e. mail, email, Facebook, etc.). The proposed newsletter would include the following content: information on general study progress, important study and clinic phone numbers, support group meeting dates and topics, patient stories, nutritional information, health and fitness information, general research articles of interest, and financial services information. This content will be used as a resource to keep in touch with study participants and their families and to build stronger relationships in an effort to increase retention rates. A mock-up of the newsletter template will be provided to the IRB under separate cover.

Participants will be notified that they will be contacted by telephone or by electronic medium (text or electronic mail) mid-year between all annual visits. To increase the likelihood that phone calls will be returned or that participants will take our calls, compensation of $10 will be awarded (mailed) when the mid-year contact is made. The content of this mid-year phone call will consist of demographic updates (changes in addresses, phone numbers, custody, schools, etc.), and reminders of the upcoming annual study assessment.

Frequent communications, including birthday cards, holiday cards, and quarterly newsletters, will be employed to facilitate the maintenance of the central database and maintain contact with participants.

5.G.3. Study branding and publicity

A study logo will be created depicting “Teen-LABS” that will establish trust, rapport and pride in participation. Study refrigerator magnets and other materials may be created and issued that contain reminders to call the toll-free number if they move or change phone numbers. The study will also maintain a World Wide Web presence at the domain www.Teen-LABS.org to publicize operational information about the study such as ancillary study guidelines and study accomplishments.

5.G.4. Strategy for those lost to follow-up

Because this is a long-term study, with extended periods of time between research-related interactions, maintaining the cohort will be a challenging element of completing the research. Various methods will be utilized to re-establish contact with study participants should a participant become lost to follow-up.

In the event that a research participant becomes lost to follow-up and traditional contact methods (i.e. telephone, email, traditional mail, etc.) are unsuccessful, the use of a nationwide electronic search strategy will be employed. This will include general use of public web-based search functionality, and the use of a 3rd party service. The use of a service to identify current contact information for a lost to follow-up research participant will be administered by the Central Study Coordinator. Only the demographic/contact information identified above will be utilized for the purpose of facilitating the search. The Central Study Coordinator will provide a report back to the research coordinator that includes the most current available contact information for the requested research participant(s). Records of each search will be maintained and be available for IRB review if needed.

Once contact is re-established, the participant will be reminded of the research study and that they voluntarily agreed to participate, interest in continued participation will be verbally confirmed and documented and the participant will return to active study participation as appropriate based on their status/time point in the research.

Study staff will also search publicly available resources including White Pages®, directories (high school, college, alumni, etc.), search engines (Yahoo, Google, etc.), credit bureau (Equifax, Experian), and social media websites to assist in ascertaining new contact information (address, phone number) for participants whose phone numbers and addresses have become obsolete. Name, social security number, date of birth, last known address, and other demographic information provided by the participant at the time of study enrollment will be used in an effort to locate participants who are lost to follow-up. Use of this retention contingency will be kept to a minimum and only used when all other methods of contact have failed.

Executing searches on online social networking sites to look for individual adolescents and/or caregivers aids our efforts in keeping in contact. Searches on online social networking sites are only to retain individual participants, not to send out blanket recruitment messages on the web. Nor are they performed to harass or trouble participants who are no longer interested in participating.

Accurate records, which reflect the frequency of use of these various strategies, will be kept and can be reported to the IRB on an annual basis, upon request.

5.G.5. Use of social networking

When connecting online with an enrolled participant, the study-specific Facebook (or other social networking profile) page will be used. Participants will be asked to "friend" our study page.

State of the art confidentiality and privacy settings will be used when "friending" occurs. Profile preferences will be set commensurate with keeping all names and identities of all other "friends" hidden. Those who have friended the study page will not be allowed to view the names or profiles of others in the study. The use of social networking with aid us in maintaining communication with participants once enrolled.

There are two broad categories of communication on social networking sites. First, study updates and general communication (i.e. Facebook posts) will be sent to participants who have "friended" the study page. For example, "*Have you moved in the past 6 months or gotten new phone numbers? Please call Teen-LABS (xxx-xxxx) or message us your new information!"*

It is possible that these communications will appear in participants' "news feeds," but the communications will be benign in nature. They are simply designed to stay in contact with adolescents and remind them that they can contact us at any time. PHI will not be communicated in these general communications unless prior authorization for use and/or disclosure of limited protected health information have been obtained from the participant. For example, our study newsletter often features study participant(s) and their name (typically only first name) and/or picture in the newsletter. Links to the newsletter will be posted on the Social Network site, but the participant's information will not be directly in the post, just the link to the newsletter. Again, prior to using participant names and photos, the required authorization to use the information and for what purpose will be obtained from the participant.

Second, since social networking is one of the most common ways adolescents use to communicate, it is possible that such site may be the preferred method of communicating with study staff. In these cases, social networking will be used to communicate directly with participants about scheduling their study visits. These individual communications (i.e. Facebook messages) will be sent securely though individual private messaging through social networking sites and will not be visible to anyone outside the study team. Hence, these communications will NOT appear in "news feeds" and are akin to email communications like those that are routinely used in studies of this kind. For example, we may write:

> *Your annual study visit is now overdue. If you would like to participate with us in Teen-LABS this year please call Teen-LABS (xxx-xxxx) or message us.*

Finally, participants will also be informed that social networking sites occasionally use personal information for targeted advertising and TL will have no control over this.

Approval of the Teen-LABS Facebook account has been obtained from CCHMC Marketing & Communications and staff members will read and follow CCHMC's Social Media Policy.

5.G.6. Contractor services for field study visits

If all opportunities to coordinate an on-site visit have failed, the study will utilize Examination Management Services, Inc. Health Services Division (EMSI) to conduct remote/home visits. EMSI will partner with the study's research coordinators at each site to gather study data at participants' homes if the participant is unable to return to the clinical center for a study visit in person. The procedures can be summarized as follows:

1. Once the DCC confirms the need for this home visit with the local site coordinator and the participant and family agrees to allow EMSI to come to their home, the home visit protocol will be activated and EMSI National Service Center (NSC) staff will contact the appropriate EMSI branch office and provide the participant contact information, location, date, and time of services.
2. Research data collection kits will be shipped from EMSI NSC Dallas, Texas to the local EMSI branch office.
3. Study coordinators will mail self-report forms to participants and will assist them with completion by phone.
4. All EMSI personnel performing services for this study will have completed the required Human Subject Certification course, the Teen-LABS study protocol training, and training for the handling of dry ice shipments before any scheduled home visit.
5. EMSI staff will drive to the participant's home to conduct study procedures
    - Collect self-report forms, perform anthropometric measurements using Tanita scale and stadiometer, perform mobile phlebotomy, blood/urine specimen processing, packaging, and shipment of biosamples to our central laboratory
    - The central laboratory will process bio-specimens for the NIDDK Repository
6. Teleforms will be completed for laboratory and anthropometric measures.
7. All forms (including completed self-report data forms) will be packaged and sent FedEx back to local site coordinator.
8. The corridor walk (400m walk test) will not be done due to safety and cost implications.

Authorization to Release PHI to EMSI and Quest: A business service agreement is in place between each study site and EMSI to allow demographic/contact information to be given to EMSI to arrange the home visit, and for participants to schedule a visit at the Quest locations.

Incident Reporting
In the event of an adverse event or unexpected problem during the home visit, the EMSI examiner must report it to EMSI NSC immediately by phone. EMSI NSC will notify the site coordinator and DCC central study coordinator immediately.

5.G.7. Other off-site mechanisms for study visit completion

In the event that a participant is not willing or able to have an EMSI home visit nor come back to the clinical site, they may go to a Quest Diagnostics Patient Service Center affiliated with the Teen-LABS study to have their anthropometric measurements, blood pressure, pulse and when possible, blood work and urine sample collected for the study. The results from this testing will be given to the Teen-LABS study as well as a copy to the participant to share with their local health care provider.

5.G.8. Retention Operations/Oversight

To oversee retention at all sites a Central Retention Coordinator has been added to the study staff. The Central Retention Coordinator at UCD will be added to the other participating sites' IRB protocols, so that they may assist the other sites with retention efforts.

A sub-committee, entitled the Retention Oversight Committee (ROC) has been established to monitor the implementation of the retention plan, evaluate the effectiveness of retention strategies, and lead efforts in any revision of the retention plan in response to study progress.

