ADIPOSITY AND CORTISOL REGULATION DURING DAILY LIVING AND EXERCISE
IN ADOLESCENTS WITH AND WITHOUT DOWN SYNDROME

by

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ABSTRACT

Adiposity and cortisol regulation during daily living and exercise in adolescents

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Down syndrome is the most common genetic form of intellectual disability. Overweight and obesity are highly prevalent among individuals with Down syndrome, representing a health disparity relative to the general population. Research on obesity in Down syndrome is limited by reliance on body mass index (BMI) to describe and classify this health condition. Measuring adiposity with dual-energy x-ray absorptiometry (DXA) allows for three-compartment analysis including estimates of total and regional body composition and provides a better representation of adiposity in this unique population. Factors that contribute to the higher prevalence of obesity in Down syndrome are not well understood, yet are critical for designing health promotion interventions to address this condition in Down syndrome. The focus of this dissertation was to examine the associations between health behaviors (i.e. physical activity, dietary energy intake), cortisol, and adiposity in adolescents with and without Down syndrome. Three studies were conducted. The first described adiposity using DXA and examined associations with physical activity and dietary energy intake. The second and third studies examined cortisol, including

diurnal patterns and responses to exercise, and associations with adiposity. The first study found significantly higher levels of body fat among adolescents with Down syndrome, particularly in the trunk region. However, the magnitude of difference compared to typically developing adolescents was considerably smaller than when examined with BMI, suggesting that BMI may be overestimating obesity in Down syndrome. Physical activity showed a moderate association with adiposity. The second study found that diurnal cortisol patterns were not significantly different between adolescents with and without Down syndrome. Differences in cortisol levels were observed between adolescents when stratified by the interaction of total body fatness and physical activity, indicating a joint effect on the association with adiposity. The third study attempted to compare the cortisol response to exercise between adolescents with and without Down syndrome, but neither group demonstrated a significant response. When stratified by total body fatness, cortisol levels were moderately higher in adolescents with greater adiposity. These higher cortisol levels may help to partially explain levels of adiposity in adolescents with Down syndrome. Due to the high levels of obesity and low levels of physical activity, directed health promotion interventions are needed in this population.

CHAPTER 1

DXA-derived body composition and health behaviors in adolescents with Down syndrome

Introduction

Childhood obesity in the general pediatric population poses a significant public health problem, with estimated rates continuing to track at epidemic levels (Karnik & Kanekar, 2012). Prevalence rates of overweight and obesity are currently estimated at 30.4% among adolescents in the United States (Ogden, Carroll, Kit & Flegal, 2014). This health condition is likely to continue into adulthood, increasing the risks of developing comorbidities and of early mortality (Reilly & Kelly, 2010). Youth with intellectual disabilities experience overweight and obesity with greater frequency than the general population (Rimmer, Yamaki, Davis, Wang & Vogel, 2011; Rimmer, Yamaki, Lowry, Wang & Vogel, 2010; Segal et al., 2016), yet obesity and health behaviors (e.g. physical activity and dietary intake) remain understudied areas of research in this 'at-risk' population.

Down syndrome is the most common genetic form of intellectual disability (Sherman, Allen, Bean & Freeman, 2007). Recent prevalence rates range from 1/691 to 1/847 infants (Parker et al., 2010; Shin et al., 2009). Within this population, overweight and obesity are highly prevalent. Approximately 60% of youth with Down syndrome are estimated to be overweight (Bandini, Fleming, Scampini, Gleason & Must, 2012; Gonzalez-Aguero, Ara, Moreno, Vicente-Rodriguez & Casajus, 2011; Grammatikopoulou et al., 2008; Harris, Rosenberg, Jangda, O'Brien & Gallagher, 2003; Myrelid, Gustafsson, Ollars & Anneren, 2002; Rimmer et al., 2010; Styles, Cole, Dennis & Preece, 2002; van Gameren-Oosterom et al., 2012); rates that far exceed the

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general population (Ogden, Carroll, Kit & Flegal, 2012; Ogden et al., 2014). Multiple studies have demonstrated greater risk for obesity in youth with Down syndrome compared to typically developing peers (OR 3.00)(Rimmer et al., 2010) and youth with non-genetic intellectual disabilities (OR 1.97 to 3.21)(Bégarie, Maïano, Leconte & Ninot, 2013; Krause, Ware, McPherson, Lennox & O'Callaghan, 2015; Pan, Davis, Nichols, Hwang & Hsieh, 2016). Thus, obesity in youth with Down syndrome cannot be attributed to the effects of cognitive impairments alone. High rates of overweight and obesity persist and expand throughout adulthood (Bhaumik, Watson, Thorp, Tyrer & McGrother, 2008; Melville, Cooper, McGrother, Thorp & Collacott, 2005; Melville, Hamilton, Hankey, Miller & Boyle, 2008; Rubin, Rimmer, Chicoine, Braddock & McGuire, 1998; Stancliffe et al., 2011). In addition to the health risks associated with obesity, decreased community participation, challenges with independent living, and reductions in overall quality of life are also associated with obesity in persons with Down syndrome (Rimmer & Yamaki, 2006).

However, the current evidence on obesity in Down syndrome is limited by over-reliance upon body mass index (BMI; kg/m^2). The large range of prevalence estimates reported across youth with Down syndrome is likely due to unique study populations and the variety of obesity definitions and cut-offs employed (van Gameren-Oosterom et al., 2012). Due to short stature, a common characteristic of Down syndrome (Roizen & Patterson, 2003), BMI may overestimate excess fatness in this population. In particular, the 85th percentile on BMI-for-age growth charts appears to misclassify a large number of adolescents with Down syndrome as overweight when excess fatness is not present (Bandini et al., 2012). Overall efficiency (e.g. proportion correctly classified) was reported as only 69% (Bandini et al., 2012). Few studies have utilized dual energy x-ray absorptiometry (DXA), an advanced body composition measurement technique, in

persons with Down syndrome. DXA scans use low levels of radiation to measure fat mass, lean soft tissue, fat-free mass, and bone mineral content, allowing for estimates of total and regional body composition (Lohman & Chen, 2005). Limited evidence indicates adolescents and adults with Down syndrome have elevated body fat proportions, with greater fat mass and lower lean mass than typically developing controls (Bandini et al., 2012; Baptista, Varela & Sardinha, 2005; Esco, Nickerson, Bicard, Russell & Bishop, 2016; Gonzalez-Aguero et al., 2011; Guijarro, Valero, Paule, Gonzalez-Macias & Riancho, 2008; Loveday, Thompson & Mitchell, 2012; Nickerson et al., 2015; Wendel et al., 2016). Greater abdominal obesity (fat in the trunk region) has also been identified in adolescents with Down syndrome, particularly in females (Gonzalez-Aguero et al., 2011). These findings suggest a unique body fat topology in Down syndrome, further violating the assumptions needed to apply BMI in this population and indicate that DXA-derived fat mass can elucidate differences that are not available from standard anthropometry. Research utilizing DXA to study obesity in Down syndrome is critical to better understand and account for these systematic differences in body fat distribution.

Despite the high prevalence of overweight and obesity among youth with Down syndrome, the factors that contribute to this health condition are not well understood. Researchers contend that low physical activity contributes to the obesity health disparity (Latash, Wood & Ulrich, 2008; Murray & Ryan-Krause, 2010; Rimmer & Rowland, 2008; van Gameren-Oosterom et al., 2012); however, this relationship has not been empirically established in Down syndrome. Moderate-to-vigorous physical activity (MVPA) is a well-defined correlate of obesity in the general pediatric population (Jiménez-Pavón, Kelly & Reilly, 2010; Ness et al., 2007; Saelens, Seeley, van Schaick, Donnelly & O'Brien, 2007). Nutritional intake and dietary composition are also thought to contribute to obesity and secondary conditions. Multiple public

health organizations have stated a need to improve dietary quality in persons with intellectual disabilities (National Center for Health Statistics, 2012; Van Riper, 2010; World Health Organization, 2000). There remains a paucity of dietary intake data in this population, in particular for adolescents with Down syndrome.

The objective data available on physical activity among individuals with Down syndrome is expanding, but currently inconclusive (Frey, Stanish & Temple, 2008; Pitetti, Baynard & Agiovlasitis, 2013). Reported estimates of youth that meet recommended physical activity guidelines (U.S. Department of Health and Human Services, 2008) range from 0% to approximately 40% (Esposito, MacDonald, Hornyak & Ulrich, 2012; Izquierdo-Gomez et al., 2014; Matute-Llorente, González-Agüero, Gómez-Cabello, Vicente-Rodríguez & Casajús, 2013a; Matute-Llorente, González-Agüero, Gómez-Cabello, Vicente-Rodríguez & Casajús, 2013b; Phillips & Holland, 2011; Shields, Dodd & Abblitt, 2009), suggesting that youth with Down syndrome engage in insufficient levels of MVPA. However, Whitt–Glover et al. (2006) found children with Down syndrome (ages 3 to 10 years) to engage in very high levels of physical activity, with over 90% engaging in 60 minutes of moderate physical activity per day. Physical activity in this population appears to be a function of age, with all studies examining cross-sectional age trends showing significant declines through adolescence (Esposito et al., 2012; Izquierdo-Gomez et al., 2014; Izquierdo-Gomez et al., 2015; Phillips & Holland, 2011; Shields et al., 2009; Shields, Hussey, Murphy, Gormley & Hoey, 2015). Additional studies have also statistically controlled for age in analyses to address this trend and had similar results (Matute-Llorente et al., 2013a; Matute-Llorente et al., 2013b; Whitt-Glover et al., 2006). Finally, it appears that youth with Down syndrome engage in less physical activity than typically developing controls (Matute-Llorente et al., 2013a; Matute-Llorente et al., 2013b; Phillips &

Holland, 2011; Whitt-Glover et al., 2006); however more studies are needed to confirm these differences.

Total caloric intake and macronutrient distribution may also impact the development of obesity. Resting metabolic rate in youth with Down syndrome is significantly lower, thus many medical professionals recommend calorie restriction to prevent excess weight gain (Murray & Ryan-Krause, 2010; Roizen, 2002). However, youth and adults with Down syndrome have been shown to already consume less caloric energy compared to typical recommended dietary allowances (Fujiura, Fitzsimons, Marks & Chicoine, 1997; Hopman et al., 1998; Luke, Sutton, Schoeller & Roizen, 1996; Sharav & Bowman, 1992) and significantly fewer calories than typically developing controls or siblings (Hopman et al., 1998; Luke et al., 1996; Samarkandy, Mohamed & Al-Hamdan, 2012; Sharav & Bowman, 1992). It is unclear whether this caloric intake is sufficiently lower to account for differences in stature and metabolic rate among individuals with Down syndrome, or whether these values still contribute to the risk for obesity.

While total energy intake is consistently lower in individuals with Down syndrome, the macronutrient profile of intake reported across studies is very consistent. Individuals with Down syndrome have been shown to consume macronutrients in typical proportions of daily calories from carbohydrate (53% to 60%), fat (28% to 30%) and protein (13% to 18%)(Braunschweig et al., 2004; Grammatikopoulou et al., 2008; Hopman et al., 1998; Luke et al., 1996; Samarkandy et al., 2012). This profile of macronutrient content does not appear to differ significantly between individuals with and without Down syndrome, nor does dietary energy intake differ greatly from recommended dietary allowance ranges (Grammatikopoulou et al., 2008; Luke et al., 1996; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015).

However, there remain multiple gaps in the literature on diet in Down syndrome. Only one study has included a sample of adolescents (Grammatikopoulou et al., 2008) and few have examined daily caloric intake relative to weight (Braunschweig et al., 2004; Grammatikopoulou et al., 2008; Hopman et al., 1998). This methodology could be further improved by calculating energy intake relative to lean mass instead of total mass (Cameron et al., 2016). Lean body mass is strongly correlated with resting metabolic rate which represents the largest component of daily energy expenditure (Blundell, Gibbons, Caudwell, Finlayson & Hopkins, 2015).

There is a need for more research describing physical activity and dietary energy intake in youth with Down syndrome, but also to understand how these health behaviors are associated with health outcomes such as obesity. A relationship between these health behaviors and body composition is the foundation for many health promotion practices intended to prevent excess weight gain. However, studies examining the association between MVPA and BMI have found surprisingly weak and non-statistically significant relationships (Esposito et al., 2012; Izquierdo-Gomez et al., 2015; Nordstrom, Hansen, Paus & Kolset, 2013; Shields et al., 2009; Shields et al., 2015). Very few studies have reported an association between dietary energy intake and BMI in Down syndrome, but findings are also weak, non-significant, and limited to adults with Down syndrome (Braunschweig et al., 2004; Fujiura et al., 1997). While it is possible that cross-sectional relationships do not exist with these health behaviors, it is equally likely that BMI is an inappropriate index of body composition for use in the Down syndrome population. Precise measures of body composition that account for unique regional fat distributions are likely needed define such a relationship. The association between these health behaviors and adiposity in Down syndrome have not been examined using DXA.

The primary purpose of this study was to measure adiposity using an advanced measurement technique (DXA) capable of examining differences between adolescents with and without Down syndrome and associations with health behaviors. Three specific aims were addressed. First, the study compared group differences between adolescents with and without Down syndrome on body composition, physical activity, and dietary energy intake. Second, differences in the proportion of samples identified as overweight or obese were compared between BMI and DXA metrics. Third, the study examined the associations between physical activity, diet, and adiposity within each group.

Methods

Participants

All methods and procedures for the study were approved by the Institutional Review Board of the University of Michigan Medical School. All parents signed written informed consent documents while participants completed written or verbal assessment prior to initiating the study. Adult participants (e.g. 18 years old) were allowed to independently provide written informed consent, however parents of adult participants with Down syndrome also provided written consent. Participants were recruited through Down syndrome parent support groups in Michigan and northern Ohio. Typically developing adolescents were recruited through family referrals, local school districts, and from previous study samples. All participants were between 12 and 18 years old, Tanner stages III to V (Marshall & Tanner, 1969, 1970), and *without* dual disability diagnosis (e.g. autism), comorbid disease (e.g. diabetes), or contraindication limiting safe engagement in physical activity.

Procedures

Phases. Each participant completed two phases of the study. First, participants completed a clinical visit with research staff at the Michigan Clinical Research Unit at the University of Michigan Hospital. The clinical visit included the informed consent process, measurement of body composition through standard anthropometry and a single DXA scan, and parental questionnaires for demographics and dietary assessment. Second, participants completed one week of accelerometry to measure habitual physical activity participation. Researchers trained both participants and parents in the accelerometer procedures during the clinical visit.

Traditional Anthropometry. All anthropometric measurements were conducted according to guidelines from Lohman et al. (1988). Height and weight were measured in duplicate to the nearest 0.1 cm with a stadiometer (SECA S-214) and nearest 0.01 kg with a digital scale (Health O Meter H-349KL), respectively. If measures were more than 0.5 cm (height) or 0.5 kg (weight) apart, a third trial was conducted. The average across all trials was used to derive BMI (kg/m^2). BMI percentile was also determined using sex-specific BMI-for-age growth references from the CDC (Kuczmarski et al., 2000). Overweight was defined as a BMI $\geq$ 85th percentile and obesity was defined as $\geq$ 95th percentile. To determine how the current sample compared to other youth with Down syndrome, BMI percentile was also determined from sex- and age-specific growth curves for youth with Down syndrome ages 2 to 20 years (Zemel et al., 2015). However, inferences regarding definitions for overweight or obesity in Down syndrome cannot be made from these charts.

Dual-Energy X-Ray Absorptiometry. Each participant completed one whole-body DXA scan (GE Lunar Prodigy Advance [DPX-IQ 240] densitometer; Lunar Radiation Corp, Madison, WI) to measure body composition. Measurement through DXA provides a three-

component analysis of body composition and is more cost-effective, time-efficient, and has minimal-risk, yet is correlated with more advanced imaging techniques (e.g. computed tomography and magnetic resonance imaging) making it a viable method for epidemiological research (Kendler et al., 2013). Reliability of the GE Lunar Prodigy has been established in pediatric populations for both total (ICC ≥ 0.98) and regional (ICC ≥ 0.97) estimates of body composition (Margulies et al., 2005). However, reliability evidence in populations with Down syndrome is not available.

The scanner was calibrated daily according to the manufacturer's instructions using a standardized block by the certified technician. Each DXA scan involves a low radiation dose (0.37 μSv); equivalent to the radiation absorbed during a typical day (Albanese, Diessel & Genant, 2003). This radiation dose is considered medically negligible. However, only one DXA scan was attempted per participant to minimize exposure as an additional safeguard.

Participants wore wear light clothing, free of metallic objects, and were positioned in a supine position with hands by the sides in a neutral position. If needed, loose-fitting Velcro around the ankles or a warm blanket were used to assist the participant with maintaining position during the scan. While minimal movement did occur during some scans among participants with DS, all participants completed a DXA scan capable of analysis with the DXA software (EnCore v.14.10). The DXA scan provided measurements of fat mass, lean mass, fat-free mass, bone content, and percent body fat. Total-body and regional segments of arms, legs, and trunk were calculated using pediatric software. Ratios of fat mass distribution were then calculated including trunk-to-total, legs-to-total, arms-to-total, and arms and legs-to-trunk. The primary variables from the DXA scan used in analyses were the total and regional estimates of body fat percentage (%BF). Obesity was classified based on age- and sex-specific pediatric cut-points for elevated

body fat percentage (Freedman et al., 2009). Total-body lean mass from the DXA scan was also used to standardize dietary energy intake (kcal/lean kg).

Questionnaires. All questionnaires were completed by the accompanying parent with the assistance of the participant to ensure consistency between participants with and without Down syndrome and minimize reporting bias due to intellectual disabilities (Emerson, Felce & Stancliffe, 2013).

S*ociodemographic survey.* Parents completed a basic demographic survey including the participant's age, sex, race and ethnicity, disability severity, current medication use and comorbidities, as well as parental age, education, and household income.

Tanner Staging. To account for differences in pubertal development across adolescents, puberty stage was measured using Tanner stages (Marshall & Tanner, 1969, 1970). Parents completed a proxy-report questionnaire using schematic line drawings of pubertal development. Reports using line drawings have been shown to significantly correlate (boys: r = .59; girls: r = .81) with physician exam (Morris & Udry, 1980). Ratings on this scale range from I (prepubertal) to V (adult). Parental reports of pubertal stage have moderate agreement (r = 0.37 to 0.71) with physician exams, but are prone to underestimate pubertal development in girls and overestimate in boys (Rasmussen et al., 2015). Due to the challenges with self-report in adolescents with Down syndrome and the invasiveness of a physical exam, the use of parental report is justified for the current study. Tanner stage was operationalized as the average of reported stages in pubic hair and genital development for males and breast and pubic hair development for females. Participants in the current study were limited to adolescents in Tanner stages III to V.

