**OREGON HEALTH & SCIENCE UNIVERSITY**
**SCHOOL OF MEIDICNE- GRADUATE STUDIES**

# AN EVALUATION OF BODY COMPOSITION AND PHYSICAL ACTIVITY IN CHILDREN AND ADOLESCENTS WITH CLASSICAL GALACTOSEMIA

By

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# TABLE OF CONTENTS

## LIST OF FIGURES

## LIST OF TABLES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| BIA | Bioimpedance Analysis |
| BMD | Bone Mineral Density |
| BMR | Basal Metabolic Rate |
| CDC | Center for Disease Control |
| CDG | Congenital Disorders of Glycosylation |
| CG | Classical Galactosemia |
| CPM | counts per minute |
| CTX | Carboxy Terminal Telopeptide |
| DCH | Doernbecher Children's' Hospital |
| DEXA | Dual Energy X-ray Absorptiometry |
| DLW | Doubly Labeled Water |
| FM | Fat Mass |
| FMI | Fat Mass Index |
| FM% | Fat Mass Percentage |
| FSH | Follicle Stimulating Hormone |
| G0 | Aglycosylated |
| G2 | Diglycosylated |

| | |
|---|---|
| Gal-1- P | galactose-1-phosphate |
| GALK | galctokinase |
| GALT | galactose-1-phosphate uridyltransferase |
| ICAD | International Children's Accelerometry Database |
| IGF-II | Insulin Growth Factor-II |
| IGF-1 | Insulin Growth Factor -1 |
| IgG | Immunoglobulin-G |
| LBM | Lean Body Mass |
| LBM% | Lean Body Mass Percentage |
| LMI | Lean Mass Index |
| LTM | Lean Tissue Mass |
| MET | Metabolic Equivalent at Task |
| MVPA | Moderate-to-Vigorous Physical Activity |
| NMJ | Neuromuscular Junction |
| NTX | Amino Terminal Telopeptide |
| OGMD | Other Galactose Metabolic Disorders |
| OHSU | Oregon Health and Science University |

| | |
|---|---|
| PA | Physical Activity |
| PCP | Primary Care Provider |
| PD | Parkinson Disease |
| RBC | Red Blood Cell |
| REE | Resting Energy Expenditure |
| SD | Standard Deviation |
| STS | Sit-to-stand test |
| TGF-β | Transforming Growth Factor-β |
| UDP | Uridine Diphosphate |
| USA | United States of America |

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## Abstract

**Background**: Classical galactosemia (CG) is an inherited disorder of galactose metabolism treated with a low galactose/lactose diet. Unfortunately, even with pre-symptomatic diagnosis and life-long dietary intervention, patients with CG experience long-term complications including low bone mineral density, poor growth and abnormal body composition. However, muscle strength and the level of physical activity (PA) have not been reported.

**Methods**: We compared PA level, muscle strength, and body composition of patients with CG (8-18 years, n=12) to an age-, sex-, Tanner stage- and ethnicity-matched population in overall good health. Body composition (LMI and FMI) was determined by Bioimpedance Analysis. Anthropometric measures were plotted on CDC growth charts. Growth was assessed from a corrected height z-score. Muscle strength was quantified from hand-grip dynamometer and a 60-second sit-to-stand test. Participants wore an ActiGraph tri-axial accelerometer for 7 days to measure PA level.

**Results**: Corrected height z-score, weight z-score, and BMI z-score were all significantly decreased in subjects with CG. There was no significant difference in LMI or FMI. All muscle strength measures were significantly decreased in subjects with CG. Control participants spent significantly more time in moderate to vigorous PA per day than participants with CG, but there was no difference in time spent in sedentary activity, raw counts per minute, or wear-time.

**Conclusions**: Patients with CG are smaller in height and weight with decreased growth compared to matched controls. Children with CG have decreased muscle strength and PA. Future studies should investigate the effects of strength training and increased PA in patients with CG.

## Chapter 1

## Specific Aims

Classical galactosemia (CG) is a rare metabolic disorder affecting an estimated 1:30,000 live births in those of European descent.[1-3] CG is an inherited autosomal recessive disorder of galactose metabolism characterized by a deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT) causing an accumulation of galactose, galactose-1-phosphate (gal-1-P) and other abnormal metabolites.

Newborns with CG usually present with neonatal toxic syndrome after ingestion of galactose from breast milk or a whey-based infant formula. Even with early detection through newborn screening, complications can still occur, including failure to thrive, hepatomegaly, renal tubular dysfunction, hypotonia, cataracts and *E. coli* sepsis. Immediate treatment with a soy formula or other lactose/galactose-free formula can reverse acute neonatal manifestations. Long-term treatment includes a galactose/lactose diet restriction. However, even with early detection and dietary treatment, long-term complications can develop including intellectual disability, verbal dyspraxia, abnormalities of motor function, and primary ovarian insufficiency in females.[4]

Given the rarity of the disease, the sparse research has focused on growth and bone mineralization showing decreased height-for-age and decreased bone mineral density (BMD) in patients with CG.[5-8] Studies suggest that one in four adults with CG will develop low BMD and an abnormal body composition.[2,3,7] Intrinsic genetic changes, dietary restrictions, low serum insulin growth factor-1

(IGF-1) concentrations, irregular endocrine status in females and defects in collagen structure are proposed mechanisms. Only two studies have investigated body composition finding a strong positive correlation between BMD and lean body mass (LBM) in those with CG[2,3]. Overall, the research concludes that the abnormalities seen in CG are most likely multifactorial in origin.

The limited research has shown that patients with CG are likely to have an abnormal body composition. The level of physical activity (PA) and muscle strength has not been documented and it is not well understood how dietary management contributes to the patient phenotype. The lack of research suggests that these factors need to be investigated further to establish improved treatment and prevention protocols.

This project aims to investigate an understudied population by enhancing our understanding of body composition of patients with CG that can contribute to improving practice recommendations and promotion of healthy lifestyles. We propose to investigate PA, muscle strength, and body composition of patients with CG, ages 8 to 20 years, followed at an established metabolic clinic in the United States of America (USA). An age-, sex-, ethnicity- and Tanner Stage-matched pediatric reference population in overall good health will be recruited from the Doernbecher Children's Hospital (DCH) General Pediatric Clinic.

**Specific Aim 1:** We will assess the body composition of patients with CG, ages 8 to 20 years, by Bioelectrical Impedance Analysis (BIA) and anthropometric measurements and compare the results to a control pediatric population in general good health.

**Hypothesis 1:** We predict that patients with CG will have a higher body fat mass index in $kg/m^2$ (FMI) and lower lean mass index in $kg/m^2$ (LMI) in comparison to the control population.

**Hypothesis 2:** We hypothesize that patients with CG will have a reduced height z-score, mid-parental corrected height z-score, weight z-score, and waist:hip circumference compared to the matched controls.

**Specific Aim 2:** We will determine the relationship between muscle strength and PA level on body composition factors in patients with CG by comparing BIA, muscle strength measures (dynamometer grip strength and sit-to-stand repetitions), and PA level measured by an accelerometer.

**Hypothesis 1:** We hypothesize LMI will positively correlate with muscle strength measures and PA level.

**Hypothesis 2:** We believe that patients with CG will have lower muscle strength measures and PA levels than age-, sex-, ethnicity- and Tanner scale-matched controls.

## Chapter 2

## Background

### *Galactose Metabolism*

The Leloir pathway of β-D-galactose metabolism is a three-step process to convert β-D-galactose into glucose-1-phosphate. After this conversion, glucose-1-phosphate can enter glycolysis. In the Leloir pathway, β-D-galactose is first converted into gal-1-P by the enzyme galactokinase (GALK). In the second step, GALT transfers a uridine monophosphate from uridine diphosphate (UDP)–glucose to galactose–1-phosphate, releasing glucose–1-phosphate and producing UDP–galactose, by a double displacement reaction involving a histidine 186 residue at the active site of the catalyst. GALT is a dimeric enzyme.[9] GALT has a specific nucleoside monophosphate transferase activity, which has been well characterized and described by ping-pong kinetics.[10] The resulting UDP–galactose is reconverted into UDP–glucose by the final Leloir enzyme, UDP–galactose 4-epimerase **(Figure 1).**

Mutations to the GALT gene disrupts galactose metabolism by creating a decrease in GALT activity, resulting in an autosomal recessive inborn error of metabolism, CG. GALT deficiency creates an accumulation of gal-1-P; ultimately causing substrate inhibition of GALK, thus increasing galactose and the production of galactitol and galactonate metabolites.[7] High concentrations of these metabolites can have long-term, adverse effects on development of skeletal, ovary and other tissues.[7]

Over 260 variants to the GALT gene are known, of which 85% have pathogenic outcomes.[10] About 60% of CG-related mutations are missense mutations that are distributed along the whole gene.[4,10,11] These mutated residues alter GALT's stability, structure, function, or the active site and thus its activity. With an attempt to understand the complex interactions of the genotype-phenotype outcomes of CG, the number of known missense variations in the GALT gene has increased by 50% in recent years.[4,9,10]

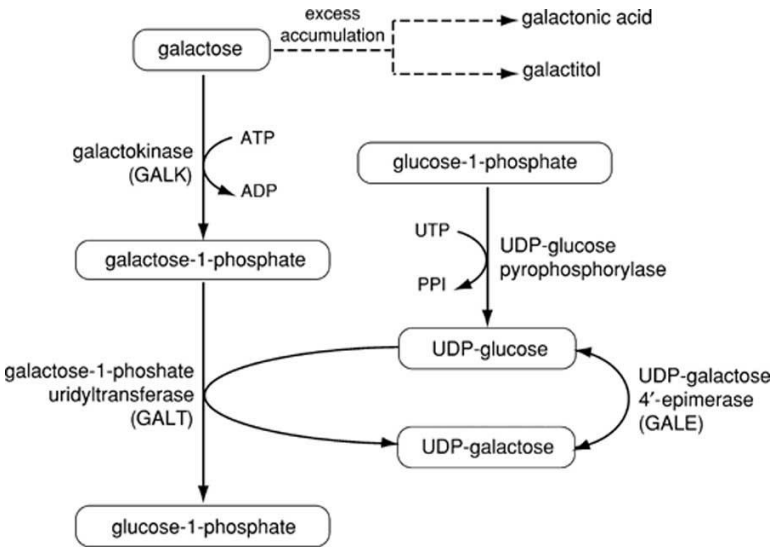



**Figure 1:** Galactose Metabolism

### *Prevalence and Genotype Worldwide*

There is a characteristic distribution of the frequency of specific GALT mutations and incidence of CG in different ethnic groups. In the USA, the incidence of CG from 1999-2009 was 0.020/1,000 births or 1:50,000 newborn infants.[4] CG seems to be more common in those of European descent. CG occurs in 1:30,000 live births in European countries.[4,12] The indigenous Irish

Travelers have the highest incidence rate of 1:480 births.[12] Turkey has a reported 1:23,000 incidence rate.[13] Genetic screening has become a valuable tool in assessing ancestry, prevalence, and detection of CG worldwide.

Common mutations in the GALT gene include Q188R, K285N, S135L, and N314D. For both Irish Travelers and those of predominant European descent the most common defect is seen at Q188R.[4,13,14] The wild type glutamine 188 residue stabilizes the uridyl-enzyme intermediate by establishing two hydrogen bonds with the α- and ß-phosphoryl oxygens on UDP-glucose. The Q188R mutation replaces the glutamine residue with an arginine residue. Arginine can only establish one hydrogen bond with the intermediate, thus decreasing its stability.[9] **(Figure 2)** More importantly, this change in hydrogen bonding slows the nucleophilic attack of UDP-glucose by galactose 1-phosphate and creates steric hindrance decreasing the hydrogen bonds between the subunit dimers of GALT.[9]

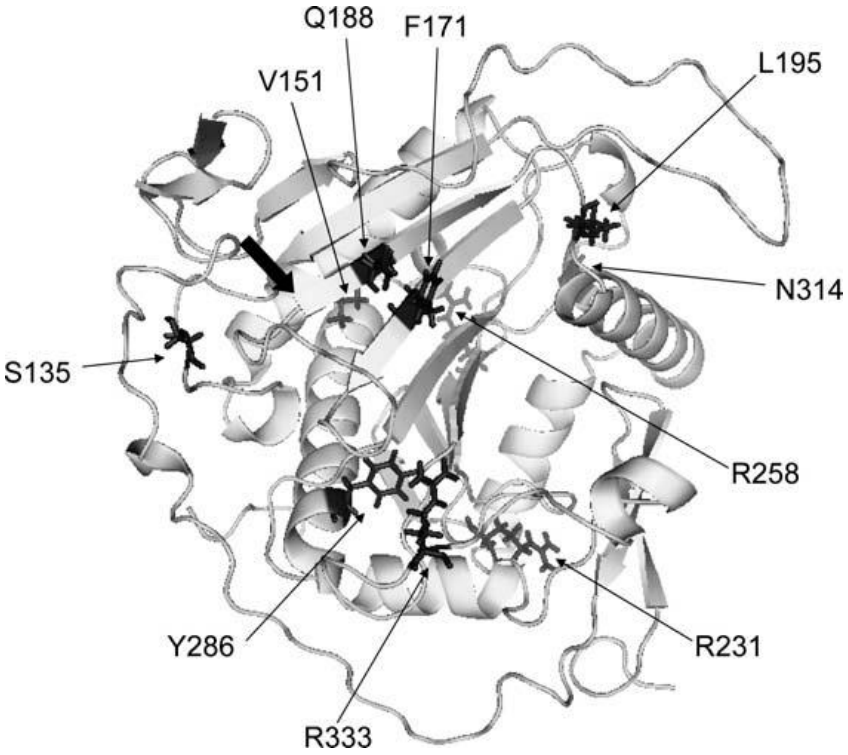



**Figure 2: Ribbon Diagram of GALT Protein and Common Mutated Residues** location of amino acids that are altered by mutations in human GALT. Variants are shown in dark grey in one subunit of the homodimer. The position of the active site histidine (His-186) is indicated by the black block arrow. The model was obtained using the online molecular modeling service and McCorvie et al review[9].

Overall, Q188R is responsible for the majority of mutant chromosomes in CG, but there are significant differences in its relative frequency in individual populations. The frequency of the Q188R mutation in different countries has been reported as follows: Ireland 93.6%; Great Britain 77%; Germany 69%; Austria 60%; Portugal 57.8%; The Netherlands 58.5%; Spain 50%; Poland 51.3%; the Czech Republic and the Slovak Republic 46%; and Hungary 45%.[13] As one can observe from these reported frequencies, the prevalence of the Q188R mutation decreases from the west to east and north to south on the European continent. Those with homoallelic Q188R mutation have no detectable enzyme activity and a severe CG phenotype.[11,14]

Conversely, the S135L mutation is the prevalent cause of galactosemia among those of South African and African American descent.[14,15] The S135L mutation causes various degrees of enzyme impairment among different tissues. The S135L mutation displays little to no activity in erythrocytes, but does cause a greater detectable GALT activity in leukocytes when compared to other missense mutations and produces an approximate 10% residual activity in the liver and small intestines.[14-17] Thus, patients homozygous for S135L may have a milder phenotype and better clinical outcome than those with other galactosemia genotypes.

Undoubtedly, there is considerable genetic heterogeneity described in the literature resulting in a high degree of observed phenotypic heterogeneity in the CG population. Examining the correlation between genotype's effect of the mutation at the protein level and the resulting clinical phenotype may help to predict the outcome of the disease, including the origin of the complex, long-term clinical deficits observed in adult patients. In addition, it is important to examine not only genetic variation but the effects of nonallelic variation and other constitutional factors on the phenotypic variability observed in this population.

### *Initial Presentation and Diagnosis*

A CG infant who ingests breastmilk or a whey-based formula will first develop poor feeding, vomiting, and lethargy. With continued ingestion of galactose, undiagnosed infants will develop liver dysfunction and jaundice followed by renal failure, cerebral impairment, cataract development, and

breakdown of gut integrity leading to an *E. coli* sepsis infection that causes a 30-50% mortality rate for those left untreated.[2,18] The mortality rate has significantly decreased with early newborn screening and subsequently immediate initiation of treatment.

In the 1980s, CG was added to the newborn screening panel in the USA. Two screening tests are used to detect galactosemia in a two-tiered sequence.[19,20] First a quantitative GALT assay is performed; the GALT enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells (RBC). An abnormal result is a diminished or absent fluorescent activity and is found in 1:2,000 infants.[20] This assay does not differentiate milder variants from severe defects in GALT activity. Thus, all infants are screened with the GALT test and any infant with an abnormal GALT activity will then be screened with the second tier, the Hill test. The Hill test is a fluorometric chemical spot test that measures galactose and gal-1-P which are greatly elevated in neonates with galactosemia. If an infant has both an abnormal GALT and abnormal Hill test, the newborn screening lab will contact the infant's Primary Care Provider (PCP) and a referral to a metabolic program to request confirmatory tests to diagnosis the infant with CG. These follow-up tests include a GALT enzyme assay, RBC gal-1-P, and mutation analysis of the GALT gene. The newborn screening tests to detect CG have 99% validity.[20]

Although complications including failure to thrive, feeding difficulties, hypotonia, brain edema, cataracts, hepatomegaly, and renal tubular dysfunction can develop before newborn screening results become available, screening has

allowed for earlier diagnosis and initiation of dietary treatment. With treatment, any acute complications resolve and RBC gal-1-P concentrations typically decreases into the treatment range within seven to nine months of age.[21]

***Diet Treatment***

Newborns diagnosed with CG have the same nutritional requirements as healthy infants.[14,22] With a suspected diagnosis of CG, the infant should immediately stop consumption of breast milk or whey-based infant formula due to the high content of lactose. Lactose is a disaccharide composed of D-galactose and β-glucose joined by a β1-4 glycosidic linkage. Thus, any food high in lactose will subsequently be high in galactose. Infants with a positive screen for galactosemia should be placed on an infant formula with minimal galactose immediately, without waiting for final confirmation of the diagnosis.[23,24]

A soy based infant formula containing soy protein isolate as the protein source is the recommended diet treatment.[14,23,24] All forms of soy based infant formulas contain negligible amounts of galactose and are appropriate for treatment. Compared to powdered soy formulas, liquid concentrate and ready-to-use soy infant formulas contain some galactose from carrageenan.[14] Carrageen molecules, found in red seaweed, form helical structures that have thickening and emulsifying properties as a food additive.[25] Carrageen is a large, polysaccharide molecule with repeating galactose, 3, 6 anhydrogalactose, and sulfate galactose units joined by alternating β 1–4 and α 1-3 glycosidic linkages. Carrageen is considered a dietary fiber and cannot be hydrolyzed by intestinal

enzymes of both humans and monogastric animals.[26] Thus, the galactose present in ready-to-use and liquid concentrate infant soy formulas are safe for consumption by infants with CG.

Soy formulas are contraindicated in premature infants, including those diagnosed with CG.[23] Soy formulas have been found to increase a preterm infant's risk for premature osteopenia by increasing serum alkaline phosphate and decreasing serum phosphorus concentrations when compared to serum concentrations in preterm infants fed cow's milk based formula.[27,28] In premature infants with CG, L-amino acid based elemental formulas are recommended. Furthermore, CG infants presenting with acute hepatic dysfunction and possibly limited absorption, casein hydrolysate infant formulas or L-amino-acid elemental formula should be considered over soy based formulas.[14,24] Elemental formulas contain no galactose and are considered for infants with erythrocyte gal-1-P concentrations that are decreasing too slowly.[14] Published case studies of 3 infants with CG showed a more rapid decrease in RBC gal-1-P after changing from a soy based to an elemental formula at 4 to 6 months of age.[21,29] Yet, no formal studies have been conducted to make an evidence-based recommendation for the routine use of elemental formulas over soy based formulas in infants with CG. They are only prescribed if there is a specific indication for their initiation.[14]

Introduction of complimentary and solid foods to infants with CG follow the same developmental stages as for the general pediatric population. Gastrointestinal symptoms of constipation and nausea are common in children

with CG with an increased incidence four to five fold greater than the general pediatric population.[30] There may be a correlation between gastrointestinal symptoms and dietary restriction in early childhood, yet this needs to be further elucidated, The benefit and extent of galactose restriction beyond infancy remains unclear and clinics have varying protocols in dietary treatment recommendations around the world.[14]

Dairy products are the major contributor of lactose and galactose in the diet after infancy; 100 ml of milk contains 2400 mg of galactose.[31] Thus, there is consensus among practitioners to eliminate dairy-based foods and ingredients from the CG diet. Yogurt, cottage cheese, and most other dairy products contain high concentrations of lactose and thus, galactose. Yet, with food processing, lactose and galactose concentrations can be significantly reduced to allow some dairy products in the diet for CG. For example, cheese is a fermented food product in which the lactose in milk is converted to lactic acid by the action of bacteria. A variety of factors in cheese production, such as the starter bacteria strain, aging temperature, and length of aging, can eliminate lactose and galactose making some aged, hard cheeses acceptable to include in the CG diet.[14]

Cheese is made through the separation of the whey and casein proteins found in milk. Cheese production begins when a starter culture of bacteria and/or renin is added to milk for acidification by converting lactose to lactic acid. This allows the milk to form a gel which is cut or broken and the gel pieces contract to release liquid whey. The solid casein fraction, also referred to as curds, is

suspended in the liquid whey. Syneresis or the process of removing moisture from the curds is enhanced by heating the mixture. This temperature can also influence the amount of lactose removed. Eventually, the solid curds are separated from the liquid whey. Since lactose is a water-soluble molecule, a majority of lactose remains in the liquid whey. Whey is an important by-product of cheese manufacturing and is added to many commercial food products. Whey has a significant amount of lactose and should be avoided by CG individuals.

Once separated, the curds are molded, shaped, and manipulated depending on the variety of cheese being produced. The casein curd fraction still contains a significant amount of residual lactose. Finally, the cheese will be aged or ripened. The conditions under which the cheese is ripened and the period of aging determines the final lactose and galactose content of the cheese.[14,32] The UK GSG Medical Advisory Panel recommends cheese should have a lactose and galactose consistently below 10 mg/100 g for its inclusion in a low galactose diet.[33]

Gruyere, Jarlsberg, mature Cheddar cheese, Comte (aged greater than 12 months), brick Italian Parmesan (aged greater than 10 months), and Emmentaler, Swiss Fondue or Swiss cheese have been found to contain negligible galactose due to their aging and production processes.[14,32,33] It is important to note, not all mature Cheddar cheese is processed in the same way and will have varying amounts of lactose. Cheddar chesses produced by small regional dairies using traditional cheese-making techniques were found to have lower galactose concentrations than similar cheeses produced by large-scale,

national manufacturers.[14] In the traditional manufacture of Cheddar cheese, the lactose content decreases as cheese dries naturally over many months in truckles covered only by a cloth or dried in blocks or covered in a rind. However, in large-scale manufacturing, the cheese will undergo maturation within its plastic wrap packaging. Thus, maturation within the package does not decrease lactose levels in the cheese.[33]

While there is consensus among clinicians to eliminate most dairy products from the CG diet, there is more variability about allowing various plant products that contain minor amounts of galactose.[34] Many fruits, vegetables, and legumes contain free and bound sources of galactose. Fresh and processed fruits and vegetables contain a free galactose content ranging from < 5 to 77 mg of galactose in 100 g of food.[14] Free galactose can be absorbed by the human GI tract and contribute to dietary galactose intake. Plants also synthesize a multitude of complex carbohydrates, mostly found in the cell wall. Cell wall polysaccharide structures are diverse and vary between individual cell types and different developmental phases. Galactose can be “bound” in various cell wall polysaccharides but these sources of galactose are not digestible in the human gastrointestinal tract. Other forms of bound galactose in plant tissues are conjugated with proteins and lipids. Upon ripening, plants produce both alpha- and beta-galactosidases that can release galactose from the cell wall.[14] The metabolizable content of free galactose and galactose oligomers in plant tissues available in the human gastrointestinal tract varies based on many factors such as the ripeness, and developmental stage of the plant.[14,32,35] **(Table 1)**

Acosta and Gross reported that various legumes contain a high concentration of free galactose, especially garbanzo beans.[36] Yet, a more recent study reanalyzed commercially canned garbanzo beans and other legumes and found significantly less free galactose in beans and canning liquid than Acosta and Gross' findings.[32] This study reported a higher level of free galactose in the canning liquid and the galactose content of legumes can be reduced by discarding the cooking liquid and rinsing canned beans before consumption.