5.H. Adjudication of Clinical Events

To accurately and objectively assess the risks of bariatric surgery to adolescents, it is critical that clinical complications observed by the Teen-LABS study be clearly classified as related to the surgical intervention or to other causes unrelated to the surgical intervention. In cycle 3 of the study, the Adjudication Committee for Teen-LABS will review and classify etiology and relatedness to bariatric surgery of the following adjudicatable events: 1) deaths and 2) abdominal surgical operations occurring after the index bariatric operation.

Events that will trigger the adjudication process will come to the Data Coordinating Center (DCC) from de-identified information contained within the routine study data collection forms, including the forms describing events related to the initial operation and hospitalization, the 30 day status forms, subsequent healthcare utilization forms, and research adverse event forms if applicable. The Teen-LABS site coordinator will collect detailed information about the adjudicatable event including (but not necessarily limited to):

- Admission note (history and physical)
- Radiology reports
- Operative and procedure reports
- Discharge summary
- Pathology reports
- Consultant's report(s)
- Death summary
- Coroner's report

- Autopsy report
- Death certificate

Records will be stripped of patient and site identifiers, documents scanned, a cover page added that provides the Teen-LABS participant ID, and documents will be sent to a secure website at the DCC. Using this information, the DCC will assemble a patient packet to send to Adjudication Committee members, for review and classification. The resulting outcome data will be used to scientifically report results.

5.I. Incentives and Reimbursements

Participants will receive monetary incentives to cover the time burden of participating in the study. In consideration of the importance of excellent retention for achieving study goals, and of the increasing competing demands on the participants' time as they age and become young adults with additional life responsibilities (e.g., work related demands, relationships, family, and/or college) we have realized a need for reasonable annual increases in reimbursement for participants' time. To cover participants increasing time demands all annual visit incentives have been increased $100.00, except for study year 7 and 9 visits which are partial, phone call visits. Also, to accommodate participants who are unable to participate 100% and complete a full annual study visit, participant reimbursement has been prorated (except for partial visit study years 7 and 9). Participants will be reimbursed based on what elements of their scheduled study visit they complete. Depending on which visit they are completing and what elements of the study visit they complete, participants will be paid up to a maximum of $300.00 for the costs, inconvenience, and time associated with participation in this research study. If a participant is unable to complete a full visit, the reimbursement amount will be prorated. The table on the next page outlines the prorated reimbursement schedule for participants continuing in the study for each study visit:

Study Visit	For completion of Self-Assessment Questionnaires	For completion of Physical Measurements	For completion of Blood Draw & Urine Collection	For completion of Phone Interview	Total Maximum Participant Amount Possible (for full study visit)
5 year full visit	$50.00	$120.00	$90.00	n/a	**$**260.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
6 year full visit	$50.00	$130.00	$100.00	n/a	**$**280.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
7 year phone call	$25.00	n/a	n/a	$25.00	**$**50.00
Midyear phone	n/a	n/a	n/a	$10.00	**$**10.00
8 year full visit	$50.00	$140.00	$110.00	n/a	**$**300.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
9 year phone call	$25.00	n/a	n/a	$25.00	**$**50.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00

10 year full visit	$50.00 $50.00 psych measures	$150.00	$120.00	n/a	**$**370.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
11 year phone call	$30.00	n/a	n/a	$30.00	**$**60.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
12 year phone call	$35.00	n/a	n/a	$35.00	**$**70.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
13 year phone call	$40.00	n/a	n/a	$40.00	**$**80.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
14 year phone call	$45.00	n/a	n/a	$45.00	**$**90.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
15 year phone call	$50.00	n/a	n/a	$50.00	**$**100.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00

Prorated reimbursement schedule for new SG cohort being recruited 2017-2019:

Study Visit	**For completion of Self-Assessment Questionnaires**	**For completion of Physical Measurements**	**For completion of Blood Draw & Urine Collection**	**For completion of Phone Interview**	**Total Maximum Participant Amount Possible (for full study visit)**
3 year full visit	$50.00	$100.00	$70.00	n/a	**$**220.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
4 year full visit	$50.00	$110.00	$80.00	n/a	**$**240.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00
5 year full visit	$50.00	$120.00	$90.00	n/a	$260.00
Midyear phone call	n/a	n/a	n/a	$10.00	$10.00
6 year full visit	$50.00	$130.00	$100.00	n/a	$280.00

Midyear phone call	n/a	n/a	n/a	$10.00	$10.00
7 year phone call	$25.00	n/a	n/a	$25.00	$50.00
Midyear phone call	n/a	n/a	n/a	$10.00	$10.00
8 year full visit	$50.00	$140.00	$110.00	n/a	$300.00
Midyear phone call	n/a	n/a	n/a	$10.00	$10.00
9 year phone call	$25.00	n/a	n/a	$25.00	$50.00
Midyear phone call	n/a	n/a	n/a	$10.00	$10.00
10 year full visit	$50.00 $50.00 psych measures	$150.00	$120.00	n/a	**$**370.00
Midyear phone call	n/a	n/a	n/a	$10.00	**$**10.00

In addition, compensation for travel to the study site may be provided for all participants continuing in the study for the full annual study visits (years 3-5, 6, 8, and 10) that occur after June 2009 and for new 2017-2019 SG cohort for full annual study visits. The compensation will be based on the federally allowable reimbursement for automobile use, based on round trip distance from the participant's home to the clinical center. The compensation schedule is below:

- Round trip travel from home to center up to 125 miles: $25 compensation
- Round trip travel from home to center 126-250 miles: $100 compensation
- Round trip travel from home to center 251-375 miles: $150 compensation
- Round trip travel from home to center 376-500 miles: $200 compensation
- Round trip travel from home to center over >500 miles: $250 compensation

Participants continuing in the study who live >250 miles round trip from the center may also be reimbursed for reasonable lodging costs up to $150.00 per visit for full study visits in years 3-5, 6, 8, and 10. SG cohort who live >250 miles round trip from the center may also be reimbursed for reasonable lodging costs up to $150.00 per visit for full study visits (years 3-5, 6, 8, and 10).

If a DXA is done, participants will receive a one-time reimbursement of $100.00.

The non-surgical cohort for DXA comparison will be reimbursed $100.00 for their one-time visit.

Since our study participants are maturing and having many "life changing events" such as marriages, births, graduations etc. they will be provided with congratulation baskets (total value not to exceed $25.00) to celebrate these milestones and acknowledge these important changes.

5.J. Data Collection and Management:

5.J.1. Overview. The Division of Biostatistics and Epidemiology at CCHMC will serve as the Data Coordinating Center (DCC) for this project. The study statistician and epidemiologist will act as the scientific director of the DCC and supervise DCC operations. The primary responsibilities of the DCC are to ensure the completeness and accuracy of the collected data while maintaining participant confidentiality, and to provide the operational infrastructure to facilitate cooperation and communication among the clinical sites. The DCC will provide a centralized information management system for collecting, editing, storing, and analyzing data. This includes development of data collection forms for the adolescent data, a manual of operations, quality control procedures, security and confidentiality of data, long-term data storage, and coordination with the LABS DCC.

Prior to the start of the study, the Principal Investigators and Site Coordinators at each adolescent clinical site will travel to Cincinnati for a meeting to go over the entire study design, collection of the variables, and other aspects of the study. Each Site Coordinator will be responsible for data collection and transfer of data to the Teen-LABS DCC.

5.J.2. Identifying Source Data.
The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. A template will be developed such that all relevant intraoperative data is captured in the surgeon's dictated operative note which will become the source document for operative data. The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical and research records and the data will be transferred to the DCC via facsimile on TeleForms®. Safety data will be recorded on forms specifically designed for this purpose. All adverse events will be reported on an adverse event report form as well as on individual data collection forms. Safety data will be reviewed periodically by the Teen-LABS DSMB (as specified in the DSMB charter and DSMP) and each participating institution's IRB. The NIDDK at the advice of the DSMB, or the IRBs, will have the authority to withdraw participants and/or terminate the study because of study-related safety problems.

5.J.3. Permitting Access to Source Data.
The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from Teen-LABS participants. Medical and research records at each site and contact information / PHI stored centrally should be maintained in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of the study, the investigational site must permit authorized representatives of the sponsor(s), the Teen-LABS DCC, and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress.

Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals. The investigational site will normally be notified before auditing visits occur.

5.J.4. Data Forms and Database. The data collection forms have been created using TeleForm®. TeleForm® is a software application that automates data capture. Historically, TeleForms are shared electronically with each clinical site in PDF format for printing and completion. The Site Coordinator is responsible for completing all necessary forms for each participant at each visit and returning completed forms to the DCC in a timely fashion. Completed forms are sent back to the DCC. Once forms are in the system, the software evaluates the form images and runs data quality checks to detect answers that may be out of

range, missing or inconsistent with skip-pattern logic. Questionable data is flagged and sent to a human verifier to be reviewed. For the psychological instruments under copyright for which there is no TeleForm® version, permission is granted before a TeleForm® version can be constructed.