Dietary Intake. To estimate dietary energy intake and macronutrient distribution, a parent completed a proxy-report of the Harvard Youth/Adolescent Questionnaire (YAQ)(Rockett, Wolf & Colditz, 1995). This food frequency questionnaire (FFQ) assesses dietary behavior over the past year based on how often specific foods are consumed. The YAQ was selected for four reasons: a) it is a simple, self-administered assessment; b) there are no dietary assessments specifically validated for individuals with intellectual disabilities; c) the YAQ produces valid ($r=0.45$) and reliable ($r=0.41$) estimates of dietary energy intake (Rockett, Berkey & Colditz, 2007; Rockett et al., 1997; Rockett et al., 1995); and d) FFQs have been used previously for persons with Down syndrome (Braunschweig et al., 2004). Parents completed the YAQ with the assistance of the participant with the researchers available to answer questions. Individual measures of total daily energy intake (kcals), macronutrient distributions from protein, available carbohydrate (total carbohydrate minus fiber), fiber, total fat, saturated fat, poly- and mono-unsaturated fat, and cholesterol were calculated from estimates of the frequency of consumption of various foods. Daily energy intake per kilogram of lean mass (Cameron et al., 2016) was also calculated using data from the DXA scan and is the primary variable for dietary energy intake. The percentage of contribution to total calories for protein (4 kcals/g), carbohydrate (4 kcals/g), and fat (9 kcals/g) were also calculated.

Physical Activity. Each participant wore an Actigraph GT3X+ (Pensacola, FL) triaxial accelerometer during waking hours for seven days at the waist to measure habitual physical activity. Waking hours were determined through a log completed by the parents and participants. All actigraphy data was collected at sampling frequency of 30 Hz with a 10-second epoch to capture intermittent movement. Criteria for minimum wear time included 10 hours per day for at least 4 days of the 7-day period, including at least one weekend day (Cain, Sallis, Conway, Van

Dyck & Calhoon, 2013). Each accelerometry file was manually cleaned to only include data collected between reported waking and bed times on each day and then assessed for non-wear time using validated algorithms (Choi, Liu, Matthews & Buchowski, 2011) with ActiLife 6 software v6.9.5 (Actigraph, Pensecola, FL). Data were then reduced into physical activity intensity categories based on counts per minute (cpm): sedentary (<100 cpm), light physical activity (101-2295 cpm), moderate physical activity (2296-4011 cpm), and vigorous physical activity (≥ 4012 cpm), validated by Evenson et al. (2008). Cut-points were selected based on recommendations from a comparative study of cut-points in youth (Trost, Loprinzi, Moore & Pfeiffer, 2011) and evidence of moderate MVPA outcomes using this threshold (Loprinzi et al., 2012).

Data Analysis

Data analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY) with an a priori α of 0.05. Demographics of the sample were described using independent t-tests and Pearson's Chi-square (X^2) tests with effect sizes (i.e. Cohen's *d* and Cramer's V). Based on these statistics, groups exhibited equivalent distributions of demographic characteristics (e.g. age, sex, Tanner stage).

First, multivariate analysis of covariance analyses (MANCOVA) were conducted to determine if groups with and without Down syndrome produced mean differences after adjusting for relevant covariates. Analyses were conducted in four sets: 1) body composition, 2) body fat distribution, 3) physical activity, and 4) dietary energy intake. The covariates included in all models were age, sex, and Tanner pubertal stage. Accelerometer wear-time was also a covariate for analyses on physical activity. Each analysis set was then repeated with separate analyses by sex groupings. Effect sizes including eta-squared (η^2) from the adjusted MANCOVA analysis

and Cohen's *d* from unadjusted group means were also calculated. Results were interpreted based on statistical significance of multivariate and univariate *F* tests and magnitude of effect sizes (Hopkins, Marshall, Batterham & Hanin, 2009; Sutlive & Ulrich, 1998). MANCOVA was used to reduce Type I error rate compared to a series of ANCOVA tests.

Second, differences in the proportion of samples identified as overweight or obese were compared between BMI and DXA metrics. Participants were coded as overweight if their BMI was greater or equal to the 85th percentile on the CDC growth reference (Kuczmarski et al., 2000), obese if their BMI was greater or equal to 95th percentile (Kuczmarski et al., 2000), and as having elevated fat mass if total body fat percentage was greater or equal to age- and sex-specific cut-points from Freedman et al. (2009). Pearson's Chi-square (X^2) tests with Cramer's V effect sizes were used to examine differences in proportions across tests. Each analysis compared the proportion identified as overweight or obese with those identified as having an elevated fat mass. Chi-square analyses were conducted separately for adolescents with and without Down syndrome.

Third, Pearson product-moment correlation coefficients and linear regression were used to examine the associations between physical activity, dietary intake, and adiposity. Pearson correlations were examined for all possible associations between body composition variables, physical activity variables, and total dietary energy intake normalized to lean body mass (kcal/lean kg). Analyses were conducted separately for males and females with Down syndrome. Correlations were interpreted based on statistical significance ($p < .05$) and the magnitude of the association (Hopkins et al., 2009; Sutlive & Ulrich, 1998). Linear regression analyses examined the association of total body fat percentage and Down syndrome diagnosis controlling for the effects of sex, age, Tanner pubertal stage, physical activity (MVPA minutes/day), and dietary

energy intake (kcal/lean kg). Results were interpreted based on the magnitude of standardized

coefficients (β), statistical significance ($p < .05$), and effect size (η^2). Semi-partial correlation (sr)

and squared (sr^2) coefficients were also calculated to examine the unique contributions of each

factor to the total variance explained in the dependent variable. Subsequent analyses examined

the same model, but separately for adolescents with and without Down syndrome to examine the

differential effects of each factor within each group.

Results

A total of 39 participants (22 with Down syndrome, 17 with typical development)

participated in the study. Descriptive statistics and demographic information for the participants

are presented in **Table 1.1.** All participants were between 12 and 18 years old and between

Tanner stages III and V. Groups had equivalent distributions for age, sex, and Tanner pubertal

stage ($p > .10$). Adolescents with Down syndrome were significantly shorter in stature than

adolescents with typical development, $t(37) = 5.82$, $p < .001$; but were similar in weight, $t(37) =$

0.51, $p = .612$. No statistical differences were observed between groups for any other

demographic characteristics ($p > .10$).

Large differences were observed in body composition between groups (**Table 1.2** and

Figure 1.1). Adolescents with Down syndrome had significantly higher BMI ($p = .001$, $d = 1.06$)

and BMI percentile based on CDC growth charts ($p < .001$, $d = 1.31$)(Kuczmarski et al., 2000).

Down syndrome-specific growth charts characterized the sample at the 64th percentile ($SE =$

4.08) on average (Zemel et al., 2015). Body fat percentages, measured via DXA, were also

significantly higher among adolescents with Down syndrome ($p < .05$); however the magnitude

of difference was considerably smaller than was observed for differences in BMI ($d < .80$).

Significant differences were observed in body fat percentage for the total body as well as fat in

regional segments at the arms, legs, and trunk. Sex was a significant covariate for multiple indices of body composition. When separated by sex (**Table 1.3** and **Figure 1.2**), significant differences in BMI and BMI percentile persisted for both males ($p < .05$, $d > .80$) and females ($p < .05$, $d > 1.00$). Body fat percentages were significantly greater among males with Down syndrome for the total body, trunk, and arms ($p < .05$, $d > .80$), and trended toward statistical significance for the legs region ($p = .052$, $d = .862$). Statistically significant differences were not observed among females ($p > .10$), but females with Down syndrome averaged higher body fat percentages of a moderate magnitude ($d > .60$). Compared to other youth with Down syndrome, males with Down syndrome were considerably more overweight (74th percentile, $SE = 4.99$) than females (45th percentile, $SE = 8.08$).

To further examine body composition, ratios between regional segments of body fat were calculated. **Table 1.4** and **Figure 1.3** present the group differences in these body fat ratios. Large and statistically significant differences were observed between group for ratios of trunk-to-total ($p < .001$, $d = 1.19$), legs-to-total ($p < .001$, $d = 1.32$), and arms and legs-to-trunk ($p < .001$, $d = 1.23$). No differences were observed in the ratio of arms-to-total body fat ($p = .600$, $d = .264$). Each of these ratios indicated greater fat mass in the trunk (e.g. abdomen) among adolescents with Down syndrome. **Table 1.5** and **Figure 1.4** show similar group differences when stratified by sex.

The differences observed in BMI and percent body fat indices affected the prevalence of adolescents categorized as being overweight and obese, depending on whether classifications were based on BMI or body fatness. **Table 1.6** presents a series of 2x2 tests comparing the proportions of the sample categorized as obese or overweight based on the CDC growth curve (Kuczmarski et al., 2000) versus proportions of the sample with normal or elevated body fat

percentage (Freedman et al., 2009) as measured by DXA. Adolescents with Down syndrome exhibited high levels of obesity and were categorized as 70.2% overweight, 41.0% obese, and 38.6% with elevated body fatness. Adolescents with typical development had lower levels of obesity and were categorized as 20.9% overweight, 17.5% obese, and 20.6% with elevated body fatness. 31.8% of adolescents with Down syndrome who were categorized as overweight based on the CDC growth charts (Kuczmarski et al., 2000) did not exhibit elevated body fatness (Freedman et al., 2009). Furthermore, 18.2% of adolescents with Down syndrome categorized as obese did not have elevated body fatness. An additional 13.6% had elevated body fatness, but were not categorized as obese. In total, only 68.2% of adolescents with Down syndrome were properly categorized. No substantial discrepancies in classification ($< 6\%$) were observed in typically developing adolescents. **Figure 1.5** shows the significant differences in adolescents with and without Down syndrome categorized as overweight, $F(1,34) = 12.67$, $p = .001$, partial $\eta^2 = .272$, $d = 1.30$; and differences in overweight and elevated body fat classification within adolescents with Down syndrome ($t(21) = 3.13$, $p = .005$, $d = .664$).

Physical activity levels were very low among most participants. Fewer than 5% of adolescents with Down syndrome and fewer than 20% of adolescents with typical development met physical activity guidelines of 60 minutes of MVPA per day (U.S. Department of Health and Human Services, 2008). **Table 1.7** and **Figure 1.6** present the group differences for each physical activity intensity category. Adolescents with Down syndrome averaged 27.83 (SE = 5.43) minutes of MVPA per day compared to 45.76 (SE = 6.21) minutes per day among adolescents with typical development, while controlling for age, sex, pubertal stage, and wear time (60 minutes per day). Significant differences between groups were observed for MVPA ($p = .041$, $d = .421$), vigorous physical activity ($p = .011$, $d = .617$), and light physical activity ($p <$

.001, $d = .110$). No differences were observed in sedentary time ($p = .104$, $d = .146$) or moderate physical activity ($p = .264$, $d = .098$). When stratified by sex (**Table 1.8** and **Figure 1.7**) only light physical activity was significantly different among both males ($p = .013$, $d = 1.15$) and females ($p = .009$, $d = .957$), while vigorous physical activity was significantly different among males only ($p = .037$, $d = .867$).

Dietary energy intake was also different between groups (**Table 1.9** and **Figure 1.8**). Estimates of raw energy intake were significantly lower among adolescents with Down syndrome including total energy (kcals/day; $p = .023$, $d = .761$), carbohydrates (g/day; $p = .035$, $d = .688$), and fat (g/day; $p = .015$, $d = .794$). However, when energy intake as normalized to lean body mass (kg), significant differences were no longer observed (kcal/kg/day; $p = .369$, $d = .382$). Macronutrient distributions were also not significantly different when normalized to total energy intake ($p > .10$, $d < .350$). On average, adolescents in the sample consumed most calories from carbohydrates (53.58%), fat (30.00%), and protein (17.40%). **Table 1.10** and **Figure 1.9** show how differences in dietary energy intake were moderated by sex. Among females, significant differences were observed in raw energy and macronutrient intake ($p < .05$, $d > .900$). Macronutrient content normalized to total energy intake were not significantly different between groups ($p > .30$, $d < .700$). Total energy intake normalized to lean body mass was not statistically different between groups ($p = 0.70$), but exhibited a large effect size ($d = .997$). Among males, there were neither significant group differences ($p > .05$) nor large effect sizes ($d < .800$).

Table 1.11 shows the correlations between measurements of body composition and physical activity for adolescents with Down syndrome and typical development. The primary purpose was to examine the association between MVPA and each index of obesity. In adolescents with Down syndrome, the associations between MVPA and BMI ($r = -.015$), and

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BMI percentile ($r = .021$) were very low. The associations between MVPA and body fat percentages including total body ($r = -.233$), trunk ($r = -.217$), legs ($r = -.237$), and arms ($r = -.230$), were stronger, but these associations did not reach statistical significance ($p > .05$). A similar pattern was observed for these associations among typically developing adolescents with larger magnitudes of association between MVPA and body fat percentages compared to associations with BMI, but these were also not statistically significant ($p > .05$). Statistically significant correlations among adolescents with Down syndrome were observed between light physical activity and all measures of body composition ($r > .500$, $p < .05$). Among typically developing adolescents, statistically significant correlations were observed between vigorous physical activity and total body, trunk, and leg region body fat percentage ($r > .400$, $p < .05$).

Finally, **Table 1.12** presents linear regression analyses examining the associations of sex, age, Tanner stage, MVPA, dietary energy intake, and presence of Down syndrome on total body fat percentage. The first analysis examined this relationship for all participants in the sample and explained a large portion of variance in total body fat percentage, $F(6,32) = 3.55$, $p = .008$, $R^2 = .400$. Significant factors affecting total body fat percentage included age ($\beta = 0.52$, $p = .031$), Tanner stage ($\beta = -0.58$, $p = .005$), and presence of Down syndrome ($\beta = 0.34$, $p = .027$). Semi-partial correlation coefficients (sr^2) suggested that the independent contribution to total variance explained was largest for Tanner stage (17.30%), Down syndrome (10.0%), and age (9.50%). The analysis was repeated separately for adolescents with and without Down syndrome. The linear model explained 60% of the variance in total body fat percentage among adolescents with Down syndrome, $F(5,21) = 4.88$, $p = .007$, $R^2 = .604$, but only Tanner stage remained as a statistically significant predictor ($\beta = -0.79$, $p = .001$), accounting for 44.5% of total variance. The same model explained 31% of the variance in total body fat percentage among adolescents

with typical development, $F(5,16) = 1.01$, $p = .457$, $R^2 = .315$, with no statistically significant predictors ($p > .05$).

Discussion

The primary purpose of this study was to examine the associations between body composition and the health behaviors of physical activity and dietary energy intake in adolescents with Down syndrome. The main findings showed that: (i) physical activity is not significantly associated with body composition; however, the magnitude of association is considerably stronger when using body fat percentage from DXA; (ii) estimates of obesity proportions are different between metrics of BMI compared to body fat from DXA; and (iii) there are significant differences in adiposity, physical activity, and dietary intake between adolescents with Down syndrome and typical development that may be clinically relevant.

Overweight and obesity are clearly an issue of health disparity in Down syndrome. Previous studies in adolescents with Down syndrome have estimated the prevalence of overweight between 32% and 60% (Bandini et al., 2012; Gonzalez-Aguero et al., 2011; Grammatikopoulou et al., 2008; Harris et al., 2003; Myrelid et al., 2002; Rimmer et al., 2010; Styles et al., 2002; van Gameren-Oosterom et al., 2012) while the current sample categorized approximately 70% of adolescents with Down syndrome as overweight based on BMI percentile. Given the high rates of overweight and obesity among adolescents with Down syndrome, targeted health promotion is of particular need for this at-risk population.

The associations presented in this study add to a small but growing body of literature examining health behaviors and obesity in youth with Down syndrome. In the general population, higher levels of physical activity have been associated with lower levels of body fatness (Katzmarzyk et al., 2015; Mitchell et al., 2013; Ortega, Ruiz & Castillo, 2013). A limited

number of studies have examined such an association in persons with Down syndrome and have largely found weak and non-statistically significant relationships (Esposito et al., 2012; Izquierdo-Gomez et al., 2015; Nordstrom et al., 2013; Shields et al., 2009; Shields et al., 2015). In the current study, the associations between MVPA and BMI for males and females with Down syndrome (r = -.17 and -.34, respectively) were similar to other reported associations ranging from r = -.05 to -.30 (Esposito et al., 2012; Shields et al., 2009; Shields et al., 2015). However, when using body fat percentage from DXA, the associations with MVPA were much stronger. Correlation coefficients (r) exceeded -.40 for males and -.50 for females, indicating a larger magnitude of association of MVPA with body fat compared to BMI. Similarly large associations were observed between MVPA and regional body fat in the trunk, legs, and arms. While correlations did not reach statistical significance, the large magnitude suggests that with greater sample size, statistical significance could be observed.

Given the improved magnitude of association between body fat and MVPA, it was important to examine the relationship of physical activity on body fat while controlling for other relevant covariates. A significant association was observed between the presence of Down syndrome and total body fat percentage, as was expected. However, other relevant factors also exhibited significant associations with total body fat percentage including Tanner pubertal stage, but not sex or age. The association of MVPA, controlling for other factors, with total body fat percentage trended toward statistical significance (p = .076), but still accounted for a large portion of the variance explained. Average caloric intake did not present significant associations with percent body fat in any model. When broken down into separate analyses for disability groups, the significant association of Tanner stage and trending association of MVPA persisted for adolescents with Down syndrome. The lack of association between body composition and sex

or age is consistent with previous findings (Krause et al., 2015), but the strong association with Tanner stage is novel and has not been previously addressed in this population. Interestingly, Tanner stage and body fat in adolescents with Down syndrome were negatively associated, suggesting lower body fatness at higher Tanner stages. Adiposity has been shown to decrease in typically developing adolescents from mid- to post-puberty, particularly among boys (Crocker et al., 2014).

To my knowledge, the only other study to examine associations between physical activity and body composition in adolescents with Down syndrome using similar methodology found no associations (Izquierdo-Gomez et al., 2015). Demographics, including age, weight, height, BMI, and body fat, were similar between studies; however, participants in the Izquierdo-Gomez et al. (2015) sample engaged in approximately 56 minutes of MVPA per day. It is possible that the high rates of adiposity and very low rates of physical activity in the current study are inflating these associations. Izquierdo-Gomez et al. (2015) suggest that physical activity levels may not contribute to fatness in this population, but longitudinal studies of this relationship are needed to make such inferences. However, given that my analysis controlled for multiple relevant factors including sex, age, and Tanner stage, the resulting association and variance explained suggest there is at least a moderate relationship to consider.