When studies finding free galactose in plant-based products were published in the 1990's, many clinicians started to restrict fruits, vegetables and legumes with higher amounts of free galactose from the CG diet. Yet, further studies did not find improved clinical outcomes for individuals who restricted these minor sources of galactose from the diet. Thus, newer recommendations do not advocate restricting these plant-based foods, [37]

Most of the galactose found in soybeans and soybean food products is bound and not digestible by the human gastrointestinal enzymes and, thus, are safe for consumption by patients with CG. Once the soybean is fermented, free galactose is released from various oligosaccharides found in the soybean. Thus, fermented soy products contain significantly greater amounts of free galactose compared to unfermented soy products. Fermented products include soy sauce, miso, natto, tempeh, and sufu (fermented soy cheese).[14,32] Most of these products are used as condiments in very small quantities in the diet. This should be considered when making recommendations to include fermented soy products in the diet for individual patients.

Dietary treatment can reverse acute neonatal manifestations. Yet, with lifelong dietary galactose restriction, elevated concentrations of galactose metabolites persist and potential long-term complications can still develop; these include intellectual disability and developmental delays,[4] abnormalities of motor function,[1] verbal dyspraxia,[4,16] speech abnormalities,[4] clinical depression, anxiety,[4] primary ovarian insufficiency in females,[8,16] abnormal growth,[5] low bone mineralization,[6,7] and abnormal body composition.[2,3] These complications do not seem to be related to dietary galactose restriction and their etiology is not completely understood.[4,38,39] Jumbo-Lucioni et al surveyed 5 continents representing 11 countries on their management of CG.[40] They found no clear correlation between differing approaches to care and long-term outcomes. Negative outcomes were present in the majority of patients and found to be independent of treatment initiation, restriction of galactose, or extent of patient follow-up. This global comparison demonstrates that there is no one best practice for managing CG, although recently published international guidelines are attempting to standardize treatment protocols for this population.[37]

| Table 1: Reported Galactose in Various Foods | |
|---|---|
| **Food Product** | **Galactose Content (mg/100 g food) Mean ± SD** |
| Cheddar Cheese | 9.5 ± 17.9 |
| Gruyere | 4.1 ± 1.2 |
| Parmesan (aged > 10 months) | 18.3 ±13.3 |
| Various Fruits (raw or processed) | 9.7 ± 7.9 |
| Various Vegetables (raw or processed) | 9.3 ± 11.4 |
| Fruit and Vegetable Juice | 18.3 ± 14.0 |
| Garbanzo Beans (cooked or processed) | 148.5 ± 197.0 |
| Other Legumes (cooked or processed) | 46.2 ± 63.1 |
| Tofu, silken | 90 (dry weight) |
| Soy Sauce (mg/100mL) | 290.7 ± 121.2 |

Van Calcar SC, Bernstein LE, Rohr FJ, Yannicelli S, Berry GT, Scaman CH. Galactose content of legumes, caseinates, and some hard cheeses: implications for diet treatment of classic galactosemia. *J Agric Food Chem.* 2014;62(6):1397-1402.

### *Endogenous Galactose Production*

Continued elevations in erythrocyte gal-1-P content in patients with CG despite elimination of lactose/galactose from the diet, as well as elevated gal-1-P concentrations found in the cord blood cells of newborns with CG who were born to women on a lactose-free diet and thus were never exposed to an exogenous source of galactose, suggests that there must be an alternative pathway to produce gal-1-P from other means than just exogenous galactose intake.[39] In 1969, Gitzelmann and Steinmann[41] were the first to propose a mechanism of endogenous galactose synthesis from turnover of glycoproteins and glycolipids. In 2001, Berry et al[38] established that there must be an alternative non-GALT oxidative pathway by feeding an oral bolus of isotope labeled galactose to a patient with CG and collecting breath samples over 24

hours to measure the amount of administered galactose that was oxidized.[38] An age matched control was able to oxidize and eliminate over 15% of the administered galactose in three hours whereas it took 20 hours for the patient with CG to eliminate 15% of the administered galactose.[38,39] The data suggests that even with 0% GALT activity, a patient with CG can oxidize the same amount of galactose as a healthy control, but only after a much longer time.

Additional studies have investigated endogenous galactose production by continuous infusion of a stable isotope tracer of D-galactose to determine the apparent rate of endogenous galactose appearance over 24 hours in CG patients.[39,42] These findings supported the theory of endogenous production of galactose from glycoprotein and glycolipid turnover by detection of unlabeled $CO_2$, galactose metabolites, and galactose in plasma. The whole body endogenous galactose synthesis rate in CG patients was originally estimated as 0.76-1.05 mg/kg/h by Berry et al.[38] This was later disputed as an overestimation due to analytical shortcomings.[42] Newer research estimated the synthesis rate for adults (n=6) with CG to be 0.58 mg/kg/h ± 0.12 SD. The production of galactose in infants and children was significantly higher (p=0.002) at 1.38 mg/kg/h ± 0.73 (n=17) suggesting that CG patients' de novo synthesis of galactose declines with age. [39]

A subsequent study[42] investigated whether the galactose release rate from endogenous sources might be growth related. It would be expected to see an accelerated turnover rate of endogenous galactose during periods of growth and a cessation in adulthood. The data fit well to a simple growth rate model, finding

an exponential decrease in endogenous production rate after puberty until reaching a constant rate in adults.[42] This further supports the theory that the primary source of endogenous galactose is from a basal glycoprotein and glycolipid turnover, necessary for tissue maintenance and integrity. This endogenous source of galactose results in elevated gal-1-P and galactose metabolites in infants and children with CG and may account for the long-term complications observed in this population.

Current research suggests that the liver clears a three-fold higher amount of galactose released from peripheral tissues in infants with galactosemia compared to adults.[42] Isselbacher hypothesized[43] that dietary galactose tolerance might increase with age by up-regulation of the hepatic UDP–glucose pyrophosphorylase (UDPGP) enzyme. UDPGP catalyzes conversion of glucose 1-phosphate to UDP-glucose, but can also use gal 1-P as a substrate to form UDP-galactose, thus providing an alternative pathway to utilize galactose in individuals with CG.[43] **(Figure 1)**

It is estimated that endogenous galactose production is approximately 10 times greater than the average 50 mg galactose in the restricted diet consumed by children and adults with CG. (**Table 2**).

***Potential nutritional consequences of a galactose restricted diet***

Since recent studies demonstrate that endogenous galactose production greatly exceeds exogenous dietary galactose consumption from plant sources, lactose from dairy sources has become the primary source of galactose that is

| Table 2: Apparent Galactose Appearance Rate | | | | |
|---|---|---|---|---|
| **Age** | **Average Rate of Endogenous Galactose Production (mg/kg/hr.)** | **Average Weight** | **Daily Milligrams of Endogenous Galactose** | **Exogenous Galactose from CG Restricted Diet** |
| **Infant** | 1.45 mg/kg/hr. | 10 kg | 350 mg | 0 mg |
| **Child/Teen** | 1.0 mg/kg/hr. | 35 kg | 840 mg | 50 mg/day* |
| **Adult** | 0.67 mg/kg/hr. | 70 kg | 1125 mg | 50 mg/day* |
| *Approximate galactose content of a typical galactose-restricted diet including 3 servings of fruits and vegetables with a galactose content of >20 mg/100 g food. | | | | |

Schadewaldt P, Kamalanathan L, Hammen HW, Wendel U. Age dependence of endogenous galactose formation in Q188R homozygous galactosemic patients. Mol Genet Metab. 2004;81(1):31-44.

restricted in the prescribed diet treatment for CG. Recent guidelines do not recommend restriction of minor sources of galactose primarily found in plant products.[37]

The primary nutritional concern from the required dietary restriction is consuming adequate calcium and vitamin D from dietary sources, which becomes more difficult once a child is no longer consuming a fortified soy formula.[1,14] It is standard practice to prescribe calcium and vitamin D supplements; but compliance issues may lead to nutritional inadequacies.

The galactose-restricted diet is considered a primary risk factor for bone loss and/or inadequate accrual of nutrients that play a key role in body composition and bone physiology. Concentrations of calcium in serum has been found to be a significant predictor of BMD in patients with CG and low total 25-

hydroxyvitamin D concentrations have been noted in several studies.[6,44] Thus, diet may have a dramatic effect on a patient's phenotype.

### *Metabolic Intoxication*

Exogenous and endogenous galactose is a major component of complex carbohydrates in glycoproteins and glycolipids in the nervous system and defective glycosylation impairs neurodevelopment and neurological function.[40] Decreased exogenous galactose may lead to poor incorporation of galactose into protein and lipid structures. Glycosylation defects in muscular dystrophies (MDs) and congenital disorders of glycosylation (CDGs) are characterized by severe neurological impairments causing hypotonia, strabismus, developmental delay, and an underdeveloped cerebellum. CG has been referred to as a secondary disorder of glycosylation since patients with CG experience many of the same neurological symptoms as individuals with CDG. There is long standing literature documenting glycosylation defects in those with CG finding abnormal patterns of glycoproteins and a deficiency of glycolipids containing galactose or N-acetylgalactosamine in postmortem brain tissue.[45,46]

With deficient GALT activity, endogenous and exogenous sources of galactose results in elevated gal-1-P concentrations and a product inhibition of GALK. Galactose concentrations increase and ultimately are diverted into the secondary pathways of aldose reductase forming galactitol and galactose dehydrogenase forming galactonate.[14] **(Figure 1)** Accumulation of these

metabolites can lead to cell intoxication and cell death, possibly playing a role in the pathophysiology observed in CG.[1,7]

Galactitol seems to play a prevalent role in the pathology in CG. The GALT knockout mouse develops similar biochemical features to those observed in humans including high concentrations of gal-1-P, but have minimal elevations of galactitol in tissues. These mice do not develop cataracts, which form in humans from an accumulation of galactitol in the cells of the ocular lens. The mice have normal reproduction and do not demonstrate neurological impairment as seen in humans with CG.[1] Thus, GALT deficiency, elevated gal-1-P and galactitol are all involved in the human phenotype.

The variability of clinical outcomes observed in patients with CG could be related to heterogeneity of the inherited GALT allele activity, individual endogenous production of galactose, individual ability to oxidize galactose, and the function of alternative enzymes in metabolic pathways resulting in different levels of metabolic intoxication. Yet, studies have not been able to demonstrate a strong correlation between RBC gal-1-P and serum galactitol concentrations to clinical outcomes.[1,47] In a retrospective cohort study of siblings[48], there were no significant differences in long term outcomes, despite differences in early neonatal course. For example one pair of siblings, the eldest sibling was diagnosed after becoming symptomatic and was not started on a therapeutic diet until 63 days of life; while the younger sibling was diagnosed and treated during the first week of life, yet these siblings do not exhibit differences in long-term outcome, Similar sibling pair studies have corroborated this finding,

demonstrating that the pathophysiology is multifactorial in origin and not solely related to metabolic intoxication.[16,47]

### *Glycosylation Deficits and Long-term Outcomes*

Abnormal elevations of galactose, gal-1-P, and galactitol in CG alters glycoprotein and glycolipid production.[46,49] Elevated cellular concentrations of gal-1-P competitively inhibit the galactose metabolism pathway, the inositol monophosphatase pathway, and hepatic glucosyltransferases.[46,50] Hepatic glucosyltransferases are critical enzymes in glycoprotein assembly and processing and glycolipid synthesis for myelin production.[40,49,50]

High gal-1-P concentrations in neonates with galactose-intoxication promotes endoplasmic reticulum (ER) stress, which, in turn, disrupts protein folding.[49,50] This severely disrupts the assembly of N-glycans in the ER of neonates with CG. Coss et al[50] observed that an untreated neonate with CG had both N-glycan assembly and processing defects from an accumulation of incorrectly glycosylated N-glycans with decreased sugar residues and altered glycan branching. Other studies[40,45,49,50] have observed similar abnormalities in glycosylation of N-glycans in serum transferrin from untreated neonates with CG. After introduction of galactose restriction, the gross assembly abnormalities are largely corrected.

In treated children and adults with CG, N-glycan assembly defects are not evident, but N-glycan processing in the Golgi body of the cell seems to be impaired. Studies have shown that treated pediatric and adult patients with CG

continue to have decreased galactosylation of the N-glycan structure relative to healthy age-matched controls suggesting ongoing processing defects, with poor incorporation of galactose and sialic acid.[43,49] These subtle, but chronic, glycosylation processing defects are observed only in some patients despite adherent dietary treatment and raises the possibility that deviations in glycosylation might contribute to the long-term complications experienced only by a subgroup of treated patients.

Coss and colleagues concluded that Immunoglobulin-G (IgG) N-glycan profiles may be a highly sensitive marker of galactose tolerance; N-glycosylation patterns in serum from treated CG patients showed consistent individual alterations in response to a partial and transient diet liberalization over a 16-week period.[46,49] There was significant individual variability in galactose tolerance levels even in patients with identical genotype and siblings with an identical genotype. At a galactose intake of 1,000 mg/day, N-glycan profiles were improved in all but one patient, with decreases in a-galactosylated (G0) and increases in di-galactosylated (G2) N-glycans. This suggests that CG is a modifiable galactosylation disorder with an alternative pathway for galactose oxidation.[40,46]

Strict galactose restriction is life-saving in the neonatal period, but it is important for future research to explore the best way to determine the optimal galactose intake for older individual patients to optimize glycosylation patterns, and perhaps prevent long-term neurological complications. It is important to target pediatric and young adults as the process of brain myelination in humans

extends into at least the first 20 years of life and complications observed in adults are irreversible.[14]

Patients experience a range of severity of neurological complications and they seem to be related to, yet independent of, glycosylation patterns suggesting that the pathophysiology is multifactorial in nature. Reduced brain myelination has been shown from neuroimaging studies.[50,51] This might be due to lower levels of galactose-containing glycolipids in myelin and reduced galactocerebroside.[50]

Decreased glycoproteins could also play a role in the neurological motor dysfunctions of ataxia and dystonia observed in CG. GALT, GALK, and UDP-glucose have been shown to play a role in the biosynthesis of the heavily glycosylated neuromuscular junction (NMJ) synaptomatrix, which has a crucial role in synaptogenesis during normal development, and its disruption from defects in glycosylation is implicated in numerous heritable disease states [40] The GALT-deficient *Drosophila* disease model has shown loss of GALT activity impairs coordinated movement, synaptomatrix glycosylation losses, altered trans-synaptic signaling pathway components, defective synaptogenesis, and structural synapse overelaboration.[40] The glycan loss in the NMJ synaptomatrix is a potential underlying pathogenesis of motor dysfunctions reported in CG.

Glycosylation can also affect protein folding, effector functioning of proteins, half-life of proteins and the biological activities of many other signaling and receptor proteins. This may have a functional systemic effect on the

dysregulation of leptin receptor glycosylation, IGF binding protein and follicle stimulating hormone (FSH) even in treated patients with CG.[49,52]

### *Diet Independent Long-term Complications in Classical Galactosemia*

Unfortunately, even with pre-symptomatic diagnosis and strict life-long dietary intervention and compliance, a majority of patients with CG experience a constellation of troubling long-term complications.[1,4] These include cognitive and/or behavioral impairment, motor dysfunction (dystonia, tremors, ataxia), poor myelination, scattered white matter (WM) abnormalities, cerebellar atrophy, and altered cerebral glucose metabolism.[4,53] As a result, at least half of all patients with CG experience learning disabilities, diminished IQ, executive functioning deficits, and speech disorders.[4] Low BMD, poor growth and abnormal body composition have been observed.[2,3,6,7] In addition, primary or premature ovarian insufficiency (POI) is noted in more than 80% of females.[4,7,16]

Most of the evidence does not suggest this sequelae to be progressive or degenerative in nature.[1] However, Waisbren et al[4] reported high levels of depression and anxiety among 39-67% of patients with CG, ranging from 18-59 years of age. There was a much higher prevalence among males. Logistic regression analyses revealed age to be a strong predictor of depression with a 3-fold increase in odds of developing depression for every 10-year increment of age.[4]

### *Gonadal Function*

The most common long term complication observed in CG is hypogonadotropic hypogonadism, affecting 80-90% of female patients.[4,8,16,54] There is a spectrum of severity. Some women have primary amenorrhea and require hormone therapy to achieve secondary sexual characteristics, while others do achieve menarche.[16] Yet, of those who are able to menstruate, most develop oligomenorrhea and eventually develop secondary amenorrhea. Similar to other complications noted in this population, the cause of the insult is unknown. It seems to be more common in those with homozygosity of the Q188R mutation with RBC gal-1-P concentrations greater than 3.5 mg/dL despite diet therapy.[16] Levels of gonadotropin are monitored and estrogen/ progesterone replacement is administered as necessary. Pregnancies without hormone replacement have been reported, but are rare.[1] Possibly significant to this pathology, follicular stimulating hormone (FSH) is quantitatively abnormal in females with CG showing reduced terminal glycosylation.[1,49]

The reproductive tract does not seem to be impaired in males. There have been preliminary reports of cryptorchidism and low semen volume.[55] There have been few reports of males with CG fathering a child, but this may be due to the high incidence of depression in males with CG rather than abnormal hormone concentrations.[4,56]

### Neurological Sequelae

Patients with CG are at risk for central nervous system (CNS) dysfunction including motor disorders, cognitive impairment, learning difficulties, psychiatric symptoms or, most commonly, speech and language problems. Motor complications have been reported in 18%–66% of patients with CG.[1,4,53] The most common feature is a progressive tremor, although a subgroup develops a more serious cerebellar ataxia and dystonia.[1,53] The cerebellum is particularly involved in targeted movements and learned motor functions. Disorders that damage the cerebellum cause ataxia with abnormal regulation of movement speed, force, and direction. In the limbs, this translates to poor targeting and poor rhythmicity of movement. In the trunk, this can create gait ataxia and poor balance. Poor coordination or "clumsiness" experienced by some patients with CG may reflect abnormally slow movements (bradykinesia), poor fine motor coordination, and stiffness (rigidity), all key features of basal ganglia dysfunction, as seen in Parkinson disease (PD), although, symptoms observed in CG are not as severe as in PD. Dystonia is an abnormal involuntary twisting posture. It is a key motor feature of disorders that impact the basal ganglia, such as PD and Huntington disease (HD). New research suggests that the cerebellum also contributes to the development of dystonia. While there is little in the literature describing dystonia in patients with CG, dystonia can cause “tremor”, which has been reported in this population.[53]

Both postmortem brains and neuroimaging studies confirm poor myelination, scattered white matter abnormalities, and cerebellar atrophy in some

patients, as well as abnormalities in glucose metabolism in many brain regions.[1,16,37,40,45] The mechanism is unknown, yet insufficient galactose donors for synthesis of myelin glycolipids, such as galactocerebroside, is a proposed theory.[50,51] Metabolic intoxication has also been suggested to affect white matter.[1] A recent study[57] reported the coexistence of Friedreich ataxia (FRDA) and CG in nine children from Irish Traveler families with the Q188R allele. This co-occurrence is most likely due to linkage disequilibrium, as the loci for these two disorders are located on either side of the centromere of chromosome 9. Thus, it is important to consider FRDA in a child with CG presenting with ataxia.

One limitation of neuroimaging research is that the diffuse white matter changes in the CNS has not been quantified through magnetic resonance imaging (MRI) studies. This makes it impossible to interpret these findings as a continuous variable in regression analyses with outcome variables, such as IQ or motor function. Also, since white matter abnormalities are present in almost all individuals with CG, it is not useful as a variable for intergroup comparisons to measure the degree of impairment. The general variable “white matter abnormalities” does not seem to be a predictor of significant IQ outcome, ataxia, or tremor.[1,16]

In 2015, Timmers et al[51] was able to quantify microstructure WM pathology in eight patients with CG and eight age- and gender-matched controls through diffusion-weighted imaging. Fractional anisotropy (FA) was measured to reflect the degree of anisotropic diffusion, which is higher in white matter than grey matter because of formed fibre bundles. FA can be decreased in white

matter from axonal degeneration or myelin breakdown depending on the disease state. FA is very sensitive, but is non-specific. A reduction in FA can be caused by a decrease in neurite (axons and dendrites) density or an increase in dispersion of orientation, and various other factors. To distinguish between the two key contributors to FA, an approach called neurite orientation dispersion and density imaging (NODDI) was also used in the study to assess WM microstructure and establish relationships with the observed cognitive profile in patients with CG.

Abnormalities in both the density and the orientation dispersion of axons of the white matter microstructure in patients with CG. The CG group had lower FA values over almost the entire cerebrum. A lower neurite density was found in the anterior of the brain bilaterally. A higher orientation and dispersion in WM was mostly left-localized. These specific regions of abnormalities relate to the cognitive profile observed in galactosemia. Motor skills for speech and language are left-lateralized in the brain and the anterior pattern of reduced neurite density is in accordance with higher order cognitive impairments and motor dysfunction. In addition, the WM properties were found to correlate with age, age of onset of the diet and with behavioral outcome such as visual working memory. Further studies are needed to corroborate these findings and provide timing of insult and mechanistic action. The authors' hypothesis that these changes in neurite density and dispersion could be due to poor myelination.

### Bone Mineral Density

CG patients often have decreased height-for-age and decreased BMD.[58] Dual energy X-ray absorptiometry (DEXA) has been useful in the investigation of BMD and body composition of CG patients. Low bone mass is most prominent in adults, but can be observed in prepubertal children.[2,3,5,58] Since bone mass increases quickly during puberty and reaches its peak in early adulthood, a low bone mass at an early age predisposes patients to osteoporosis and an increased risk of fractures later in life. One in four adults with CG will have low bone mineral density.[3] A Dutch cohort of 3-17 year old patients with CG were found to have significantly decreased weight and height z-scores, mean volumetric BMD, and mean aerial BMD z-score of the lumbar spine when compared to the reference population.[6] The underlying pathophysiological system of skeletal losses in CG patients is not well understood. There are several proposed mechanisms including nutritional deficiencies, decreased galactosylation of key bone mineralization proteins, alterations in the endocrine axis, and intrinsic factors related to bone metabolism.[50,58]

Normal bone physiology is a dynamic process of development and maintenance of skeletal integrity. Bone remodeling is a continuous, lifelong process of bone breakdown and renewal. Bone remodeling (formation), and bone resorption (removal) occur sequentially at the same anatomical location to preserve skeletal size, shape, and structural integrity, and to regulate mineral homeostasis. In childhood and adolescence, bone modeling and linear growth is

greater than bone remodeling. After peak bone mass has been reached, at approximately 26 years of age, bone resorption predominates.

Bone remodeling and formation is an intricate process involving endocrine and immune regulation. B-cells and T-cells play a role in osteoclastgenesis. Osteoclasts are cells responsible for bone remodeling or the removal of bone. Megakaryocytes promote osteoblasts, which are specialized bone forming cells that express parathyroid hormone (PTH) receptors and osteoclastogenic factors as well as produce bone matrix proteins for mineralization.

Bone resorption is activated by structural changes to bone or an endocrine signal, such as PTH. In response to a systemic change, PTH binds to an osteoblast and stimulates secretion of osteoclast precursors and matrix metalloproteinases (MMP). MMPs degrade the osteoid bone surface and expose RGD (Arg-Gly-Asp) adhesion sites for osteoclast binding. The osteoclast binds to the RGD tripeptide and creates a sealed zone microenvironment. Hydrogen ions are pumped into this space and in this acidic environment, bone demineralization occurs. The remaining bone matrix, mostly composed of collagen type-1 protein, is then degraded by collagenolytic enzymes. Collagen type-1 is a triple helical protein comprised of an amino terminal telopeptide (NTX) and a carboxy terminal telopeptide (CTX), non-helical ends that are linked by pyridinium cross-links to a nearby helical fibril.[59] Thus, urinary NTX and CTX are direct measures of the bone resorption process.[6]

Unknown reversal cells prepare the bone resorption site for transition into bone remodeling. While this process is not fully understood, insulin growth factor-1, insulin growth factor-II, and transforming growth factor-β (IGF-1, IGF-II, and TGF-β) secretion by hepatocytes is thought to be responsible for the signaling of osteoblast progenitors at the site of resorption.[6] Collagen type-1 is the primary organic compound of bone followed by numerous non collagenous material deposits, and an optimal extracellular concentration of inorganic phosphate allows mineralization to proceed. Once an equal quantity of resorbed bone has been replaced, the remodeling cycle is terminated.