Use of TeleForm® allows scanned information to be entered directly into a database. Going forward, data will be captured via REDCap. The DMC will develop the data capture system using a web-based data collection system, REDCap, as the primary source of data entry and storage. REDCap is a software toolset and workflow methodology for electronic collection and management of research and clinical trial data developed by Vanderbilt University, with collaboration from a consortium of institutional partners including the University of Cincinnati Academic Health Center. The DMC will develop, test and maintain the REDCap data entry system, the data management plan, data quality checks and query management, and preparation of the data for analysis.

The REDCap system provides a secure, web-based application that is flexible and provides: 1) an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry; 2) HIPAA-compliant and 21 CFR Part 11-ready audit trails for tracking page views, data manipulation and export procedures; 3) record locking and electronic signature functions; 4) fine grained control of user rights to view and manipulate data, and tool to sequester data access for multiple sites; 5) a report builder for reporting, monitoring and querying patient records; and 6) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

REDCap is hosted on a network specially designed to support the rigorous security and compliance requirements of basic, clinical and translational research projects. Administered by the Division of Biomedical Informatics (BMI), this network features multiple firewalls as well as a central facility for managing hosted systems and users. The result is another layer of access control and audit capability on top of what REDCap already provides. For example, a user's ability to access a REDCap study or even a specific questionnaire can be monitored and controlled at the network level without making any changes within REDCap itself. These capabilities are available to authorized BMI network administrators and REDCap study owners only, and all user access changes are documented by an automatic audit trail.

Data files from the analyzed DXA scans will be transferred electronically ("data dumps") for creation of data analysis files.

<u>Miscellaneous Forms.</u>

- The Off-Protocol Form will be completed by the study coordinator to report deviation(s) from study protocol (e.g., decision not to pursue surgery, missed visit, incomplete data collection).
- An Inactivation Form will be utilized to report patient drop-outs or inactivations and reason(s) for dropping out or inactivation.
- The Mortality Form will be completed by the site surgeon co-investigator. This form will be sent to the DCC.
- An Enrollment Form will be completed to report those participants who are enrolled into Teen-LABS. This form reports whether participants provided consent to Teen-LABS, along with date of consent. If the patient does not consent, the reason is reported.
- When a liver biopsy is taken as part of usual care during surgery, a liver sample will be processed and a sample stored frozen by the pathologist at the clinical site. Unstained

histology slides will be sent to a central pathology laboratory for Teen-LABS, at which time, a liver Pathology Evaluation form will be completed by the central hepatopathologist.

5.J.5. Data Storage. All paper data collection forms will be stored under lock and key in file cabinets kept in the DCC offices. Only study personnel will have access to the offices and file cabinets. Electronic data will be stored on a network server that is backed up nightly. Access to the stored electronic data will be limited to study personnel and password protected. The server is maintained and all backups are conducted by the Informatics Division of Cincinnati Children's Hospital. PHI data (social security number, phone numbers, social networking site contacts, e-mail addresses, home address), collected for sole purposes of the centralized retention effort (see section 5.G.1.), will be stored in a password protected database at UCD which is on a network server that is backed up nightly and only centralized retention study staff will have access to these PHI in the database.

5.K. Data Analysis and Statistical Power.

5.K.1. General Analysis Plans.

The following is a description of our general approach to statistical analyses. Once data have been cleaned, descriptive statistics are generated. Although the analysis dataset will have previously been scrutinized using quality control measures, additional descriptive statistics are calculated to further identify any outliers and errors. Means, ranges, standard deviations, frequencies, and percentages are computed and graphs are generated. The assumption of normality is checked. If there is skewness in the data, either a transformation of the data is done and analyses run on the transformed variable or non-parametric methods are used. After using descriptive techniques to identify outliers and questionable data, these techniques are employed to illuminate data patterns and guide modeling. Graphical techniques such as histograms, density plots, and boxplots are examined to assess distributional forms, variability, and extent of outliers. Change over time (e.g., extent of weight loss), and bivariate relationships are examined by scatter plots, side-by-side boxplots, and contingency tables, depending on the types of variables.

Specific Aim 1: Assessment of mid-term (5 year) outcomes of adolescent compared to adult bariatric surgery, assessment of long-term (10 year) outcomes of adolescents bariatric surgery, and assessment of differences in outcome for RYGB and SG procedures performed in adolescence.

Study staff will rigorously document anthropometric changes, remission/relapse of comorbidities, and new incident comorbidities to assess long-term efficacy of adolescent bariatric surgery. Variables describing anthropometric changes include weight, BMI, body fat and waist circumference. Other variables broadly describing health include systolic blood pressure, diastolic blood pressure, fasting insulin, fasting glucose, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and C-reactive protein. Comorbidity status (Y/N) variables will be derived from multiple variables, including those just mentioned, but also including participant-reported medications taken. The status is determined by following individual pre-defined criteria. All of these variables may be either the outcome / dependent variable of interest, or an independent variable within the model, dependent upon the hypothesis being addressed.

Descriptive statistics will be calculated overall and by subgroups (including surgery groups), for example, crude measures of remission, incidence, and prevalence of health conditions (comorbidities) and complications, as well as their cumulative incidence and rates (person time). The initial step will be to examine the association between each independent variable

and the dependent variable by inserting only one variable into the model at a time. As an example, the outcome "total cholesterol" will be classified as normal / abnormal and a log-binomial regression (or logistic) model will be constructed. Multiple linear regression modeling will be used when outcomes are continuous in nature. Multivariate prevalence ratios and 95% confidence intervals will be calculated. Similar models will be fitted for the other pre-operative measures of health.

For continuous outcomes in longitudinal analyses (baseline, 6 months following surgery, 12 months, and annually thereafter), linear mixed modeling will be performed. Prior to modeling, descriptive statistics will be studied, as well as plots of outcomes over time since surgery. In instances where longitudinal outcome variables are dichotomous in nature (i.e., normal / abnormal), generalized linear mixed modeling will be utilized.

Hypothesis 1.1: Adolescents will experience a higher comorbidity remission and lower comorbidity relapse rate at 5 years compared to adults enrolled in LABS-2.

To address this hypothesis, baseline, through 5 year follow up data from the adult LABS-2 study who underwent RYGB will be compared to data collected at the same follow-up time-points (since surgery) from the Teen-LABS cohort of RYGB participants. Dependent variables of interest include type 2 diabetes, hypertension, and dyslipidemia. Univariate analyses using logistic regression as these are binary outcome variables, will first be run to compare change in weight, BMI, waist circumference, with remission and relapse at 5 years for each comorbidity between the two cohorts. Based on preliminary examination of the data, we anticipate similar changes in BMI over time for the two cohorts. However, there appear to be greater proportions of adolescents with remission of diabetes at 2 years compared to adults, and we believe that differences will be even greater at 5 years. To control for confounding variables, multivariable logistic regression models will be constructed to compare the two cohorts for each dependent variable (comorbidity remission and comorbidity relapse rate at 5 years). The primary independent variable will be cohort membership (Teen-LABS, LABS-2). The primary covariates would be baseline BMI, change in BMI, sex, and race, however, others may be considered.

Hypothesis 1.2: Adolescents undergoing bariatric surgery will demonstrate durable improvements in BMI and cardiovascular status over long-term follow-up.

Based on our 3 year outcomes and 5 and 10 year outcomes from longitudinal adult bariatric studies such as the Swedish Obese Subjects Study, we anticipate that most adolescents will maintain BMI loss at later time-points (5 and 10 years) but some will experience major weight regain, and some may well experience heterogeneous comorbidity outcomes, including complete relapse of hypertension and diabetes, while others will successfully maintain remission of these conditions.

Exploratory analyses of longitudinal adolescent data, as well as adolescent subgroups, will be conducted. An example is assessing the relationship between maximal weight loss, maximal loss of fat mass and nadir BMI following surgery with improvement in cardiovascular disease risk factors. Multivariable linear regression models will be used to examine predictors (both baseline and follow-up variables) of BMI reduction at 5 and 10 years respectively. Linear mixed modeling will be performed to examine BMI reduction longitudinally (6 months following surgery, 12 months, and annually thereafter). Covariates evaluated include, but are not restricted to: time, time^2, age at surgery, baseline BMI, race, and sex. For longitudinal outcome variables of cardiovascular status, that are dichotomous in nature (i.e., normal / abnormal), generalized linear mixed modeling with logit as link function will be utilized.