The second purpose of the study was to compare differences in body composition using traditional anthropometry with BMI and estimates of body fat percentage from DXA. The literature on youth with Down syndrome consistently shows greater BMI and higher proportions of overweight and obesity compared to typically developing youth (Bandini et al., 2012; Grammatikopoulou et al., 2008; Harris et al., 2003; Izquierdo-Gomez et al., 2015; Myrelid et al., 2002; Rimmer et al., 2010; Styles et al., 2002; van Gameren-Oosterom et al., 2012). The results

from DXA measurements tell a similar, but different story. When controlling for sex, age, and Tanner pubertal stage there were statistically significant differences in BMI and BMI percentile between groups with very large effect sizes. Differences in total body fat percentage from DXA were also statistically significant between groups, but the effect size was only moderate (Hopkins et al., 2009). The decrease in magnitude suggests that differences in adiposity between adolescents with and without Down syndrome based on the CDC growth charts may be inflated.

I examined a similar issue comparing BMI categories with DXA adiposity in adolescents and young adults with Down syndrome. Substantial discrepancies were reported, in particular a low efficiency of the 85th percentile compared to elevated fat mass. While I did not address all aspects of classification function (i.e. specificity, sensitivity, etc.), the present analysis also showed substantial misidentification of overweight at the 85th percentile compared to elevated fat mass. Nearly 32% of adolescents with Down syndrome categorized as overweight based on the CDC growth chart 85th percentile did not present with elevated fat mass. These results are consistent with findings of low overall efficiency in overweight and obese classification among adolescents with Down syndrome (Bandini et al., 2012). A control group was not included in the Bandini et al. (2012) analysis on elevated body fat and BMI classifications, but the classification of typically developing adolescents in the present study appears to be appropriate.

The high body fat percentage observed in this sample of adolescents with Down syndrome (33.20%) is consistent with previous studies that have measured adiposity with DXA in persons with Down syndrome (26.99% - 39.94%)(Bandini et al., 2012; Baptista et al., 2005; Esco et al., 2016; Guijarro et al., 2008; Loveday et al., 2012; Nickerson et al., 2015; Wendel et al., 2016). However, Gonzalez-Aguero et al. (2011) reported a lower body fat percentage

(24.7%) compared to other DXA studies in Down syndrome. This was among a sample of youth with Down syndrome ages 10 to 19 years matched on BMI. Despite the lack of group matching in the present study, similar differences in regional body fat distribution were observed. Adolescents with Down syndrome had significantly higher body fat percentages in the trunk region and both body fat distribution ratios suggest a greater proportion of total body fat is stored in the abdomen. Gonzalez-Aguero et al. (2011) found a similar trend toward abdominal obesity with greater fat mass among adolescents with Down syndrome, particularly among girls. These findings reflect a unique body fat topology in Down syndrome that BMI is not capable of describing. While significant differences are evident using both traditional anthropometry and the advanced DXA measurement, the differences seen across regional segments and distribution ratios confirm that more detailed measurements are highly useful in this population. When feasible and cost-effective (Kendler et al., 2013), the use of DXA to measure body composition in adolescents with Down syndrome is suggested.

The third purpose of the study was to examine differences in physical activity and dietary behaviors between adolescents with and without Down syndrome. These findings continue the description of physical activity in adolescents with Down syndrome. While more research efforts need to be funneled toward understanding correlates of physical activity and designing interventions to increase physical activity behavior in adolescents with Down syndrome, there is still insufficient literature to thoroughly describe the physical activity patterns of this population (Frey et al., 2008; Pitetti et al., 2013). In the current sample, only four of the 39 participants (10%) including one adolescent with Down syndrome (4.5%) and three adolescents with typical development (17.65%) met the guidelines of 60 minutes of MVPA per day (U.S. Department of Health and Human Services, 2008). Previous studies have also shown a very low proportion of

adolescents with Down syndrome engaging in adequate physical activity (Esposito et al., 2012; Izquierdo-Gomez et al., 2014; Matute-Llorente et al., 2013a; Matute-Llorente et al., 2013b; Phillips & Holland, 2011; Shields et al., 2009), including three studies that found 0% of the sample with Down syndrome met guidelines (Matute-Llorente et al., 2013a; Matute-Llorente et al., 2013b; Phillips & Holland, 2011). The average MVPA among the adolescents with Down syndrome was approximately 27 minutes per day after controlling for sex, age, Tanner stage, and wear time. The typically developing adolescents also engaged in largely insufficient levels of MVPA as well, averaging only 44 minutes of MVPA per day. Measured MVPA in this sample was consistent with previous reports among adults with Down syndrome (25 to 30 minutes per day)(Nordstrom et al., 2013; Phillips & Holland, 2011), but below previously reported levels for adolescents with Down syndrome (56 to 85 minutes per day)(Izquierdo-Gomez et al., 2014; Izquierdo-Gomez et al., 2015; Shields et al., 2009). Age may be relevant in this relationship, as MVPA has been shown to decrease with increasing age both within youth (Esposito et al., 2012; Izquierdo-Gomez et al., 2014; Shields et al., 2009) and into adulthood (Phillips & Holland, 2011) in individuals with Down syndrome. Interpreting differences in physical activity across studies is challenging due to the variety of accelerometers used to measure physical activity and cut-points employed to categorize time spent in different intensities. Furthermore, all physical activity data from accelerometers should be interpreted cautiously due to the lack of validated cut-points for this population. While cut-points from the general population may be useful when applied to other populations, the known differences in heart rate response, energy expenditure, and gait in individuals with Down syndrome (Pitetti et al., 2013) may limit the usefulness of general population cut-points in this population. This issue has been demonstrated in adults (Agiovlasitis et al., 2011; Agiovlasitis, Motl, Foley & Fernhall, 2012), but is likely also relevant in youth with

Down syndrome. Despite these methodological issues, increasing levels of phyical activity among adolescents with Down syndrome continues to be an area of great need.

The low levels of physical activity in adolescents with Down syndrome may also have implications for physical fitness. In the general population, obesity is strongly associated with lower levels of physical fitness (Rauner, Mess & Woll, 2013). Cardiovascular and muscular fitness are both significantly lower in Down syndrome (Gonzalez-Aguero et al., 2010; Izquierdo-Gomez, Martinez-Gomez, Fernhall, Sanz & Veiga, 2016; Izquierdo-Gomez et al., 2013; Izquierdo-Gomez et al., 2015). Recent studies have found that physical activity levels are positively associated with cardiovascular and muscular fitness in adolescents with Down syndrome (Izquierdo-Gomez et al., 2015; Matute-Llorente et al., 2013a). Significant negative associations between BMI, waist circumference, and cardiovascular fitness have also been shown (Shields et al., 2015), while other studies have not found significant relationships within samples of youth with Down syndrome (Izquierdo-Gomez et al., 2016; Izquierdo-Gomez et al., 2015). While these relationships have not been examined through longitudinal studies, there is at least cross-sectional evidence that a healthy profile characterized by low body fat, greater levels of physical activity, and higher physical fitness is possible in youth with Down syndrome.

Finally, the present study adds to a very small body of literature on dietary energy intake patterns of people with Down syndrome. Raw values of daily caloric and macronutrient intake were significantly lower among adolescents with Down syndrome compared to typical development. This is consistent with nearly all research on diet in youth and adults with Down syndrome (Fujiura et al., 1997; Hopman et al., 1998; Luke et al., 1996; Samarkandy et al., 2012; Sharav & Bowman, 1992). The novel approach of this study was to also examine caloric intake normalized to lean body mass. Once caloric intake was normalized, differences between Down

syndrome and typical development were no longer present. Although there were no differences in weight between groups, lean mass was significantly lower in adolescents with Down syndrome. This difference was sufficient to account for the gap in energy intake. Previous studies in Down syndrome have also normalized calorie intake to total body mass (Braunschweig et al., 2004; Grammatikopoulou et al., 2008; Hopman et al., 1998). However, the only other study to both normalize energy intake to body weight and compare kcals/kg between youth with and without Down syndrome (Hopman et al., 1998) also found significantly lower kcal but similar kcal/kg intake in children with Down syndrome. However, this study was conducted in children between the ages of 1 and 4 years, so additional comparisons have limited meaning to the current sample of adolescents. Even after controlling for lean mass, adolescents with Down syndrome had lower average kcal/kg intake than typically developing peers. This suggests that dietary practices alone are unlikely to be a primary contributor to excess fatness.

Macronutrient consumption levels were also lower among adolescents with Down syndrome, but when normalized to total energy there were no longer significant differences. Estimated macronutrient intake levels (53% from carbohydrates, 30% from fat, and 18% from protein) were also within the recommended daily allowances (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). This macronutrient profile was not significantly different from adolescents with typical development and was consistent with previous literature in person with Down syndrome (Braunschweig et al., 2004; Grammatikopoulou et al., 2008; Hopman et al., 1998; Luke et al., 1996; Samarkandy et al., 2012; Soler Marin & Xandri Graupera, 2011). However, the current analysis does not account for the quality of dietary intake. Previous studies have shown that individuals with Down syndrome do not consume appropriate portions of healthy foods such as fruits and vegetables (Braunschweig

et al., 2004). It also remains unclear if the lower caloric intake observed is sufficient to account for the lower resting metabolic rate observed in persons with Down syndrome (Murray & Ryan-Krause, 2010; Roizen, 2002). Given the paucity of studies in this area, additional research on diet and obesity in this population are needed.

The current study is limited by a number of important factors that should be considered when interpreting the findings presented. First, the study includes a relatively small convenience sample, which limited statistical power. While groups were statistically similar for age, sex, and Tanner pubertal stage, there was a considerable difference in the distribution of sex between groups. This discrepancy could have influenced the effect of sex observed in multiple analyses. Furthermore, while statistically significant results were observed in many analyses using the full sample, many of the follow-up analyses stratified by sex did not present significant differences. Dividing a limited sample size into separate groups further reduced statistical power. The data is also cross-sectional in nature, so casual inferences cannot be made. Given the small sample, differences observed across subgroupings may also be skewed. There were multiple issues with recruiting participants for this study due to the time and effort needed to complete the DXA scan at the MCRU. Therefore, the sample may not be representative and the findings generalizable to the broader population of adolescents with Down syndrome.

Second, physical activity was measured using accelerometers with psychometric properties and cut-point procedures for the general population. The current physical activity literature in Down syndrome should be viewed cautiously, as psychometric evidence for accelerometer use in this population is limited. Due to differences in energy expenditure (Agiovlasitis et al., 2011; Agiovlasitis et al., 2012), the validity of accelerometer cut-points derived from the general population for individuals with Down syndrome is highly questionable.

Alternative cut-points have been proposed for adults with Down syndrome (Agiovlasitis et al., 2011; Agiovlasitis et al., 2012), but still require external validation. Examinations of cut-points in youth with Down syndrome have not been published to date. Additionally, the selection of the Evenson et al. (2008) cut-points may also influence the interpretation of findings, as the time classified in MVPA may have been very different using an alternative set of cut-points (e.g. Freedson, Pober & Janz, 2005). Loprinzi et al. (2012) found that, based on accelerometer data from NHANES 2003-2006, youth participated in 44.5 minutes of MVPA per day using the Evenson cut-points, but 59.4 minutes of MVPA using the Freedson cut-points. This translates to interpreting the same data as 45.7% or 59.3% of youth meeting physical activity guidelines. Differences between cut-points and the wide range of cut-points used in the literature hinder the ability to compare findings across studies.

Third, both diet and Tanner stage were measured through questionnaires and with parental proxy reporting. These procedures were selected to address issues with self-report (Emerson et al., 2013). Dietary assessments such as the YAQ and other FFQs have not been validated in youth with disabilities, so it is not possible to determine how accurately the dietary information reflects actual behavior (Braunschweig et al., 2004). However, all participants in the study lived with the parent completing the questionnaire and assisted with the process. Similar issues limit the potential accuracy of the Tanner stage assessment. While parents have been shown to provide acceptable estimates of Tanner stage using this methodology (Rasmussen et al., 2015), these validity studies did not include adolescents with disabilities. Given that Tanner stage was an important covariate in the linear regression analysis, a future study with a direct physician exam of Tanner stage is recommended to confirm the current findings.

Despite these limitations, this study demonstrated significantly higher levels of overweight, obesity, and excess fatness among adolescents with Down syndrome, provided additional evidence for unique body fat distribution in this population, highlighted the pervasive low levels of physical activity, added another example of consistent dietary profile, and demonstrate that a moderate associative relationship can be observed between adiposity and physical activity levels if adiposity is properly measured. Future research should continue to examine the causes and consequences of greater obesity in Down syndrome and develop novel interventions to address health disparities.

In conclusion, it is clear that overweight and obesity are pervasive among adolescents with Down syndrome, representing a source of health disparity. Due to unique body fat topology, BMI is an inadequate metric of adiposity. Future studies should utilize DXA or other methodologies capable of capturing abdominal obesity. The results also show a moderate association between physical activity and adiposity. Though causal nature cannot be inferred, this is the first study to show even a mild association between these variables in persons with Down syndrome, suggesting a potential relationship between this health behavior and health condition. Future studies should continue to create innovative interventions and health promotion programming to increase physical activity levels in adolescents with Down syndrome. The information presented here may also be relevant to parents, teachers, and health professionals who are in a position to promote healthy behaviors.

Table Captions

Figure Captions

Figure 1.1.   Group differences in body composition

Figure 1.2.   Group differences in body composition by sex

Figure 1.3.   Group differences in body fat distribution

Figure 1.4.   Group differences in body fat distribution by sex

Figure 1.5.   Group differences in prevalence of obesity and elevated fat mass

Figure 1.6.   Group differences in physical activity

Figure 1.7.   Group differences in physical activity by sex

Figure 1.8.   Group differences in dietary intake

Figure 1.9.   Group differences in dietary intake by sex

Table 1.1.   Characteristics of participants with and without Down syndrome

	Down syndrome ($n = 22$)	Typical development ($n = 17$)	Group difference	p	ES
Age (years)	14.96 (1.92)	15.08 (2.12)	$t(37) = 0.19$	.850	.088
Sex	14 - Male (63.6%)	7 - Male (41.2%)	$X^2(1,39) = 1.95$	.163	.223
	8 - Female (36.4%)	10 - Female (58.8%)			
Race/Ethnicity	20 - Caucasian (90.9%)	17 - Caucasian (100%)	$X^2(2,39) = 1.63$	.443	.204
	1 - African-American (4.55%)				
	1 - Hispanic (4.55%)				
Tanner stage	3.64 (1.09)	3.68 (1.22)	$t(37) = 0.11$	.915	.093
Height (cm)	145.91 (8.41)	164.68 (11.73)	$t(37) = 5.82$	**<.001**	1.379
Weight (kg)	55.69 (10.69)	57.94 (16.76)	$t(37) = 0.51$	.612	.147
Siblings (#)	2.09 (1.06)	2.29 (1.36)	$t(37) = 0.52$	.603	.206
Mother's age (years)	48.77 (6.44)	48.65 (3.67)	$t(37) = 0.07$	.943	.066
Father's age (years)	51.14 (7.57)	49.65 (3.28)	$t(36) = 0.76$	.454	.249
Parent education	3 - HS or GED (13.6%)	2 - HS or GED (11.8%)	$X^2(5,39) = 2.28$	.809	.242
	2 - Associates/Vocational (9.1%)	0 - Associates/Vocational (0%)			
	8 - Bachelors (36.4%)	6 - Bachelors (35.3%)			
	8 - Masters (36.4%)	7 - Masters (41.1%)			
	1 - Doctorate/Professional (4.5%)	2 - Doctorate/Professional (11.8%)			
Family income level	1 - <$25,000 (4.5%)	0 - <$25,000 (0%)	$X^2(6,39) = 2.20$	.900	.238
	6 - $50,000-$74,999 (27.3%)	3 - $50,000-$74,999 (17.6%)			
	4 - $75,000-$99,999 (18.2%)	3 - $75,000-$99,999 (17.6%)			
	3 - $100,000-$149,999 (13.6%)	3 - $100,000-$149,999 (17.6%)			
	5 - >$150,000 (22.7%)	5 - >$150,000 (29.4%)			
	3 - Missing/DNR (13.6%)	3 - Missing/DNR (17.6%)			

Note: Data are Means (Standard Deviations) or Frequencies, n (proportions, %)
ES = effect size (Cohen's d for t-tests, Cramer's V for X^2 tests)
$p < .05$, bolded

Table 1.2.   Group differences in body composition

Body Composition [a]	DS (n=22)	TD (n=17)	F (1,34)	p	partial η^2	d [b]
CDC growth reference [c]						
BMI (kg/m^2)	25.84 (.807)	21.32 (.921)	13.26	**.001**	.281	**1.06**
BMI %ile	86.47 (4.43)	53.80 (5.05)	23.05	**< .001**	.404	**1.31**
DS growth reference [d]						
BMI %ile	64.09 (4.08)	--	--	--	--	--
DXA (% BF)						
Total %	33.20 (1.80)	25.90 (2.00)	7.26	**.011**	.176	.692
Arms %	33.90 (1.80)	26.30 (2.00)	7.65	**.009**	.184	.603
Legs %	36.10 (1.60)	29.90 (1.80)	6.25	**.017**	.155	.570
Trunk %	32.40 (2.10)	23.30 (2.40)	7.60	**.009**	.183	.772

MANCOVA controlling for age, sex, and Tanner pubertal stage
$F(6,29) = 4.235$, $p = .004$, Wilks' $\Lambda = .533$, partial $\eta^2 = .467$
[a] Adjusted group means and standard errors (SE)
[b] Cohen's d effect size (unadjusted), **d > .80, bolded**
[c] CDC growth reference (Kuczmarski et al., 2000)
[d] DS growth reference (Zemel et al., 2015)
p < .05, bolded

Table 1.3.   Group differences in body composition by sex

Body Composition [a]	DS	TD	F	p	η^2	d [b]
Male	n = 14	n = 7	(1,17)			
CDC growth reference [c]						
BMI (kg/m^2)	26.76 (1.15)	21.88 (1.64)	5.80	**.028**	.254	**.890**
BMI %ile	90.74 (5.38)	58.44 (7.66)	11.68	**.003**	.407	**1.35**
DS growth reference [d]						
BMI %ile	74.84 (4.99)	--	--	--	--	--
DXA (% BF)						
Total %	31.80 (2.30)	23.00 (3.30)	4.74	**.044**	.218	**.879**
Arms %	31.00 (2.20)	21.90 (3.20)	5.40	**.033**	.241	**.929**
Legs %	34.00 (2.20)	26.10 (3.10)	4.37	.052	.205	**.862**
Trunk %	31.60 (2.80)	21.00 (4.00)	4.67	**.045**	.215	**.883**
Female	n = 8	n = 10	(1,14)			
CDC growth reference [c]						
BMI (kg/m^2)	24.73 (1.10)	20.52 (0.98)	7.82	**.014**	.358	**1.19**
BMI %ile	80.49 (6.47)	49.36 (5.76)	12.32	**.003**	.468	**1.20**
DS growth reference [d]						
BMI %ile	45.28 (8.08)	--	--	--	--	--
DXA (% BF)						
Total %	33.60 (2.30)	29.50 (2.10)	1.59	.228	.102	.726
Arms %	35.40 (2.40)	32.20 (2.10)	0.97	.340	.065	.653
Legs %	37.00 (1.80)	34.60 (1.60)	0.94	.349	.063	.622
Trunk %	32.10 (3.00)	26.10 (2.70)	2.07	.172	.129	.777

MANCOVA controlling for age and Tanner pubertal stage
Male: $F(6,12) = 1.66$, $p = .214$, Wilks' $\Lambda = .546$, partial $\eta^2 = .454$; Female: $F(6,9) = 2.73$, $p = .085$, Wilks' $\Lambda = .355$, partial $\eta^2 = .645$
[a] Adjusted group means and standard errors (SE); [b] Cohen's d effect size, (unadjusted), **d > .80, bolded**; [c] CDC growth reference (Kuczmarski et al., 2000); [d] DS growth reference (Zemel et al., 2015); **p < .05, bolded**.