One proposed mechanism for low BMD in CG is that patients may have abnormal collagen formation as a result of deficient galactose residues.[8,60] Biosynthesis of collagen type 1 requires several post-translational modifications, including lysine modifications that are critical to the structure and biological functions of this protein. Lysine modification of collagen is a highly complicated cascade process catalyzed by several groups of enzymes leading to the final step of covalent, intermolecular cross-linking. Before this final step, hydroxylysine residues located in the helical domain of collagen are glycosylated by the addition of galactose or glucose-galactose.[59]

In CG, it has been proposed that restriction of dietary galactose may lead to an abnormal collagen type-1 formation by decreasing levels of galactose available for glycosylation.[7] This theory is supported by a study in which calcium supplementation was able to improve, but not normalize bone density in patients

with CG, suggesting that other intrinsic abnormalities in bone and mineral metabolism may be involved.[54]

Endocrine abnormalities are another proposed mechanism for low BMD and decreased height in this population. Panis et al[6] found significantly decreased carboxylated osteocalcin, NTX, CTX, and IGF-1 z-scores in patients with CG compared to a Dutch reference population. Decreased IGF-1 z-scores were significantly correlated to decreased height and weight z-scores in thesepatients.[2] Panis et al speculated that lower IGF-1 concentrations may play a role in the decreased height and abnormal body composition observed in this study. The decreased serum IGF-1 concentration in children with galactosemia might be a result of abnormal glycosylation of IGF binding protein. The decreased serum IGF-1 concentration found in children with congenital disorders of glycosylation (CDG) is consistent with these findings in CG.

Batey et al[7] observed a higher percentage of adults with CG exhibit BMD Z-scores ≤−2.0 at the spine compared to the hip. Trabecular bone found in the spine is more hormonally responsive than cortical bone, the type of bone found in the hip. Another study found a significant decrease in osteocalcin and a significant inverse correlation of CTX and osteocalcin with BMD z-scores in female subjects with CG.[7] The 2016 international clinical guidelines for the management of CG[37] recommends BMD screening with DXA starting at age 8-10 years old.

## Body Composition and Low Lean Mass in Classical Galactosemia

While bone heath and growth patterns have been investigated in those with CG, the research on body composition in this disorder is extremely sparse. To date, there are only two studies[2,3] directly aimed at investigating body composition in this population. While both studies noted a significant lower lean tissue mass (LTM) in the CG group compared to the normal reference population, both found very different overall body composition, growth patterns, and diet treatments that may have affected the variability in outcomes and makes it difficult to directly compare the two groups.

In 2005, Panis et al[2] from The Netherlands evaluated body composition by DXA scan in 38 patients with CG, ages 3-17 years. The CG participants' mean FMI z-score ($\bar{x}$=0.32 vs control $\bar{x}$= 0.44, p<0.001) and LMI z-score ($\bar{x}$=1.20 vs control $\bar{x}$= 1.29, p=0.013) were significantly lower than reference data.[2] All adolescent females with delayed puberty were treated with hormone replacement. Separate analyses for prepubertal and adolescent patients showed equivalent results. There were no differences in FMI in either the male or female participants. LMI was significantly decreased in the females, but not in the males, possibly because of the low number of male participants (males n=13, females n=27) in the study. In linear regression analysis, height, weight and LMI z-scores were correlated with IGF-1 z-score. Weight-for-height z-score and FMI were correlated with soy intake but not IGF-1 z-score. The intake of vitamins, minerals, energy, and protein were adequate in this group, so deficiencies did not seem to play a role in the decreased height and abnormal body composition.

In 2014, Doulgeraki et al[3] from Greece evaluated body composition in 14 patients with CG and 8 patients with other galactose metabolic disorders (OGMD) ages 5-16 years, finding slightly different results than Panis et al.[2] Body composition was assessed using DXA scans and values were compared to published pediatric reference data. Muscle mass was calculated by subtracting total body bone mineral content from fat-free mass and converted to a z-score. Z-scores within -2 to 2 were considered normal, a z-score of -1 to -2 was considered low-normal, and <-2 was considered abnormal. To estimate adiposity, fat mass index (FMI) was calculated for each subject, using the formula: FMI=FM in kg/height in $m^2$. There were no statistically significant differences in growth ($p > 0.05$) between the study subjects when plotted on growth charts for the Greek population. LMI z-score was significantly decreased with the median at -1.92 and a range of -2.6 to 0.8 in CG subjects.[3] This suggests that the subjects had sarcopenia; a finding that was also reported in the Panis study. However, this body composition parameter has its limitations, as it does not reflect muscle function and muscle load. Thus, it cannot directly provide information on muscle strength.

Finally, nearly half of the study population in the Doulgeraki paper [3] had normal fat mass for age and sex. Five of the eleven CG patients had normal percent body fat. The remaining six subjects were classified as either overweight or obese based upon body fat percentage. Given the observed sarcopenia, an imbalance between muscle and fat mass was evident. Panis et al[2] reported low fat mass in subjects with CG as well, but that study population was comprised of

patients with poor growth and thus, are not directly comparable to the participants in the study[3] from Greece.

In the Doulgeraki study [3], the subjects with CG were all on a lactose-free, non-soy, casein based formula during infancy. Median BMD z-scores were normal (even with height-age corrections). There was no statistical difference in BMD or bone strength compared to a normal reference population. Participants in the Panis et al[2] study were on a soy-based formula during infancy. These subjects had decreased height z-scores and corrected target height z-scores.[5] Weight-for height z-score and FMI was correlated with soy intake, but no correlation was found between soy intake and IGF-1 z-score. No nutritional deficiencies were found in this group of patients.[2,5]

In both studies, there were positive correlations between FMI and bone strength and soy intake as well as a positive relationship between BMI and bone strength.[2,3,5,7] In the CG group from Greece[3], BMD was strongly correlated to LMI z-score, stressing the importance of assessing the muscle-bone unit in future research. These studies demonstrate that patients with CG are at high risk for abnormal body composition, yet the etiology remains elusive. Finally, few studies have explored the overall nutrition status of individuals with CG, including body composition, and various macro- and micronutrients that can impact bone metabolism. By addressing the relationships between these factors, this project aims to provide useful recommendations that can be applied in clinical practice to promote healthy outcomes in this population.

### *Assessing Physical Activity in Classical Galactosemia*

As PA is a positive determinant of muscle density and reduced muscle adiposity, it is important to measure PA when assessing body composition.[61] PA is defined as any bodily movement by skeletal muscles that results in energy expenditure.[62] There are several ways to measure PA, all with their own inherent limitations. The goal of assessing PA is to determine the intensity, duration, frequency, and type of activity. This can be achieved through direct observation, objective measurement devices, or self-report from validated questionnaires. Direct observation along with doubly labeled water (DLW) is the gold standard for PA research. Objective measurement devices include heart rate monitors, pedometers, accelerometers, and multiple sensor devices. These devices have become increasingly popular by decreasing subjectivity that is inherent to direct observation and self-report. Self-administered PA questionnaires have been traditionally used, but in general have shown to provide poor data in young children because of their limited recall ability and poor cognition of time.[62] Furthermore, children tend to have sporadic play versus a planned duration of PA which can make it difficult to observe and quantify in a report.

Given the complexity and cost of the gold standard of DLW technique research studies have increasingly adopted the use of objective measurement technologies such as accelerometers to assess children's PA.[63-69] The ActiGraph accelerometer models are the most widely used and cited throughout international literature.[63,67,68] Their widespread use in free-living studies is attributed to their ability to measure frequency, intensity and duration of activity

with precision and minimal invasiveness.[67] Accelerometers have shown a wide range of correlation with measures of oxygen consumption during validation in laboratory and field settings using standardized activities against portable calorimeters (r=0.62-0.93, r=0.45-0.93).[62,67] This large range is a product of protocol-related variations in the use of different monitors, monitor placement (e.g. hip, low back, thigh, ankle), the specific activities performed during measurement (i.e. ambulatory PA is more accurately measured), and the setting of activity (free living vs laboratory). ActiGraph accelerometers have demonstrated acceptable technical reliability and have been validated in children, adolescents, and adults.[66] Ekelund et al.[70] validated the ActiGraph in free-living children against energy expenditure measured by DLW. The accelerometer counts per min (cpm) correlate to PA level by $r = 0.58$ ($P < 0.01$).[70] This can be compared with correlations of self-report versus gold standard DLW measurements that are in the range of r = 0.00-0.2.[68] Thus, ActiGraph accelerometers are considered relatively precise and useful in community based, free-living research.

The ActiGraph GT3X® (ActiGraph, Pensacola, FL., USA) is a lightweight, solid state, tri-axial accelerometer used to collect motion data on 3 planes: vertical up and down (Y); horizontal right and left (X); and horizontal front and back (Z). When the ActiGraph is accelerated, a voltage signal is generated proportional to the intensity of the acceleration. The ActiGraph uses a piezoelectric acceleration sensor to filter collected samples at 30 hertz/second.[67,68] The samples are summarized over an investigator-specified

time sampling interval (1 sec, 4 sec, 15 sec, or 60 sec), called an “epoch”. Accelerations over a given epoch are converted into “counts” and recorded to the internal memory of the accelerometer. The ActiGraph will report counts which are linearly related to the intensity of the subject's PA during wear time.

The World Health Organization and Center for Disease Control (CDC) recommends children and adolescents engage in 60 minutes or more a day of moderate to vigorous physical activity (MVPA), including muscle strengthening activities at least three times a week.[58,69,71] According to NHANES 2003-2004 data which used accelerometers to measure PA, only 42% of 6-11-year-old, 8% of 12-15-year-old, and 7.6% of 16-19 year old children and adolescents in the US are meeting this recommendation.[69] Activity level differs by gender, age group, and ethnicity. Males, younger children, and non-white ethnicities are more active than their female, older, and white ethnicity counterparts. The prevalence of adherence to the recommendations from the NHANES data by gender was 48% of boys compared with 35% of girls ages 6-11 years old.[69] The gender difference increased during adolescence. For ages 12-15 years, adherence rates for boys was 12% and for girls was only 3%.[63,69] Among 5–17 year old children in the International Children’s Accelerometry Database (ICAD), only 9.0% of boys and 1.9% of girls achieved the CDC recommendations with the highest percentage of 13% among Norwegian boys.[68]

It is clear that the lack of PA is a global health concern among children and adolescents. Decreased participation in PA is associated with obesity and decreased physical fitness.[71] Various social environmental and child factors may

contribute to the amount of PA a child engages in. Some of the child factors include coordination, lack of confidence, exclusion from activities by peers, and withdrawal from feelings of incompetence or lack of necessary skill to participate. The literature on children with developmental coordination disorders shows a significant decrease in PA compared to healthy control subjects.[72]

PA has yet to be evaluated in the CG population. Yet, motor complications have been reported in 18%–48% of patients with CG.[53] Most common is the development of tremors with a smaller percentage experiencing ataxia and dystonia. Furthermore, there is also concern for social withdrawal and depression, especially in males with CG.[4] This increases the likelihood that children with CG are at risk for decreased participation in PA which could result in fewer opportunities to develop proficient motor skills and an increased risk of weight problems and lack of physical fitness.[72] In order to develop effective interventions for the observed body composition abnormalities in CG, it is important to evaluate patients' level of PA and barriers to their involvement in PA.

Accelerometer data must be processed to have meaningful interpretation of intensity of an activity over a period of time. Traditionally a 60 second epoch was used with older accelerometers.[67,68] Newer technology allows for more precision in a 10 second epoch interval. After import of data, files can be converted into a standard 60 second epoch which can then be applied to cut-points to determine the amount of time a participant spent in sedentary, light, moderate, MVPA, or vigorous PA.

### *Accelerometer Data Cleaning*

The first step in preparing accelerometer data for interpretation is to separate valid raw data from potentially corrupt data from a malfunctioned unit. Files with corrupt data, temporally shifted data, or a potentially spurious data that does not return to baseline (zero), or a malfunctioned unit should be excluded from further analysis.[68] A 'temporally shifted' file shows the time stamping of the data is shifted with a greater than expected consecutive zeros during the day and activity counts during the night on each day of monitoring. A plateau or 3 consecutive counts at the same number at a count ≥ 10 are a good indicator of technical fault.[68] Spurious data is considered greater than or equal to 30,000 counts per minute (cpm) or greater than 10 minutes of the same repeated non-zero counts.[69]

Next, data collected while the device was not being worn must be excluded from further calculation. This is called establishing wear-time. Wear-time is determined by subtracting non-wear time from 24 hours. Non-wear time is usually defined by an interval of at least 60 consecutive minutes of zero activity intensity counts, with allowance for 1-2 minutes of counts between 0 and 100 counts.[63,68,69] Non-wear time counts are coded as "missing" to separate the non-wear zeros from legitimate zeros that occur in periods of sedentary behavior.

### *Accelerometer Cut Points for Determination of Physical Activity Intensity*

A MET is a physiological measure of energy expenditure during a PA. One MET is considered the resting energy expenditure (REE) of an average person at rest. METs can be thought of as an index of intensity of different physical activities, such that an activity with a MET of 5 means that during that activity an individual will be expending five times the amount of energy they would at rest.

Calibration studies focus on creating a link between accelerometer counts and PA intensity. These studies establish “cut-points” or thresholds by categorizing accelerometer output (counts per a given epoch) into time spent in various physical activity intensity levels (i.e., sedentary, light, moderate, and vigorous). The epoch is traditionally 60 seconds and PA intensity categories are developed from accelerometer output in cpm. In cut-point calibration studies, participants perform various types of field or laboratory activities while wearing an accelerometer along with the concurrent measurement of energy expenditure from a criterion measure, such as indirect calorimeter. Accelerometer activity counts are then compared to the criterion measure (e.g., metabolic equivalent of task, METs) to establish cpm into corresponding defined values for energy expenditure of MVPA. For example, moderate intensity, 2020 cpm, or METs=3 are all regarded as equivalent “cut-points” for moderate intensity PA.[67,69] Based on accelerometer cpm at or exceeding the defined cut-points for moderate intensity but below vigorous intensity are considered time in moderate intensity PA. MVPA is used to refer to the amount of time a participant spends at or above the moderate activity cut point, indicating “significant” activity.

### *Pediatric Accelerometer Cut Points*

Accelerometers are used extensively to assess PA in children and adolescents. ActiGraph accelerometers are the most commonly used device in these studies and an ICAD has been established.[68] Various cut points have been developed and used by researchers when interpreting PA intensity in children and adolescents. There is no consensus on the best cut point to use to determine PA intensity in the pediatric population (3-17 years of age).[67] Thus there is variability of cut points used throughout the literature and inconclusive comparative validation reviews of pediatric PA cut points.[64,67]

Activity intensity estimates can result in variable outcomes when applying different cut points to the same raw data. This can affect outcomes and interpretation of the data making cross study comparisons impossible. For example, a review of European studies assessing PA with accelerometers reported that the proportion of children meeting guidelines for sufficiently active youth ranged between 3 to 100%, depending on the accelerometer intensity thresholds applied to the raw data.[66] The ICAD and comparative cut-point review focuses on six of the most common cut points used in pediatric PA research, Pate et al. (PT), Puyau et al. (PY), Freedson equation et al. (where the MVPA threshold can be either 3 METs (FR3) or 4 METs (FR4)), Van Cauwenberghe et al. (VC), and Evenson et al. (EV) are shown in Table **3** [64,68,69] demonstrates the discrepancies of different threshold measurements. Yet, recently Brazendale et al[64] formulated a conversion system from the 6 cut points listed above to

standardize minutes of MVPA to allow for comparisons across different research studies.

The Freedson et al equation is the most commonly used equation in pediatric and adult PA research.[63,64,69,73-76] This equation relates accelerometer cpm to a MET. Freedson et al[75] established their age-specific MET algorithm by measuring oxygen consumption by indirect calorimetry from 50 adults wearing an accelerometer while walking or running at various speeds on a treadmill. Regression analysis showed a strong relationship between cpm and oxygen consumption (r=88). From various analyses an algorithm was developed[73-75]:

MET= 2.757 + (0.0015 x counts/min) – (0.08957 x age [yr]) - (0.000038 x counts/min/age [yr])

$R^2$=0.74

The regression equation for estimating METs from cpm was used to establish count ranges corresponding to MET level categories typically used in the literature to define light (<= 2.99 METs), moderate (3.0-5.99 METs), vigorous (6.0-8.99 METs), and very vigorous (>= 9.0 METs). The cpm range corresponding to each intensity level was determined by solving the rearranged regression equation for counts and inserting the lower and upper limits for METs.

The commonly cited Troiano et al[69] technique was developed from analysis of NHANES 2003-2004 data and has been used in assessing NHANES studies since that time.[63] Troiano applied the Freedson equation with a 4 MET age-specific PA intensity cut point, where 4 METs is considered moderate

| Table 3: Accelerometer cutpoints associated with moderate-to-vigorous physical activity (MVPA) in children and adolescents aged 3–18 years. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Abbreviaito | Age (yrs.) | | | | | | | | | | | | | | | |
| MVPA Cutpoint | n from text | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| Pate et al.[73] | PT | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 | 1680 |
| Puyau et al. | PY | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 | 3201 |
| Freedson et al. (3 METS | FR3 | 369 | 446 | 527 | 614 | 706 | 803 | 906 | 1017 | 1136 | 1263 | 1400 | 1547 | 1706 | 1880 | 2068 | 2274 |
| Freedson et al. (4 METS | FR4 | 1090 | 1187 | 1290 | 1400 | 1515 | 1638 | 1770 | 1910 | 2059 | 2220 | 2392 | 2580 | 2781 | 3000 | 3239 | 3499 |
| Van Cauwenberghe et a | VC | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 | 2340 |
| Evenson et al. | EV | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 | 2296 |
| Brazendale K, Beets MW, Bornstein DB, et al. Equating accelerometer estimates among youth: The Rosetta Stone 2. J Sci Med Sport. | | | | | | | | | | | | | | | | | |

All cut-points are presented as counts per minute (CPM).

intensity and 7 METs is considered vigorous activity. This accounts for the higher REE in children. For participants 18 and older, Troiano et al[69] developed a cut point based on a weighted average of four calibration studies that used treadmill and track walking criteria data. The resulting Troiano cut point criteria is 2020 counts for moderate intensity (equivalent to 3 METs) and 5999 counts for vigorous intensity (6 METs).

### *Future Research Needed*

CG is a rare metabolic disorder. Dietary treatment is lifesaving in the neonatal period, yet does not prevent a constellation of long-term complications, including reduced height, weight, BMI, LBM, and BMD in individuals with CG. The insult leading to altered anthropometrics and body composition in this population is not well understood. Furthermore, it has not been investigated how diet, muscle function/strength, or PA attributes to this phenotype. The goal of this study is to evaluate body composition in patients with CG compared to control subjects as well as the relationship between body composition, diet, and PA to improve practice recommendations.

## Chapter 3

**Methods**:

### *Study Participants and Recruitment*

Approval was obtained for this research from the Oregon Health and Science University (OHSU) Institutional Review Board (IRB #00011812). Healthy subjects diagnosed with classical galactosemia by newborn screening, ages 8 to 20 years, were recruited from patients followed at the Metabolic Program at OHSU. Children less than eight years were not recruited since they would likely be unable to remain still for the duration of certain examinations involved in the study protocol. All recruited subjects needed to be healthy, able to stand for anthropometric measurements, and not have any other medical conditions that would have limited their ability to participate in the study protocol. Adolescent females included in the study needed to be followed by an endocrine specialist and treated with hormone replacement therapy, if prescribed, given the known negative effect of ovarian insufficiency on growth, bone mineral metabolism and other endocrine outcomes.[7] Additional exclusion criteria included pregnant and lactating females and any patient with an implanted defibrillator since the BIA has not been tested in individuals with this cardiac device (**Table 4a).**

Recruitment letters with a copy of the consent form was mailed to all patients with the diagnosis of CG followed at the OHSU Metabolic Program who met the inclusion criteria. Three weeks after the letters were mailed, these

patients and/or their caregivers received a follow up phone call to review the protocol, answer questions, and determine interest in the study. Additional time was provided if potential subjects and/or caregivers needed to further consider participation. If this was the case, the subject was contacted within two weeks of the first call to assess interest. Additional participants with CG were recruited at the 2016 Galactosemia Foundation conference in Atlanta, Georgia from July 14-16, 2016.

**Table 4a: Inclusion and Exclusion Criteria for Study Subjects with CG**

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| **1.** Age of 8-20 years<br>**2.** Diagnosis of Classical Galactosemia<br>**3.** Treated at a Metabolic Clinic at an established medical center<br>**4.** Females over the age of 11 years followed by an endocrine specialist<br>**5.** Able to stand and lie still for data collection<br>**6.** Willing to participate in the study<br>**7.** English speaking | 1. Diagnosis of pregnancy<br>2. Lactating female<br>3. Females over the age of 11 years who are not followed by an endocrine specialist and treated for hormone insufficiency, if prescribed.<br>4. One or more missing limbs<br>5. Implanted defibrillator<br>**6.** Non-English speaking |

**Recruitment of the Control Group:**

An age-, sex-, Tanner stage- and ethnicity-matched control group was recruited from patient's in general good health scheduled for an appointment at the General Pediatric Clinic at DCH. Control subjects had to be able stand for anthropometric measurements. Pregnant or lactating females were excluded. Patients with, abnormal weight gain or loss, eating disorders, any disorder

affecting body composition, one or more missing limbs, an implanted defibrillator, milk allergy, or were following a lactose-restricted diet were excluded from this study **(Table 4b).**

The clinic schedule was provided to the study coordinator with the following week's clinic schedule. One day prior to the clinic visits, potential study subjects were screened by a chart review conducted by the study coordinator to determine if patients met the criteria for an appropriate matched control. On the day of their clinic visit, the study coordinator met the patient and legal guardian to review the study, answer questions, and obtain informed consent. Once consented, the necessary data was collected.

**Table 4b: Inclusion and Exclusion Criteria for Controls**

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| **1.** Age-matched to test subject (+/- 6 months; aged 8 years to 20 years)<br>**2.** Sex-, ethnicity-, and Tanner scale-matched to test subjects<br>**3.** Treated at DCH Pediatric clinic<br>**4.** Able to stand and lie still for data collection<br>**5.** Willing to participate in the study<br>6. English speaking | 1. Diagnosis of pregnancy<br>2. Lactating females<br>3. One or more missing limbs<br>4. Abnormal weight gain/loss<br>5. Diagnosis of milk allergy or an eating disorder<br>6. Dietary lactose restriction or any dairy restricted diet<br>7. Implanted defibrillator |

***Protection of confidentiality:***

Subjects and controls were assigned a confidential study number that did not contain any patient health information. This number was used on all study

data (hard copy or electronic). Hard copies of any data gathered during the study appointment were stored in a filing cabinet in the PI's office, which remained locked when unoccupied. Only OHSU-approved computers with necessary security and encryption were used to record and store electronic data and access to data required OHSU ID/password authentication. The key associating the codes and the personal information was restricted to the PI and study staff. The key remained secure on a restricted OHSU network drive and in a limited access folder.

### *Assent of Children and Parent Permission*

Prior to any study measurements or data collection, the consent forms were reviewed in person with the potential subject and/or caregiver and signatures were collected. The study coordinator or PI reviewed all study components with the potential subject and/or parent/legal guardian and all questions were answered before any study activities were started. Only one parent/legal guardian was required to consent for any subject under the age of 18 years by signing the Consent and Authorization Form **(Appendix 1)**. Child Assent was obtained for all children over 7 years of age. For those over the age of 18 years, only the subject needed to sign the consent form.

### *Anthropometric Measurements*

Subjects with CG were evaluated at the OHSU Graduate Program in Human Nutrition Metabolic Assessment Room or in a private research room at the Galactosemia Foundation conference. All study measurements were collected

on the same day. After participants arrived and consent forms were signed in person, study subjects were directed into a private room where anthropometric measurements were taken.

During the subject's visit, the study coordinator measured height, weight, hip circumference and waist circumference for comparison to standard growth curve measurements. Measurements were documented on the encoded Data Collection Form. For control subjects, measurements were collected at the outpatient General Pediatric Clinic at DCH.

**Standing Height**

A wall-mounted stadiometer was used to measure the subjects' height. The participant was asked to remove shoes, socks, hair ornaments and any bulky outer clothing. Each subject was instructed to stand with his/her back against the stadiometer with heels together and toes pointed directly forward. The head, shoulder blades, buttocks, and heels made contact with the backboard. The head was aligned in the Frankfort horizontal plane. The study coordinator gently lowered the headboard of the stadiometer until it was placed on the crown of the head and perpendicular to the wall. The study coordinator read and recorded the height to the nearest 0.1 cm. The measurement was repeated until two measurements were consistent within 0.4 cm. Between measurements the subject was instructed to step away from the board, stretch, relax and then reposition. The two values in agreement were averaged and recorded to the nearest 0.01 cm.