Covariates to be considered, but not restricted to: time, time^2, age at surgery, baseline BMI, race, sex.

Hypothesis 1.3: Compared to adolescents who underwent SG, those treated with RYGB will experience a greater reduction in BMI and greater cardiovascular risk factor reduction (increase in HDL cholesterol, decrease in triglycerides and blood pressure) over long-term follow-up.

Similar to our approach to hypothesis 1.2, for hypothesis 1.3 Linear mixed modeling will be used to examine the contribution of surgical membership (SG vs. RYGB) to the dependent longitudinal variable (6 months following surgery, 12 months, and annually thereafter). The dependent variable will be change from baseline in BMI, HDL cholesterol, triglycerides and blood pressure respectively. The primary independent variable will be surgical group (SG vs. RYGB). Covariates evaluated include, but not restricted to: time, time^2, age at surgery, baseline BMI, race, and sex. For longitudinal outcome variables of change in comborbidity, they are dichotomous, generalized linear mixed modeling with logit as link function will be utilized. The primary independent variable will be surgical group (SG vs. RYGB). Covariates are, but not restricted to: time, time^2, age at surgery, baseline BMI, race, and sex. Based on power calculations (see below), the new SG participants will permit examination of differences in outcome by cohort membership as early as year 5.

Hypothesis 1.4: Improvement in depressive symptoms and weight-related quality of life will be greater in RYGB compared to SG participants over long-term follow-up.

Similar to our approach for hypothesis 1.3, linear mixed modeling will be used to compare depressive symptoms (Beck Depression Inventory (BDI)) and weight-related quality of life (IWRQOL) between two surgical groups (SG vs. RYGB). The dependent variables are BDI and IWRQOL. The primary independent variable will be surgical group (SG vs. RYGB). BDI is a continuous variable scored 0 to 63, with predefined categories: 0-13, 14-19, 20-28 and $\geq$29 defining none, mild, moderate and severe depression. Additionally $\geq$17 is considered as a clinical marker for depression. Choice of analysis as a continuous or categorical outcome (multinomial, or binomial) will depend upon deviation from the assumption of underlying multivariate normality. The IWRQOL will be evaluated as total score. The approach will involve multistage or hierarchical modeling to initially examine the changes over time for the dependent variables, in order to establish if there are higher order terms beyond linear required. The independent variable of interest surgical group will then be added into the model and interaction with the time variables, such as: time, time^2 etcetera assessed. Covariates evaluated include, but not restricted to: age at surgery, baseline BMI, race, and sex. The interaction between surgical group and the covariates will be checked. The same approach will be used for the three domains of IWRQOL as for the total score.

For hypotheses related to micronutrients, linear mixed modeling will be used to compare SG and RYGB in folate, 25-OH vitamin D, and transferrin levels over long-term follow-up. The dependent variable will be folate, 25-OH vitamin D, and transferrin levels respectively and the primary independent variable will be group (RYGB, SG). Covariates evaluated include, but not restricted to: time, time^2, age, race, sex. Multivariable linear regression modelling will be used to assess the association between bone mineral density z scores with 25-OH vitamin D levels, reported intake of calcium and parathyroid hormone levels. For the time-to-event safety outcomes such as time from bariatric surgery to death, first hospital re-admission, abdominal reoperations, hypoferritinemia, low Vitamin B12, Kaplan-Meier method will be used to estimate survival functions. Median survival and 95% CI will be provided. Cox models

will be performed to check potential predictors: bariatric surgery group, baseline variables and follow-up variables (as time-dependent covariates). For bariatric surgery group and baseline variables, the proportional hazard (PH) assumption will be checked (using the ASSESS Statement in PROC PHREG). If PH assumption is violated for a variable, the interaction between the variable and time will be added in the model.

Specific Aim 2: Assessment of long term bone health by assessment of bone mineral density.

A cross-sectional study design will be employed to compare bone density of RYGB and SG patients relative to a weight -similar, non-surgical control group. Controls will be similar to TL participants in respect to age, sex, race, and weight at the time of the DXA measurements (5 year post-operative time point). A frequency matching approach will be used. This study design addresses the clinically relevant question of whether bariatric surgery has a long-term adverse impact on bone health, namely, is BMD several years following surgery lower than expected for body weight? Multivariable linear regression models will be used to test whether the surgery groups have a lower bone density than controls. The dependent variable will be the bone outcome measure BMD and the primary independent variable will be group (RYGB, SG, control). Covariates (age, race, sex, study site, height and weight) will be fitted in regression models to reduce variability in the outcome measures and adjust for potential imbalances among groups. Distributions of serum 25OH-vitamin D and PTH, calcium intake and physical activity will be analyzed among groups. Multivariable linear regression models will be used to test whether the surgery groups have higher serum parathyroid hormone concentrations (PTH) than weight-similar controls. The dependent variable will be PTH and the primary independent variable will be group (RYGB, SG, control). The covariates would be race, sex and compliance with calcium supplements.

Exploratory Aim 3: To evaluate cognitive functioning and behavioral constructs during the first decade following adolescent bariatric surgery. In particular, cognitive function, cognitive control, affect regulation, reward processing, risk taking behaviors (including problems with alcohol), and problematic eating behaviors will be assessed.

Statistical analyses will examine between-group differences on the Total Composite Score, with corrected post-hoc comparisons for each cognitive domain (Executive Function, Attention, Episodic Memory, Language, Processing Speed, and Working Memory), these are all contained in the NIH Toolbox for Assessment of Neurological/ Behavioral Function. As for Aim 1, multivariable linear regression models will be used to test whether the surgery groups (RYGB, SG) have different cognitive function from the weight -similar, non-surgical control group described in Aim 2. The dependent variable will be cognitive function and the primary independent variable will be group (RYGB, SG, control). The initial approach will be to examine the total surgery group compared to controls, and then examine the difference between the individual surgery groups and controls. Covariates evaluated include, but not restricted to: age, BMI, race, sex and study site.

To test whether impaired cognitive control, affect regulation dysfunction, and impaired reward processing will impact negatively on weight outcomes and increase the risk for problematic eating behaviors and alcohol problems, univariate analyses will be performed first. If the p-value is less than 0.2, the independent variable will be used in the multivariable linear regression models. The variable for surgery groups (RYGB, SG) and its interaction with the independent variables selected from univariate analyses will be fitted in the multivariable linear regression models. To control for confounding, age, race, sex and study site will be adjusted in regression models.

Exploratory Analyses: Exploratory analyses of longitudinal adolescent data, as well as adolescent subgroups, will be conducted. Linear mixed model is a very appealing approach. However, the normality assumption for the random effects, which is automatically made by the linear mixed model might not hold. To extend the normality assumption about the random-effects distribution to a very broad class of distributions (unimodal and multimodal, symmetric and skewed), heterogeneity model(57, 58) assuming the random effects to be sampled from a mixture of normal distributions (rather than from just one single normal distribution) will be used for classification of participants based on the longitudinal profiles. Additionally, steps will be taken to clarify what are the most appropriate or useful weight loss outcome measures (e.g., BMI units, percent excess weight loss, z-scores, etc.). Various other exploratory and preliminary analyses will also be conducted – for example, comparison of weight loss outcomes and safety profiles across multiple bariatric surgical procedures in adolescents (RYGB, SG) in total and in subgroups.

5.K.2. Missing Data. Missing data are ubiquitous in longitudinal research, frequently due to item or question nonresponse, missed follow-up visits, and participant attrition. The optimal strategy for addressing missing data is to make every effort to obtain complete data during the conduct of the study. As described in the companion clinical application, the Consortium uses a variety of methods to minimize the amount of missing data in the study. Nevertheless, there will be a small portion of data that are missing. The first step in addressing missing data in the analyses will be to assess the pattern of missing data (e.g., missing completely at random, missing at random, missing not at random, etc.). The reasons why data are missing will be documented. Knowing the reason for missing data will help assess the pattern. Once missing data patterns are deciphered, concurrent analyses will be completed using complete and available case data or multiple imputation techniques. Additionally, pattern-mixture and selection models(59) will be used for sensitivity analyses.