Table 1.4.   Group differences in body fat distribution

Body Fat Ratios [a]	DS (n=22)	TD (n=17)	F (1,34)	p	η^2	d [b]
Trunk / Total ratio	.484 (.013)	.402 (.014)	17.66	**< .001**	.342	**1.19**
Legs / Total ratio	.357 (.009)	.421 (.010)	23.96	**< .001**	.413	**1.32**
Arms / Total ratio	.110 (.004)	.114 (.004)	0.28	.600	.008	.264
Arms & Legs / Trunk ratio	.993 (.056)	1.37 (.064)	18.84	**< .001**	.357	**1.23**

MANCOVA controlling for age, sex, and Tanner pubertal stage
$F(4,31) = 5.68$, $p = .002$, Wilks' $\Lambda = .577$, partial $\eta^2 = .423$
[a] Adjusted group means and standard errors (SE)
[b] Cohen's d effect size (unadjusted), $d > .80$, **bolded**
$p < .05$, **bolded**

Table 1.5.   Group differences in body fat distribution by sex

Body Fat Ratios [a]	DS	TD	F	p	η^2	d [b]
Male	n = 14	n = 7	(1,17)			
Trunk / Total ratio	.495 (.017)	.404 (.024)	9.59	**.007**	.361	**1.18**
Legs / Total ratio	.345 (.011)	.414 (.016)	12.99	**.002**	.433	**1.35**
Arms / Total ratio	.109 (.005)	.109 (.007)	0.01	.940	.000	.063
Arms & Legs / Trunk ratio	.944 (.068)	1.34 (.098)	10.89	**.004**	.390	**1.26**
Female	n = 8	n = 10	(1,14)			
Trunk / Total ratio	.471 (.019)	.397 (.017)	8.49	**.011**	.377	**1.16**
Legs / Total ratio	.373 (.015)	.431 (.013)	8.41	**.012**	.375	**1.23**
Arms / Total ratio	.111 (.006)	.118 (.005)	0.74	.404	.050	.314
Arms & Legs / Trunk ratio	1.05 (.094)	1.41 (.083)	7.89	**.014**	.360	**1.13**

MANCOVA controlling for age and Tanner pubertal stage
Male: $F(4,14) = 2.75$, $p = .070$, Wilks' $\Lambda = .560$, partial $\eta^2 = .440$
Female: $F(4,11) = 2.29$, $p = .125$, Wilks' $\Lambda = .546$, partial $\eta^2 = .454$
[a] Adjusted group means and standard errors (SE)
[b] Cohen's *d* effect size (unadjusted), ***d* > .80, bolded**
p **< .05, bolded**

Table 1.6.   Differences in proportions of overweight / obese and elevated fat mass

Down syndrome	Elevated Body Fat (DXA)	Not Elevated		Elevated Body Fat (DXA)	Not Elevated
Obese (95th percentile)	27.3%	18.2%	Overweight (85th percentile)	40.9%	31.8%
Not Obese	13.6%	40.9%	Not Overweight	0%	27.3%
Chi-square	$X^2 = 2.76, p = .094, V = .354$			$X^2 = 5.71, p = .017, V = .510$	

Typically Developing	Elevated Body Fat (DXA)	Not Elevated		Elevated Body Fat (DXA)	Not Elevated
Obese (95th percentile)	11.8%	0%	Overweight (85th percentile)	17.6%	0%
Not Obese	5.9%	82.3%	Not Overweight	0%	82.3%
Chi-square	$X^2 = 10.58, p = .001, V = .789$			$X^2 = 17.00, p < .001, V = 1.00$	

Note: Overweight (85th percentile) and obese (95th percentile) based on CDC growth curves (Kuczmarski et al., 2000); Elevated body fatness based on pediatric cut-points (Freedman et al., 2009).

Table 1.7.   Group differences in physical activity

Physical Activity [a]	DS (n=21)	TD (n=17)	F (1,33)	p	η^2	d [b]
Sedentary (min/day)	531.74 (9.30)	555.90 (10.64)	2.80	.104	.078	.146
Light (min/day)	200.99 (7.00)	158.89 (8.01)	14.96	**< .001**	.312	**1.10**
Moderate (min/day)	21.05 (2.42)	25.32 (2.77)	1.29	.264	.038	.098
Vigorous (min/day)	6.78 (3.25)	20.44 (3.72)	7.32	**.011**	.182	.617
MVPA (min/day)	27.83 (5.43)	45.76 (6.21)	4.52	**.041**	.120	.421

MANCOVA controlling for age, sex, Tanner pubertal stage, and accelerometer wear time.

MVPA = moderate-to-vigorous physical activity. All physical activity data are minutes per day.

$F(3,31) = 6.75$, $p = .001$, Wilks' $\Lambda = .605$, partial $\eta^2 = .395$

[a] Adjusted group means and standard errors (SE)

[b] Cohen's d effect size (unadjusted), **d > .80, bolded**

p < .05, bolded

Table 1.8.   Group differences in physical activity by sex

Physical Activity [a]	DS	TD	F	p	η²	d [b]
Male	(n=14)	(n=7)	(1,16)			
Sedentary (min/day)	509.69 (11.00)	533.97 (15.88)	1.50	.239	.086	.245
Light (min/day)	212.87 (8.25)	171.52 (11.90)	7.74	**.013**	.326	**1.15**
Moderate (min/day)	23.46 (2.45)	25.75 (3.53)	0.27	.611	.017	.000
Vigorous (min/day)	7.58 (3.61)	22.36 (5.21)	5.17	**.037**	.244	**.867**
MVPA (min/day)	31.04 (5.73)	48.12 (8.27)	2.73	.118	.146	.546
Female	(n=8)	(n=10)	(1,13)			
Sedentary (min/day)	553.78 (17.54)	584.48 (15.60)	1.63	.224	.112	.272
Light (min/day)	192.09 (12.19)	140.53 (10.84)	9.53	**.009**	.423	**.957**
Moderate (min/day)	18.09 (5.10)	24.00 (4.54)	0.72	.413	.052	.284
Vigorous (min/day)	4.70 (6.32)	19.64 (5.62)	2.98	.108	.186	.453
MVPA (min/day)	22.79 (11.12)	43.65 (9.89)	1.87	.194	.126	.395

MANCOVA controlling for age, Tanner pubertal stage, and accelerometer wear time

MVPA = moderate-to-vigorous physical activity. All physical activity data are minutes per day.

Male: $F(3,14) = 3.81$, $p = .035$, Wilks' $\Lambda = .551$, partial $\eta^2 = .449$

Female: $F(3,11) = 3.59$, $p = .050$, Wilks' $\Lambda = .505$, partial $\eta^2 = .495$

[a] Adjusted group means and standard errors (SE)

[b] Cohen's d effect size (unadjusted), **d > .80, bolded**

p < .05, bolded

Table 1.9.　　　Group differences in energy intake and micronutrient distribution

Dietary Intake [a]	DS (n=22)	TD (n=17)	F (1,34)	p	η^2	d [b]
kcal (per day)	2135.98 (159.06)	2717.79 (181.56)	5.68	**.023**	.143	.761
kcal per lean mass (kcal/kg)	63.11 (5.09)	70.23 (5.81)	0.82	.369	.024	.382
Protein (g/day)	93.59 (7.83)	114.62 (8.93)	3.06	.089	.082	.640
% Protein	18.10% (0.7%)	16.5% (0.8%)	2.31	.138	.064	.314
Carbohydrates (g/day)	288.09 (22.23)	363.26 (25.37)	4.84	**.035**	.125	.688
% Carbohydrates	53.30% (1.0%)	53.90% (1.1%)	0.16	.635	.005	.032
Fat (g/day)	70.33 (5.44)	91.78 (6.21)	6.58	**.015**	.162	.794
% Fat	29.70% (0.9%)	30.40% (1.0%)	0.23	.635	.007	.125
Saturated (g/day)	24.76 (2.14)	33.46 (2.44)	7.00	**.012**	.171	**.838**
Monounsaturated (g/day)	24.30 (1.83)	31.35 (2.09)	6.26	**.017**	.155	.756
Polyunsaturated (g/day)	13.70 (1.18)	17.93 (1.35)	5.46	**.026**	.138	.731
Cholesterol (mg/day)	256.92 (19.68)	282.15 (22.46)	0.70	.410	.020	.249
Fiber (g/day)	21.60 (1.97)	26.51 (2.25)	2.64	.114	.072	.539

MANCOVA controlling for age, sex, and Tanner pubertal stage
$F(13,22) = 1.98$, $p = .077$, Wilks' $\Lambda = .461$, partial $\eta^2 = .539$
[a] Adjusted group means and standard errors (SE)
[b] Cohen's d effect size (unadjusted), **$d > .80$, bolded**
$p < .05$, bolded

Table 1.10.   Group differences in energy intake and micronutrient distribution by sex

Dietary Intake [a]	DS	TD	F(df)	p	η^2	d [b]
Male	(n=14)	(n=7)	(1,17)			
kcal (per day)	2332.99 (150.61)	2373.52 (214.52)	0.02	.880	.001	.059
kcal per lean mass (kcal/kg)	63.47 (5.43)	55.88 (7.74)	0.63	.437	.036	.555
Protein (g/day)	95.27 (6.21)	95.93 (8.85)	0.01	.953	.000	.032
% Protein	16.50% (0.7%)	16.10% (0.9%)	0.11	.743	.006	.239
Carbohydrates (g/day)	318.03 (24.71)	326.54 (35.20)	0.04	.847	.002	.101
% Carbohydrates	53.80% (1.4%)	55.30% (2.0%)	0.38	.545	.022	.353
Fat (g/day)	78.69 (5.17)	79.13 (7.36)	0.01	.962	.000	.011
% Fat	30.80% (1.2%)	29.70% (1.8%)	0.26	.616	.015	.258
Saturated (g/day)	27.26 (2.09)	28.14 (2.98)	0.06	.815	.003	.096
Monounsaturated (g/day)	27.41 (1.79)	27.56 (2.54)	0.01	.961	.000	.012
Polyunsaturated (g/day)	15.56 (1.24)	15.32 (1.77)	0.01	.916	.001	.020
Cholesterol (mg/day)	289.08 (18.63)	241.99 (26.53)	2.07	.168	.109	.720
Fiber (g/day)	23.69 (2.36)	21.65 (3.37)	0.24	.629	.014	.162
Female	(n=8)	(n=10)	(1,14)			
kcal (per day)	1789.20 (312.47)	2958.19 (277.98)	7.46	**.016**	.348	**1.19**
kcal per lean mass (kcal/kg)	58.214 (9.50)	83.69 (8.45)	3.83	.070	.215	**.997**
Protein (g/day)	84.44 (16.87)	132.66 (15.01)	4.35	.056	.237	**.922**
% Protein	19.70% (1.4%)	17.70% (1.2%)	1.16	.300	.076	.698
Carbohydrates (g/day)	239.49 (40.78)	385.92 (36.28)	6.87	**.020**	.329	**1.16**
% Carbohydrates	53.0% (1.5%)	52.50% (1.3%)	0.06	.814	.004	.698
Fat (g/day)	57.41 (10.29)	99.26 (9.15)	8.81	**.010**	.386	**1.28**
% Fat	28.60% (1.4%)	30.30% (1.3%)	0.86	.369	.058	.670
Saturated (g/day)	20.63 (4.07)	36.97 (3.62)	8.60	**.011**	.380	**1.28**
Monounsaturated (g/day)	19.72 (3.45)	33.31 (3.07)	8.29	**.012**	.372	**1.27**
Polyunsaturated (g/day)	10.89 (2.08)	19.39 (1.85)	8.88	**.010**	.388	**1.27**
Cholesterol (mg/day)	198.16 (36.63)	312.22 (32.59)	5.17	**.039**	.270	**.974**
Fiber (g/day)	16.99 (2.95)	30.66 (2.63)	11.42	**.004**	.449	**1.11**

MANCOVA controlling for age and Tanner pubertal stage

Male: $F(5,13) = 2.56$, $p = .153$, Wilks' $\Lambda = .131$, partial $\eta^2 = .869$; Female: $F(2,13) = 0.49$, $p = .829$, Wilks' $\Lambda = .238$, partial $\eta^2 = .762$

[a] Adjusted group means and standard errors (SE)

[b] Cohen's d effect size (unadjusted), **d > .80, bolded**

p < .05, bolded

Table 1.11.   Correlations between body composition and physical activity levels in adolescents with Down syndrome

Variable	BMI	BMI percentile	Total %BF	Trunk %BF	Legs %BF	Arms %BF	Sedentary PA	Light PA	Moderate PA	Vigorous PA	MVPA
BMI	–	.676*	.731*	.762*	.650*	.630*	-.259	-.143	-.056	-.335	-.174
BMI percentile	.878*	–	.643*	.648*	.622*	.636*	-.129	.217	.069	-.542*	-.170
Total %BF	.784*	.671	–	.996*	.983*	.973*	.073	-.166	-.288	-.559*	-.429
Trunk % BF	.802*	.727*	.987*	–	.964*	.966*	.020	-.180	-.273	-.535*	-.408
Legs %BF	.580	.407	.922*	.853*	–	.962*	.170	-.142	-.314	-.602*	-.464
Arms %BF	.887*	.748*	.971*	.964*	.857*	–	.098	-.162	-.279	-.532	-.412
Sedentary PA	-.521	-.635	-.047	-.132	.229	-.221	–	.316	-.060	-.104	-.084
Light PA	.101	.363	.082	.128	.004	.096	-.543	–	.750*	.193	.607*
Moderate PA	-.322	-.201	-.457	-.432	-.431	-.408	-.418	.762*	–	.596*	.946*
Vigorous PA	-.363	-.320	-.547	-.518	-.527	-.488	-.403	.600	.965*	–	.824*
MVPA	-.343	-.255	-.501	-.474	-.478	-.447	-.415	.697	.993*	.989*	–

Note. Values above the diagonal (grey background) represent **males** with Down syndrome; Values below the diagonal (white background) represent **females** with Down syndrome; BMI = body mass index; %BF = percent body fat; PA = physical activity; MVPA = moderate-to-vigorous physical activity.
*$p < .05$

Table 1.12.   Linear regression analyses on total body fat percentage

Variable	b	SE	β	p	sr
Total Body Fat %					$R^2 = .400$
Sex	.015	.031	.079	.635	.066
Age	.025	.011	.521	**.031**	.309
Tanner stage	-.048	.016	-.580	**.005**	-.416
MVPA (min/day)	-.001	.001	-.271	.076	-.251
kcal / lean mass (kg)	.000	.001	.136	.430	.110
Down syndrome	.064	.028	.340	**.027**	.317
Total Body Fat % for Down syndrome					$R^2 = .604$
Sex	.015	.038	.075	.695	.063
Age	.019	.013	.369	.152	.237
Tanner stage	-.072	.017	-.789	**.001**	-.667
MVPA (min/day)	-.002	.001	-.302	.095	-.279
kcal / lean mass (kg)	.000	.001	.111	.590	.087
Total Body Fat % for Typically Developing					$R^2 = .315$
Sex	.072	.049	.492	.167	.370
Age	.003	.022	.072	.912	.028
Tanner stage	.008	.034	.125	.826	.056
MVPA (min/day)	-.001	.001	-.263	.414	-.212
kcal / lean mass (kg)	.000	.001	-.060	.870	-.042

Note: Sex = reference is female; Down syndrome = reference is adolescents with Down syndrome;
MVPA = moderate-to-vigorous physical activity;
sr = semipartial (part) correlation;
p < .05, **bolded**

Figure 1.1.   Group differences in body composition
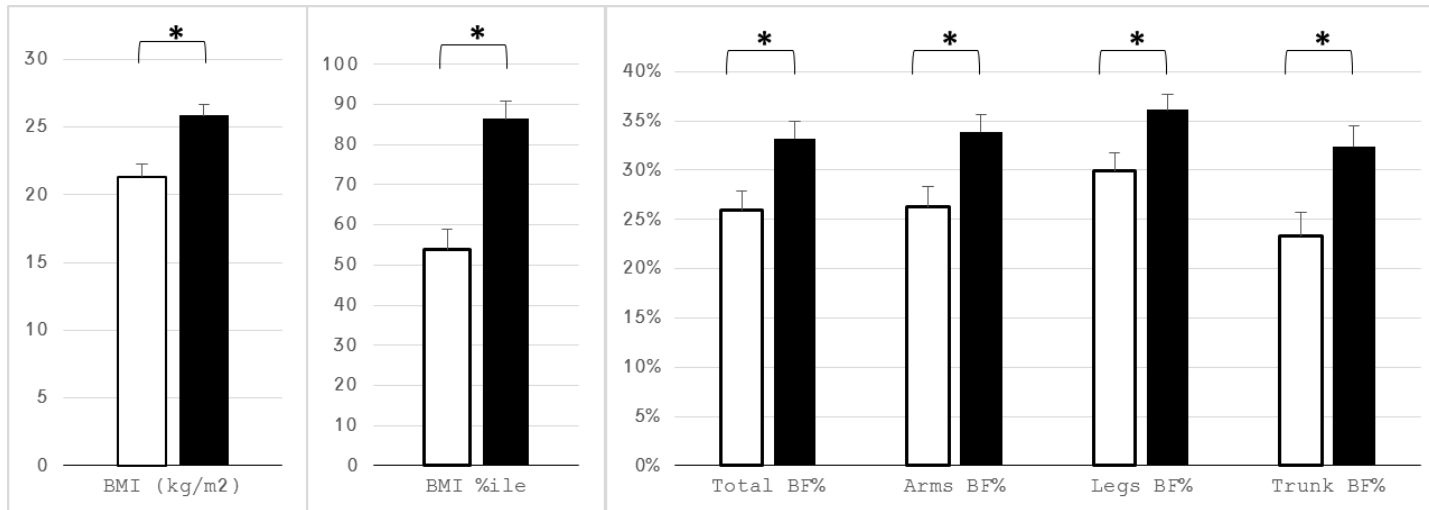
Estimated marginal means and standard errors of body composition controlling for age, sex, and Tanner pubertal stage
Typical development (□); Down syndrome (■)
* *p* < .05