Target height was determined using mid-parental height by parent report of maternal and paternal heights. Participants' heights and mid-parental target height values were standardized to obtain z-scores using the Centers for Disease Control and Prevention (CDC) 2000 growth charts for reference population norms of stature by age and sex. Mid–parental target height was plotted on the CDC growth chart using the sex of the participant and the age of a 20-year-old to determine a target height z-score. Height z-score was corrected for target height z-score by the following equation:

Corrected Height Z-score= height Z-score – target height Z-score.

**Weight**

Next, the participant's weight was taken in the same condition without baggy outwear, jewelry and shoes as described above. Weight was measured on an electronic scale with a digital display. Two sequential weights were ascertained to the nearest 0.1 kg. The two closest weights were averaged. Body mass index (BMI) was calculated as the participant's weight in kg divided by their height in meters squared. Weight and BMI for age were recorded on a sex-specific CDC growth chart and expressed as a z-score.

**Waist and Hip Circumference**

The subject was asked to stand up straight and lift their shirt enough to expose their waist. A measuring tape was placed around the bare skin of the subject's abdomen so that the tape lied across the waist just above the iliac crest and hip circumference was measured around the widest part of the buttocks

following procedures of the National Health and Nutrition Examination Survey. Waist circumference was measured and recorded to the nearest 0.1 cm. A waist:hip ratio was calculated by dividing waist circumference by hip circumference.

### *Pubertal Stage via Tanner-Scale*

Pubertal development, based on the Tanner scale, was obtained per chart review for subjects over nine years of age. If a recent pubertal development had not been documented within the past 6 months, the caregiver and/or subject assessed pubertal development using the Tanner stage self-assessment tool.[77]

Subjects completed the pubertal maturation self-assessment in a private area equipped with sex specific Tanner stage pictures. Two sexual maturation self-assessment questions were asked: females selected one breast color image and one pubic hair image that looked the most like their body, and males selected one image representing genital and pubic hair development from a color image that looked the most like their body **(Figure 3)**. These pubertal stage values along with age, ethnicity, and sex was used to match a reference pediatric control participant.

### *Body Composition*

Resistance, body cell mass, extracellular mass, LBM, FM, basal metabolic rate (BMR), BMI, intracellular water, extracellular water, and total body water was ascertained by bioimpedance analyzer (Biodynamics, model 450, Biodynamics Corporation, Seattle, WA).

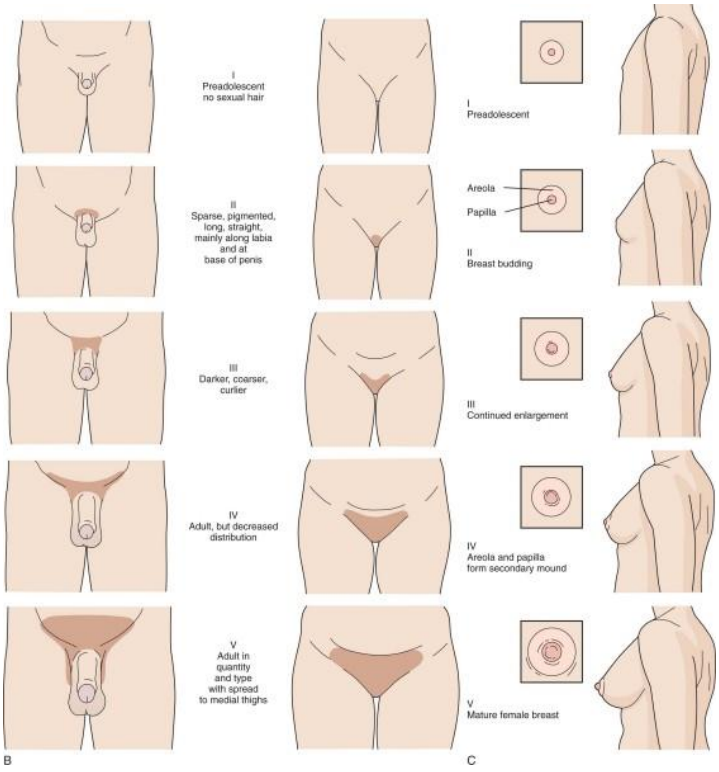



**Figure 3: Tanner Stage Self-assessment**

For this test, the subject remained barefoot with light clothing and removed any additional jewelry on their right arm or right foot. Subjects laid down in a supine position on the exam table, with arms by their sides, and with arms and thighs not touching. The subject's right wrist, hand, ankle, and foot was gently cleaned with alcohol wipes. The study coordinator attached red electrodes to the subject's right wrist and ankle and black electrodes to the right hand and foot. The leads were attached to the four electrodes, the subjects lied completely still abstaining from movement until the test was complete, about 5 minutes. Using the analyzer keypad, the patient's sex, age, height, and weight was entered. Resistance was recorded in ohms. Body cell mass, extracellular mass, LBM, and FM will be recorded in kilograms (kg) and as a percent. BMR was measured in calories

(kcals), Intracellular water, extracellular water, and total body water was recorded in liters (L) and as a percent. A lean mass index (LMI) and a fat mass index (FMI) was calculated to correct for height by dividing the mass of the body composition component in kg by the participant's height in meters squared.

### *Strength Measurements*

#### Upper Body Muscle Strength via Hand Grip Dynamometer:

To assess upper body muscle strength, a Digital Jamar® Plus hand-grip dynamometer was employed (Performance Health, Warrenville, IL). Each subject was positioned using the recommendations of the American Society of Hand Therapists: shoulder adducted and in neutral rotated position, elbow in about 90° flexion, forearm semi-prone and wrist in a neutral resting position. Subjects gripped the dynamometer (equipped with a smaller handle for children) for 10 seconds to measure hand grip strength in pounds.

This was repeated 3 times on both hands, switching off between hands allowing a ten second rest interval between the tests on the same hand. All values of the 6 consecutive hand grip tests was recorded in pound units. The mean and standard deviation was calculated for each hand. These results are compared to standards determined by age from Jamar and the subject's matched controls to refine the analysis of body composition.

#### Lower Body Strength via Sit-to-Stand

The one minute chair sit-to-stand (STS) test was completed using a standard padded chair without armrests. Participants sat in the chair with

approximately 90-degree flexion of the knee, with both arms crossed against the chest. Starting from the seated position, the subject stood up (legs straight) and sat down (full weight on the chair) as many times as possible during a one minute period. The study coordinator demonstrated this to the participant before the STS was performed. The number of STS a participant completed was recorded by the study coordinator.

### *Physical Activity Assessment via Accelerometer*

An ActiGraph tri-axial accelerometer (ActiGraph, Pensacola, FL.) was provided to each participant at the study visit. Subjects signed a contract to return the accelerometer to OHSU. Participants were instructed to wear the accelerometer on their right hip in a straight line above their right knee with the black button facing up, making direct contact with the body. Subjects were asked to start wearing their accelerometer the morning after their study visit for a total of 7 days with 10 hours of wear time a day. The accelerometer was worn continuously except for swimming, showering, or sleeping. A log was provided to participants to document their waking time and wear time each day. Participants were told to log swimming activities or other activities when the accelerometer could not be worn (e.g. gymnastics or impact sports). After 7 days of wear time participants mailed back their accelerometer and log in a prepaid envelope.

#### Physical Activity Data Platform

ActiLife 6 Data Analysis Software (ActiGraph, Pensacola, FL., USA) an ActiGraph data analysis and management platform was used to prepare ActiGraph

devices for data collection. ActiLife software downloaded, processed, and securely managed the collected data.

### Physical Activity Data Acquisition and Cleaning

Data was downloaded with a 10 second epoch from the ActiGraph and imported onto the Actilife software. First, the data was analyzed to identify valid days with valid accelerometer wear-time. To be considered valid wear time, a participant must have worn the accelerometer for at least 8 hours (≥480 minutes) a day. Non-wear time was defined as an interval of 60 minutes with zero activity intensity counts, with allowance of 1-2 minutes of counts between 0 and 100 counts. Next, an individual's data was screened to determine the number of valid days within the 7-day collection period. Participants had to have a minimum of 5 days with at least 8 hours/day of wear-time/valid day to be included in the final analysis. Data meeting this criterion were used in different ways to assess PA. First, using mean counts per minute (cpm) we evaluated the raw data, which represents the average intensity of PA without imposing cut-point determinants. This is important for comparison purposes for accelerometer and other PA data. Raw mean cpm was derived by dividing the sum of the total counts measured during valid wear-time by the total number of minutes that the accelerometer was worn on each compliant day. The participants' intensity of PA level and METs was determined by applying the Freedson et al[75] Children's algorithm cut-points **(Table 5)**.

**Table 5: Cut Point for Freedson et al Children's Algorithm**

| | Minimum Counts | Maximum Counts |
|---|---|---|
| **Sedentary** | 0 | 149 |
| **Light** | 150 | 499 |
| **Moderate** | 500 | 3999 |
| **Vigorous** | 4000 | 7599 |
| **Very Vigorous** | 7600 | < |
| **MVPA Minimum** | 500 | < |

***Dietary Assessment***

Recruited patients were not required to change their usual diet for the study. The participants' family completed a three-day diet record starting the day after their outpatient study visit. During the study visit, instructions to fill out the diet record log was provided along with a stamped/addressed envelope to send the record back to OHSU. If a three-day diet record was not fully completed or contained insufficient information for analysis, the study coordinator contacted the subject to obtain a 24-hour recall using a 24-hour multi pass technique. Each participant was also asked about his/her current supplement use, including calcium and Vitamin D. Energy, macronutrient distribution, calcium (mg) and Vitamin D (IU) were all assessed using Food Processor software (ESHA, Salem, OR).

***Withdrawal from Study:***

During recruitment, it was emphasized to the patient that he/she would continue to receive routine medical care at OHSU if he/she did not want to participate in the study. Subjects could decide at any time to stop participation in the study. If the participant withdrew before any significant measurements were collected, their records were destroyed. If a participant decided to withdraw after significant data was collected, the collected data was used for partial analysis, but no further data was collected.

***Statistical Analysis***

Data was plotted and deemed to be normally distributed. Metric data was expressed as mean +/- standard deviation (SD) with 95% confidence intervals. Z-scores were used over percentiles for anthropometric measurements as this is preferred in the literature and pediatric clinical practice. Using z-scores allows for comparison among different age groups. Paired t-tests were used to determine significant differences ($p < 0.05$) between the CG study subjects' values and Tanner stage-, ethnicity-, sex- and age-matched control participants. A Bonferroni Correction was used as this study was analyzing so many variables with a small sample size; there was a chance that a false positive result could occur on chance alone. For analysis, height z-score, weight z-score, BMI z-score, and LBM% were all considered primary variables; all other variables were considered exploratory in nature because of the lack of previous research in the literature. A Bonferroni correction of 4 was applied to the primary variables to

determine a significant difference ($p<0.05$) between subjects with CG and their matched controls. No correction was applied to any other variables.

A paired t-test was also completed for all female participants to determine if the results from this cohort different from the entire group. Since females with CG tend to have a more severe clinical phenotype than males, there was potential for a sex-related effect on the results. All dietary variables were normalized to account for the large age range. Unpaired t-tests were used to determine if there was a significant difference in dietary intakes between subjects with CG and controls.

Spearman's correlation was employed to find any correlations between the different parameters of body composition, muscle strength, physical activity and diet. A Spearman's correlation was selected over a Pearson's correlation because of the small sample size.

| Table 6: Statistical Analysis Summary | | |
|---|---|---|
| **Specific Aim** | **Hypothesis** | **Statistical Test** |
| **Specific Aim 1:** We will assess the body composition of patients with CG, ages 8 to 20 years, by Bioelectrical Impedance Analysis (BIA) and anthropometric measurements and compare the results to a control pediatric population in general good health. | **Hypothesis 1:** We predict that patients with CG will have a higher percent body fat mass (FM %) and lower LBM% in comparison to the control population. | A paired t-test will be used to determine if there is a significant difference ($p < 0.05$) between measured body composition variables in the CG population compared to their matched controls. Bonferroni correction was applied to primary variables. |
| | **Hypothesis 2:** We hypothesize that patients with CG will have a reduced height for age, mid-parental height, weight-for-age percentiles, and waist circumference compared to the matched controls. | A paired t-test will be used to determine if there is a significant difference ($p < 0.05$) between measured anthropometric variables and standardized z-scores. Bonferroni correction was applied to primary variables. |
| **Specific Aim 2:** We will determine the relationship between muscle strength and PA level on body composition factors in patients with CG by comparing BIA, muscle strength measures (dynamometer grip strength and sit-to-stand repetitions), and PA level measured by an accelerometer. | **Hypothesis 1:** We hypothesize LBM will positively correlate with muscle strength measures and PA level. | Spearman's linear regression to determine a significant correlation ($p<0.05$) between LMI with muscle strength and PA level. |
| | **Hypothesis 2:** We believe that patients with CG will have lower muscle strength measures and PA levels than age-, sex-, ethnicity- and Tanner scale--matched controls. | A paired t-test will be used to determine if there is a significant difference ($p < 0.05$) between measured muscle strength measures and PA levels in the CG population compared to their matched controls. |

## Chapter 4

## Results

### *Recruitment*

Study measurements were completed for 19 participants with CG and 19 control subjects. Only 12 of the controls matched the subjects with CG for age, sex, ethnicity, and Tanner stage pubertal development. Thus, only 12 pairs were used for the complete set of analyses (n=24). Characteristics of these 12 pairs are given in **Table 7**. Of these 12 pairs, nine pairs were female subjects. Female pairs were analyzed separately to determine if there was a gender-related effect as females with CG tend to be more clinically affected than males primarily because of effects of ovarian insufficiency on metabolism. A separate analysis for male participants was not feasible with this study population distribution given the small number.

### *Anthropometric Measurements*

There were no significant differences in height, weight, BMI, waist circumference, hip circumference, and waist:hip circumference ratio between subjects with CG and healthy control subjects. Height z-score was significantly decreased in subjects with CG; however, this was no longer significant after applying the Bonferroni Correction (-0.68 ± 0.87 in subjects vs. 0.42 ± 1.18 in controls, p=0.10 after correction). Weight z-score was significantly lower in subjects with CG than their matched controls (-1.04 ± 0.79 in subjects vs. 0.29 ± 0.99 in controls, p=0.04 after correction). Participants with CG had a significantly lower BMI z-score than matched controls, but this observation only trended

towards a significant difference after applying the Bonferroni Correction (-0.78 ± 0.66 in subjects vs -1.08 ± 1.27 in controls; p=0.05 after correction). It seems that subjects with CG are significantly shorter and weigh less than their matched controls when comparing them to the USA population on the CDC growth charts. **(Figure 4)** Subjects with CG are not attaining their target growth potential compared to healthy matched controls based on comparison of corrected height z-score (-1.36 ± 0.69 in subjects vs 0.21 ± 1.20 in controls; p=0.01). Furthermore, this difference is not due to differences in pubertal status or ethnicity since these parameters were controlled by our matching criteria.

When analyzed by gender, there was a significant difference in height in cm, height z-score, corrected height z-score, weight z-score, and BMI z-score in female participants **(Table 8).** However, BMI z-score was not significant in the female cohort after applying the Bonferroni Correction. This loss in significance is most likely due to a loss in statistical power with the decreased sample size. These results suggest that females with CG are shorter and weigh less than their matched controls when comparing their growth to the USA population on the CDC growth charts, but not in their weight-for- height comparisons by BMI z-score. **Figure 6** and **Figure 7** shows that growth of the female participants with CG was more affected than that of male participants given that the height z-score was significantly lower in the female cohort even with the Bonferroni Correction (-1.02 ± 0.45 in female subjects vs -0.28 ± 1.00 female controls; p=0.03), which was not observed in the whole group. BMI z-score distribution was similar among the whole cohort and female participants. The observed change in the trend

towards significance observed in the whole cohort, but not females is most likely a loss of statistical power and not one related to gender.

**Table 7: Study Population Global Data (age and anthropometry)**

| Variable | CG Subjects | Controls | Δ subject-control | | |
|---|---|---|---|---|---|
| n= | 12 (9 female, 3 male) | 12 (9 female, 3 male) | 12 pairs (9 female, 3 male) | Paired T-Test p-value | Bonferroni Correction p-value |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | Mean ± SD (95% CI) | | |
| Age (years) | 12.19 ± 2.79 (10.42 to 13.97) | 11.88 ± 3.05 (9.94 to13.8) | 0.31 ± 4.26 (-2.39 to 3.02) | 0.80 | -- |
| Tanner Stage n (1,2,3,4,5) | 5, 5, 0, 1, 1 | 5, 5, 0, 1, 1 | -- | -- | -- |
| Ht (cm) | 143.54 ± 12.85 (135.50 to 151.50) | 148.23 ± 16.04 (138.00 to 158.40) | -4.69 ± 11.15 (-11.77 to 2.39) | 0.17 | -- |
| Ht z-score* | -0.68 ± 0.87 (-1.233 to -0.126) | 0.42 ± 1.18 (-0.33 to 1.17) | -1.10 ± 1.46 (-2.36 to 0.16)ǂ | 0.02 | 0.10 |
| Corrected Ht z-score* | -1.36 ± 0.69 (-1.79 to -0.92) | 0.21 ± 1.20 (-0.55 to 0.98) | -1.59 ± 1.22 (-2.34 to -0.79) | 0.01 | |
| Wt (kg) | 34.54 ± 8.60 (29.07 to 40.00) | 45.19 ± 22.20 (31.09 to 59.30) | -10.65 ± 18.45 (-22.38 to 1.07) | 0.07 | -- |
| Wt z-score** | -1.04 ± 0.79 (-1.54 to -0.54) | 0.29 ± 0.99 (-0.34 to 0.92) | -1.33 ± 1.49 ( -2.61 to -0.05)ǂ | 0.01 | 0.04 |
| BMI (kg/m2) | 16.48 ± 1.57 (15.48 to 17.48) | 19.82 ± 6.31 (15.80 to 23.83) | -3.34 ± 6.00 (-7.15 to 0.47) | 0.08 | -- |
| BMI z-score* | -0.78 ± 0.66 (-1.20 to 0.36) | 0.31 ± 0.88 (-0.25 to 0.86) | -1.08 ± 1.27 (-2.17 to 0.01)ǂ | 0.01 | 0.05 |
| Waist Circum (cm) | 61.34 ± 6.62 (57.13 to 65.55) | 64.45 ± 13.70 (55.75 to 73.16) | -3.11 ± 12.28 (-10.92 to 4.69) | 0.40 | -- |
| Hip Circum (cm) | 74.67 ± 8.53 (69.25 to 80.08) | 81.84 ± 17.12 (70.96 to 92.72) | -7.18 ± 12.94 (-15.40 to 1.05) | 0.08 | -- |
| Waist:Hip | 0.82 ± 0.07 (0.78 to 0.87) | 0.79 ± 0.07 (0.75 to 0.83) | 0.03 ± 0.07 (-0.01 to 0.08) | 0.10 | -- |

*CG significantly different than matched control in paired T-test $p<0.05$
**primary variable remains significantly different with Bonferroni Correction
--exploratory variable does not need to be adjusted with Bonferroni Correction
ǂ 95% Confidence Interval based off Bonferroni Correction alpha=0.0125

## Table 8: Study Female Population Global Data (age and anthropometry)

| Variable<br>n= | CG Subjects<br>9 Females<br>Mean ± SD<br>(95% CI) | Controls<br>9 Female<br>Mean ± SD<br>(95% CI) | ∆ subject-control<br>9 pairs<br>Mean ± SD<br>(95% CI) | Paired<br>T-Test<br>p-value | Bonferroni<br>Correction<br>p-value |
|---|---|---|---|---|---|
| **Age (years)** | 12.07 ± 3.25<br>(9.57 to 14.57) | 11.86 ± 3.55<br>(9.13 to14.59) | 0.21 ± 0.73<br>(-0.35 to 0.77) | 0.40 | -- |
| **Tanner Stage n (1,2,3,4,5)** | 5, 5, 0, 1, 1 | 5, 5, 0, 1, 1 | -- | -- | -- |
| **Ht (cm)** | 139.50 ± 11.24<br>(130.90 to 148.10) | 145.60 ± 14.96<br>(134.10 to 157.10) | -6.11 ± 6.85<br>(-11.38 to -0.84) | 0.03 | -- |
| **Ht z-score**** | -1.02 ± 0.45<br>(-1.40 to -0.65) | -0.28 ± 1.00<br>(-0.48 to -1.04) | -1.30 ± 1.12<br>(-2.26 to -0.443)ǂ | 0.01 | 0.03 |
| **Corrected Ht z-score*** | -1.37 ± 0.79<br>(-1.98 to -0.77) | 0.24 ± 1.07<br>(-0.58 to 1.06) | -1.65 ± 1.43<br>(-2.75 to -0.34) | 0.01 | -- |
| **Wt (kg)** | 32.43 ± 8.55<br>(25.86 to 39.00) | 44.84 ± 24.86<br>(25.74 to 63.95) | -12.42 ± 19.05<br>(-27.06 to 2.23) | 0.09 | -- |
| **Wt z-score**** | | 0.38 ± 1.08<br>(-0.45 to 1.22) | -1.65 ± 1.43<br>(-2.88 to -0.42)ǂ | 0.01 | 0.04 |
| **BMI (kg/m2)** | 16.37 ± 1.79<br>(15.00 to 17.75) | 20.22 ± 7.25<br>(14.65 to 25.79) | -3.85 ± 6.76<br>(-9.05 to 1.35) | 0.13 | -- |
| **BMI z-score*** | -0.82 ± 0.73<br>(-1.38 to -0.26) | 0.35 ± 0.915<br>(-0.36 to 1.05) | -1.16 ± 1.34<br>(-2.19 to -0.13)ǂ | 0.03 | 0.12 |
| **Waist Circum (cm)** | 60.06. ± 6.27<br>(55.24 to 64.88) | 65.58 ± 15.20<br>(53.90 to 77.26) | -5.53 ± 10.82<br>(-13.84 to 2.79) | 0.16 | -- |
| **Hip Circum (cm)** | 73.58 ± 9.39<br>(66.37 to 80.80) | 82.17 ± 19.46<br>(67.21 to 97.13) | -8.58 ± 13.13<br>(-18.67 to 1.515 | 0.09 | -- |
| **Waist:Hip** | 0.82 ± 0.08<br>(0.76 to 0.88) | 0.80 ± 0.07<br>(0.75 to 0.86) | 0.02 ± 0.06<br>(-0.27 to 0.06) | 0.37 | -- |

*CG significantly different than matched control in paired T-test $p<0.05$
**primary variable remains significantly different with Bonferroni Correction
--exploratory variable does not need to be adjusted with Bonferroni Correction
ǂ 95% Confidence Interval based off Bonferroni Correction alpha=0.0125