5.K.3. Power Analyses. To properly determine the required sample size for achieving study aims, the DCC previously conducted extensive power and sample size calculations to support the initial cycle for Teen-LABS. These calculations represented the required number of participants receiving RYGB surgery that are necessary to detect statistical differences in outcomes of primary interest. In short, approximately 160 RYGB adolescent subjects were necessary to yield adequate power (≥80%) to detect anticipated baseline differences between adolescents and adults for most outcomes of interest identified in hypothesis 1.1.

Aim 1 Hypothesis 1.1: For the current submission we are examining longitudinal comparisons, the procedure in nQuery that was employed to calculate power was the repeated measures analysis of variance for two groups. From this program the power is computed for the main effect of groups, the main effect of time, and their interaction. The hypothesis we proposed was that adolescents and adults will have different rates of change over time, so only the power for interaction is presented in Table 7.

Table 7. Power for the interaction term using repeated measures of variance when comparing adolescents to adults.*

	N		
Variable	200	160	100
Cholesterol	99	97	85
Triglycerides	99	99	99
HDL	**	**	**
LDL	98	96	85
Glucose	95	90	72

* For the calculations, the level of significance was set at 0.05 for a two-sided test.
** Power was very low for the interaction term, but greater than 90% for main effect of groups and 99% for the main effect of time.

Aside from HDL cholesterol, we predict that the power will be sufficient for detecting clinically significant differences, based on the mean and standard deviations available for adult bariatric patients in the literature. From the power calculations provided, we are satisfied that the major scientific goals of Teen-LABS will be readily achieved with a sample size of 160 gastric by-pass participants in each group. Even if the attrition is as high as 10%, the major comparisons will be possible and valid.

Aim 1 Hypotheses 1.2 to 1.4: For the longitudinal comparisons of Teen-LABS gastric bypass and sleeve gastrectomy participants (Aim 1), the procedure in nQuery that was employed to calculate power was the repeated measures analysis of variance for two groups. Values for these calculations were taken from the Teen-LABS study database for visits through the fourth post-operative year. The power is computed for the main effect of groups, the main effect of time, and their interaction. The hypothesis we proposed was that gastric bypass and sleeve gastrectomy will have different rates of change, so only the power for interaction is presented in Table 8. Power estimates are presented by various sample sizes per group, including n=67 as that was the original number of SG cases enrolled, n=80, and n=100. These calculations indicate adequate power is achieved for many endpoints with 67 participants per group, but reaches 80% or greater when 80 participants per group are available. Thus, recruitment of 38 additional SG participants will increase this cohort size to n=108 (original 67+38=108), so that an anticipated 20% (n=20) could miss any particular visit and we would still retain n=80 and thus have sufficient power to compare outcomes between SG and RYGB cohorts.

Table 8. Power for the interaction term using repeated measures of variance when comparing Teen-LABS gastric bypass and sleeve gastrectomy participants.*

	Power with specified N per group		
Variable	N=100	N=80	N=67
Body Mass Index	88%	80%	72%
Systolic blood pressure	93	86	78
Diastolic blood pressure	99	99	99
HDL cholesterol	91	83	75
Triglycerides	95	90	83
Folate	99	97	94
Transferrin	99	99	99
Parathyroid hormone	93	86	79
Serum albumin	96	90	83
Vitamin D	98	95	90
IWQOL-Total score	89	80	72
Beck depression index	99	97	94

Aim 2: Statistical power for aim 2 is bounded by the existing sample size of TL participants at the three sites that are likely to return to the clinical center for bone measurements in addition to the new SG patients that will be recruited. We estimated that we could obtain bone density measurements on 65-77 RYGB patients and 82-95 SG patients. One hundred BMI-similar control participants will be recruited. We used hip bone mineral density (BMD) data reported by Maghrabi et al, 2015(34) on 2 year post-surgery (mean =1.112) and standard deviations (SD= 0.128) to estimate the size of difference in hip BMD that we could detect with sufficient power. For all calculations we assume a 2-sided two-sample equal variance t- test and type 1 error of 0.05. There will be sufficient power to detect a 5%

reduction (0.4 SD) in hip BMD (Table 9). This difference is clinically meaningful given that a 1 SD decrease in hip BMD is associated with a 2-fold increased risk of hip fracture(25).

Table 9. Power calculation estimates for detecting differences in hip BMD.

Number per surgery group	Number in control group	Difference in hip BMD		Power
65	100	5%	0.4 SD	77.3%
82	100	5%	0.4 SD	82.6%
95	100	5%	0.4 SD	85.4%

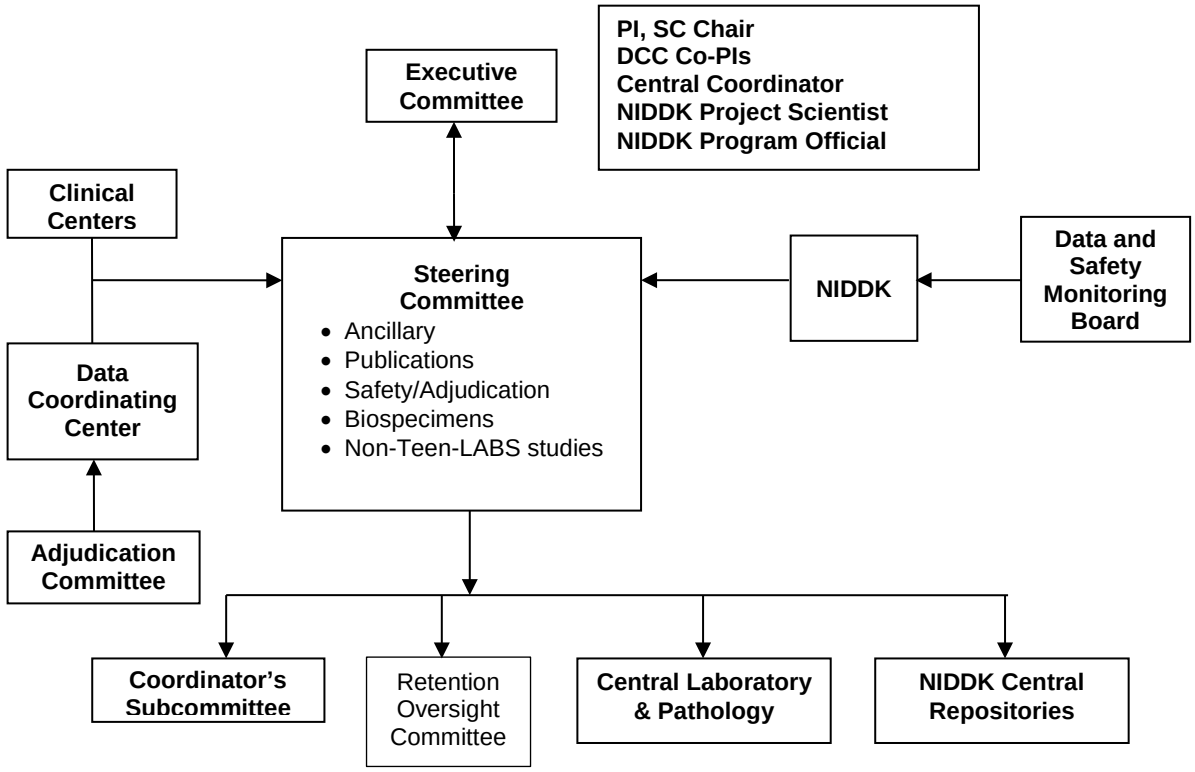
Exploratory Aim 3: Due to lack of preliminary data there are no formal power analyses for Aim 3. However Table 10 below from the Clinical application provides effect sizes for pre- versus post-operative tests. Given the sample sizes of between 67 and 100 per surgery group and 100 for the control group, we shall have 80% power using a 2-sided test and type 1 error of 0.05 to detect an effect size of .40, or greater.

Table 10. Pre- and post-operative cognitive function in adolescent bariatric surgery patients

Domain	Test	Pre-op	Post-op	Effect Size	
Memory	Learning	32.1 ± 5.7	34.0 ± 5.4	0.32	
Attention	Digit Span	5.6 ± 2.6	8.0 ± 2.9	0.98	
Executive Function	Switching of Attention	50.0 ± 16.1	42.1 ± 13.2	0.54	
Executive Function	Verbal Interference	13.8 ± 2.6	16.2 ± 3.4	0.79	

6. Study Organization

6.A. Overview. Teen-LABS General Organization Structure is shown below.



Annually, the Central Study Coordinator will travel to each site and randomly select 10% of the charts (or a minimum of 5 records) to review for data completion and accuracy. A greater than 5% error rate will result in further review of the source documents for the clinical site. If this error rate for the site does not decrease, a conference call between the appropriate study personnel will be made to discuss the issue and devise a solution.