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Figure 1.2.   Group differences in body composition by sex

Estimated marginal means and standard errors of body composition by sex controlling for age and Tanner pubertal stage. **Figure A:** males, **Figure B:** females; Down syndrome (■); Typical development (□); males (diagonal); females (horizontal); * *p* < .05

Figure 1.3.   Group differences in body fat distribution

Estimated marginal means and standard errors of body fat distribution controlling for age, sex, and Tanner pubertal stage
Typical development (□); Down syndrome (■)
* $p < .05$

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Figure 1.4.   Group differences in body fat distribution by sex

Estimated marginal means and standard errors of body fat distribution by sex controlling for age and Tanner pubertal stage
Figure A: males, **Figure B:** females; Down syndrome (■); Typical development (□); males (diagonal); females (horizontal); * *p* < .05

Figure 1.5.   Group differences in prevalence of obesity and elevated fat mass
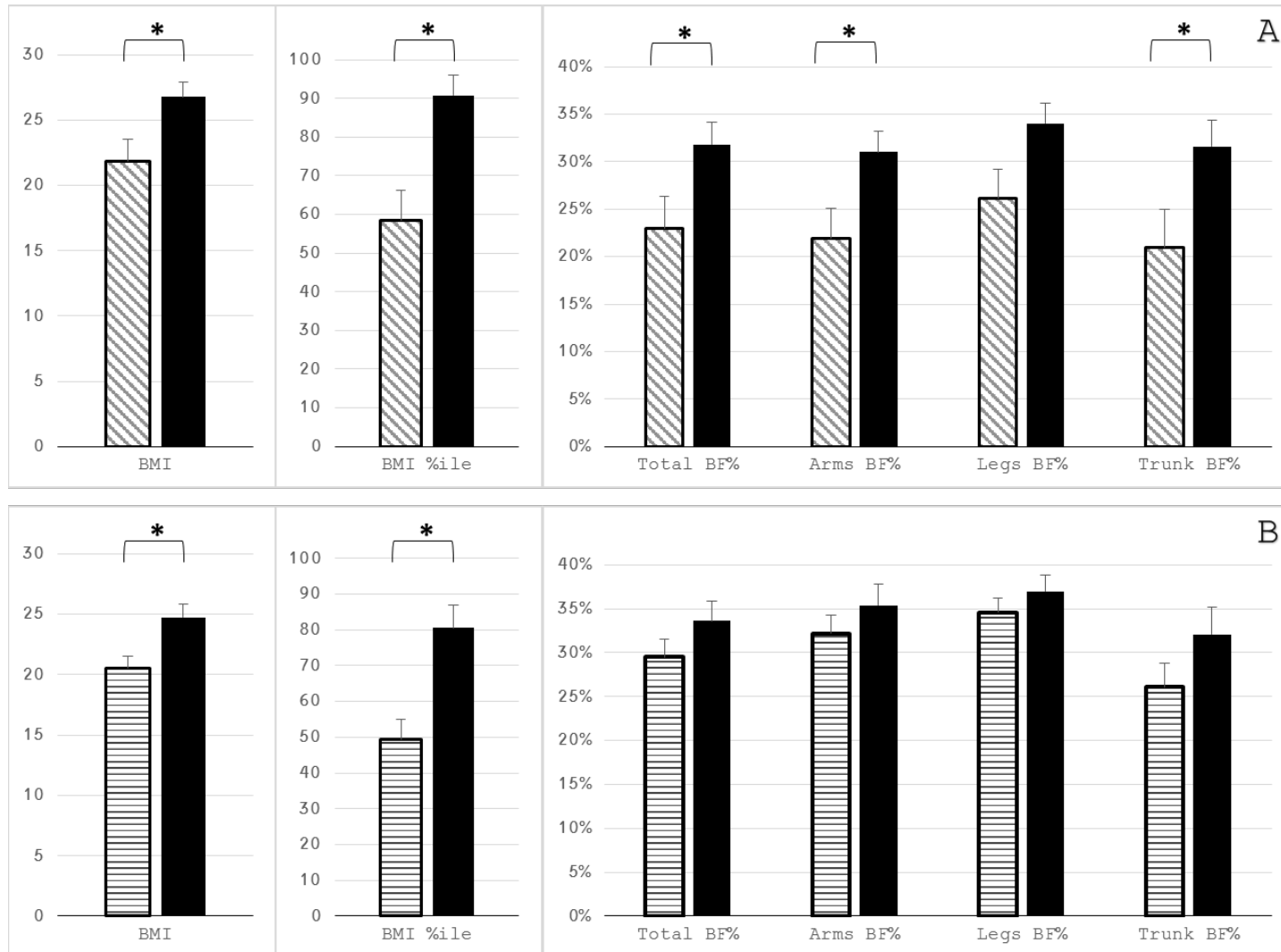

Estimated marginal means and standard errors of obesity prevalence controlling for age, sex, and Tanner pubertal stage
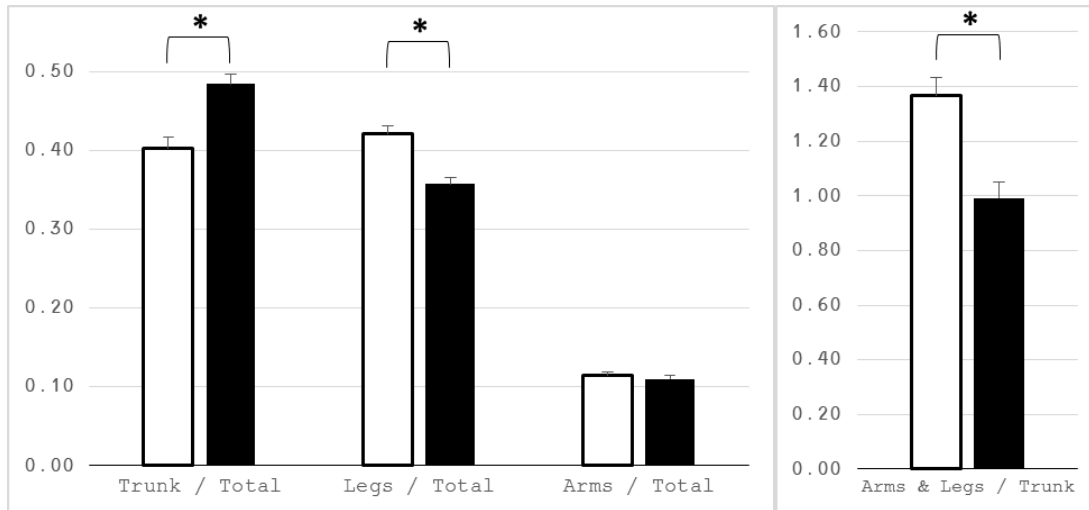Typical development (□); Down syndrome (■)
* Group differences, $p < .05$
[†] Difference between BMI and elevated fat mass, $p < .05$

Figure 1.6.   Group differences in physical activity
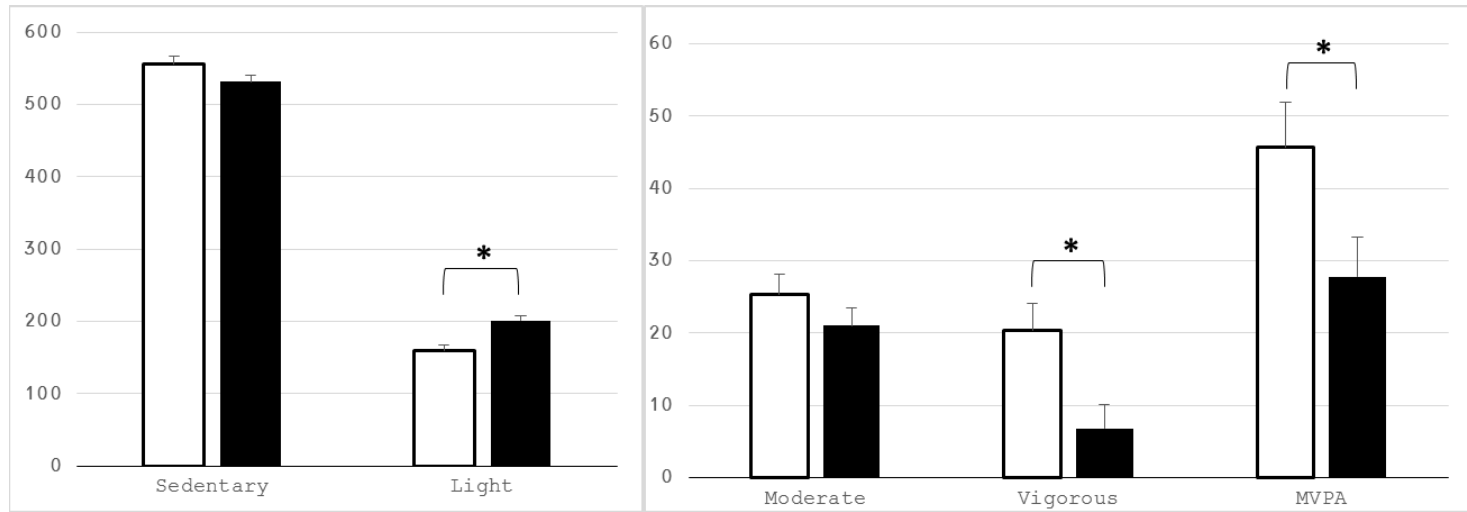
Estimated marginal means and standard errors of physical activity controlling for age, sex, Tanner pubertal stage, and Actigraph wear-time; Typical development (□); Down syndrome (■)
* $p < .05$

Figure 1.7.   Group differences in physical activity by sex
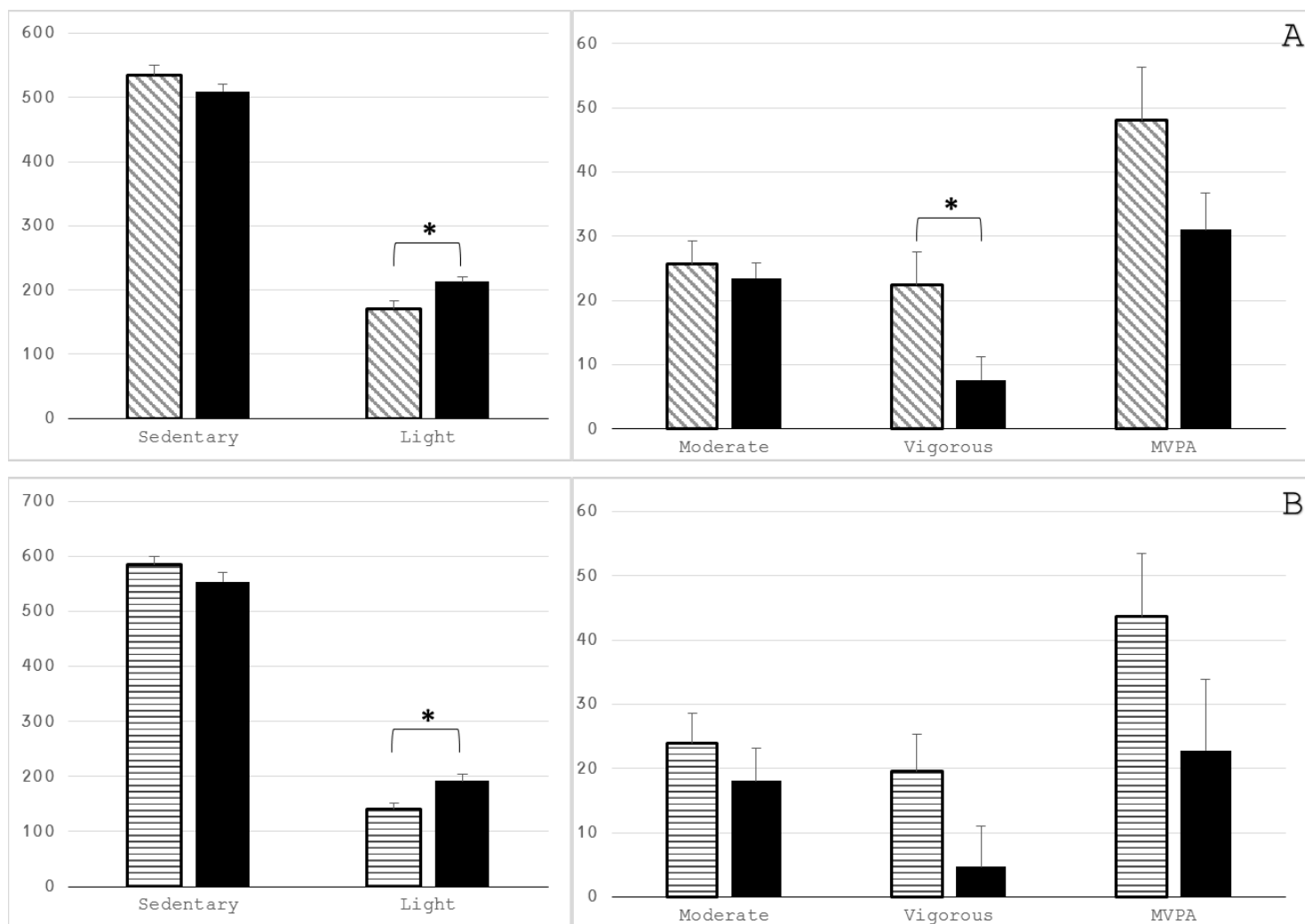
Estimated marginal means and standard errors of physical activity by sex controlling for age, Tanner pubertal stage, and Actigraph wear-time; **Figure A:** males, **Figure B:** females; Down syndrome (■);Typical development (□); males (diagonal); females (horizontal);* *p* < .05

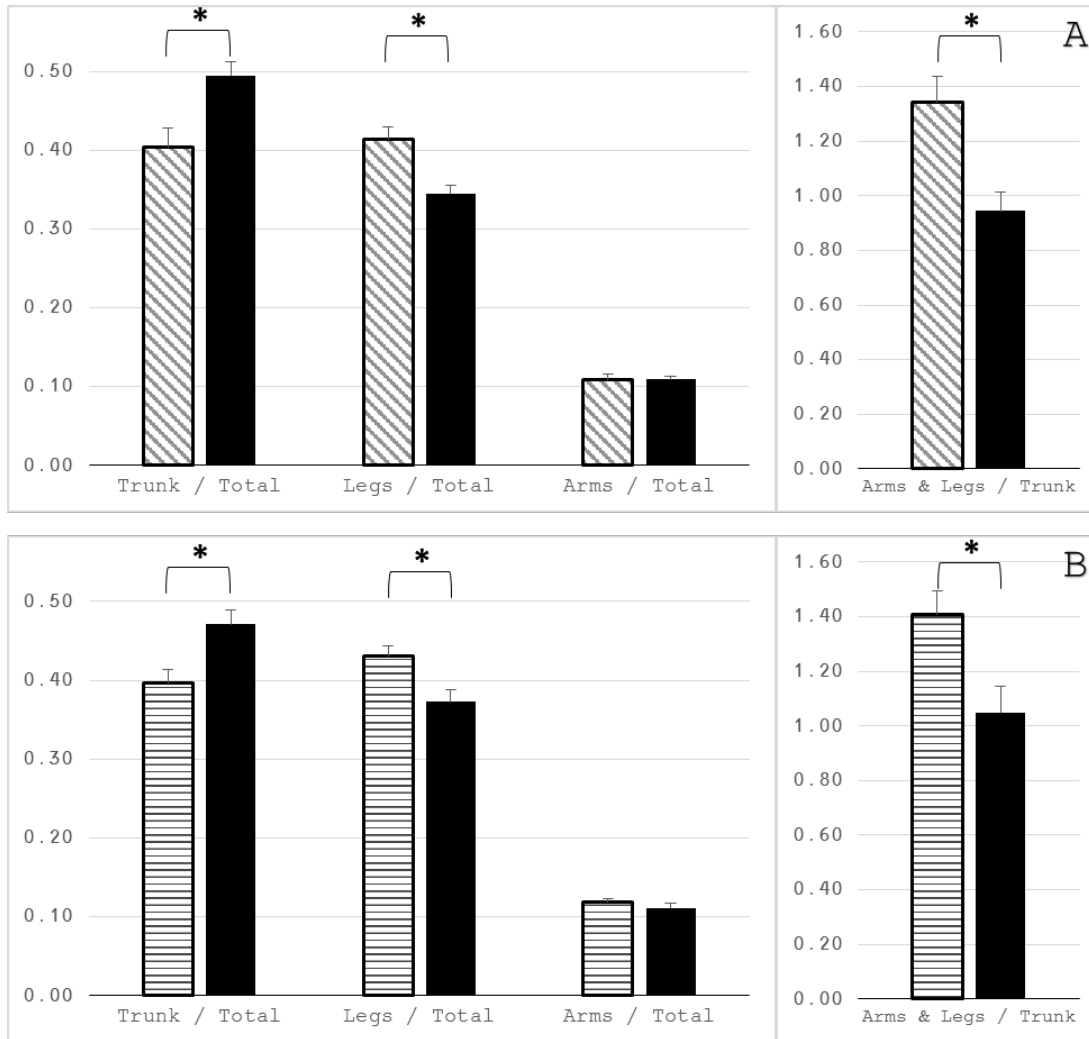Figure 1.8.   Group differences in energy intake and micronutrient distribution
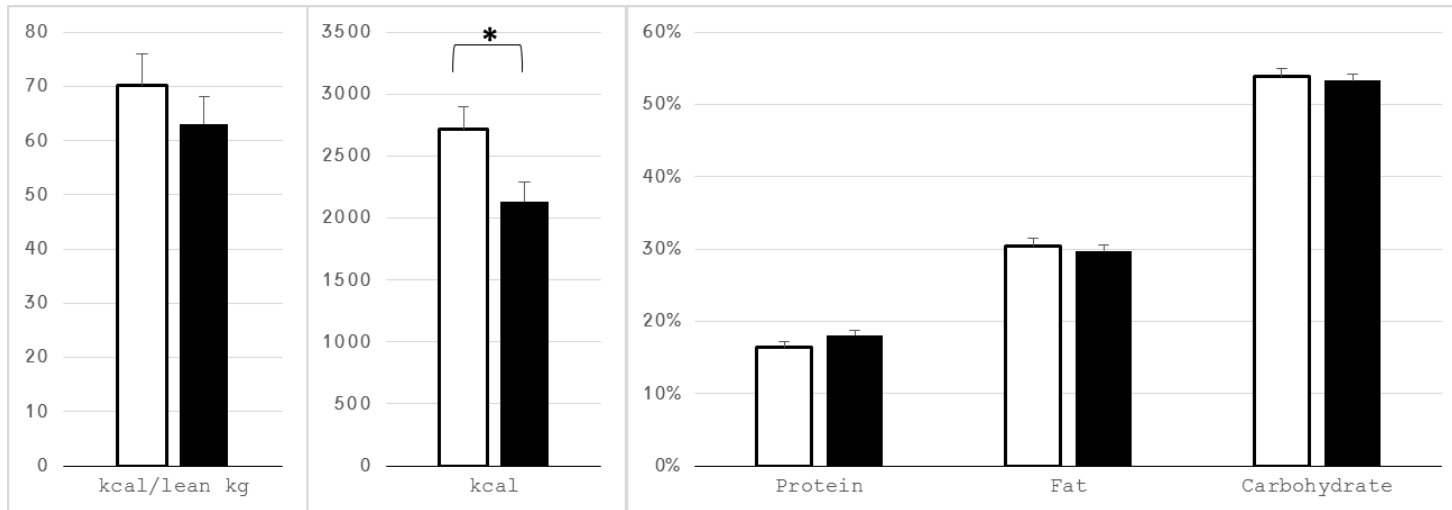
Estimated marginal means and standard errors of diet controlling for age, sex, and Tanner pubertal stage
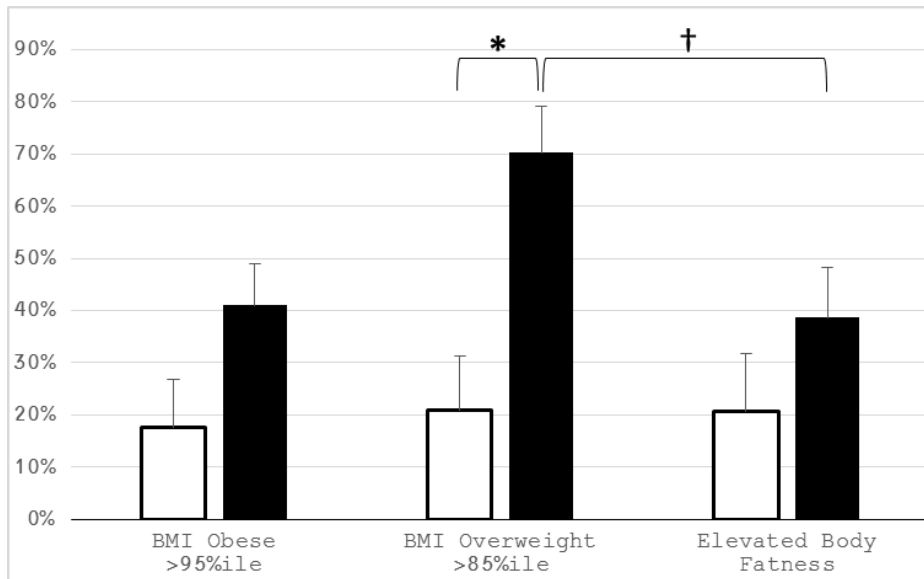Typical development (□); Down syndrome (■)
* *p* < .05