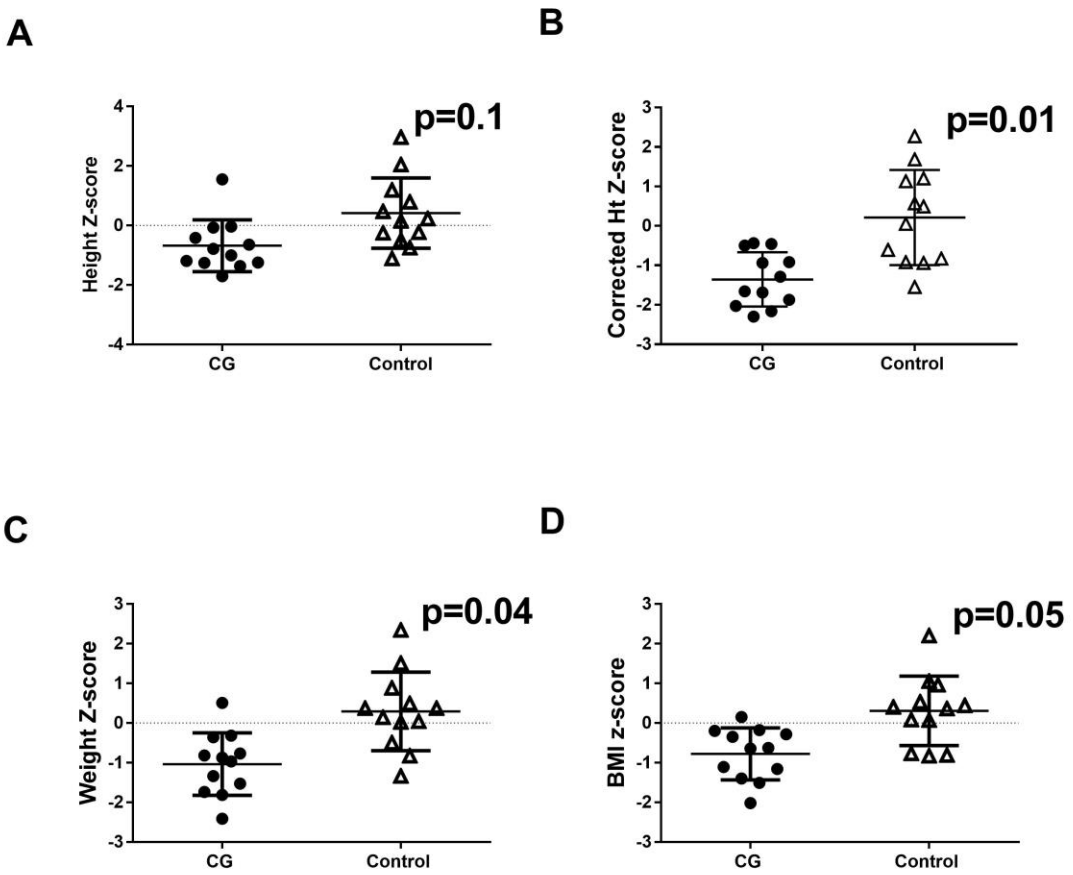



**Figure 4. A:** Comparison of height z-score between subjects with CG and controls (-0.68 ± 0.87 [95% CI-1.23 to -0.13] vs 0.42 ± 1.18 [95% CI -0.33 to 1.17]; p=0.10) **B:** Comparison of corrected height z-score between subjects with CG and controls (-1.36 ± 0.69 [95% CI-1.79 to -0.92] vs 0.21 ± 1.20 [95% CI -0.55 to 0.98]; p=0.01) **C:** Comparison of weight z-score between subjects with CG and controls (-1.04 ± 0.79 [95% CI -1.54 to -0.54] vs 0.29 ± 0.99 [95% CI -0.34 to 0.92]; p=0.04) **D:** Comparison of BMI z-score between subjects with CG and controls (-0.78 ± 0.66 [95% CI -1.20 to 0.36] vs 0.31 ± 0.88 [95% CI -0.25 to 0.86]; p=0.05)

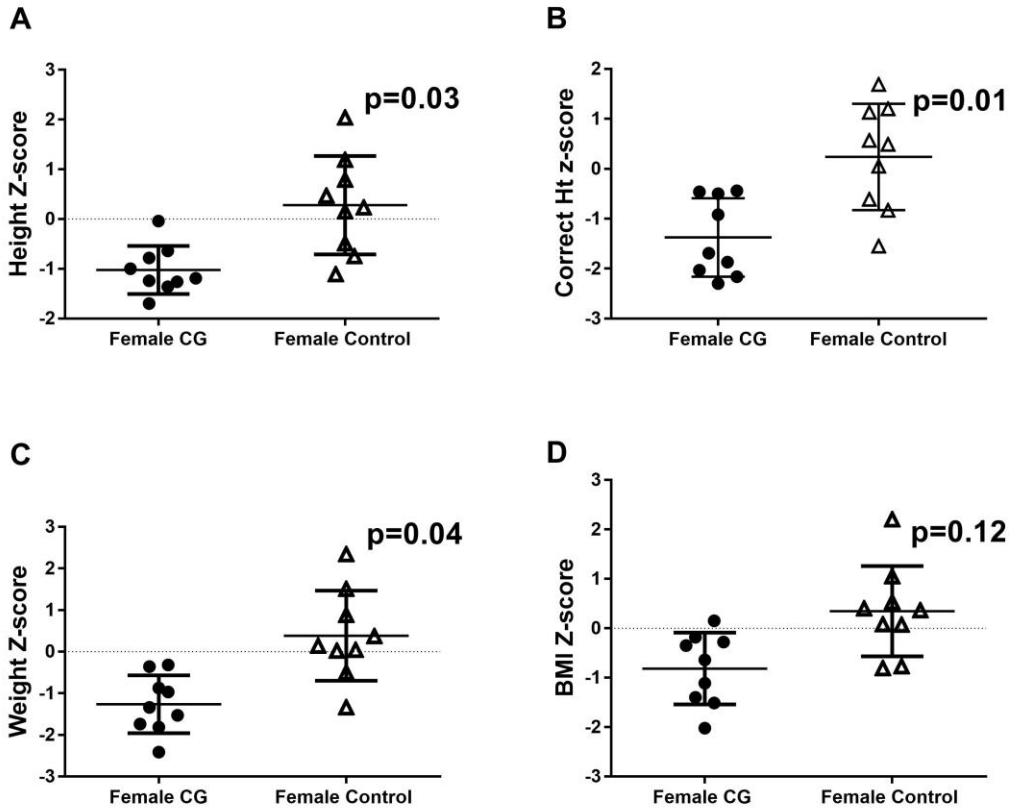



**Figure 5. A:** Comparison of height z-score between female subjects with CG and female controls (-1.02 ± 0.45 [95% CI -1.40 to -0.65] vs -0.28 ± 1.00 [95% CI -0.48 to -1.04]; p=0.03) **B:** Comparison of corrected height z-score between female subjects with CG and female controls (-1.37 ± 0.79 [95% CI-1.98 to -0.77] vs 0.24 ± 1.07 [95% CI -0.58 to 1.06]; p=0.01) **C:** Comparison of weight z-score between female subjects with CG and female controls (-1.26 ± 0.70 [95% CI-1.80 to -0.70] vs 0.38 ± 1.08 [95% CI -0.45 to 1.22]; p=0.04) **D:** Comparison of BMI z-score between female subjects with CG and female controls (-0.82 ± 0.73 [95% CI -1.38 to -0.26] vs 0.35 ± 0.92 [95% CI -0.36 to 1.05)]; p=0.12)

**Figure 6. Comparison of z-scores between subjects with CG and controls.** From left to right: Height z-score of subjects with CG and controls (-0.68 ± 0.87 [95% CI-1.233 to -0.13] vs (0.42 ± 1.18 [95% CI -0.33 to 1.17]; p=0.10). Corrected height z-score of subjects with CG and controls (-1.36 ± 0.69 [95% CI-1.79 to -0.92] vs (0.21 ± 1.20 [95% CI -0.55 to 0.98]; p=0.01). Weight z-score between subjects with CG and controls (-1.04 ± 0.79 [95% CI -1.54 to -0.54] vs (0.29 ± 0.99 [95% CI -0.34 to 0.92]; p=0.04). BMI z-score between subjects with CG and controls (-0.78 ± 0.66 [95% CI -1.20 to 0.36] vs (0.31 ± 0.88 [95% CI -0.25 to 0.86]; p=0.05).

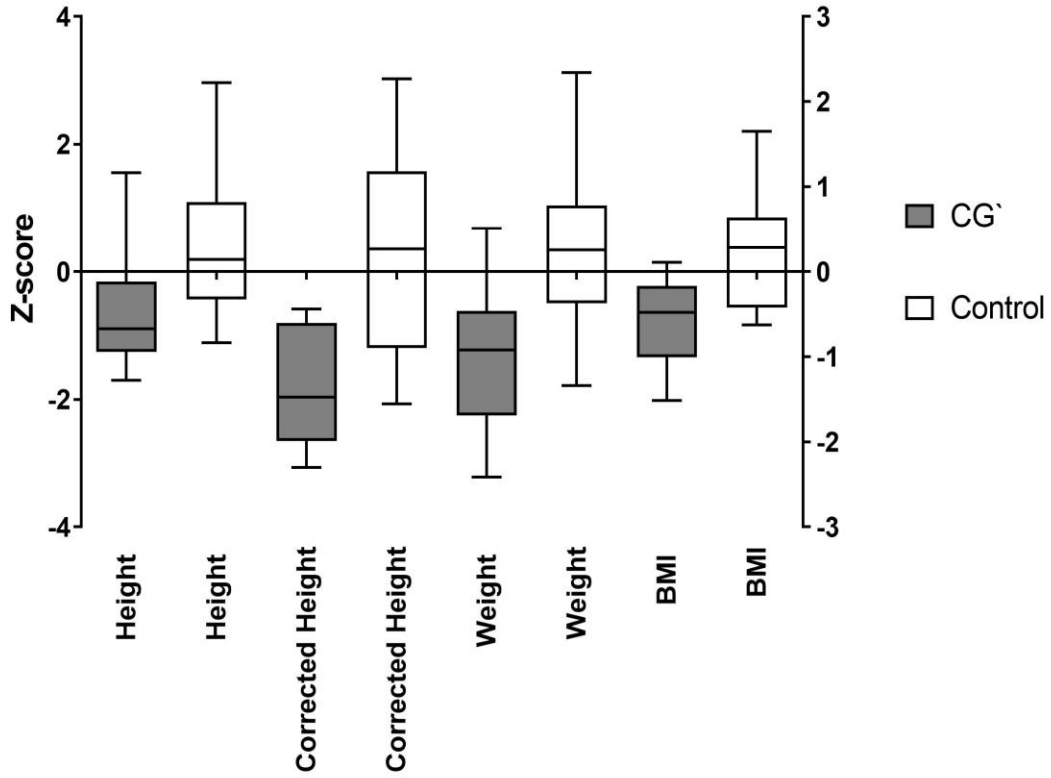
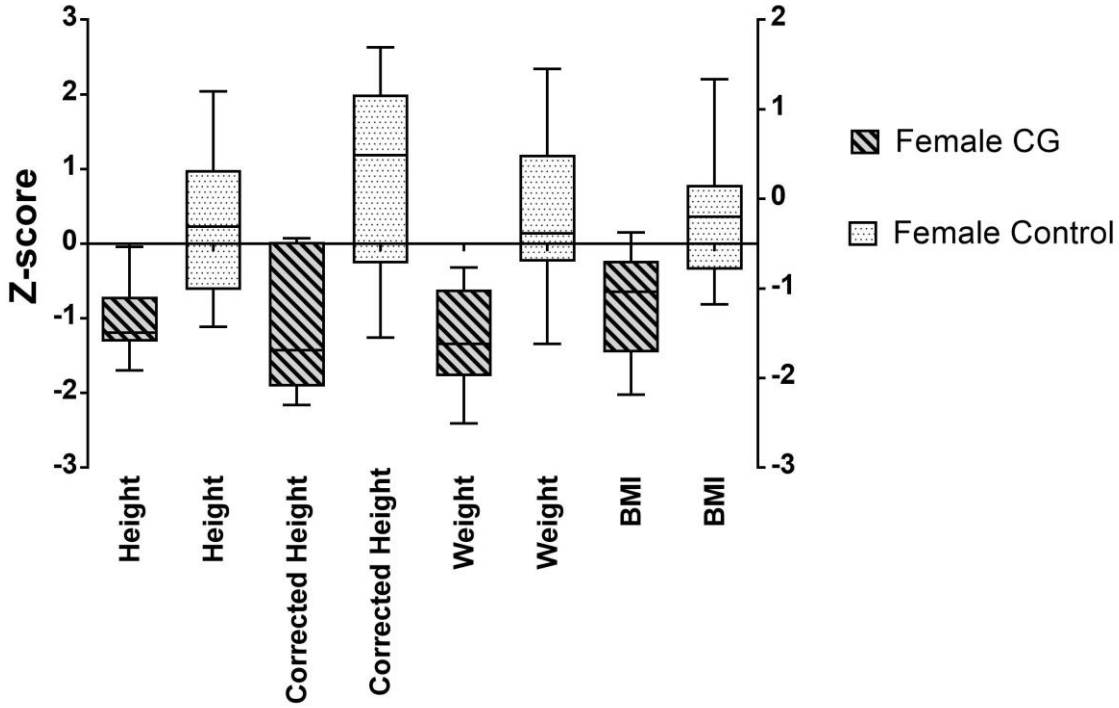


**Figure 7. Comparison of z-scores between female subjects with CG and female controls.** From left to right: Height z-score between female subjects with CG and female controls (-1.02 ± 0.45 [95% CI -1.40 to -0.65] vs -0.28 ± 1.00 [95% CI -0.48 to -1.04]; p=0.03). Corrected height z-score between female subjects with CG and female controls (-1.37 ± 0.79 [95% CI-1.98 to -0.77] vs 0.24 ± 1.07 [95% CI -0.58 to 1.06]; p=0.01). Weight z-score between female subjects with CG and female controls (-1.26 ± 0.70 [95% CI-1.80 to -0.70] vs 0.38 ± 1.08 [95% CI -0.45 to 1.22]; p=0.04). BMI z-score between female subjects with CG and female controls (-0.82 ± 0.73 [95% CI -1.38 to -0.26] vs 0.35 ± 0.92 [95% CI -0.36 to 1.05)]; p=0.12)



### *Body Composition*

LBM% and FM% were not found to be statistically different between participants with CG and their matched controls (**Table 9**). When corrected for height, LMI ($kg/m^2$) trended towards statistical significance (13.57 ± 1.50 in subjects vs 15.48 ± 2.77 in controls, p=0.06), but this was not significant after correcting fat mass for height. It is possible that we are seeing a false negative result in differences between LMI due to the small sample size or this may be a gender-specific difference that is not being elucidated by the sex distribution of this study population. By gender, female LMI did not trend towards significance (13.16 ± 1.46 in female subjects vs 15.35 ± 2.97 female controls, p=0.10) as observed in the whole group statistics (**Table 10**), yet this could be due to a loss in statistical power. It is likely that we would have observed a statistical difference in LMI with a larger study population since nine of the twelve pairs (75%) showed a lower LMI in the subjects with CG than their matched controls **(Figure 10)**.

## Table 9: Measures of Body Composition of All Study Subjects

| Variable | CG Subjects | Controls | ∆ subject-control | | |
|---|---|---|---|---|---|
| n= | 12 (9 female, 3 male) | 12 (9 female, 3 male) | 12 Pairs (9 female, 3 male) | Paired T-Test p-value | Bonferroni Correction p-value |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | Mean ± SD (95% CI) | | |
| LBM % | 83.28 ±7.45 (78.54 to 88.01) | 79.98 ± 8.06 (74.85 to 85.10) | 3.30 ± 7.10 (-0.10 to 6.70)ǂ | 0.14 | 0.54 |
| LMI (LM kg/m2) | 13.57 ± 1.50 (12.62 to 14.53) | 15.48 ± 2.77 (13.71 to 17.24) | -1.90 ± 3.15 (-3.90 to 0.1) | 0.06 | -- |
| FM % | 16.73 ±7.45 (11.99 to 21.46) | 20.03 ± 8.06 (14.90 to 25.15) | -3.30 ± 7.10 (-7.81 to 1.21) | 0.14 | -- |
| FMI (FM kg/m2) | 2.76 ± 1.32 (1.92 to 3.60) | 4.34 ± 3.82 (1.91 to 6.77) | -1.58 ± 3.25 (-3.64 to -0.49) | 0.12 | -- |

ǂ 95% Confidence Interval based off Bonferroni Correction alpha=0.0125
--exploratory variable does not need to be adjusted with Bonferroni Correction

## Table 10: Measures of Body Composition of Female Subjects

| Variable | CG Subjects | Controls | ∆ subject-control | | |
|---|---|---|---|---|---|
| n= | 9 Females | 9 Females | 9 Pairs | Paired T-Test p-value | Bonferroni Correction p-value |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | Mean ± SD (95% CI) | | |
| LBM % | 81.42 ± 6.22 (76.64 to 86.21) | 78.39 ± 8.69 ( 71.71 to 85.07) | 3.03 ± 7.53 ('-1.00 to 7.60)ǂ | 0.26 | 1.00 |
| LMI (LM kg/m2) | 13.16 ± 1.46 (12.04 to 14.28) | 15.35 ± 2.97 (13.07 to 17.64) | -2.19 ± 3.54 (-4.91 to 0.53) | 0.10 | -- |
| FM % | 18.58 ± 6.23 (13.79 to 23.36) | 21.61 ± 8.69 (14.93 to 28.29) | -3.03 ± 7.53 (-8.82 to 2.76) | 0.26 | -- |
| FMI (FM kg/m2) | 3.05 ± 1.16 (2.17 to 3.94) | 4.85 ± 4.34 (1.52 to 8.19) | -1.58 ± 3.25 (-3.64 to -0.49) | 0.18 | -- |

ǂ 95% Confidence Interval based off Bonferroni Correction alpha=0.0125
--exploratory variable does not need to be adjusted with Bonferroni Correction

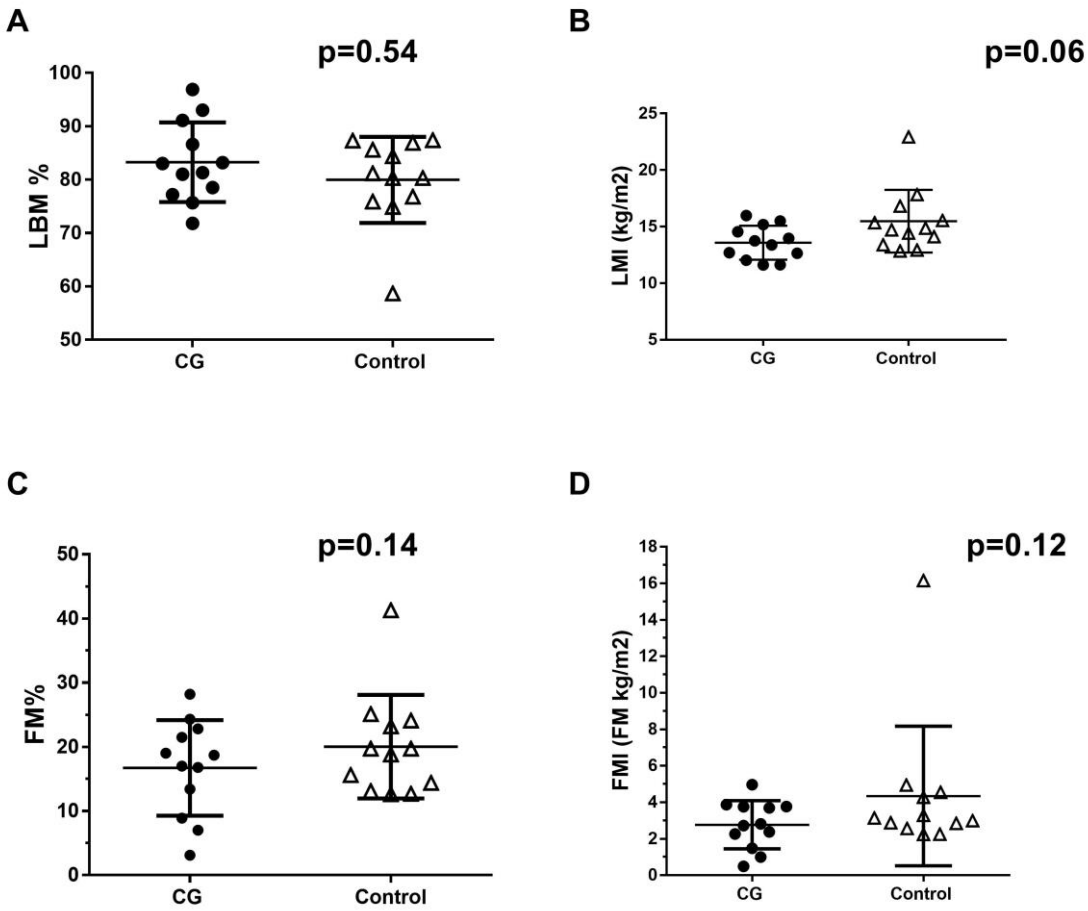



**Figure 8. A:** Comparison of LBM% between subjects with CG and controls (83.28 ± 7.45 [95% CI 78.54 to 88.01] vs 79.98 ± 8.06 [95% CI 74.85 to 85.10]; p=0.54) **B:** Comparison of LMI (LM (kg)/m$^2$) between subjects with CG and controls (13.57 ± 1.50 [95% CI 12.62 to 14.53] vs 15.48 ± 2.77 [95% CI 13.71 to 17.24]; p=0.06) **C:** Comparison of FM% between subjects with CG and controls (16.73 ± 7.45 [95% CI 11.99 to 21.46] vs 20.03 ± 8.06 [ 95% Ci 14.90 to 25.15]; p=0.14) **D:** Comparison of FMI (FM (kg)/m$^2$) between subjects with CG and controls (2.76 ± 1.32 [95%1.92 to 3.60] vs 4.34 ± 3.82 [1.91 to 6.77];p=0.12)

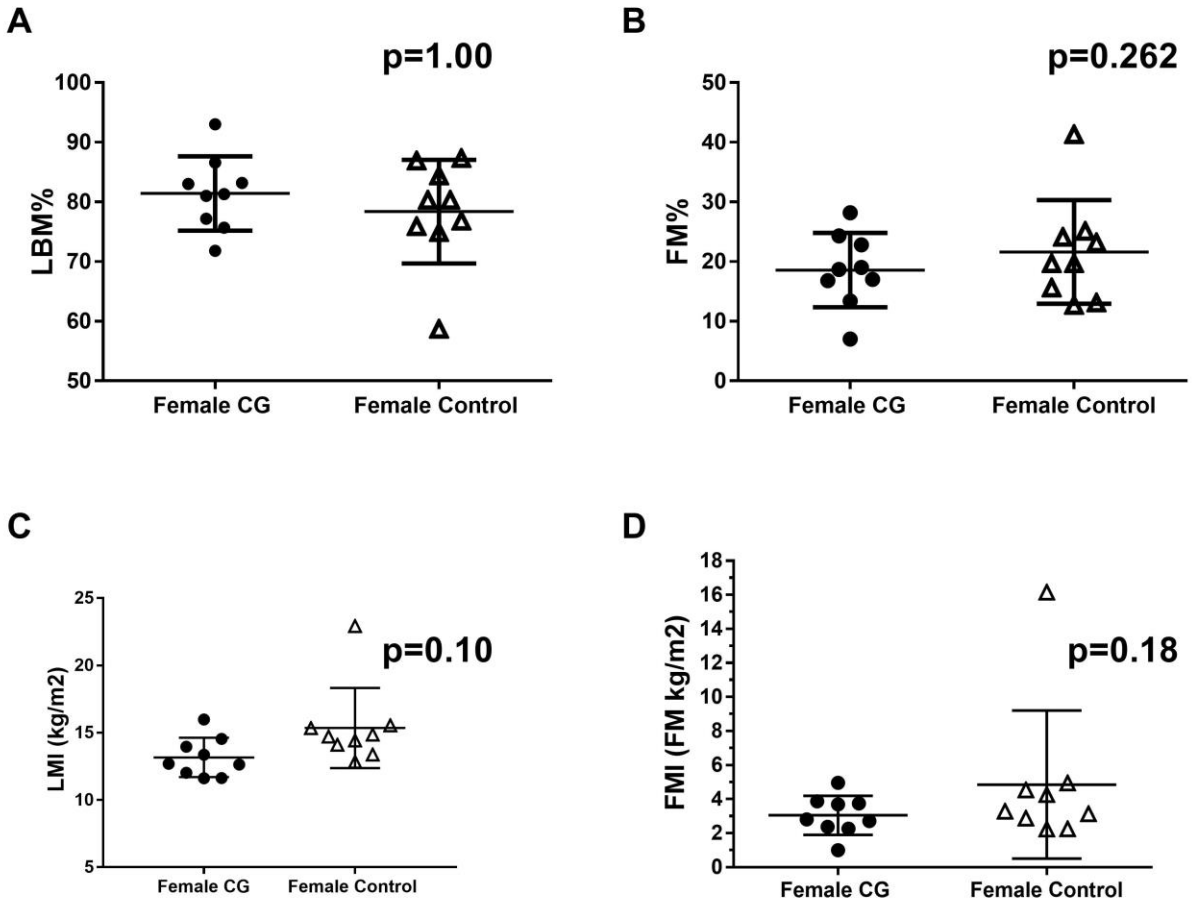
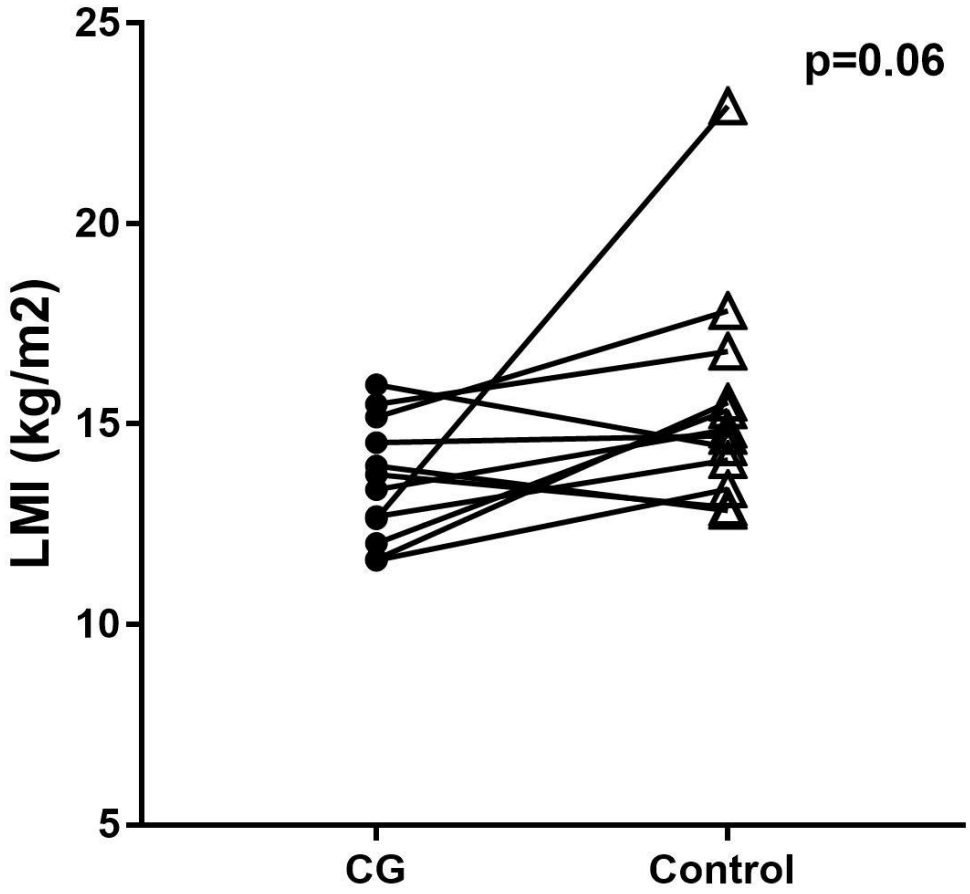



**Figure 9. A**: Comparison of LBM% between female subjects with CG and female controls (81.42 ± 6.22[95% CI 76.64 to 86.21] vs78.39 ± 8.69 [ 71.71 to 85.07]; p=1.00) **B:** Comparison of LMI (LM (kg)/$m^2$) between female subjects with CG and female controls (13.16 ± 1.46 [95% CI 12.04 to 14.28] vs 15.35 ± 2.97 [95% CI 13.07 to 17.64]; p=0.10) **C:** Comparison of FM% between female subjects with CG and female controls (18.58 ± 6.23 [95% Ci 13.79 to 23.36] vs 21.61 ± 8.69 [95% CI 14.93 to 28.29]; p=26) **D:** Comparison of FMI (FM (kg)/$m^2$) between female subjects with CG and female controls (3.05 ± 1.16 [95% CI 2.17 to 3.94] vs 4.85 ± 4.34 [95% CI 1.52 to 8.19];p=0.18)

**Figure 10: Comparison of the differences in LMI (LM (kg)/m$^2$)** between subjects with CG and controls (Δ subject-control -1.90 ± 3.15 [95% CI -3.90 to 0.1]; p=0.06). Black lines connect a subject with CG's LMI to their matched control's LMI. Nine (75%) of CG subjects had a lower LMI than controls.