The Teen-LABS DCC staff will meet weekly and the Executive Committee will conduct biweekly conference calls to ensure that all aspects of study initiation, data collection and abstraction are progressing without problems. The frequency of Steering Committee phone calls will vary between weekly and monthly.

An important component of the DCC and the sites' collaboration is communication. The DCC will seek and welcome ongoing input from the sites on any of the operating procedures that present challenges. This input is imperative because these individuals are the most familiar with the capabilities and limitations in the data collection efforts at each site. The DCC will also place calls on a frequent and routine basis to facilitate communications. For example, a problem may occur at a site that the site staff believe can be resolved. The Central Study Coordinator will further facilitate communications by serving as a point contact for the Site Coordinators to answer procedural questions as they arise involving the DCC as necessary.

6.B. Sites
Clinical & Consulting Centers:

- Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
- Texas Children's Hospital Baylor Medical Center, Houston, Texas
- Children's Hospital of Alabama, University of Alabama, Birmingham, Alabama
- University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania
- Nationwide Children's Hospital, Columbus, Ohio
- Neuropsychiatric Research Institute, Fargo, North Dakota
- University of Colorado, Denver, Colorado

Data Coordinating Center (DCC). Division of Biostatistics and Epidemiology (DBE) at CCHMC.

6.C. Website. The DCC has designed and manages the study website for research use and for public consumption www.teen-labs.org.The latter includes general information about obesity and bariatric surgery, a description of Teen-LABS, including the goals of the study, the core information database, clinical projects and ancillary study guidelines; and contact information at the clinical sites for persons interested in enrolling in the LABS. Teen-LABS is also listed on http://clinicaltrial.gov

6.D. Committees:
6.D.1. Executive Committee. Manages day-to-day issues of the study; makes decisions required between the Steering Committee meetings as needed for efficient progress of the study, and reports its actions to the Steering Committee on a regular basis; organizes and sets agendas for Steering Committee meetings. Members consist of the Steering Committee chairman, the DCC Co-PIs, the Central Coordinator and the NIDDK Project Scientist.

6.D.2. Steering Committee. Serves as the primary governing body of the study; responsible for policy decisions; votes on and approves all major decisions, provides oversight in planning the overall study design, approves protocols and subsequent amendments, facilitates study conduct and reporting of study results.

Coordinators Subcommittee. The Coordinators Subcommittee of the Steering Committee attends to the day-to-day operations of the study including recruitment and retention, protocol adherence, and consistent and complete data collection at each clinical center. The Central Study Coordinator will be on the Committee, as well as all coordinator at Teen-LABS Clinical Centers preparing strategies that can be implemented study wide to maximize recruitment and to maintain participants in the study. The Central Study Coordinator will serve as the chair. The committee will report to the Steering Committee regularly and will make recommendations regarding any study issues that may require modification or resolution.
Safety. The Steering Committee has responsibility for safe conduct of the study and for regularly reporting study progress and concerns to the Data and Safety Monitoring Board (DSMB).

- The Steering Committee appointments a safety monitor for the study and establishes safety parameters and procedures for collecting data pertaining to the safety of participation in the Teen-LABS protocols and related Ancillary studies. Relevant information gathered at clinical sites is collated by the DCC for review by the Steering Committee, safety monitor, and Data and Safety Monitoring Board (DSMB).

- The Steering Committee establishes the Data and Safety Monitoring Plan that requires approval by the DSMB.
- Individuals involved in study leadership are responsible for reporting to the DSMB on a regular basis and for seeking ongoing approval for continuation of study operations and sharing the results of this process with affiliated Institutional Review Boards.
- The Steering Committee will address human subject issues that arise related to participant interactions, safety, and confidentiality.

6.D.3. Ancillary Studies Committee. Teen-LABS will consider proposals from investigators which seek to enhance the ability of Teen-LABS to document the efficacy and complications of bariatric surgery and its role in the overall management of pediatric obesity and to address other important questions related both to clinical aspects of pediatric obesity and its co-morbidities and underlying mechanistic and other basic science issues. The steering committee will appoint two Teen-LABS investigators who will perform initial evaluation of proposals for ancillary studies. The primary goals of this screening is to determine that proposed studies do not impose an unacceptable burden on Teen-LABS staff or study participants or conflict with the aims of Teen-LABS. Proposals that meet specific ancillary studies criteria will be forwarded to the Steering Committee, which must approve all ancillary studies. Data collection for funded ancillary studies may not proceed without the approval of the Steering Committee. The Steering Committee must solicit and oversee sponsored research agreements, materials transfer agreements and cooperative research and development agreements. All data from ancillary studies must be submitted to the DCC upon completion of data analysis.

The Ancillary Study guidelines also describe the conduct of other scientific studies in the population eligible and/or recruited for Teen-LABS at each participating institution, including ancillary studies and other studies that are not affiliated with the Teen-LABS study.

6.D.4. Publications and Presentations. The Publications and Presentations Committee will develop policies for publications regarding preparation of abstracts, presentations, and manuscripts. Policies pertaining to requests for data analysis, authorship, and other issues related to publications will be shared with the research community in a formal publication policy for full manuscripts and abstracts. The committee will also maintain a list of publications that arising from the study.

6.D.5. Adjudication Committee. The Adjudication Committee provides on-going review of specific clinical events related to the project, including the following:

- The Adjudication Committee will review and classify deaths and specified post-surgical events or interventions (criteria for confirmation are detailed in the Teen-LABS Adjudication Committee Handbook)
- To develop reports, with the assistance of the Teen-LABS DCC, for presentation to the Data and Safety Monitoring Boards of Teen-LABS related to participant safety.

6.D.6. Retention Oversight Committee. The Retention Oversight Committee (ROC) was created to create and monitor the effectiveness of retention strategies, and lead efforts in any revision of the retention plan in response to study progress. The committee will consist of the Teen-LABS Principal Investigator (PI), the Central Retention Coordinator, the Teen-LABS Central Study Coordinator, one surgeon PI representative, one Site Coordinator representative, the other individuals with subject matter expertise to advise on best practices for participant retention.

6.D.7. Data and Safety Monitoring Board. The Data and Safety Monitoring Board (DSMB) will oversee study progress, safety, and productively and will provide recommendations to the study sponsor. The NIDDK and Teen-LABS DSMB will develop a DSMB Charter and will approve the Data and Safety Monitoring Plan (DSMP).

7. Special Considerations

7.A. Human Subjects Issues.

Teen-LABS is a prospective, longitudinal multi-center study, and involvement of human subjects being used to describe long-term surgical risks and changes in clinical, metabolic, and psychosocial measures among patients undergoing bariatric surgery. Individuals of all race and ethnic groups will be eligible for study participation. The racial, gender, and ethnic characteristics will reflect the demographics of the patient population of the study institutions. No exclusion criteria shall be based on race, gender, ethnicity, or HIV status. The participant population includes individuals who are at least 13 years of age that had primary bariatric surgery by a Teen-LABS certified surgeon. 242 patients have been recruited from the 5 clinical sites. Individuals are excluded from participation if informed consent was not obtained or if deemed unlikely to comply with the follow-up protocol or unable to communicate with local study staff. Approximately 138 new participants will be enrolled in cycle 3 from the same clinical sites to address new study questions (Aims 1c and Aim 2).

7.A.1. Institutional Review Board Approval. The study protocol, consent forms, DSMB charter, data and safety monitoring plan, and data collection forms will be submitted to each clinical center's Institutional Review Board (IRB). A site may not initiate any patient contact for Teen-LABS until the site has IRB approval. All study personnel will have completed training in the Protection of Human Subjects per NIH guidelines. It is the clinical site investigator's responsibility to ensure that the Teen-LABS protocol and informed consent documents are reviewed and approved by the appropriate IRB. Each clinical site must obtain a letter of approval from the IRB prior to enrolling participants into this study. Sites must provide the DCC with copies of the initial IRB approval notice prior to enrolling the first patient, and subsequent renewals, as well as copies of the IRB approved consent. Additionally, the NIDDK must review the IRB approved informed consent prior to enrollment.

The relevant IRBs must also review and approve any other written information provided to the patient prior to any registration of participants.

If, during the study, it is necessary to amend either the protocol or any of the informed consent documents, study investigators will be responsible for ensuring that the local IRB reviews and approves the amended documents. IRB approval of the amended informed consent documents must be obtained before new participants consent to participate in the study using the new version of the consent.

The informed consent documents will inform participants of their right to refuse any release of their protected health information.