Figure 1.9.   Group differences in energy intake and micronutrient distribution by sex
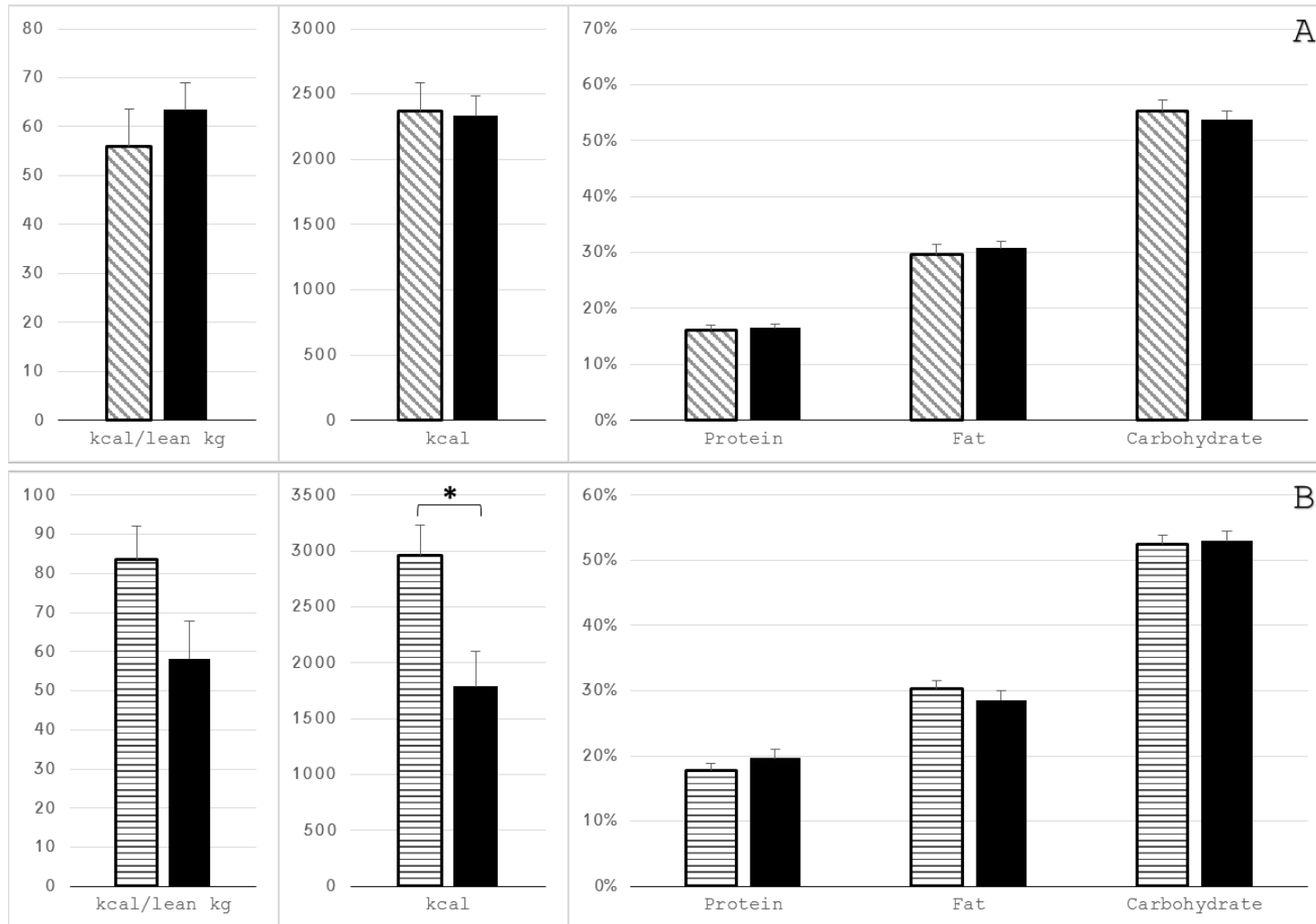
Estimated marginal means and standard errors of diet by sex controlling for age and Tanner pubertal stage
Figure A: males, **Figure B:** females; Down syndrome (■); Typical development (□); males (diagonal); females (horizontal)
* *p* < .05

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CHAPTER 2

Diurnal cortisol, obesity, and physical activity in adolescents with and without Down syndrome

Introduction

Down syndrome is the most common genetic form of intellectual disability with a prevalence estimated at 14.47 per 10,000 live births (Parker et al., 2010). Individuals with Down syndrome typically exhibit phenotypic characteristics including cognitive impairment, unique body and facial features, growth stature differences, muscle hypotonia, joint laxity, significant delays in motor development, and a variety of medical conditions including congenital heart disease (Bull & Committee on Genetics, 2011; Latash, Wood & Ulrich, 2008; Roizen & Patterson, 2003). Life expectancy of persons with Down syndrome has increased substantially due to advancements in health care, but morbidity and early mortality remain significantly higher than the general population (Bittles, Bower, Hussain & Glasson, 2007; Glasson, Dye & Bittles, 2014; Torr, Strydom, Patti & Jokinen, 2010). Addressing secondary health conditions is critical to improving long-term health outcomes.

Obesity is a prevalent health condition among persons with Down syndrome. Approximately 55% of youth with Down syndrome are overweight or obese (Rimmer, Yamaki, Lowry, Wang & Vogel, 2010) compared to 31% of youth in the general population (Ogden, Carroll, Kit & Flegal, 2012; Ogden, Carroll, Kit & Flegal, 2014). The odds of being obese are over three times greater for youth with Down syndrome than youth without a disability (Rimmer et al., 2010). Estimates of overweight and obesity for adults with Down syndrome in the United States continue to increase from childhood levels, ranging from 45% to 72% (Rubin, Rimmer,

Chicoine, Braddock & McGuire, 1998; Stancliffe et al., 2011). Smaller studies have reported the prevalence of obesity and overweight as high as 89% (Braunschweig et al., 2004; Fujiura, Fitzsimons, Marks & Chicoine, 1997; Rimmer & Wang, 2005; Sohler, Lubetkin, Levy, Soghomonian & Rimmerman, 2009). However, the current evidence is limited by the use of body mass index (BMI), which may overestimate excess fatness in Down syndrome (Bandini, Fleming, Scampini, Gleason & Must, 2012). Adolescents and adults with Down syndrome have been shown to exhibit greater body fat percentages, more total fat mass, less total lean mass, and greater abdominal obesity than the general population (Bandini et al., 2012; Baptista, Varela & Sardinha, 2005; Gonzalez-Aguero, Ara, Moreno, Vicente-Rodriguez & Casajus, 2011; Guijarro, Valero, Paule, Gonzalez-Macias & Riancho, 2008; Loveday, Thompson & Mitchell, 2012; Nickerson et al., 2015). When feasible, measurement of body composition should employ technology capable of detecting these systematic differences in body fat topology.

The foundation for obesity is energy imbalance between caloric intake (i.e. diet) and energy expenditure (i.e. physical activity). However, many factors contribute to the development of excess fat mass. The hypothalamic-pituitary-adrenal (HPA) axis regulates a variety of endocrine processes including energy storage and energy expenditure (Adam & Epel, 2007; Bjorntorp, 2001; De Vriendt, Moreno & De Henauw, 2009; Rodriguez et al., 2015). Activation of the HPA axis begins with the release of corticotrophin releasing hormone (CRH) from the hypothalamus, which stimulates release of adrenocorticotrophic hormone (ACTH) from the pituitary gland, which then increases glucocorticoid (i.e. cortisol) secretion from the adrenal gland (Adam & Epel, 2007; Bjorntorp, 1996, 2001; De Vriendt et al., 2009). Cortisol can affect circulating energy substrate levels in the blood by increasing gluconeogenesis and lipolysis (Adam & Epel, 2007; Bjorntorp, 2001; Bjorntorp & Rosmond, 2000). Adipose tissue in the

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abdominal region, in particular the visceral depot, is especially sensitive to cortisol due to increased density of glucocorticoid receptors and regulating enzymes (11β-HSD) in this type of adipose tissue. Glucocorticoids, like cortisol, enhance the size and number of fat cells and may play a role in the redistribution of adiposity into the visceral (abdominal) depot (Adam & Epel, 2007; Bjorntorp, 1996, 2001; De Vriendt et al., 2009; Rodriguez et al., 2015).

Cortisol dysregulation can influence the development of obesity through multiple pathways. First, higher cortisol levels (hypercortisolemia) upon waking are commonly associated with greater BMI (Bjorntorp, 1996, 2001; De Vriendt et al., 2009), but findings are not ubiquitous (Rodriguez et al., 2015). Second, blunted cortisol reactivity and circadian rhythms throughout the day are associated with greater abdominal obesity (Adam & Epel, 2007; Bjorntorp, 1996, 2001; De Vriendt et al., 2009). A blunted or less reactive diurnal cortisol pattern likely results in greater total cortisol output across the day. Third, cortisol is also associated with greater insulin and leptin resistance, increased neuropeptide Y release, and multiple markers of the metabolic syndrome, thus promoting metabolic dysregulation and increased food intake (Adam & Epel, 2007; Bjorntorp, 2001; Nieuwenhuizen & Rutters, 2008; Pasquali, Vicennati, Cacciari & Pagotto, 2006). It has also been suggested that altered cortisol regulation and weight gain may perpetuate as a positive feedback loop (Adam & Epel, 2007; Anagnostis, Athyros, Tziomalos, Karagiannis & Mikhailidis, 2009; Foss & Dyrstad, 2011).

HPA axis hormones, including cortisol, are characterized by a circadian diurnal pattern with high levels in the morning and decay of output throughout the day (Pasquali et al., 2006). Although most research has been conducted in adults, significant associations have been shown between aspects of the diurnal cortisol pattern (e.g. morning levels, repeated measures, cortisol intercept and slope) and indices of obesity (e.g. BMI, BMI percentile, waist circumference,

abdominal fat mass) in children and adolescents. Multiple studies have identified associations suggesting that cortisol levels are higher in individuals with greater adiposity (Barat et al., 2007; Guzzetti, Pilia, Ibba & Loche, 2014; Hill, Eisenmann, Gentile, Holmes & Walsh, 2011; Misra et al., 2008; Reinehr et al., 2014; Rosmalen et al., 2005; Weigensberg, Toledo-Corral & Goran, 2008). It should be noted that these associations are highly variable and many significant findings are limited to specific sub-groups within studies (e.g. obese girls; Hill et al., 2011, Rosmalen et al., 2005). Positive associations between cortisol and aspects of elevated body fatness appear to be strongest among obese adolescents. However, two large studies found the opposite direction of association with lower cortisol levels associated with higher adiposity (Ondrak, McMurray, Hackney & Harrell, 2011; Ruttle et al., 2013). Independent of BMI or adiposity, cortisol has strong positive associations with insulin resistance and other markers of metabolic syndrome (Adam et al., 2010; Barat et al., 2007; Guzzetti et al., 2014; Misra et al., 2008; Prodam et al., 2013; Reinehr et al., 2014; Rubin, McMurray, Hackney & Harrell, 2005; Weigensberg et al., 2008), indicating further relevance of this hormonal to overall physical health.

Habitual exercise (e.g. physical activity) can promote health benefits through improving adrenal efficiency and lowering basal cortisol levels (Hackney, 2006; Viru & Viru, 2004). Evidence is limited in youth, but higher levels of physical activity appear to be associated with lower salivary cortisol levels in adolescents and with less cortisol stress reactivity in children (Martikainen et al., 2013; Martikainen et al., 2014; Rubin et al., 2005). However, DuBose and McKune (2014) found a significant positive association between levels of vigorous physical activity with 30-minute post-waking cortisol and an association trending toward significance with overall cortisol output. This could indicate that high-intensity physical activity is needed to

influence cortisol function. The direction of this relationship is further obscured as both increases and decreases in cortisol have been observed in response to exercise interventions in obese children (Karacabey, 2009; Ounis et al., 2011)

There is limited research on cortisol function in persons with Down syndrome (Anneren, Sara, Hall & Tuvemo, 1986; Arnell, Gustafsson, Ivarsson & Anneren, 1996; Bricout et al., 2008; Hestnes et al., 1991; Murdoch, Giles, Grant & Ratcliffe, 1979), but the most recent evidence suggests that persons with Down syndrome may exhibit characteristics of cortisol dysfunction that could promote obesity (Bricout et al., 2008). Significantly lower cortisol levels and less reactivity to rest and exercise were observed in young adult males with Down syndrome compared to controls (Bricout et al., 2008). However, other studies have reported normal cortisol values or differences that did not reach statistical significance (Anneren et al., 1986; Arnell et al., 1996; Hestnes et al., 1991; Murdoch et al., 1979).

Multiple research questions remain regarding cortisol in Down syndrome: **1)** To my knowledge, no studies have tracked changes in cortisol across the day in this population, despite the known diurnal fluctuation of cortisol (Pasquali et al., 2006). Describing this diurnal pattern among individuals with Down syndrome is a first step in examining the association between this hormone and health outcomes. An understanding of the cortisol output pattern is also essential to contextualize specific tests (e.g. exercise, stress) of cortisol response. **2)** Cortisol function has not been examined within the scope of obesity in Down syndrome. Given the influence of cortisol on body fat (Bjorntorp & Rosmond, 2000), the high rates of obesity and greater fat mass observed in adolescents with Down syndrome (Gonzalez-Aguero et al., 2011; Rimmer et al., 2010) may be associated with dysregulation of cortisol. Furthermore, only two studies have measured cortisol function in youth with Down syndrome (Anneren et al., 1986; Arnell et al., 1996), both with

measures with limited generalizability. Growth velocity in adolescents with Down syndrome can be 25% to 50% lower than in typically developing adolescents (Cronk et al., 1988), putting adolescents with Down syndrome at increased risk for obesity during this period (van Gameren-Oosterom et al., 2012). Changes in endocrine function during this period may also be important to understanding the greater rates of obesity in Down syndrome (Alberga, Sigal, Goldfield, Prud' homme & Kenny, 2012; Daniels et al., 2005). More cortisol research is needed, particularly in adolescents during the sensitive period of puberty, to understand the developmental nature of this obesogenic hormone. **3)** Physical activity levels are often lower among youth with Down syndrome compared to peers without disabilities (Pitetti, Baynard & Agiovlasitis, 2013), although more objective physical activity data are needed. While the association between BMI and physical activity is weak and non-significant among youth with Down syndrome (Esposito, MacDonald, Hornyak & Ulrich, 2012; Izquierdo-Gomez et al., 2015; Shields, Dodd & Abblitt, 2009), the joint effects of adiposity and physical activity may have implications for cortisol function.