### *Muscle Strength Measures*

Mean right grip strength in pounds (34.12 ± 11.24 in subjects vs 45.42 ± 19.81 in controls; p=0.02), mean left grip strength (31.96 ± 11.74 in subjects vs 41.78 ± 16.31 in controls; p=0.01) and mean dominant hand grip strength (33.81 ± 11.77 in subjects vs 45.57 ± 19.69 in controls; p=0.01) were all significantly reduced in subjects with CG compared to their matched controls **(Table 11).** This was also observed in the female cohort **(Table 12)**. Decreased grip strength indicates decreased upper body strength and functional whole body muscle strength**.** This finding does not seem to be due to differences in LBM% among the groups because this was not found to be statistically different. Sit-to-stand repetitions/min were found to be significantly decreased in individuals with CG compared to their matched controls (35.67 ± 8.98 in subjects vs 46.08 ± 11.75 in controls; p=0.02) **(Figure 11 D)**. Sit-to-stand repetitions are an indirect measure of lower body and core strength. However, when further analyzed by gender, this finding did not remain significant among female individuals with CG and their matched controls (37.11 ± 9.816 vs 44.22 ± 12.85; p=0.11) **(Figure 12 D)**. This is an interesting finding as it resembles the LMI which also trended towards decreased significance in the whole group, but not among the female participants. This could be a false negative finding with the loss of statistical power with the smaller female cohort or we could be observing a gender driven decrease in LMI and decreased muscle strength in the male subjects.

## Table 11: Measures of Muscle Strength

| Variable | CG Subjects | Controls | ∆ subject-control | |
|---|---|---|---|---|
| n= | 12 (9 female, 3 male) | 12 (9 female, 3 male) | 12 Pairs (9 female, 3 male) | Paired T-Test p-value |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | Mean ± SD (95% CI) | |
| Ave R Grip (lbs)* | 34.12 ± 11.24 (26.98 to 41.26) | 45.42 ± 19.81 (32.83 to 58.00) | -11.3 ± 13.64 (-19.97 to -2.63) | 0.02 |
| Ave L Grip (lbs)* | 31.96 ± 11.74 (24.5 to 39.43) | 41.78 ± 16.31 (31.42 to 52.14) | -9.815 ± 10.74 (-16.64 to -2.99) | 0.01 |
| Ave Dominant Grip (lbs)* | 33.81 ± 11.77 (26.34 to 41.29) | 45.57 ± 19.69 (33.06 to 58.08) | -11.76 ± 13.23 (-20.16 to -3.36) | 0.01 |
| Sit-to-Stand* | 35.67 ± 8.98 (29.96 to 41.37 | 46.08 ± 11.75 (38.62 to 53.55) | -10.42 ± 12.52 (-18.37 to 2.47) | 0.02 |

*CG significantly different than matched control in paired T-test p<0.05

## Table 12: Measures of Muscle Strength of Female Participants

| Variable | CG Subjects | Controls | ∆ subject-control | |
|---|---|---|---|---|
| n= | 9 Females | 9 Females | 9 Pairs | Paired T-Test p-value |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | Mean ± SD (95% CI) | |
| Ave R Grip (lbs)* | 32.15 ± 11.71 (23.15 to 41.15) | 44.35 ± 20.2 (28.82 to 59.87) | -12.20 ± 13.87 (-22.86 to -1.537) | 0.03 |
| Ave L Grip (lbs)* | 29.89 ± 12.27 (20.46 to 39.32) | 39.99 ± 15.79 (27.86 to 52.10) | -10.10 ± 10.36 (-18.06 to -2.14) | 0.02 |
| Ave Dominant Grip (lbs)* | 31.74 ± 12.33 (22.27 to 41.21) | 44.55 ± 20.05 (29.14 to 59.96) | -13.38 ± 14.13 (-22.36 to -4.40) | 0.02 |
| Sit-to-Stand | 37.11 ± 9.82 (29.57 to 44.66 | 44.22 ± 12.85 (34.34 to 54.10) | -7.11 ± 12.04 (-2.14 to 16.36) | 0.11 |

*CG significantly different than matched control in paired T-test p<0.05

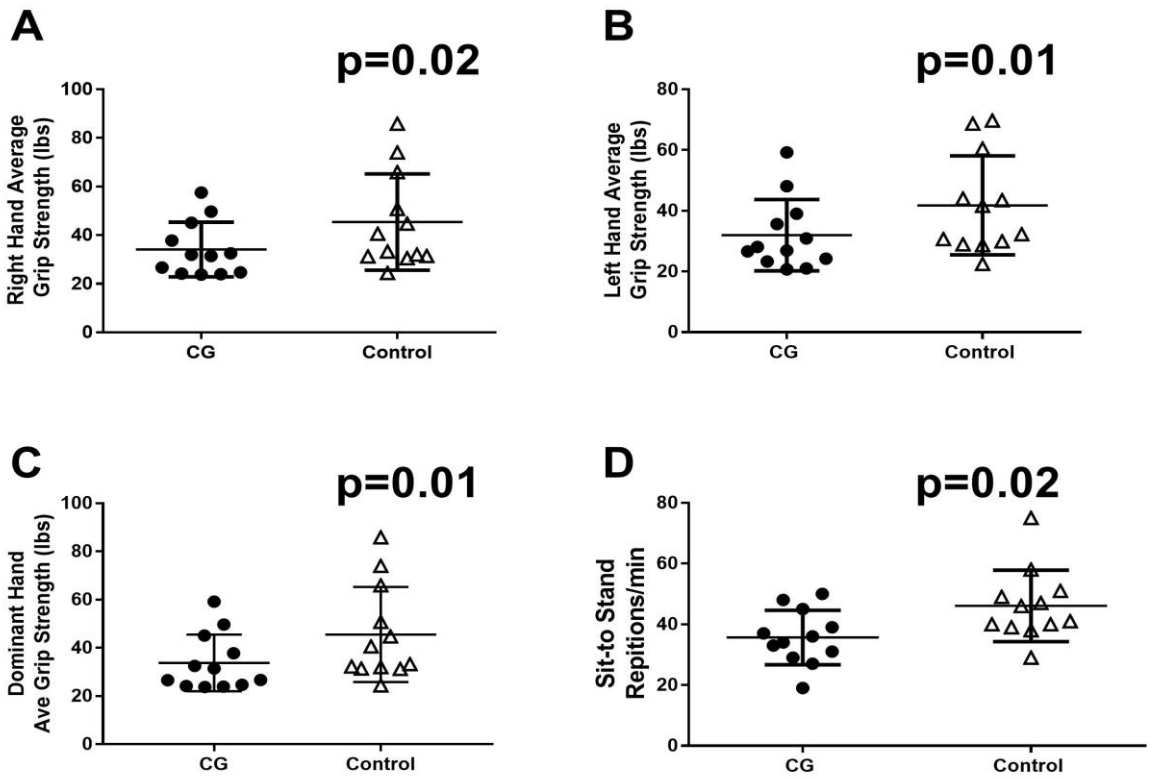



**Figure 11. A:** Comparison of Right Average Grip Strength (lbs.) between subjects with CG and controls (34.12 ± 11.24 [95% CI 26.98 to 41.26] vs 45.42 ± 19.81 [95% CI 32.83 to 58.00]; p=0.02) **B:** Comparison of Left Average Grip Strength (lbs.) between subjects with CG and controls (33.81 ± 11.77 [95%CI 26.34 to 41.29] vs 41.78 ± 16.31 [31.42 to 52.14];p=0.01) **C:** Comparison of Average Dominant Grip Strength between subjects with CG and controls (32.19 ± 11.53 [95% CI 24.86 to 39.52] vs 45.57 ± 19.69 [33.06 to 58.08]; p=0.01) **D:** Comparison of sit-to-stand repetitions/minute between subjects with CG and controls (35.67 ± 8.978 [95% CI 29.96 to 41.37] vs 46.08 ± 11.75 [95% CI 38.62 to 53.55]; p=0.02)

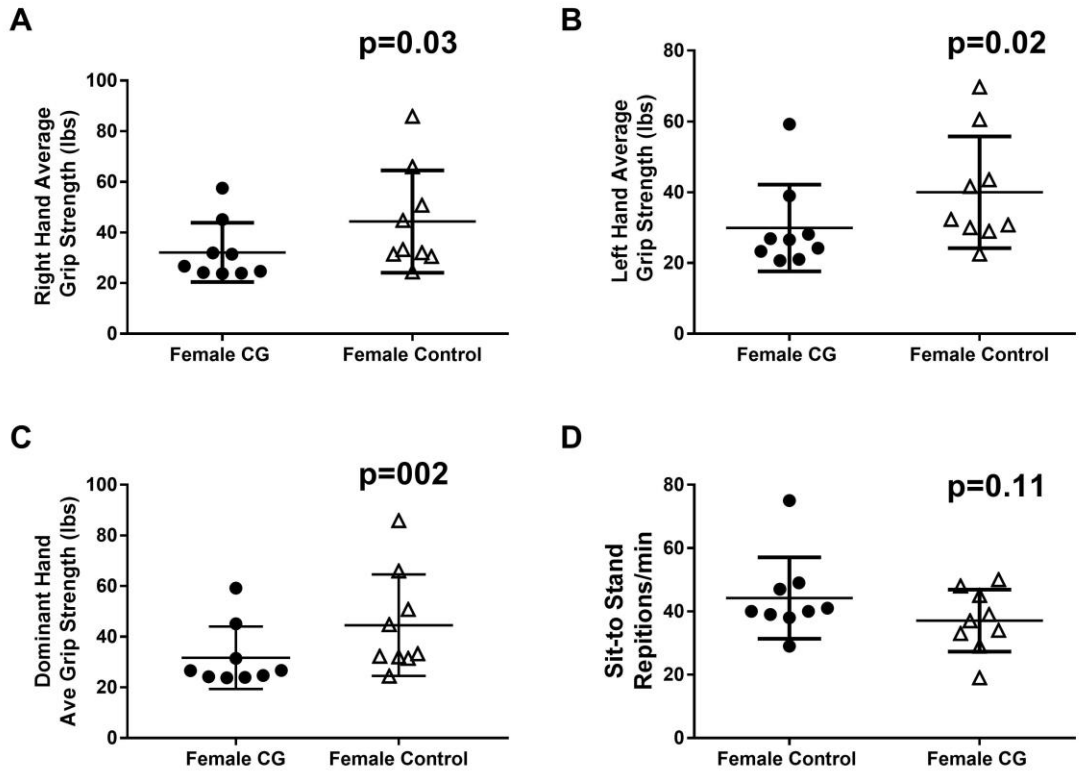
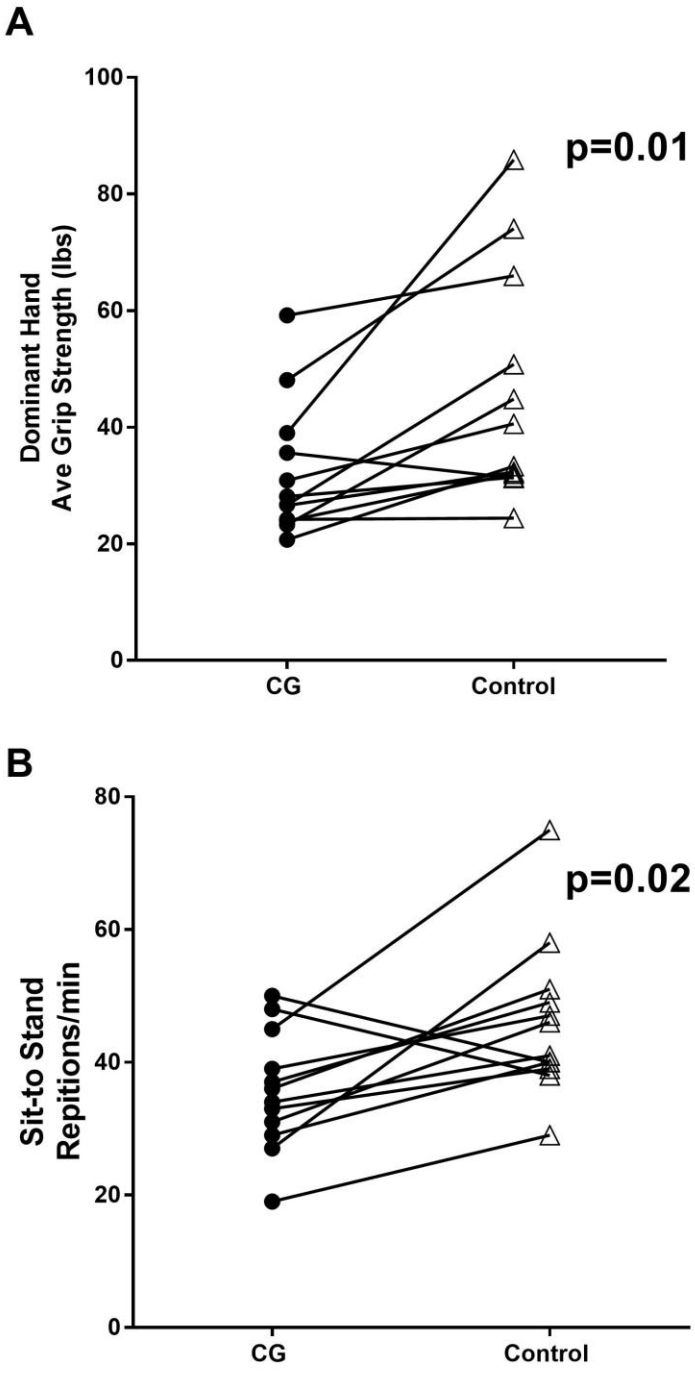



**Figure 12. A:** Comparison of Right Average Grip Strength (lbs.) between female subjects with CG and female controls (32.15 ± 11.71 [95% CI 23.15 to 41.15] vs 44.35 ± 20.2 [95% CI 28.82 to 59.87]; p=0.03) **B:** Comparison of Left Average Grip Strength (lbs.) between female subjects with CG and female controls (29.89 ± 12.27 [95% CI 20.46 to 39.32] vs 39.99 ± 15.79 [95% CI 27.86 to 52.10] vs 10.1 ± 10.36 [95% CI -18.06 to -2.14]; p=0.02) **C:** Comparison of Average Dominant Grip Strength between female subjects with CG and female controls (31.74 ± 12.33 [95% CI 22.27 to 41.21] vs 44.55 ± 20.05 [95% CI 29.14 to 59.96]'p=0.02) **D:** Comparison of sit-to-stand repetitions/minute between female subjects with CG and female controls (37.11 ± 9.816 [95% CI 29.57 to 44.66] vs. 4.22 ± 12.85 [95% CI 34.34 to 54.10]; p=0.11)

**Figure 13: A.** Comparison of the differences in Average Dominant Grip Strength (lbs.) between subjects with CG and matched controls (∆ subject-control (-13.38 ± 14.13 [95% CI -22.36 to -4.404]; p=0.01) **B.** Comparison of the differences in Average Sit-to-stand repetitions/minute between subjects with CG and controls (∆ subject-control {-10.42 ± 12.52 [95% CI -18.37 to 2.47]; p=0.02).
Black lines connect a subject with CG's muscle strength measure to their matched control's muscle strength measure.



### *Physical Activity Level*

**Table 13** describes the amount of wear time, cpm, METs, and amount of time spent preforming PA in minutes per day at varying levels of intensity. Participants were instructed to wear the accelerometer for 7 consecutive days during all waking hours except for when swimming or showering. Of the 12 pairs, nine subjects with CG and eleven controls had valid wear-time (defined as wearing the accelerometer ≥5 days with ≥480 minutes/valid day) leaving 9 pairs for the final analysis. There was no difference in wear-time between participants with CG and their matched control. The average number of days that participants with CG wore the accelerometer ≥480 minutes/day was 6.22 days ± 0.67 and the average number of days that controls wore the accelerometer was 6.00 days ± 0.87. There was not a significant difference in minutes of wear time per valid-day between the CG group and matched controls (740.70 ± 79.80 in subjects vs 784.20 ± 85.90 in controls; p=0.22).

There was a trend towards significance in raw cpm between subjects with CG and their matched controls (389.30 ± 90.31 vs 581.50 ± 235.90; p=0.05) suggesting a lower mean PA intensity in subjects with CG compared to matched controls before cut-points were imposed. METs for subjects with CG was significantly less than their matched controls (1.64 ± 0.17 vs. 1.91 ± 0.32; p=0.03), suggesting a difference in the intensity of PA throughout the wear-time between the two groups. Only one subject with CG had a higher MET value than their matched control (**Figure 15**).

All 18 participants with a valid accelerometer wear-time met the CDC recommended PA of 60 minutes of MVPA/day for children. When analyzed further by the intensity level cut-points, there was no significant difference in the amount of sedentary minutes/day between subjects with CG and matched controls (546.00 ± 79.89 in subjects vs 539.80 ± 80.24 in controls; p=0.85). Subjects with CG spent significantly less time (minutes/day) preforming light PA than matched controls (66.48 ± 12.71 in subjects vs 75.98 ± 15.12 in controls; p=0.03) (**Figure 14**). Subjects with CG performed fewer minutes of MVPA/day (129.2 ± 21.10 in subjects vs 168.5 ± 40.99 in controls; p=0.02) and less time was spent in MVPA as a percent of total wear-time (17.59 ± 20.13 in subjects vs 21.41 ± 4.28 in controls; p= 0.04) than their matched controls (**Figure 17**). It appears the key difference in time spent in MVPA between subjects with CG and their matched controls was the number of minutes/day of vigorous PA (11.20 ± 5.41 vs 20.33 ± 9.63; p=0.03) **(Figure 16)**. There was no significant difference found in minutes/day spent preforming moderate or very vigorous PA. Minutes/day of very vigorous PA trended towards being significantly less than matched controls (1.55 ± 2.32 in subjects vs 8.41 ± 9.00 in controls; p=0.06). To simplify the categories of PA intensity, vigorous activity was defined as the sum of minutes/day in vigorous and very vigorous PA, which was significantly lower in subjects with CG (12.75 ± 6.38 in subjects vs. 28.74 ± 17.79 in controls; p=0.03). **(Table 13)**

The participants were asked to only remove the accelerometer during waking hours to shower or swim. The participants recorded date, day of the

week, waking time, time of day starting and stopping the accelerometer, time of day they went to sleep, and any activity preformed without the accelerometer on**.** All participants completed a log **(Appendix 2)**.

All subjects with CG were recruited during the summer months (May-August 2016); almost all the subjects with CG removed the accelerometer while swimming. All the control subjects were recruited during winter and fall months (November 2016-January 2017), and did not report any swim-time. One of the nine control subjects reported activity while not wearing the accelerometer, but this time was not equivalent to the unreported activity of their matched subjects with CG. Thus, the time in activity while not wearing the accelerometer was not approximately equal between the two groups and cannot be ignored **(Table 14)**. Yet, there is not enough information about length of time of activity or intensity level to add to our accelerometer analysis. This is a huge limitation to our interpretation of PA in this study.

**Table 13: Accelerometer wear-time and physical activity intensity**

| Variable | CG Subjects | Controls | Δ subject-control | |
|---|---|---|---|---|
| n= | 9 | 9 | 9 pairs | Paired |
| | Mean ± SD (95% CI) | Mean ± SD (95% CI) | Mean ± SD (95% CI) | T-Test p-value |
| Wear-time (minutes) | 740.70 ± 79.80 (679.40 to 802.00) | 784.20 ± 85.90 (718.20 to 850.30) | -43.52 ± 98.47 (-119.20 to 32.16) | 0.22 |
| Counts per minute | 389.30 ± 90.31 (319.80 to 458.70) | 581.50 ± 235.90 (400.20 to 762.80) | -192.30 ± 254.40 (-387.80 to 3.29) | 0.05 |
| Metabolic Equivalents of Task (METs)* | 1.64 ± 0.17 (1.51 to 1.78) | 1.91 ± 0.32 ( 1.66 to 2.16) | -0.27 ± 0.30 (-0.50 to -0.03) | 0.03 |
| Sedentary (minutes/day) | 546.00 ± 79.89 (484.60 to 607.50) | 539.8 ± 80.24 (478.10.to 601.50) | 6.23 ± 94.14 (-66.13 to 78.60) | 0.85 |
| Light (minutes/day)* | 66.48 ± 12.71 (55.21 to 75.25) | 75.98 ± 15.12 (64.36 to 87.60) | -10.50 ± 11.887 (-19.63 to -1.37) | 0.03 |
| Moderate (minutes/day) | 116.40 ± 17.55 (102.90 to 129.90) | 139.70 ± 38.71 (109.90 to 169.50) | -23.26 ± 39.06 (-6.76 to 53.28) | 0.11 |
| Vigorous-Very Vigorous (minutes/day)* | 12.75 ± 6.38 (7.84 to 17.66) | 28.74 ± 17.79 (15.07 to 42.42) | -15.99 ± 17.68 (-29.58 to -2.40) | 0.03 |
| MVPA (minutes/day)* | 129.20 ± 21.10 (113.00 to 145.40) | 168.50 ± 40.99 (136.90 to 200.00) | -39.28 ± 41.86 (-71.48 to -7.10) | 0.02 |
| MVPA (% total wear-time)* | 17.59 ± 3.13 (15.18 to 20.00) | 21.41 ± 4.28 (18.12 to 24.70) | -3.82 ± 4.90 (-7.59 to -0.05) | 0.04 |

*CG significantly different than matched control in paired T-test $p<0.05$
MVPA: Moderate to Vigorous Physical Activity (i.e. any activity greater than moderate intensity)

**Table 14: Reported sum of physical activity in which an accelerometer was not worn during the week of physical activity data collection**

| Pair* | Subject with CG | Control |
|---|---|---|
| **1** | 120 minutes of ballet | 0 |
| **2** | 0 | 0 |
| **3** | 740 minutes of swimming | 0 |
| **4** | 120 minutes of swim | 120 minutes of gymnastics bars |
| **5** | 0 | 0 |
| **6** | 360 minutes of swim | 0 |
| **7** | 0 | 0 |
| **8** | 180 minutes of swimming | **0** |
| **9** | 150 minutes of swimming | **0** |

*All CG subject are in the same row as their sex-, ethnicity, age-, Tanner stage-matched control. Numbering of pairs stays consistent throughout all figures.