7.A.2. Informed Consent.

7.A.2.a. Informed Consent Documents. A sample of each informed consent or assent document relevant to this study has been provided at the end of this protocol (see Consents section). Each clinical site, according to local IRB requirements, is allowed to modify this informed consent document and make any necessary editorial changes as long as the meaning or intent of any section is not changed.

7.A.2.b. Informed Consent Process. The investigator or his/her designee (i.e., research coordinator or study nurse) will inform the study participant or their legally authorized representative, as applicable, of all aspects of the study pertaining to the participant's participation in the study. Study participants who become 18 years of age while enrolled in the study will be re-consented as adults.

The process for obtaining informed consent will be in accordance with all applicable regulatory requirements. Once a candidate for Teen-LABS has been identified, details will be carefully discussed with the participant. The participant (or a designated proxy/caregiver, if applicable) will be asked to read and sign the IRB-approved assent and informed consent documents, as appropriate, BEFORE the participant can participate in the study. The informed consent grants permission to collect information on a participant's health contained in the medical record, or via questionnaires, laboratory values and urine samples. If the participant is under the age of 18, a caregiver/designated legal representative has to sign the primary consent form giving permission for adolescent participation and the adolescent has to assent and sign the form. The participant and/or caregiver as appropriate will receive a copy of all signed and dated documents. The original will be placed in the participant's research binder, one copy will be given to the participant, and another copy will be placed in the patient's medical record. Where local regulatory requirements differ, clinical sites will abide by local requirements.

7.A.3. Research Study Costs: There will be no cost for participation in this research study. Research procedures and testing done for this protocol will be paid for by funding provided by the NIDDK. Some clinical information which is collected for clinical reasons during the surgical evaluation and follow-up of participants may be collected by research coordinators by abstraction from clinical records.

7.A.4. Confidentiality of Patient Data. The clinical site is responsible for the confidentiality of the data associated with participants enrolled in this study in the same manner that it is responsible for the confidentiality of any patient information within its sphere of responsibility. All forms used for the study data will be identified by coded identification number, which will be generated at the clinical center, to maintain participant confidentiality. All records will be kept in locked file cabinets at the clinical centers with access limited to Teen-LABS study staff, and all study staff will identify participants via their unique identifier. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB or Data & Safety Monitoring Board (DSMB). Clinical information may be reviewed during site visits by the DCC and the NIDDK Project Scientist. The participant grants permission to share de-identified research data with the entities identified in the consent document, including the LABS Data Coordinating Center in Pittsburgh, the NIDDK, and any other authorized investigator. For the years 2016-2021, data will not be shared with the LABS DCC since the LABS study has been discontinued. Federal regulations govern the protection of patient's rights relative to data confidentiality and use of research data. Teen-LABS has an NIH Certificate of Confidentiality.

Consent procedures and forms, and the communication, transmission of participant data will comply with individual site IRB and NIH requirements for compliance with The Health Insurance Portability and Accountability Act (HIPAA). The Privacy Rule of HIPAA governs the protection of an individual's identifiable health information. The DCC will ensure that clinical centers associated with the project are complying with HIPAA regulations by requiring documentation from the IRBs with the appropriate authorization or consent form. The DCC will maintain copies of all relevant documents from each clinical center. If IRB approvals are not current, data will not be accepted by the DCC. The Teen-LABS data management

system will ensure the confidentiality of electronic protected health information. The DCC will work with the NIDDK Data and Specimen Repositories to determine their requirements for maintaining participant confidentiality.

7.A.5. Risk/Benefit Assessment. This study is formally classified as "minimal risk without expected benefit" for participants. The risk of physical harm associated with participating in the Teen-LABS study is limited. Blood drawing can cause temporary discomfort or bruising at the skin puncture site and in, rare instances (less than 1%), fainting or an infection can occur. The 400 meter corridor walk may cause chest pain, tightness or pressure in the chest, shortness of breath, feeling faint, lightheaded or dizzy, or leg pain. The test will be stopped immediately if any of these symptoms do occur. During the 400 m walk tests, a fully stocked crash cart will be available with all necessary emergency equipment (drugs, defibrillator, and airway management). Of minimal risk to participants is the possible inconvenience of reporting medical status to the research coordinator. Some of the questions may be upsetting. For example, questions will be asked regarding alcohol and drug abuse, and emotional problems such as depression. Participants will be informed that they can decline to answer any questions they do wish not to answer. Another possible risk is a breach of confidentiality, although steps have been taken to minimize such an occurrence. All information collected for this research study will be kept confidential. Participants' names will be used only for the informed consent form and medical chart reviews. Participants will be given unique study identifiers, which will be written on all data collection forms. In addition, data collection forms will be kept in a locked file cabinet or locked room and a secure database that can only be accessed by the investigators (and their research staff) listed on the consent form. Patient names will not be recorded in the computerized study database. There will be close communication between the PI, the data entry personnel and the clinic and research staff to ensure the quality and accuracy of the data collected. Each member of the study team will meet with the PI and review confidentiality issues, prior to having contact with research participants. Blood and urine samples will be labeled with unique patient identifiers and not participants' names before shipment to central facilities. To help us protect participants' privacy, a Certificate of Confidentiality was obtained from the National Institutes of Health (http://grants.nih.gov/grants/policy/coc/). Known breaches of confidentiality will be reported to the relevant IRBs, NIDDK, and the Teen-LABS DSMB.

There are no anticipated direct benefits to participants who participate in Teen-LABS. *Potential* benefits include early identification of undiagnosed conditions through additional laboratory testing (metabolic and micronutrient analyses) done as part of the study that may not be done routinely for clinical reasons. Clinically relevant measurements will be made available to the patient and their physician with their permission. Identification of a suicidal tendency and referral to appropriate treatment would also be a benefit, although it is not expected that the study personnel would be aware of this type of event before clinical staff are aware of the tendency. The information gained from participation may benefit others who suffer from extreme obesity who are undergoing evaluation for weight-control surgery in the future.

7.A.6. Data and Safety Monitoring Plan (DSMP). A DSMP has been prepared as a separate document and was approved by the study's DSMB. Among other things this document includes information about how the DCC and study staff will monitor study operations and safety. This document includes action plans that should be executed in the event of problems encountered during use of the Beck Depression Inventory, the Suicidal Behavior Questionnaire, and also outlines risks of the phlebotomy, walk test, and loss of confidentiality.

7.B. Quality Control

Overview. The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. Study-wide quality control is the ultimate responsibility of the Clinical Sites and the Coordinating Center. The principal investigator, the central study coordinator, steering committee, and the sponsor are responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data. The Teen-LABS Study Coordinator at each site must be familiar with Teen-LABS study requirements and schedule clinic activities to allow adequate time for the research activities to be carried out while meeting quality standards. This section will address issues related to duplicate measures, equipment, issues related to quality control monitoring by the Coordinating Center and the Central laboratory, and site visits.

7.B.1. Quality Control Principles.

The purposes of quality control are:

- to ensure the highest quality of the Teen-LABS data;
- to provide constructive feedback to Teen-LABS staff performing data collection for quality performance in their data collection efforts; and
- to document the quality of the data for historical record.

The Teen-LABS Data Coordinating Center (DCC) has primary responsibility for development and implementation of quality control measures including:

- preparation and revising/updating of a comprehensive study **Manual of Operations**; training and certification of staff in standardized protocols prior to beginning data collection; maintenance of databases of completed certification;
- designing and implementing protocols and procedures for periodic site visits;
- developing quality control report forms and protocols for regular use by the clinical centers and the Teen-LABS affiliated laboratories;
- analyzing data collected with quality control protocols to: (i) ensure the quality of the performance of interviewers, technicians or other staff, (ii) ensure standardized data collection equipment, and (iii) ensure prompt notification of deviations from clinical centers;
- identifying problems in reporting or handling data from Teen-LABS affiliated laboratories and repository;
- reporting of pertinent information to the Data & Safety and Monitoring Board (DSMB), Executive & Steering Committee, as well as other pertinent groups when necessary;
- maintaining current or historical data and documents to describe the quality and performance of the entire Teen-LABS study.

The role of the clinical centers in the Quality Assurance/Quality Control (QA/QC) plan for Teen-LABS is to implement quality control protocols, and to collaborate with and assist the DCC in the performance of its responsibilities by maintaining required records and logs and by notifying the DCC of any problems/issues that require assistance.
The role of the Coordinators Subcommittee is to monitor retention rates and evaluate protocol and data quality issues as they relate to recruitment and retention. Specific responsibilities include:

- regular review of monthly reports generated by the DCC;
- review of the specific quality control issues identified either by the sites, the Executive Committee or the DCC and the recommendations from the Steering Committee (SC) for resolution of such issues;

- regular communication with the DCC, the SC regarding efficacy of quality control procedures and protocol.