The purpose of this study was to identify clinical differences in diurnal cortisol patterns, adiposity, and physical activity levels in adolescents with Down syndrome and with typical development. Furthermore, this study sought to examine the independent and interactive associations between Down syndrome, elevated body fatness, and engagement in physical activity with diurnal cortisol function. These associations may be clinically relevant to understanding the potential contributions of cortisol dysregulation to the high rates of obesity in this at-risk population.

Methods

Participants

Participants were adolescents with Down syndrome or typical development between 12 and 18 years old and Tanner stages III to V (Marshall & Tanner, 1969, 1970). Exclusion criteria to participation included **a)** documented history of hormonal insufficiency (e.g. hypothyroid); **b)** use of medication that could alter metabolic functions (e.g. prednisone, central nervous system stimulants, growth hormone, thyroid hormone); **c)** comorbid disease (e.g. diabetes); **d)** contraindication limiting ability to safely perform physical exercise (e.g. cardiac insufficiency); and/or **e)** dual disability diagnosis (e.g. autism). Participants were recruited through Down syndrome parent support groups in Michigan and northern Ohio, family referrals, local school districts, and from previous study participants. Groups of adolescents with and without Down syndrome were similar on sex, age, and Tanner pubertal stage ($p > .05$). Matching groups on cognitive function or other developmental indices was considered, but would have resulted in a pre-pubertal control group with different hormone profiles.

All methods and procedures for the study were approved by the Institutional Review Board of the University of Michigan Medical School. All parents provided written informed consent documents while participants completed written or verbal assent prior to initiating the study. Adult participants (e.g. 18 years old) independently provided written informed consent. However, parents of adult participants with Down syndrome also provided written consent.

Procedures

Phases. Each participant completed the study in two phases. Participants first completed a clinical visit at the Michigan Clinical Research Unit at the University of Michigan Hospital. The clinical visit included the informed consent process, measurement of body composition

through standard anthropometry and a single dual-energy X-ray absorptiometry (DXA) scan, and parental questionnaires. Following the clinical visit, each participant provided a total of nine saliva samples (3 times per day for 3 days) to measure cortisol patterns and one week of accelerometry to estimate habitual physical activity levels. Participants and parents were trained in the saliva sampling and accelerometer procedures during the clinical visit.

Demographics. Each participant's parent completed a proprietary demographic survey with assistance from the participant. Proxy reporting was employed to reduce response bias due to intellectual disabilities (Emerson, Felce & Stancliffe, 2013). The survey documented the age, race and ethnicity, current medication use, and comorbidities of participants.

Puberty. Tanner's stages of pubertal development were used to describe the maturational state of participants (Marshall & Tanner, 1969, 1970). Pubertal stage was estimated via parental report using schematic line drawings (Morris & Udry, 1980). Strong correlations ($r > 0.60$) have been shown in Tanner assessment between line drawings and physical exams (Morris & Udry, 1980). Parental reports can provide an acceptable estimate of pubertal stage (Coleman & Coleman, 2002) and are less invasive and stressful than traditional physical assessment. Minimizing stress, which directly influences cortisol levels (Bjorntorp, 2001; De Vriendt et al., 2009), was critical to not skew study outcomes. Tanner stage was calculated as the average of reported stages for each participant (i.e. females: breast and pubic hair development; males: genital and pubic hair development). All participants were adolescents in Tanner stages III to V.

Anthropometry. All anthropometric measurements were conducted according to established guidelines (Lohman, Roche & Martorell, 1988). Height was measured to the nearest 0.1 cm with a stadiometer (S-214; SECA, Hanover, MD). Weight was measured to nearest 0.01

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kg with a digital scale (H-349KL; Health O Meter, McCook, IL). Measurements were taken in duplicate, with a third trial taken if measures differed by more than 0.5 cm or 0.5 kg, respectively. The average of all trials were used to calculate BMI (kg/m^2) and BMI percentile from the CDC growth reference (Kuczmarski et al., 2000). Participants with Down syndrome were also categorized based on population-specific BMI-for-age curves (Zemel et al., 2015).

Dual-Energy X-Ray Absorptiometry. Each participant completed one DXA scan (Lunar Prodigy Advance [DPX-IQ 240] densitometer; GE Healthcare, Madison, WI). DXA scans provided a three-component (fat mass, lean body mass, and bone mass) analysis of body composition. This technology is cost-effective, time-efficient, and has minimal risk (0.37 μSv radiation), yet is highly correlated with advanced imaging techniques (e.g. computerized tomography scan, magnetic resonance imaging) and reliable (ICC ≥ 0.97) in pediatric populations (Albanese, Diessel & Genant, 2003; Kendler et al., 2013; Margulies et al., 2005). The Lunar Prodigy was calibrated daily according to manufacturer's instructions using a standardized block.

Participants wore light clothing and were positioned in a supine position with hands by the sides in a neutral position. A warm blanket was used to assist the participant with maintaining position during the scan, if needed. Pediatric software (enCore 14.0; GE Healthcare, Madison, WI) estimated body fat percentage for total body and regional segments (i.e. trunk, legs, and arms). Obesity was classified based on age- and sex-specific cut-points for elevated body fat percentage (Freedman et al., 2009).

Saliva Sampling. To measure diurnal patterns in cortisol, each participant provided three saliva samples per day for three consecutive days (nine samples total). Salivary cortisol is highly correlated with free serum cortisol (Dorn, Lucke, Loucks & Berga, 2007) and is the most

common approach to naturalistic cortisol measurement. The three daily samples were scheduled respective to the participant's waking times on that day. Sampling times included: **1)** immediately after waking; **2)** an afternoon measurement occuring when the participant returned home from school or daily activity (approximately 3:00 to 5:00pm); and **3)** immediately prior to bedtime. These timepoints were consistent with methodology for diurnal salivary cortisol measurement (Hoyt et al., 2014; Keiver, Bertram, Orr & Clarren, 2015; Ruttle et al., 2013; Shirtcliff et al., 2012). Participants were instructed not to eat, drink, or brush their teeth in the 30 minutes before each sample. The participant's wake time, bedtime, and saliva sample collection times were reported on a brief questionnaire.

Saliva was collected with an oral swab (Salimetrics, State College, PA). Participants placed the swab underneath the tongue to absorb saliva for 3 to 5 minutes while seated. The saturated swab was then stored in an individually-numbered polypropylene vial and frozen for storage. Cortisol concentration (µg/dL) was measured using enzyme-linked immunosorbent assay (ELISA) techniques with the Expanded High Range Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, State College, PA). The assay had a lower limit of sensitivity (minimum detectable concentration) of 0.007 µg/dL (Shirtcliff, Granger, Schwartz & Curran, 2001) and acceptable inter-assay (5.53%) and intra-assay (5.14%) coefficients of variance (%CV). These indices of precision and repeatability are acceptable based on recommended guidelines for bioassays (Reed, Lynn & Meade, 2002).

Physical Activity. Each participant wore an Actigraph GT3X+ (Pensacola, FL) triaxial accelerometer at the waist during waking hours for seven days to measure physical activity levels. Criteria for minimum wear time included 10 hours per day for at least 4 days of the 7-day period, including at least one weekend day (Cain, Sallis, Conway, Van Dyck & Calhoon, 2013).

Data were assessed for non-wear time using validated algorithms (Choi, Liu, Matthews & Buchowski, 2011) and classified into physical activity intensity categories from Evenson et al. (2008) based on counts per minute (cpm) using ActiLife 6 software (v6.9.5; Actigraph, Pensecola, FL). Physical activity was operationalized as minutes per day of moderate-to-vigorous physical acitivty (MVPA; $\geq$ 2296 cpm). Participants were divided post hoc into groups based on whether or not they averaged 30 minutes of MVPA per day. The threshold of 30 minutes was selected due to low levels of physical activity within the sample. This limited the ability to group participants based on meeting the current World Health Organization recommendation of 60 minutes of MVPA per day (World Health Organization, 2010).

Statistical Analysis

All statistical analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY) with an a priori α of 0.05. Characteristics of the sample were described using independent t-tests for continuous data and Pearson's Chi-square (X^2) tests for dichotomous data with effect sizes (i.e. Cohen's *d*).

To be included in analyses, a participant needed to have at least 5 valid cortisol measurements across 2 or more days. A total of 286 cortisol data points from the 33 participants were included in analyses, with 11 (3.7%) data points missing. Cortisol data were not normally distributed, thus all analyses were performed on logarithmically transformed cortisol values. However, figures were designed with raw cortisol values to aid in interpretation.

Linear Mixed Modeling (LMM) techniques were employed to examine associations between Down syndrome, adiposity, and physical activity on the diurnal pattern of cortisol. This approach is relevant as LMM allows for random parameters estimates, is not limited by non-independent (e.g. repeated) observations, and does not require an equal number of data points,

thus accommodating missing values. All models include random intercepts to estimate cortisol concentration at wakening and repeated time parameters to account for decreases in cortisol across the day. Sex and hours since waking were included as a covariates in all models. Age and Tanner pubertal stage were also examined as covariates, but neither factor was statistically significant in any models and both were removed in favor of parsimony and maximizing statistical power. Each model examined specific associations with diurnal cortisol pattern: **Model 1**) presence of Down syndrome; **Model 2**) elevated body fat and interaction with Down syndrome; **Model 3**) engaging in more MVPA per day and interaction with Down syndrome; and **Model 4**) interaction between Down syndrome, adiposity, and MVPA. Models 2 through 4 were then repeated with separate analyses for adolescents with and without Down syndrome.

Results

The final sample included participants with valid cortisol data and all covariates ($n = 33$). Characteristics of the sample are presented in **Table 2.1**. Groups had similar compositions based on age, sex, and Tanner pubertal stage ($p > .05$).

Table 2.2 shows the group differences in body composition. Large differences were observed across all measures of body composition, except for weight, $t(31) = -.255$, $p = .801$, $d = .090$. Differences in total body fat percentage approached statistical significance, $t(31) = 2.03$, $p = .051$, $d = .676$. Adolescents with Down syndrome had higher body fat percentage in the trunk region (32.33%) than with typical development (23.52%), $t(31) = 2.44$, $p = .020$, $d = .792$; and a higher ratio of truncal fat to total fat ($M = 0.49$, S$D = .13$) than with typical development ($M = 0.40$, $SD = .09$), $t(31) = 4.52$, $p < .001$, $d = 1.24$. The proportion of overweight, defined as $\geq 85^{th}$ percentile on CDC growth curves (Kuczmarski et al., 2000), was significantly greater among adolescents with Down syndrome (81.2%) than with typical development (17.6%), $X^2(1) =$

13.35, $p < .001$, $d = 1.25$. However, 62.5% of adolescents with Down syndrome were below the 75th percentile on BMI-for-age curves specific to youth with Down syndrome ($M_{percentile} = 67.66$, $SD = 22.19$) (Zemel et al., 2015). This indicates that despite large differences in BMI compared to controls, the sample with Down syndrome adequately represents the population.

Table 2.3 shows the group differences in physical activity level between groups. No differences in MVPA were observed between groups, $t(31) = -1.32$, $p = .196$, $d = .455$. Physical activity levels were very low in the sample ($M = 35.99$ minutes/day, $SD = 28.12$). Few participants met the recommended guidelines of 60 minutes of MVPA per day (typical development = 17.6%, Down syndrome = 0.0%) and only half of participants averaged 30 minutes (typical development = 47.0%, Down syndrome = 50.0%).

Cortisol followed the expected diurnal pattern with high levels in the morning and low levels at night. LMM (Model 1) presented in **Table 2.4** showed a significant linear decrease in log-cortisol across hours of the day (b = -0.11, $p < .001$). This corresponds to an average decrease in cortisol concentration of 0.026 µg/dL per hour. Adolescents with Down syndrome exhibited higher morning cortisol concentration (0.36 µg/dL) than adolescents with typical development (0.34 µg/dL). However, there were not significant differences in log-cortisol at the intercept (b = -0.22, $p = .171$) or in slope across time (b = 0.70, $p = .363$). Diurnal cortisol trajectories across the day are shown in **Figure 2.1**. The random intercept and within-subject variance of repeated measures were significant ($p < .001$), justifying the inclusion of random effects in the model.

The LMM analyses for Models 2 and 3 are presented in **Table 2.5** and **Table 2.6**, respectively. A significant linear decrease in log-cortisol was observed across hours in all models ($p < .001$). Model 2 examined the interaction between Down syndrome and elevated body fat

percentage (Freedman et al., 2009). No significant differences were observed at the intercept between adolescents with and without Down syndrome (b = -0.16, *p* = .365) or elevated body fatness (b = 0.14, *p* = .461). Interactions between the presence of Down syndrome and body fatness across the day were not statistically significant (*p* > .500). Model 3 examined the interaction between Down syndrome and participating in 30 or more minutes of MVPA per day. Significant differences at the intercept were not observed between adolescents with and without Down syndrome (b = -0.23, *p* = .158) nor between adolescents that did or did not meet 30 minutes of MVPA per day (b = -0.17, *p* = .296). Interactions in slope between disability and MVPA groupings were also not significant (*p* > .100).

Model 4 examined the interaction between all groupings (**Table 2.7**). A significant linear effect across time points (b = -0.43, *p* = .022) was observed independent of the effect of hours since waking (*p* < .001). While no group differences in log-cortisol were observed between Down syndrome, body fatness, or MVPA groups at the intercept (*p* > .300), multiple significant interactions were observed across time. Compared to adolescents in the reference group (i.e. adolescents with Down syndrome, normal body fat percentage, and < 30 minutes of MVPA per day), log-cortisol slope was significantly higher in adolescents with Down syndrome, with normal body fat, and higher MVPA (b = 0.59, *p* < .001), and typically developing adolescents with normal body fat and either low MVPA (b = 0.21, *p* = .050) or higher MVPA (b = 0.27, *p* = .034). The difference in slope for adolescents with Down syndrome, elevated body fat, and low MVPA also trended toward statistical significance (b = 0.19, *p* = .091). Diurnal cortisol patterns between groups by Down syndrome, body fatness, and MVPA are shown in **Figure 2.2**. Significant pairwise comparisons were observed across multiple time points within adolescents with Down syndrome (*p* < .05), but not typical development. In all models, the random intercepts

and within-subject variances were significant (p < .001), again reinforcing the need to account for random effects in diurnal cortisol.

Discussion

The current study examined whether diurnal cortisol pattern was associated with the presence of Down syndrome, adiposity, and levels of physical activity. To my knowledge, this is the first study to track diurnal cortisol in adolescents with Down syndrome. Previous studies in persons with Down syndrome have examined cortisol in single measure (Anneren et al., 1986; Arnell et al., 1996; Hestnes et al., 1991; Murdoch et al., 1979), during stress tests (Murdoch et al., 1979), and during periods of rest and exercise (Bricout et al., 2008). Examining the diurnal cortisol patterns through multiple measurements across the day may provide additional information about HPA axis activity and its associations with obesity.

The main finding of this study is that adolescents with Down syndrome do not appear to have different diurnal cortisol trajectories than adolescents with typical development. A diurnal pattern with high morning cortisol levels and a log-linear decrease in concentration across the day was observed for both groups. This pattern is consistent with the expected nature of this hormone (Pasquali et al., 2006). Cortisol levels were higher in adolescents with Down syndrome at each time point compared to adolescents with typical development. However, neither the intercept ($p > .100$) nor slope ($p > .300$) were statistically different between groups. The lack of significant group differences is consistent with studies utilizing single measurements of cortisol (Anneren et al., 1986; Arnell et al., 1996; Hestnes et al., 1991; Murdoch et al., 1979). However, the observed diurnal pattern, characterized by a significant negative slope across hours ($p < .001$), confirms that repeated measurements are required to properly describe cortisol function. While Bricout et al. (2008) found lower cortisol levels during rest and a blunted response to

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exercise among young adult males with Down syndrome, it remains unclear how these short periods of time fit into the diurnal cortisol pattern. Absolute differences in cortisol concentration between adolescents with Down syndrome and typical development in the present study, regardless of statistical significance, were larger in the morning than at other time points. The significant differences observed by Bricout et al. (2008) all occurred in the morning, but the time from waking was not reported. Thus, the discrepancy in cortisol patterns between the two studies may be due to differences in the scope of measurement. The current study included adolescents while Bricout et al. (2008) included young adults suggesting there may also be systematic differences due to age or pubertal status (De Vriendt et al., 2009; Tsai, Seiler & Jacobson, 2013).

Differences in cortisol pattern began to emerge when accounting for body fatness and engagement in physical activity. Model 4 compared adolescents with Down syndrome who had normal body fat percentages, but did not average at least 30 minute of MVPA per day to other combinations of these factors. Significant (or trending towards significant; $p < .10$) differences were observed within adolescents with Down syndrome based on both physical activity and elevated body fatness. Typically developing adolescents with normal body fatness were significantly different than the reference group, regardless of physical activity participation. The general pattern within each group was that cortisol was lower among adolescents with normal body fat and/or with higher levels of physical activity. Given these interactions, the high prevalence of overweight in adolescents with Down syndrome in the sample may be affecting the ability to identify differences between groups in Model 1.

While significant associations have been shown in typically developing children and adolescents between diurnal cortisol patterns and indices of obesity (Barat et al., 2007; Hill et al., 2011; Ruttle et al., 2013) or physical activity (DuBose & McKune, 2014; Martikainen et al.,

2014), the direction of these associations are variable. Most studies have found positive associations between cortisol and adiposity, particularly in youth with obesity (Barat et al., 2007; Guzzetti et al., 2014; Hill et al., 2011; Misra et al., 2008; Reinehr et al., 2014; Rosmalen et al., 2005; Weigensberg et al., 2008). The current study is consistent with a positive association between cortisol and adiposity. The high level of abdominal obesity among adolescents with Down syndrome was also consistent with the relationship between cortisol and visceral fat (Barat et al., 2007; Misra et al., 2008). Conversely, Ruttle et al. (2013) and (Ondrak et al., 2011) found strong negative associations between cortisol and BMI. These differences may be due to differences in the measurement of adiposity.

Similar variability in the existing literature is present for the relationship between cortisol and physical activity. DuBose and McKune (2014) found that physical activity was positively associated with cortisol levels in girls, but only the association between vigorous physical activity and 30-minute post waking cortisol was statistically significant. Conversely, Martikainen et al. (2014) found a negative association between cortisol and both overall physical activity and vigorous physical activity, but this was only statistically significant in girls. The current study suggests a negative association, with lower cortisol levels in adolescents who participate in more MVPA. It should be noted, that an interesting relationship appears in adolescents with Down syndrome. Morning cortisol was significantly higher in those with more physical activity, but the slope was much steeper resulting in significantly lower levels at night. This is yet another example of how cortisol measurement (e.g. one time point vs. diurnal pattern) can affect interpretations.