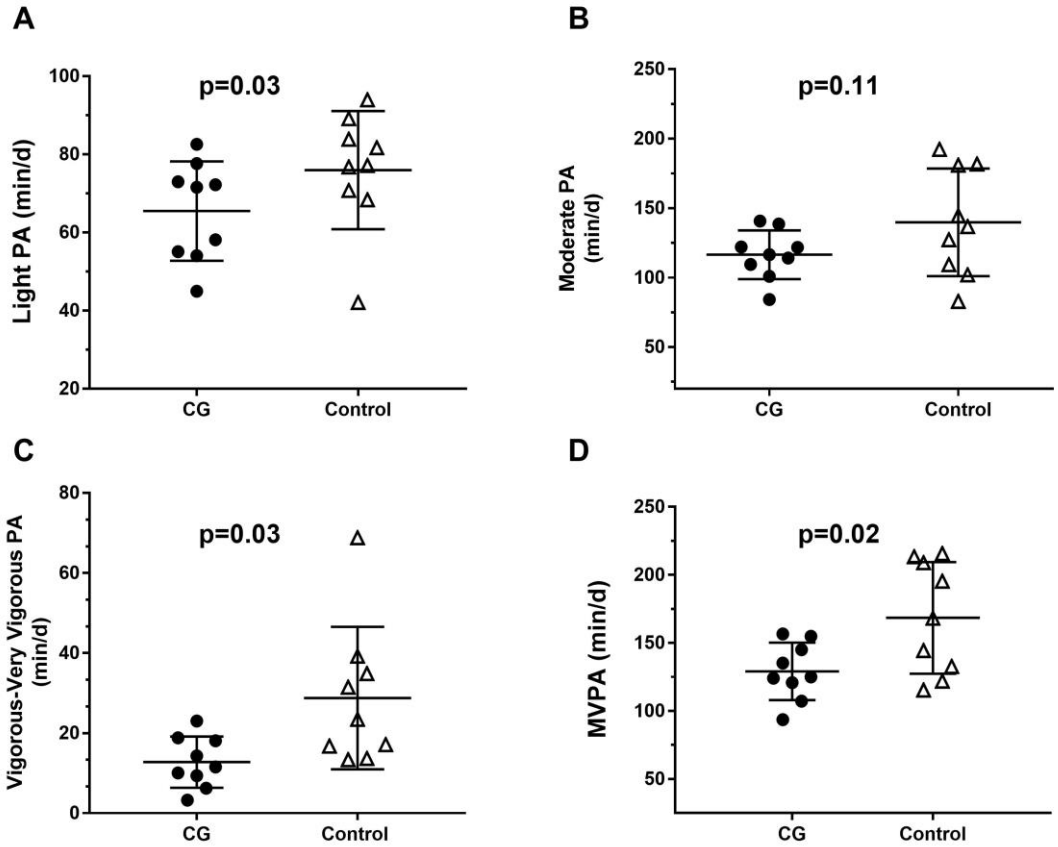



**Figure 14: A.** Comparison of the differences in light physical activity (minutes/day) between subjects with CG and matched controls (66.48 ± 12.71 [95% CI 55.21 to 75.25] vs. 75.98 ± 15.12 [95% CI 64.36 to 87.60]; p=0.03) **B.** Comparison of the differences in moderate physical activity (minutes/day) between subjects with CG and matched controls (116.40 ± 17.55 [95% CI 102.90 to 129.90] vs. 139.70 ± 38.71 [95% CI 109.90 to 169.50]; p=0.11) **C.** Comparison of the differences in vigorous-very vigorous physical activity (minutes/day) between subjects with CG and controls (12.75 ± 6.38 [95% CI 7.84 to 17.66] vs. 28.74 ± 17.79 [95% CI 15.07 to 42.42]; p=0.03) **D.** Comparison of the differences in moderate to vigorous physical activity (MVPA minutes/day) between subjects with CG and matched controls (129.20 ± 21.10 [95% CI 113.00 to 145.40] vs. 168.50 ± 40.99 [95% CI 136.90 to 200.00]; p=0.02).

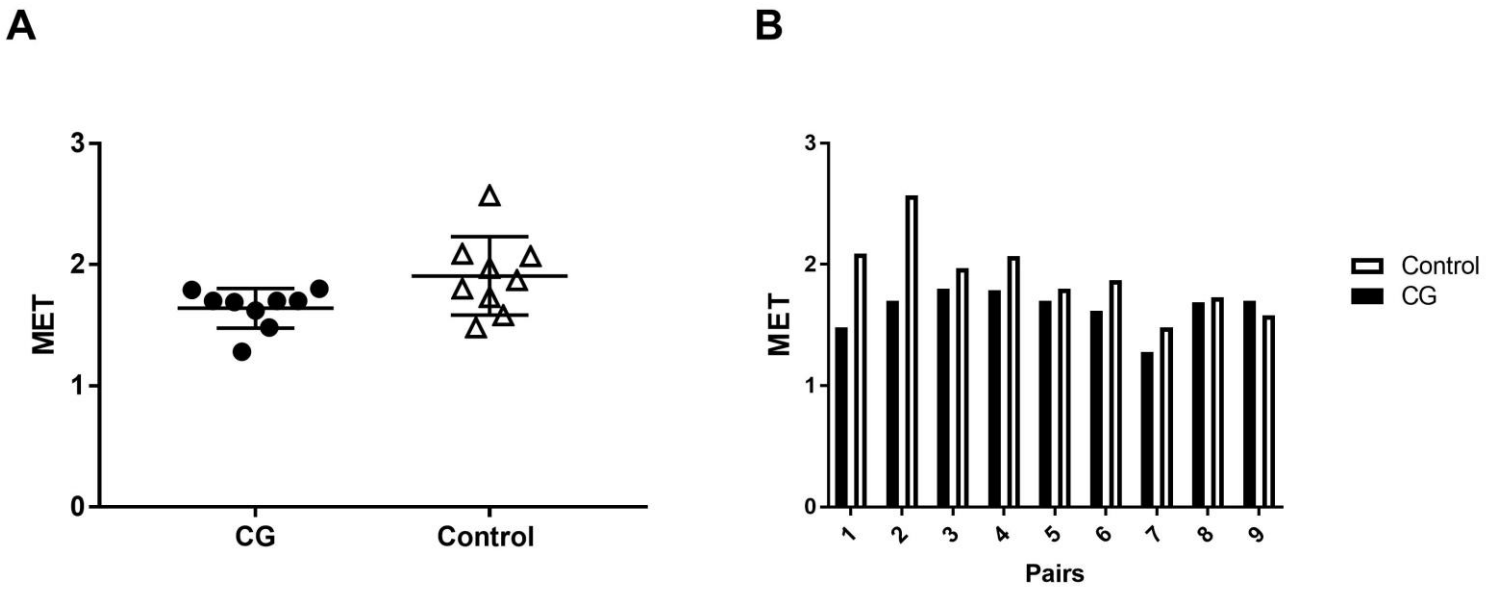



**Figure 15. A:** Comparison of Metabolic Equivalents of Task (METs) between subjects with CG and controls (1.64 ± 0.17 [95% CI 1.51 to 1.78 vs 1.91 ± 0.32]95% CI 1.66 to 2.16]) **B:** Comparison of the differences between subjects with CG and their matched controls (∆ subject-control: -0.27 ± 0.30 [95% CI -0.50 to 0.03]; p=0.03). Subject with CG is to the left of their matched control. The numbering of the pairs stays consistent through all figures.

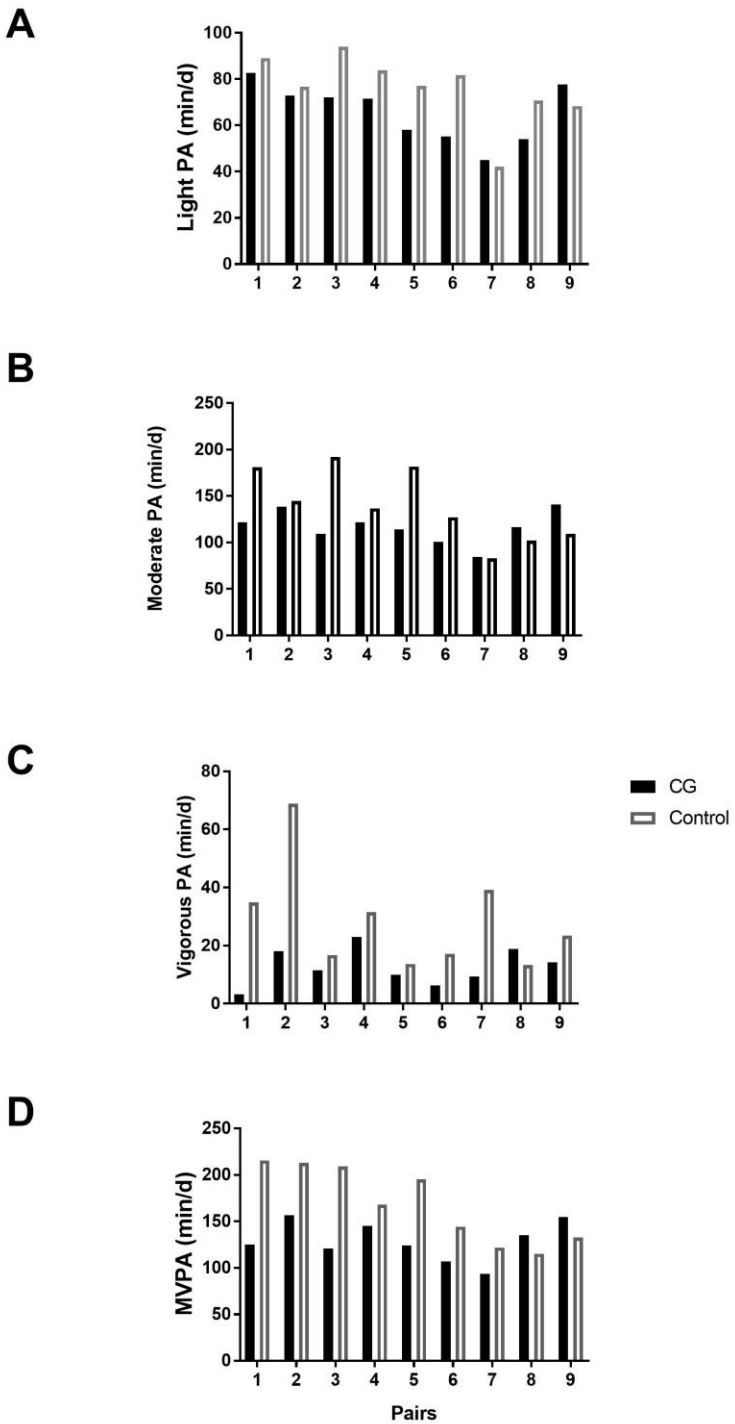



**Figure 16: A.** Comparison of the differences in light physical activity (minutes/day) between subjects with CG and matched controls (Δ subject-control: -10.50 ± 11.887 [95% CI -19.63 to -1.37]; p=0.03) **B.** Comparison of the differences in moderate physical activity (minutes/day) between subjects with CG and matched controls (Δ subject-control: 23.26 ± 39.06 [95% CI -6.76 to 53.28]; p=0.11) **C.** Comparison of the differences in vigorous-very vigorous physical activity (minutes/day) between subjects with CG and controls (Δ subject-control: -15.99 ± 17.68 [95% CI -29.58 to -2.40]; p=0.02) **D.** Comparison of the differences in moderate to vigorous physical activity (MVPA minutes/day) between subjects with CG and matched controls (Δ subject-control: -39.28 ± 41.86 [95% CI-71.48 to -7.10]; p=0.02). Subject with CG is to the left of their matched control. The numbering of the pairs stays consistent through all figures.

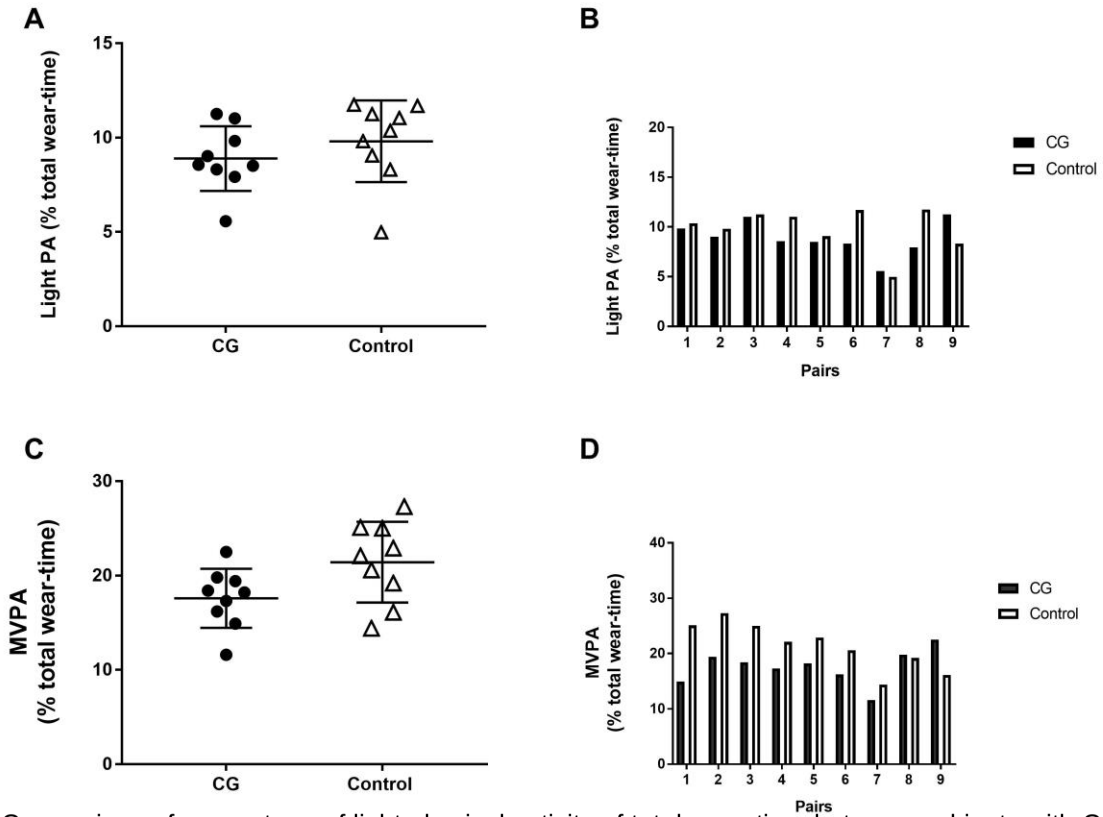



**Figure 17: A.** Comparison of percentage of light physical activity of total wear-time between subjects with CG and controls (8.89 ± 1.72 [95% CI 7.57 to10.21] vs 9.81 ± 2.16 [95% CI 8.15 to 11.47]) **B.** Comparison of the differences in percentage of light physical activity between subjects with CG and matched controls (∆ subject-control: -0.92 ± 2.09 [95% CI -2.52 to 0.69]; p=0.22).**C.** Comparison of percentage of moderate to vigorous physical activity (MVPA} of total wear-time between subjects with CG and matched controls (17.59 ± 3.13 [95% CI 15.18 to 20.00] vs 21.41 ± 4.28 [95% CI 18.12 to 24.70]) **D.** Comparison of the differences in percentage moderate to vigorous physical activity (MVPA} of total wear-time between subjects with CG and matched controls (∆ subject-control: -3.82 ± 4.9 [95% CI -7.59 to -0.05]; p=0.04). Subject with CG is to the left of their matched control. The numbering of the pairs stays consistent through all figures.

### *Dietary Analysis*

Eleven of the twelve control subjects and eight of the twelve subjects with CG completed a 3-day diet record. Due to the large age range in study participants, we normalized the dietary intake expressed as kcalories per kilogram body weight (kcal/kg), grams of protein per kilogram body weight (g/kg), macronutrient distribution as a percent of total kcal intake, milligrams calcium/1000 kcalories, and %Vitamin D (IU) of Recommended Dietary Allowance (RDA). Any reported supplementation of calcium or vitamin D was included with dietary intake. A summary of the study participants' dietary intakes are given in **Table 15.**

There was no significant difference in energy intake (kcal/kg), protein intake (g/kg), or macronutrient distribution between the participants with CG and the control group's dietary intake. When asked, only 2 of the 8 (25%) participants with CG who completed a diet record reported supplementing with calcium and vitamin D daily. The study participants with CG who completed a diet record had significantly lower calcium intake (mg/1000 kcals) than control participants (269.4 ± 155.0 in subjects vs 503.1 ± 185.4 controls; p=0.01). There was no significant difference in Vitamin D intake (IU) between the two groups. Yet, the intake of only 2 participants (one with CG and one control) met or exceeded the RDA of 600 IU Vitamin D. The median intake of vitamin D (IU), based on the percent of RDA for Vitamin D (IU) among participants with CG was 6.0% with a range of 0.0%-236.7%. The median percent intake of RDA for Vitamin D (IU) among control participants was 9.89% with a range of 0.6%-144.2%. There was no

significant difference in the percent of RDA for Vitamin D (IU) between the two groups (39.3 ± 87.5 in subjects vs 28.1 ± 43.1in controls; p=0.72)

**Table 15: Dietary Intake Data**

| | **Subject with CG (n=8)** mean ± SD | **Control (n=11)** mean ± SD | **p-value** |
|---|---|---|---|
| **Total Energy** | 2080.0 ± 606.6 | 2184.0 ± 532.9 | 0.70 |
| **Energy (kcal/kg)** | 70.6 ± 31.9 | 52.5 ± 14.6 | 0.11 |
| **Protein (g/kg)** | 2.3 ± 1.0 | 2.0 ± 0.7 | 0.38 |
| **Protein (%)** | 13.6 ± 3.6 | 14.9 ± 2.4 | 0.36 |
| **CHO (%)** | 52.6 ± 7.6 | 46.5 ± 12.3 | 0.23 |
| **Fat (%)** | 35.0 ± 8.3 | 35.8 ± 5.0 | 0.78 |
| **Calcium (mg/1000 kcal) *** | 269.4 ± 155.0 | 503.1 ± 185.4 | 0.01 |
| **Vitamin D IU (%RDA)** | 39.3 ± 87.5 | 28.1 ± 43.1 | 0.72 |

*CG significantly different than matched control in unpaired T-test p<0.05
(%) represents the percentage of macronutrient kcalorie in total kcalorie intake

***Correlations***

Height z-score, BMI z-score, and LMI was positively correlated with weight z-score. As predicted in our second specific aim, LBM% positively correlated with minutes of vigorous-very vigorous PA/day, yet did not correlate to any other PA or strength measures. LBM% trended towards being significantly correlated with LMI (p=0.07). Furthermore, LMI positively correlated with mean right grip

strength, mean left grip strength, mean dominant grip strength, and minutes of vigorous-very vigorous PA minutes/day. LMI may be a better predictor of muscle strength and time spent in PA; thus, a better measure of comparing LBM functionality. MVPA minutes/day correlated with % of total wear time spent in MVPA. Raw cpm positively correlated with MVPA minutes/day and % of total wear time spent in MVPA, demonstrating raw cpm can be a good predictor of overall intensity of PA before imposing cut-points. **(Appendix 3)**

## Chapter 5

## Discussion

This is the first study to examine muscle strength and PA in relationship to body composition in children with CG. To our knowledge, only two other studies have reported an abnormal body composition with decreased LBM in individuals with CG. Yet, the role altered body composition had on muscle strength was not assessed by these studies. Furthermore, PA was not reported in either paper. PA may positively impact the reduced LBM, weight, and overall health in children with CG. It is important to determine potential relationships between these variables to establish beneficial clinical interventions for optimal patient outcomes.

One of the major findings of this study is that individuals with CG may have decreased strength as our subjects with CG had significantly decreased strength measures in comparison to their matched controls. Skeletal muscle strength is the force-producing capacity of muscle. There is an association between muscle strength and functional impairments or motor dysfunction.[78] Motor function is controlled by the cerebral cortex, basal ganglia, and cerebellum in the brain.[40,51] The decreased strength observed in subjects with CG may be a consequence of the white matter abnormalities observed in most individuals with galactosemia and an underdeveloped cerebellum observed in some patients with CG.[16,37,51]Expressing muscle strength per unit of muscle mass could provide an estimate of the contribution of neuromuscular factors to changes in muscle

strength; however, our two-compartment body composition model does not make this assessment feasible.

Other confounding factors of muscle strength include age, sex, body weight, sex hormones, genetics, and level of physical activity training. Our subjects were matched for age, sex, ethnicity, and pubertal status eliminating many of these factors. There was a significant difference in PA between subjects with CG and their matched controls. The difference in strength we observed may be due to participants with CG spending significantly less time in MVPA. Yet, this does not seem to fully explain their reduced strength. This was illustrated by two subjects with CG who spent more time in MVPA than their matched controls, yet the same two subjects had the largest difference in reduced dominant hand grip strength compared to their matched controls.

Both subjects and controls in this study were very active, exceeding the CDC recommendations for PA for children of ≥60 min/day of MVPA. The interpretation of PA data is complicated since the accelerometer output data for subjects with CG did not account for their total activity because some of these subjects spent time swimming when the accelerometer was not worn, which was not observed in the control group **(Table 14)**. Thus, we cannot conclusively say that the subjects did in fact spend less time in MVPA than their matched controls.

The observed reduction in strength in subjects with CG could also be related to anthropometrics since they were both smaller for weight and weight for height than matched controls. This smaller body habitus may mean the subjects

were unable to generate as much force as their larger matched controls. Though there was not a significant correlation between weight z-score and our strength measures; right grip strength, left grip strength, and dominant grip strength were all significantly reduced in the subjects with CG **(Table 11)** and significantly correlated to LMI **(Appendix 3)**.

Loss of muscle mass or sarcopenia is correlated with functional limitations and decreased muscle strength.[79] Reduced strength in subjects with CG is likely due to a reduced LMI compared to matched controls, although, our cohort only shows a trend towards a decrease in LMI (p=0.06). This could be a false negative finding due to the method used to measure body composition, a small cohort with a lack of statistical power, or a selection bias since only individuals interested in measuring their body composition and PA were willing to participate.

Panis et al[2] and Doulgeraki et al[3] both found subjects with CG to have significantly reduced LMI z-scores obtained from DXA scans in comparison to DXA software reference data. Neither study reported LMI or LBM% which makes our two-compartment body composition assessment by BIA not feasible for inter-study comparisons. Our cohort had a LMI that trended towards a significant decrease (p=0.06) compared to controls and we would likely have found a significantly lower LMI z-score, as z-scores can further normalize body composition measures for a stronger statistical comparison among different groups of differing age and height.[80] Furthermore, subjects with CG in the study by Panis had similar height and weight z-scores when compared to our subjects

with CG, so it is likely that our cohort would have a similar decrease in LMI z-scores if we had been able to assess this.

Doulgeraki et al[3] found a normal FMI in their cohort, but this group included more overweight and obese individuals with CG than our study. More importantly, Doulgeraki's[3] study subjects with CG did not have a lower mean height z-score when compared to DXA reference data. Our subjects with CG did not have a significantly different FMI than their matched controls, but did have reduced height z-scores. FM is more variable between individuals than FFM; thus, the two components of weight cannot be assumed to be normalized for height in the same way. To overcome the marked differences in height, body proportions and body composition, a larger sample size would be necessary to gain meaningful insight into this disease's effect on body composition.[80] Panis et al[2] used a FMI z-score to compare FM in children with CG (n=40) to DXA reference data and found individuals with CG to have a reduced FMI z-score. The same cohort was found to have a reduced height z-score, similar to our subjects

It has been widely reported that individuals with CG have reduced weight, height, and BMI z-scores.[4-6] As hypothesized, our study subjects with CG had a reduced weight z-score, height z-score, BMI z-score, and corrected height z-score compared to controls. However, after applying the Bonferroni correction, height z-score and BMI z-score were no longer significantly reduced. These are likely false negative results due to our small sample size and lack of statistical power. BMI z-score continued to trend towards significance after correction

(p=0.05). Height z-scores of female subjects with CG remained significantly reduced after correction (p=0.03). We did not observe any difference in waist circumference, hip circumference, or waist:hip ratio suggesting that while smaller in size than matched controls, those with CG are proportional to their body size.