7.B.2. Duplicate Measures. Duplicate (and when necessary triplicate) physical measures are performed on Teen-LABS participants by site personnel. All information is entered on the Anthropometrics Form. The Teen-LABS personnel will measure each patient twice. If the first two measures are within defined limits as given in the instruction section, the third measure is not necessary. Where the Manual of Operations (MOP) mentions "laboratory" duplicate measures, it is referring to testing done at the Central Laboratory and not at the sites.

Duplicate physical and laboratory measurements for calibration will be performed annually to assist in documenting study-wide quality and assessments of inter- and intra-rater reliability. The timing of the calibration will be when the Central Coordinator visits the site. Each site's personnel conducting physical measurements will perform duplicate sets of measurements on volunteers.

Specific quality control activities to be carried out at the Teen-LABS study centers include:

- Certification/recertification of clinic staff.
- Monitoring of regular equipment calibration and maintenance;
- Local clinical lab certificates and lab normal values to DCC annually.
- Recording of participant identifiers on the top of each questionnaire/data collection form prior to their completion at all clinic visits.
- Regular observation and monitoring of clinical procedures including specimen collection.
- Review of all questionnaires and data collection forms prior to faxing the form(s) to the DCC (and before the participant leaves the clinical center).
- Compilation and review of data on lost laboratory samples, packaging problems, errors in packing, shipping, and labeling of specimens.
- Reporting of quality control concerns or problems to the Teen-LABS study coordinating center and/or the appropriate central resource center for prompt resolution.

The site Co-Investigators will regularly monitor clinical center procedures to be sure that they are being carried out properly and with consideration for the Teen-LABS study participant. Corrective action should be taken immediately if problems are observed.
The Teen-LABS personnel are encouraged to communicate with the Coordinating Center about quality control or other concerns or problems.

7.B.3. Equipment. The Teen-LABS study investigators have standardized certain equipment for the study as well as providing minimum requirements for remaining equipment. Standardization (and the attendant maintenance and calibration of the equipment) assures a level of reliability (repeatability and accuracy) across Teen-LABS study centers. (see list in MOP). Each center is responsible for the proper operation and maintenance of equipment used in the study. Some of the equipment is subject to standard calibrations and inspections (e.g., scales). It is suggested that responsibility for monitoring these standards be assumed by a specific individual. All staff should report any real or suspected equipment problems to that individual promptly.
All standard maintenance should be documented by date in a permanent log at the study center. Problems and solutions should also be recorded. Copies of calibration records must be kept on file. The log and calibration records will be inspected during periodic site visits, or copies may be requested by the Teen-LABS study Coordinating Center at periodic intervals.

7.B.4. Data Quality. Clinical Site Coordinators are asked to review all of the participants' questionnaires and data collection forms prior to ending each clinic visit. Forms must be completed neatly and accurately, and every question should be answered. Written responses to any items on the questionnaires/forms should be legible. Confirmation of review is documented at the end of each form.
Before scanning each form into the database, the DCC will perform verification. The Coordinating Center will regularly perform internal comparisons of the entered data to detect missing records or suspicious or invalid data. These comparisons will include logical consistency checks of data within and across forms/questionnaires. When inconsistencies are detected, the clinical center will be notified through edit reports, and will be asked to verify, if possible, some entries. Prompt action with these verification requests is essential for an efficient quality control system. Quality control reports will be available for review by the Steering Committee.

7.B.5. Quality Control for Central Laboratory. Results of quality control procedures carried out at the central laboratory and regularly reported to the Steering Committee. Quality monitoring will be conducted internally (within the laboratory)
Internal quality control procedures include functional and calibration checks of instrumentation as well as monitoring:

- Temperature-dependent equipment and water quality;
- Assay performance of quality control pools with each run of specimens for each analyte;
- Assay performance of blind split duplicate specimens;
- Accuracy of lipid measurements by comparison with reference methods;
- Computer-generated error lists; and Specimen turnaround times

Performance in assay of quality control pools by the various methods is determined and monitored using the database application's quality control functions. Target values have been established for pools; statistics relative to target values are calculated monthly along with Levy-Jennings graphs, and these data are analyzed by operators and by the laboratory director. Trends are noted and calibrations are made as necessary.

7.B.6. Periodic Reports. Periodic reports are submitted summarizing performance in assay of internal quality control, laboratory proficiency survey materials and/or reference materials, e.g., College of American Pathologists and Centers for Disease Control and Prevention, performance in assay of blind split duplicates, and long-term drift monitoring will be reported to the Teen-LABS Steering Committee. Additionally, data on the number of samples received by clinical site, number of samples contained in long-term storage, and turn-around times between samples being obtained and arriving at the laboratory are provided to the Coordinating Center.

7.B.7. Coordinating Center Activities. Quality assurance will be a major activity of the Coordinating Center throughout the study. Activities will include:

- Training/retraining of clinical center staff in data collection procedures
- Monitoring data entry activities

Monitoring of the Teen-LABS study data will take place at the DCC. These activities include data control and report generation. Some of the monitoring and quality control reports will be transmitted to the centers for immediate action and attention; other quality control and monitoring reports will be generated for the Steering Committee. For example, these reports will include data on:

- Recruitment yields at each clinical center

- Summaries of certifications
- Site visit summaries
- Adverse events
- Deviations from protocol
- Missed visits, refusals, losses to follow-up
- Adherence
- Errors in collection, labeling, storage, shipping of laboratory specimens or other materials to central reading centers

7.B.8. Site Visits. The Coordinating Center will arrange a site visit program to each center to promote communication, answer questions, and ensure that study procedures are understood and correctly carried out. The site visit program will provide a mechanism to encourage the effective and standardized delivery of recruitment efforts and the collection of appropriate and valid data within each of the Teen-LABS study clinic sites. Members of the site visit team will be selected by the Coordinating Center and will include the Central Coordinator from the Coordinating Center and may include other personnel from the DCC, the NIDDK, along with one or more personnel from selected centers. Site visits may also be performed if consistent departures from the Protocol and Manual of Procedures are detected. Retraining may be done as needed during these visits, depending on the availability of staff.

7.C. General Clinical Research Center. This study may utilize the General Clinical Research Center resources on a site by site basis for phlebotomy and preparation of blood for send out to the central laboratory and the NIDDK repository.

7.D. Radiation. Bone Imaging: Every person is exposed on a daily basis to a certain amount of background radiation originating from soil, rocks, outer space and within the body itself. Proposed DXA studies would expose females to <20 μSv, which is about the amount an individual receives over approximately 2 days from background radiation.

7.E. Investigational Drugs/Devices. This study will not involve drugs or devices that have not been approved by the FDA.

7.F. Investigational Pharmacy. Not applicable

7.G. Data and Specimen Sharing

7.G.1. NIDDK Data Repository.

Teen-LABS will share data and biospecimens in accordance with NIH and NIDDK data and biospecimen sharing policies, as follows:

De-identified clinical data collected during conduct of the study will be provided to the NIDDK Data Repository so that it can be shared after the publication of the data. All data elements which have been collected on the cohort will be provided to the NIDDK Data Repository so that it can be shared within two years after the end of each funding period/renewal. When the study ends (study investigators are no longer obtaining data directly from study participants), all data will be provided to the NIDDK Data Repository by the end of the funding period, which may include no-cost extensions.

7.G.2. Biospecimens.

With permission, a portion of the adolescent's plasma, serum, DNA, and urine that is not used immediately by Teen-LABS will be banked at the NIDDK Biospecimen repository or the study's central laboratory for future investigations. If a liver biopsy is taken during the adolescent's surgery for clinical indications, several histological slides of the liver specimen

will be sent to the NIDDK central hepatopathologist for comparison with specimens derived from adult LABS participants. At no time will identifying information be noted on these specimens.

Teen-LABS investigators and ancillary study investigators may request samples upon obtaining approval through the Steering Committee and/or Ancillary Studies Committee according to study policies. Once the study ends (including no-cost extension periods), biospecimen distribution will be managed by the NIDDK Repository in accordance with NIDDK policies.

Within the context of this protocol, participants will not be informed about new information that is derived from genetic or other analyses conducted with de-identified biospecimens. This stipulation can be modified in the future by action of Institutional Review Boards affiliated with the study or the DSMB.

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