The smaller stature and weight in our subjects with CG cannot be fully explained by their familial genetic potential, but these findings may be related to their diagnosis of CG. Our subjects with CG seem to have reduced growth when compared to their target growth potential based on the anthropometrics of a healthy matched control group of the same age. Three of twelve (25%) subjects with CG had height z-scores that differed from their mid–parental target height z-score by ≥ 2 standard deviations, indicating abnormal growth.[5, 77] These three individuals were within 2 standard deviations of the population mean height, indicating the importance of considering parental height when studying pediatric stature. Furthermore, this difference is not due to differences in pubertal status or ethnicity since these parameters were controlled by our matching criteria. Four of twelve (33%) subjects with CG had height z scores that differed from their mid–parental target height by -1≥x≥-2 standard deviations. Panis et al observed a reduced corrected height z-score in their study cohort as well.[2]

To our knowledge, this is the first evaluation of PA in individuals with CG. Raw cpm was significantly correlated with time in minutes/day of MVPA indicating that raw cpm is a good measure of level of PA intensity before imposing algorithmic cut-points which can provide inconsistent results from one equation to another.[67] In this study, raw cpm and wear-time in minutes/day was

not found to be significantly different between subjects and controls. Due to the differences in time of recruitment and in seasonal activities between the control group and the CG group, only the children with CG reported extensive time in PA while not wearing the accelerometer since the accelerometer cannot be worn while swimming. Thus, this PA could not be included in our analysis. While this is a limitation in our ability to definitively interpret differences in level and amount of PA, there was no significant difference in time worn or cpm so we can conclude that the children with CG were at least as active as their matched controls.

When cut-points were applied, we did observe that our subjects had significantly reduced PA in minutes/day of light activity, MVPA, and vigorous-very vigorous activity. There was not any difference in time spent in sedentary activity in minutes/day, in percent of total wear time spent in light PA, or time spent in moderate activity in minutes/day. The largest observed difference in PA between the two groups was time spent in minutes/day in vigorous-very vigorous PA. This amount of vigorous PA may or may not have been achieved by subjects with CG during their reported swimming activities when they were unable to wear the accelerometer. However, promoting high intensity activities could be an area of intervention for children and adolescents with CG to increase LMI z-score, weight z-score, and muscle strength.

Participants who completed a diet record met the Dietary Recommended Intake for energy and macronutrient intake for their age **(Table 15)**. Both subjects with CG and controls had low intakes of calcium and Vitamin D. Subjects with CG had a significantly lower intake of calcium (mg/1000 kcal), likely due to

avoidance of dairy foods within the group. Vitamin D intake was extremely low in both groups. Since most study participants were recruited in Portland, Oregon where endogenous vitamin D cannot be produced for a majority of the year, this is a public health concern and should be addressed by pediatric providers. Recommendations for food sources high in vitamin D and/or supplementation are needed. Only two subjects with CG reported supplementing with both calcium and vitamin D which is part of the standard practice recommendations to increase BMD in this population.[37] This appears to be a national concern for treatment of individuals with CG as many of the subjects with CG were recruited at the Galactosemia Foundation Conference and receive care across the USA.

We analyzed the data from the females separately as females with CG tend to be clinically more affected because of endocrine abnormalities. In our study, the female subjects with CG may have a more profoundly reduced height z-score than males with CG since the females had a significantly reduced height z-score even after the Bonferroni correction. BMI z-score did not remain significantly reduced in female subjects after correction, but this was most likely due to a loss in power. All grip strength measures remained significantly reduced when data from female participants was analyzed separately. However, sit-to-stand repetitions/min did not remain significantly reduced and LMI no longer trended towards significance in the female cohort compared to all subjects. A larger study cohort, including additional male participants would be needed to determine if there are further gender differences in these measures.

### *Study Limitations*

The greatest limitation of this study was the small sample size. Even though CG is a rare disease, the number of subjects in this study was too few to provide statistical power for meaningful interpretation of data, determine correlations, and identify possible predictors of outcome variables. This small sample size was further complicated by the extensive exploration of this study. Many outcome variables and few participants could easily lead to false positive conclusions. We employed the Bonferroni correction in an attempt to control for this statistical problem. Yet, we were only able to control for four variables because most of the study outcomes had not been previously reported in the literature and were exploratory in nature. This small sample size also led to poor gender distribution in our study population (9 females, 3 males with CG) preventing accurate comparisons between male and female cohorts.

Selection bias was another limitation of our study population as only children and adolescents interested in their PA level and body composition agreed to participate. This was demonstrated by the high activity level of our participants in both groups who were all involved in sports and included above average levels of PA in their daily routine. Thus, our group was not representative of the USA population as reported by NHANES 2003-2004 data in which accelerometers were used to measure PA. In this report, only 42% of 6-11 year old, 8% of 12-15 year old, and 7.6% of 16-19 year old children living in the USA were meeting the CDC recommendations of ≥60 mins/day of MVPA.[69] All of our study participants, both subjects and controls, with valid wear time far

exceeded the CDC recommendations. MVPA of those with CG ranged from 93 to157 min/d and MVPA of their matched controls ranged from 116 to 216 min/d. It is likely that the recruited subjects who were more clinically affected by CG did not wear the accelerometer or did not have valid wear time required for full analysis, but a review of medical records needs to be completed before this conclusion can be confirmed. For future studies, a PA questionnaire may be more useful to determine intra-comparisons of those with CG and allow for enrollment of participants who are less active.

Another limitation to our analysis of PA was that subjects and controls were recruited at different times of the year with different seasonal activities and school schedules. Most of the subjects with CG were recruited during the summer and participated in swimming activities when the accelerometer could not be worn. In contrast, control subjects were recruited during the winter and were not involved in water sports and did not have to remove the accelerometer for PA. Future research should use a more detailed report log/questionnaire to capture activity during times when the accelerometer could not be worn and/or collect accelerometer data in specified time-frames to limit seasonal variability between subjects and controls.

While BIA is considered a reliable measure of body composition, DXA is the gold standard. DXA provides information on BMD which is an important factor in this population. Our data is not comparable to past research on body composition in CG because we did not have LBM data variable that did not include bone mass to allow us to determine a LMI z-score. It would be important

for future research analyzing body composition in CG to use DXA for inter-study comparisons.

### *Study Strengths and Future Research Needs*

This study examined many factors affecting body composition, growth, muscle strength, and PA. It is the first study to examine muscle strength and PA in relationship to body composition in CG. We not only looked at muscle mass, but muscle function of various large muscle groups and joints to determine overall muscle strength of study subjects. We had strong matching criteria where subjects with CG were matched to a healthy control by age, sex, ethnicity, and puberty status eliminating many confounding variables of body composition and muscle development. We also considered parental height to adjust for the genetic potential in growth. We used a tri-axial accelerometer to measure PA which is the gold standard to measure PA in children. Our participants had excellent compliance and wear-time during the week of accelerometer data collection.

Future research should enroll a larger number of subjects with CG and attempt to include individuals who are clinically more affected by their disorder. A larger cohort with the use of DXA could determine the effects of CG on bone and body composition and provide a greater number of predictors for multiple regression analysis. Future research needs to address the significant reduction in muscle strength found in our subjects with CG, including a strength training program to assess strength and body composition before and after participation

This design could provide not only additional data but also assess the potential to increase strength and improve anthropometric measurements for those with CG. This additional data would expand our ability to provide clinical recommendations for PA for those with CG.

### *Summary and Conclusion*

In conclusion, children and adolescents with CG appear to have reduced growth potential, and muscle strength, likely influenced by their diagnosis of CG. Participation in vigorous PA may also be reduced in children and adolescents with CG. Interventions to improve strength and PA could influence weight, lean mass, and muscle strength in those with CG. Reported supplementation of calcium and vitamin D among those with CG was very low. This should be a point of clinical intervention to improve education about non-dairy sources of calcium and vitamin D and to recommend additional supplementation if intake remains suboptimal.

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## Appendix 1: Consent and Authorization Forms

IRB Approved: 12/12/2016
Approval Expires: 12/11/2017

**OREGON HEALTH & SCIENCE UNIVERSITY**
**Child Assent Form**

**TITLE**: An evaluation of body composition and energy needs in children and adolescents with galactosemia

**PRINCIPAL INVESTIGATOR**: Sandy Van Calcar, PhD, RD 503-474-5500

**CO-INVESTIGATORS**:
Esther Moe, PhD (503) 494-8051
Melanie Gillingham, PhD (503) 494-1682
Antoinette Kruger, BS (310) 529-7700

Part I
A researcher has explained this research study to me. I know that it may not help me. I also know that this study will help doctors know more about body composition and energy needs in people with galactosemia.

1. The investigator will ask me to explain what I will do and what will happen in this study to be sure I understand the study.

2. The investigator will ask me if I have any questions or want to know anything else about this study or body composition.

3. The investigator will ask me to explain some of the good and bad things that might happen to me if I enter this study.

Part II
I have thought about being a part of this study. I have asked and received answers to my questions. I agree to be in this study. I know that I don't have to agree to be in the study. Even though I agree to be in it now, I know I may feel differently later on and can ask to stop being in the study. I know that I may talk with my parents and/or doctor about not being in this study at any time.

Name/signature:_____ Date:_____



Document Control No.: IRB-CAS-01
Original Date: 12/04/2002; Revision Date: 02/10/2004

## OREGON HEALTH & SCIENCE UNIVERSITY
## Consent and Authorization Form

**TITLE**: An evaluation of body composition and energy needs in children and adolescents with galactosemia

**PRINCIPAL INVESTIGATOR**: Sandy Van Calcar, PhD, RD 503-474-5500

**CO-INVESTIGATORS**: Diane Stadler, PhD 503-494-0168
Melanie Gillingham, PhD (503) 494-1682
Antoinette Kruger, BS (310) 529-7700

**SPONSOR:** Oregon Health & Science University – Doernbecher Children's Hospital

**PURPOSE:**
In this form, "you" means you or your child. You have been invited to be in this research study because you have galactosemia. The purpose of this study is to investigate body composition, muscle strength, physical activity and calorie needs in children and adolescents with galactosemia.

This study requires one visit and will enroll 40 children, including 10 children and adolescents with galactosemia, ages 8-20 years, who are currently being followed at the OHSU Metabolic Clinic.

**PROCEDURES**:

This study requires one 3-hour visit to the OHSU Graduate Program in Human Nutrition Metabolic Assessment Room after fasting overnight. You must not eat anything or drink anything but water after you go to bed the night before your visit.

You will complete a 3-day food diary after your visit. You will arrive at OHSU at your normal waking time without eating or drinking anything that morning. Your calorie requirements will then be measured. Your muscle mass, muscle strength, and body composition will be measured at the same visit.

**3-day diet record:**
You will complete a 3-day food diary after your scheduled visit. The food diary is a record of all foods and drinks you consume over a 3 day period. It will be used to see how much vitamin D, calcium and other nutrients are in your diet. A food diary form will be provided for you. You will be provided with a stamped, addressed envelope to mail the diet record back to OHSU.

**Anthropometrics:**
We will measure your weight, height, and waist circumference at the visit.

**Tanner Scale:**
If you are 9 or older, you will be complete a pubertal stage self-assessment using the Tanner scale.

The Tanner scale pubertal self-assessment is a series of pictures that you will chose from. You will choose the image that looks most like your body. Two sexual maturation self-assessment questions will be asked: girls will select one breast image and one pubic hair image that looks the most like their bodies, and boys will select one genital image and one pubic hair image that looks the most like their bodies. The pictures you choose are your self-assessed pubertal stage of development. We need to assess your pubertal stage so that we can compare your data results to children or adolescents who are at the same developmental stage as you.

**Resting Energy Expenditure (REE):** REE is a measurement of how many calories you burn when you are at rest using a canopy air-collection system. For this measurement, you will rest comfortably on a bed. After 30 minutes of resting, a clear Plexiglass canopy will be fitted over your head and upper chest to collect expired air. You will continue to rest while the amount of expired air is measured for 30 minutes. There are no risks or discomforts associated with measuring REE. The procedure takes about one hour to complete.

**Bioimpedance Analyzer (BIA):**
A study investigator will use a BIA to determine how much muscle mass and fat mass your body has. BIA is a commonly used method for estimating body composition. It is a non-invasive, quick and painless procedure. You will lie still on a bed. Two electrodes will be placed on your right wrist and two electrodes will be placed on your right ankle. The BIA machine will be turned on, but you will not feel anything. The entire test will take approximately 5-10 minutes to complete.

**Physical Activity Level Assessment:**
You will be given an Actigraph GT3X accelerometer to wear after your study visit for 7 days for at least 10 hours a day to measure and categorize your daily physical activity into an intensity level of: Sedentary, Light, Moderate, or Vigorous. You will be given a log to document the time you wake up and what time you put on the accelerometer and when you take it off each day. The accelerometer is small, noninvasive, and worn around your waist on an elastic belt. After 7 days of wear, you will mail back the accelerometer and log back to OHSU.

**Grip Strength:**
To assess your upper body muscle strength, your grip strength will be measured. For this test, you will grip an instrument called a dynamometer for ten seconds in one hand. You will repeat this on your other hand, switching off, until you have completed three grip strength measurements on each hand. This will take a total of 2-5 minutes.

**Sit-to-Stand Test:**

A sit-to-stand test will be used to assess your lower body strength. In a seated position with your arms crossed over your chest, you will stand and sit as quickly as possible and as many times as possible in 30 seconds. The number of times you sit-to-stand will be counted in that 30 second time period.

If you have any questions regarding this study now or in the future, contact Dr. Sandy Van Calcar at 503-474-5500.

**RISKS AND DISCOMFORTS**:
All of the physical measurements taken in this study are non-invasive. The bioimpedance analyzer provides a small low-voltage current of electricity which you should not feel. This device has not been tested in people with implanted defibrillators for their heart. If you have an implanted defibrillator, you cannot participate in this study.

You may feel embarrassed during the selection of the pubertal Tanner stage pictures.

Some people with claustrophobia (fear of closed spaces) may find the REE test uncomfortable. If the Plexiglass canopy that will be placed over your face during the REE test makes you uncomfortable, the study team will stop the testing.

Although we have made every effort to protect your identity, there is a minimal risk of loss of confidentiality.

**BENEFITS**:
You may or may not personally benefit from being in this study. However, by serving as a participant, you may help us learn how to benefit other patients with galactosemia in the future.

**ALTERNATIVES**:
You may choose not to participate in this study and you can continue to receive your medical care for galactosemia at the OHSU Metabolic Clinic.

**CONFIDENTIALITY AND PRIVACY OF YOUR PROTECTED HEALTH INFORMATION:**
We will take steps to keep your personal information confidential, but we cannot guarantee total privacy.

We will create and collect health information about you as described in the Purpose and Procedures sections of this form. Health information is private and is protected under federal law and Oregon law. By agreeing to be in this study, you are giving permission (also called authorization) for us to use and disclose your health information as described in this form.

The investigators, study staff, and others at OHSU may use the information we collect and create about you in order to conduct and oversee this research study.

We may release this information to others outside of OHSU who are involved in conducting or overseeing research, including:

- The Office for Human Research Protections, a federal agency that oversees research involving humans

Those listed above may also be permitted to review and copy your records, including your medical records.

We will not release information about you to others not listed above, unless required or permitted by law. We will not use your name or your identity for publication or publicity purposes, unless we have your special permission.

We may continue to use and disclose your information as described above indefinitely.

Some of the information collected and created in this study may be placed in your OHSU medical

record. While the research is in progress, you may or may not have access to this information. After the study is complete, you will be able to access any study information that was added to your OHSU medical record. If you have questions about what study information you will be able to access, and when, ask the investigator.

**COMMERCIAL DEVELOPMENT**:
Information about you or obtained from you in this research may be used for commercial purposes, such as making a discovery that could, in the future, be patented or licensed to a company, which could result in a possible financial benefit to that company, OHSU, and its researchers. There are no plans to pay you if this happens. You will not have any property rights or ownership or financial interest in or arising from products or data that may result from your participation in this study. Further, you will have no responsibility or liability for any use that may be made of your information.

**COSTS**:
It will not cost you anything to participate in this study.

**LIABILITY**:
If you believe you have been injured or harmed while participating in this research and require treatment, contact the principal investigator, Sandy van Calcar at 503-494-5500.

If you are injured or harmed by the study procedures, you will be treated. OHSU does not offer any financial compensation or payment for the cost of treatment if you are injured or harmed as a result of participating in this research. Therefore, any medical treatment you need may be billed to you or your insurance. However, you are not prevented from seeking to collect compensation for injury related to negligence on the part of those involved in the research. Oregon law (Oregon Tort Claims Act (ORS 30.260 through 30.300)) may limit the dollar amount that you may recover from OHSU or its caregivers and researchers for a claim relating to care or research at OHSU, and the time you have to bring a claim.

If you have questions on this subject, please call the OHSU Research Integrity Office at (503) 494-7887.

**PARTICIPATION**:
If you have any questions, concerns, or complaints regarding this study now or in the future, contact Dr. Sandy Van Calcar at 503-474-5500.

This research is being overseen by an Institutional Review Board ("IRB"). You may talk to the IRB at (503) 494-7887 or irb@ohsu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research subject.
- You want to get more information or provide input about this research.

You may also submit a report to the OHSU Integrity Hotline online at https://secure.ethicspoint.com/domain/media/en/gui/18915/index.html or by calling toll-free (877) 733-8313 (anonymous and available 24 hours a day, 7 days a week).

Your participation in this study is voluntary. You do not have to join this or any research study. You do not have to allow the use and disclosure of your health information in the study, but if you do not, you cannot be in the study.

If you do join, and later change your mind, you have the right to quit at any time. This includes the right to withdraw your authorization to use and disclose your health information. If you choose not to join any or all parts of this study, or if you withdraw early from any or all parts of the study, there will be no penalty or loss of benefits to which you are otherwise entitled, including being able to receive health care services or insurance coverage for services. Talk to the investigator if you want to withdraw from the study.

If you no longer want your health information to be used and disclosed as described in this form, you must send a written request or email stating that you are revoking your authorization to:

***Sandy Van Calcar, PhD, RD***
***OHSU***
***Mail Code: GH207***
***3181 SW Sam Jackson Park Rd.***
***Portland, OR 97239***
vancalca@ohsu.edu.

Your request will be effective as of the date we receive it. However, health information collected before your request is received may continue to be used and disclosed to the extent that we have already acted based on your authorization.

If in the future you decide you no longer want to participate in this research, we will remove your name and any other identifiers from your information, but the material will not be destroyed and we will continue to use it for research.

We will give you any new information during the course of this research study that might change the way you feel about being in the study.

Your health care provider may be one of the investigators of this research study, and as an investigator is interested in both your clinical welfare and in the conduct of this study. Before entering this study or at any time during the research, you may ask for a second opinion about your care from another doctor who is in no way involved in this project. You do not have to be in any research study offered by your physician.

You may be removed from the study if the investigator stops the study or the sponsor stops the study.

We will give you a copy of this form now.

A Child Assent Form should be attached to the consent form, and should be filled out by your child.

IRB Approved: 12/12/2016
Approval Expires: 12/11/2017

**SIGNATURES:**
Your signature below indicates that you have read this entire form and that you agree to be in this study.

_____ _____
Subject signature Date

AND/OR

_____ _____ _____
Parent/Guardian Signature Relationship to subject Date

_____ _____ _____
Study coordinator/PI Signature Study coordinator/PI Printed Name Date

## Appendix 2: Physical Activity Data Collection Log

Study ID: _____

**Activity Monitor Log**

| Day | Circle the Day of the Week | Date monitor put on | Time out of bed in the morning | Time monitor put on in the morning | Time monitor removed at night | Time into bed for the night | List times during the day the monitor was not worn and state reason for not wearing (e.g., showering). Any additional comments? |
|---|---|---|---|---|---|---|---|
| 1 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |
| 2 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |
| 3 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |
| 4 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |
| 5 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |
| 6 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |
| 7 | M T W TH F SA SU | ___/___/___ dd/mm/yy | ___:___ am pm | ___:___ am pm | ___:___ am pm | ___:___ am pm | _____ _____ |

Thank you for completing this form. Please return it with the activity monitor.

## Appendix 3: Correlation p-values of subjects with CG

| | Height z-score | Weight z-score | BMI z-score | Corrected Ht Z-score | R. Grip Ave | L. Grip Ave | Dominant Grip Strength (lbs) | Sit-to Stand | LBM % | LBI (kg/m2) | METs | Light PA min/day | Vig PA min/day | MVPA Per day | CPM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Height z-score** | | **0.004** | 0.23 | 0.11 | 0.29 | 0.23 | 0.37 | 0.51 | 0.82 | 0.14 | 0.40 | 0.98 | 0.55 | 0.46 | 0.91 |
| **Weight z-score** | **0.004** | | **0.002** | 0.44 | 0.50 | 0.53 | 0.51 | 0.87 | 0.54 | **0.04** | 0.28 | 0.46 | 0.34 | 0.74 | 0.81 |
| **BMI Z-score** | 0.23 | **0.002** | | 0.42 | 0.85 | 0.96 | 0.70 | 0.32 | 0.72 | 0.08 | 0.33 | 0.49 | 0.29 | 0.84 | 0.41 |
| **Corrected Height z-score** | 0.11 | 0.44 | 0.42 | | 0.09 | 0.15 | 0.09 | 0.49 | 0.53 | 0.15 | 0.84 | 0.49 | 0.46 | 0.91 | 0.81 |
| **Right Grip Ave** | 0.29 | 0.50 | 0.85 | 0.09 | | **0.000*** | **0.000*** | 0.59 | 0.94 | **0.03** | 0.52 | 0.98 | 0.61 | 1.02 | 1.02 |
| **Left Grip Ave** | 0.23 | 0.53 | 0.96 | 0.15 | **0.000*** | | **0.000*** | 0.68 | 0.89 | **0.05** | 0.57 | 0.64 | 0.68 | 0.61 | 0.95 |
| **Dominant Grip Strength** | 0.37 | 0.51 | 0.70 | 0.09 | **0.000*** | **0.000*** | | 0.89 | 0.99 | **0.03** | 0.52 | 0.98 | 0.61 | 1.02 | 1.02 |
| **Sit-to-Stand** | 0.51 | 0.87 | 0.32 | 0.49 | 0.59 | 0.68 | 0.89 | | 0.10 | 0.62 | 0.43 | 0.61 | 0.21 | 0.52 | 0.91 |
| **LBM %** | 0.82 | 0.54 | 0.72 | 0.53 | 0.94 | 0.89 | 0.99 | 0.10 | | 0.07 | 0.08 | 0.58 | **0.05** | 0.31 | 0.10 |
| **LMI (kg/m2)** | 0.14 | **0.04** | 0.08 | 0.15 | **0.03** | **0.05** | **0.03** | 0.62 | 0.07 | | 0.61 | 0.78 | **0.04** | 0.91 | 0.91 |
| **METs** | 0.40 | 0.28 | 0.33 | 0.84 | 0.52 | 0.57 | 0.52 | 0.43 | 0.08 | 0.61 | | 0.40 | 0.26 | 0.26 | 0.09 |
| **Light PA min/day** | 0.98 | 0.46 | 0.49 | 0.49 | 0.98 | 0.64 | 0.98 | 0.61 | 0.58 | 0.78 | 0.40 | | 0.58 | 0.11 | 0.55 |
| **Vig min/day** | 0.55 | 0.34 | 0.29 | 0.46 | 0.61 | 0.68 | 0.61 | 0.21 | **0.05** | **0.04** | 0.26 | 0.58 | | 0.95 | 0.95 |
| **MVPA/ day** | 0.46 | 0.74 | 0.84 | 0.91 | 1.02 | 0.61 | 1.02 | 0.52 | 0.31 | 0.91 | 0.26 | 0.11 | 0.95 | | **0.02** |
| **CPM** | 0.91 | 0.81 | 0.41 | 0.81 | 1.02 | 0.95 | 1.02 | 0.91 | 0.10 | 0.91 | 0.09 | 0.55 | 0.95 | **0.02** | |

*p<0.0001