As seen in Figure 1, one group within adolescents with Down syndrome appears to behave differently (solid black line: Down syndrome with normal body fatness and met 30

minutes of MVPA). Cortisol levels were significantly higher and flatter ($p < .001$) in this group, despite having less body fat and more physical activity. However, this sub-group includes only two participants; both are males in Tanner stage IV. Males have been shown to produce more cortisol than females and cortisol production is positively associated with Tanner staging (Tsai et al., 2013). This may explain the phenomenon within this sample and would suggest that this group may not be representative of adolescents with Down syndrome who have favorable adiposity and physical activity characteristics. However, Hill, Eisenmann, Holmes and Heelan (2010) also found high levels of cortisol among a small group of children with normal body composition that was not congruent with other findings.

A secondary finding of this study was the differences in body composition between adolescents with Down syndrome and typical development. Compelling evidence has consistently shown that youth with Down syndrome experience obesity at higher rates than the general population (Bandini et al., 2012; Gonzalez-Aguero et al., 2011; Izquierdo-Gomez et al., 2015; Rimmer et al., 2010). The current study also found a significantly higher prevalence of overweight (81.2%) among adolescents with Down syndrome ($p < .001$, $d = 1.25$). Statistically significant differences were observed between groups for both BMI percentile ($p < .001$, $d = 1.31$) and percent body fat from the DXA scan ($p = .051$, $d = 0.68$). The magnitude of difference was considerably smaller with body fat percentage, further suggesting that BMI may overestimate the extent of excess fatness in youth with Down syndrome (Bandini et al., 2012). Body fat distribution was also significantly different in adolescents with Down syndrome. A higher proportion of body fat was stored in the trunk region ($p < .001$), consistent with findings from Gonzalez-Aguero et al. (2011). Greater fat mass in the abdominal region is also consistent with dysregulation of the HPA axis. Cortisol levels are associated with fat mass in the abdomen,

particularly in overweight and obese youth, when measured through waist circumference or DXA (Barat et al., 2007; Hill et al., 2011). These differences in body fat are important to obesity research in Down syndrome as they identify the abdomen as a particularly useful area for clinical measurement, reinforce the limitations of BMI for understanding obesity in this population, and illustrate the additional information we can ascertain from DXA. While DXA scanners have considerable costs and are not feasible for field research, the additional measurement potential is worthwhile when possible.

The lack of participation in physical activity in youth with Down syndrome is problematic. Adolescents with Down syndrome averaged approximately 36 minutes of MVPA per day, with only 50% averaging more than 30 minutes per day during the measurement week. No participants with Down syndrome met the recommended guidelines of 60 minutes per day (World Health Organization, 2010). However, it should be noted that physical activity levels in the control group with typical development (42.20 minutes per day) were also below recommendations. Previous studies have found similarly low levels of physical activity in Down syndrome (Pitetti et al., 2013), but these results are not ubiquitous. For example, Esposito et al. (2012) found only 20.6% of youth with Down syndrome met physical activity guidelines, with a strong negative association between MVPA and age. However, other studies have reported levels of MVPA that were near or exceeding the guidelines of 60 minutes of MVPA per day (Izquierdo-Gomez et al., 2015; Shields et al., 2009). Still, less than half of youth with Down syndrome (42.1%) consistently met the 60-minute recommendation across a week (Shields et al., 2009). Differences in cut-points used to classify physical activity intensity across studies may explain some of the variation in reported MVPA levels. It should be noted that the validity of these cut-points for individuals with Down syndrome are highly questionable due to differences

in energy expenditure (Agiovlasitis, Motl, Foley & Fernhall, 2012), thus, reported levels of MVPA should be viewed cautiously. Validated cut-points specific to persons with Down syndrome for use in physical activity epidemiology research are of great need (Pitetti et al., 2013).

The physical activity levels reported in the current study are among the lowest reported for youth with Down syndrome (Pitetti et al., 2013). Coupled with the high rates of obesity and elevated fat mass, there are evident health disparities with the sample. Health promotion and direct intervention efforts are clearly needed for this at-risk population (Pitetti et al., 2013; Rimmer et al., 2010; Shields et al., 2009). Study designs that allow researchers to examine relationships between health behaviors and health conditions at baseline before intervening to increase healthy behaviors may be particularly beneficial.

Despite the high prevalence of excess adipose tissue, additional health conditions including metabolic syndrome, type II diabetes, and hypertension are surprisingly absent in Down syndrome (De Winter, Bastiaanse, Hilgenkamp, Evenhuis & Echteld, 2012; Draheim, McCubbin & Williams, 2002; Real de Asua, Parra, Costa, Moldenhauer & Suarez, 2014). The mechanism behind this phenomenon is currently unknown, but sympathetic hypoactivity and endocrine balances unique to Down syndrome have been proposed (Adelekan, Magge, Shults, Stallings & Stettler, 2012; Agiovlasitis et al., 2010; Corsi et al., 2009). Even with the lack of metabolic and atherosclerotic complications, obesity remains a serious health issue for persons with Down syndrome due to detrimental effects on mobility, independent living, community participation, and quality of life (Rimmer & Yamaki, 2006).

The current study has a number of limitations that are important to consider when interpreting these results. First, the current sample size is small and limits the type of analyses

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that could be conducted. However, statistically significant results were obtained. The LMM procedures were able to converge with the variance components covariance structure and were appropriate for the data. Schwarz's Bayesian Criterion (BIC) ranged from 265.03 to 608.24. The smallest BIC value observed for Model 4 (BIC = 265.03), represented the best fit. Interpretation of Model 4 is limited by the small number of participants in each group. This issue is illustrated by the small subgroup with Down syndrome, normal body fat, and ≥ 30 minutes of MVPA per day. While the results of this study are novel, it is critical to reexamine these research questions with a sample capable of producing balanced subgroups. Second, the current sample had a greater proportion of overweight participants in the group with Down syndrome. Analyses with equal representation in BMI obesity status between groups may alter results. However, matching groups for obesity status, whether through BMI or body fat (Gonzalez-Aguero et al., 2011), is only possible with a leaner group of adolescents with Down syndrome and a more overweight group of adolescents with typical development. Such a sample would then have questionable generalizability to either population. If possible, future studies may attempt to recruit unique equally distributed sample of adolescents with and without Down syndrome as well as with and without obesity to examine the independent and joint effects of these factors. Third, physical activity levels were very low among both groups. Low physical activity levels could potentially affect the observed associations. The lack of validated cut-points for youth with Down syndrome continue to limit the interpretation of physical activity data. Finally, this research was cross-sectional and thus cannot infer or establish causation. Despite these limitations, the current results provide useful preliminary evidence to guide future research.

An extensive body of evidence from the general population supports the association between cortisol dysfunction and obesity (Adam & Epel, 2007; Barat et al., 2007; Bjorntorp &

Rosmond, 2000; De Vriendt et al., 2009; Hill et al., 2011; Pasquali et al., 2006; Rodriguez et al., 2015; Ruttle et al., 2013). Physical activity appears to be a health behavior capable of modifying hormonal patterns (Aucouturier et al., 2013; De Vriendt et al., 2009; DuBose & McKune, 2014; Martikainen et al., 2014), but more prospective studies are needed. To my knowledge, this is the first study to describe the diurnal pattern of cortisol and demonstrate associations with adiposity and physical activity in Down syndrome. A larger study is needed to better analyze this relationship, but these preliminary results justify the continuation of this research. Understanding the unique factors that contribute to health disparities in obesity among individuals with Down syndrome is a critical step in efforts to design and implement health promotion interventions.

Table Captions

Table 2.1.   Characteristics of participants with and without Down syndrome

Table 2.2.   Group comparisons for body composition

Table 2.3.   Group comparisons for physical activity

Table 2.4.   Linear mixed model on diurnal cortisol between adolescents with and without

               Down syndrome (Model 1)

Table 2.5.   Linear mixed model on diurnal cortisol, Down syndrome, and elevated fat mass

               (Model 2)

Table 2.6.   Linear mixed model on diurnal cortisol, Down syndrome, and physical activity

               level (Model 3)

Table 2.7.   Linear mixed model on diurnal cortisol, Down syndrome, elevated fat mass, and

               physical activity level (Model 4)

Figure Captions

Figure 2.1.   Diurnal cortisol pattern in adolescents with and without Down syndrome

Figure 2.2.   Association between diurnal cortisol pattern, adiposity, and physical activity in

adolescents with typical development

Figure 2.3.   Association between diurnal cortisol pattern, adiposity, and physical activity in

adolescents with Down syndrome

Table 2.1.   Characteristics of participants with and without Down syndrome

Demographics	Total (n=33)	Down syndrome (n=16)	Typically developing (n=17)	p	d
Female, *n*(%)	16 (48.5%)	6 (38.0%)	10 (59.0%)	.221 [a]	.420
Age (years)	14.91 (1.94)	14.72 (1.78)	15.08 (2.12)	.593 [b]	.190
Tanner (III-V)	3.62 (1.14)	3.56 (1.09)	3.68 (1.22)	.780 [b]	.099
Caucasian, *n*(%)	32 (97.0%)	15 (93.8%)	17 (100%)	.295 [a]	.359

Note: Values are Mean (Standard Deviation) unless otherwise noted. Abbreviations: *n* = frequency, % = proportion of column sample, *d* = Cohen's *d* effect size
[a] Pearson's chi-square test (X^2)
[b] Independent samples t-test (*t*)
* $p < .05$, **bolded**
† $p < .10$, *italicized*

Table 2.2.　　　　Group comparisons for body composition

Body Composition	Total (n=33)	Down syndrome (n=16)	Typically developing (n=17)	p	d
Weight (kg)	57.34 (13.61)	56.70 (9.98)	57.94 (16.31)	.801 [b]	.090
Height (cm)	155.95 (13.40)	146.62 (7.99)	164.68 (11.42)	**<.001** [b*]	1.33
BMI (kg/m^2)	23.59 (4.81)	26.34 (3.98)	21.01 (4.05)	**.001** [b*]	1.09
BMI %ile (CDC)	70.47 (27.72)	89.58 (10.69)	52.49 (26.79)	**<.001** [b*]	1.32
BMI%ile (DS)	--	67.66 (22.19)	--	**--**	--
Total Percent Body Fat	29.52% (9.6%)	32.88% (10.8%)	26.35% (7.4%)	*.051* [b†]	.676
Trunk %BF	27.92 (11.45)	32.59 (12.51)	23.52 (8.55)	**.020** [b*]	.792
Legs %BF	32.79 (8.90)	35.18 (9.97)	30.55 (7.36)	.137 [b]	.521
Arms %BF	29.84 (10.24)	32.69 (10.60)	27.16 (9.43)	.123 [b]	.540
Elevated Body Fat, n(%)	13 (39.4%)	10 (62.5%)	3 (17.6%)	**.008** [a*]	.904

Note: Values are Mean (Standard Deviation) unless otherwise noted. Abbreviations: n = frequency, % = proportion of column sample, BMI = body mass index, %ile = BMI percentile, CDC = Centers for Disease Control growth chart (Kuczmarski et al., 2000), DS = Down syndrome growth chart (Zemel et al., 2015), %BF = percent body fat, d = Cohen's d effect size
[a] Pearson's chi-square test (X^2)
[b] Independent samples t-test (t)
* $p < .05$, **bolded**
† $p < .10$, *italicized*

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Table 2.3.   Group comparisons for physical activity

Physical Activity	Total (n=33)	Down syndrome (n=16)	Typically developing (n=17)	p	d
Sedentary (min/day)	549.61 (62.77)	550.74 (56.33)	548.55 (70.02)	.922 [b]	.034
Light (min/day)	184.57 (53.89)	221.30 (42.76)	149.99 (38.41)	**<.001** [b]*	1.32
Moderate (min/day)	23.38 (11.39)	23.18 (8.67)	23.57 (13.75)	.923 [b]	.034
Vigorous (min/day)	13.30 (17.70)	7.64 (4.84)	18.63 (23.29)	*.074* [b†]	.621
MVPA (min/day)	36.68 (27.81)	29.41 (13.30)	42.20 (36.49)	.246 [b]	.455
≥ 30 min/day MVPA, n(%)	16 (48.5%)	8 (50.0%)	8 (47.1%)	.866 [a]	.058
≥ 60 min/day MVPA, n(%)	3 (9.1%)	0 (0%)	3 (17.6%)	*.078* [a†]	.604

Note: Values are Mean (Standard Deviation) unless otherwise noted. Abbreviations: n = frequency, % = proportion of column sample, BMI = body mass index, MVPA = moderate to vigorous physical activity, min/day = minutes per day, d = Cohen's d effect size
[a] Pearson's chi-square test (X^2)
[b] Independent samples t-test (t)
* $p < .05$, **bolded**
† $p < .1$

Table 2.4.   Linear mixed model on diurnal cortisol between adolescents with and without Down syndrome (Model 1)

Dependent Variable: Ln Cortisol (µg/dL)	Model 1	
Predictor	b	*p*
Intercept	-0.781	**<.001***
Fixed effects		
Down syndrome (DS)	*REF*	
Typical development (TD)	-0.220	.171
Sex	0.077	.435
Hours	-0.111	**<.001***
Time	-0.153	.376
DS * Time	0.070	.363
Random Effects		
var (morning)	0.158	**<.001***
var (afternoon)	0.276	**<.001***
var (night)	0.493	**<.001***
Intercept	0.142	**<.001***

Note: DS = Down syndrome ; TD = typical development Sex = reference is female.
* *p* < .05, **bolded**

Table 2.5.　　Linear mixed model on diurnal cortisol, Down syndrome, and elevated fat mass (Model 2)

Dependent Variable: Ln Cortisol (μg/dL)	**Model 2a** Total		**Model 2b** Down syndrome		**Model 2c** Typical Development	
Predictor	**b**	*p*	**b**	*p*	**b**	*p*
Intercept	-0.856	**<.001***	-0.710	**.008***	-1.237	**<.001***
Fixed effects						
Down syndrome (DS)	*REF*		--	--	--	--
Typical development (TD)	-0.163	.365	--	--	--	--
Sex	0.095	.349	-0.048	.722	0.257	*.081*[†]
Hours	-0.108	**<.001***	-0.100	**.002***	-0.122	**.001***
Time	-0.136	.449	-0.202	.375	0.006	.980
Elevated BF%	0.137	.461	0.043	.831	0.250	.447
DS * Normal BF% * Time	*REF*		*REF*		--	--
DS * Elevated BF% * Time	-0.056	.573	-0.022	.843	--	--
TD * Normal BF% * Time	0.034	.722	--	--	*REF*	
TD * Elevated BF% * Time	0.044	.775	--	--	-0.025	.862
Random Effects						
var (morning)	0.160	**<.001***	0.077	**.028***	0.252	**<.001***
var (afternoon)	0.276	**<.001***	0.214	**<.001***	0.352	**<.001***
var (night)	0.496	**<.001***	0.611	**<.001***	0.370	**<.001***
Intercept	0.143	**<.001***	0.139	**.001***	0.133	**.009***

Note: DS = Down syndrome; TD = typical development; BF% = body fat percentage; Sex = reference is female.

-- not included in model

* *p* < .05, **bolded**

† *p* < .10, *italicized*

Table 2.6.   Linear mixed model on diurnal cortisol, Down syndrome, and physical activity level (Model 3)

Dependent Variable: Ln Cortisol (µg/dL)	**Model 3a** Total		**Model 3b** Down syndrome		**Model 3c** Typical Development	
Predictor	**b**	**p**	**b**	**p**	**b**	**p**
Intercept	-0.710	**.001***	-0.715	**.007***	-1.015	**.001***
Fixed effects						
Down syndrome (DS)	*REF*		--	--	--	--
Typical development (TD)	-0.226	.158	--	--	--	--
Sex	0.095	.358	0.011	.942	0.225	.116
Hours	-0.112	**<.001***	-0.101	**.001***	-0.123	**.001***
Time	-0.220	.221	-0.244	.286	-0.046	.851
MVPA (< 30 min/day)	-0.167	.296	0.005	.981	-0.342	.158
DS * Less MVPA * Time	*REF*		*REF*		--	--
DS * More MVPA * Time	0.147	.112	0.077	.459	--	--
TD * Less MVPA * Time	0.110	.226	--	--	*REF*	
TD * More MVPA * Time	0.177	.113	--	--	0.109	.323
Random Effects						
var (morning)	0.154	**<.001***	0.081	**.024***	0.226	**<.001***
var (afternoon)	0.273	**<.001***	0.203	**<.001***	0.365	**<.001***
var (night)	0.509	**<.001***	0.620	**<.001***	0.381	**<.001***
Intercept	0.143	**<.001***	0.135	**.001***	0.137	**.008***

Note: DS = Down syndrome; TD = typical development; MVPA = moderate to vigorous physical activity (minutes per day);
Sex = reference is female.
-- not included in model
* *p* < .05, **bolded**
† *p* < .10, *italicized*

Table 2.7.   Linear mixed model on diurnal cortisol, Down syndrome, elevated fat mass, and physical activity level (Model 4)

| **Dependent Variable:** Ln Cortisol (µg/dL)	**Model 4a** Total		**Model 4b** Down syndrome		**Model 4c** Typical Development	
Predictor	**b**	**p**	**b**	**p**	**b**	**p**
Intercept	-0.756	**.002***	-0.654	**.014***	-1.040	**.002***
Fixed effects						
Down syndrome (DS)	*REF*		--		--	
Typical development (TD)	-0.183	.301	--		--	
Sex	0.187	*.056†*	0.178	.149	0.247	*.097†*
Hours	-0.094	**<.001***	-0.599	**.010***	-0.121	**.001***
Time	-0.432	**.022***	-0.066	**.034***	-0.710	.779
Elevated BF%	0.165	.367	0.120	.564	0.059	.869
MVPA (< 30 min/day)	-0.114	.472	0.098	.638	-0.322	.237
DS * Normal BF% * Less MVPA * Time	*REF*		*REF*		--	--
DS * Normal BF% * More MVPA * Time	0.588	**<.001***	0.530	**<.001***	--	--
DS * Elevated BF% * Less MVPA * Time	0.193	*.091†*	0.221	*.076†*	--	--
DS * Elevated BF% * More MVPA * Time	0.106	.411	0.047	.728	--	--
TD * Normal BF% * Less MVPA * Time	0.212	**.050***	--	--	*REF*	
TD * Normal BF% * More MVPA * Time	0.265	**.034***	--	--	0.125	.310
TD * Elevated BF% * Less MVPA * Time	0.231	.142	--	--	0.048	.765
TD * Elevated BF% * More MVPA * Time	a	a	--	--	a	a
Random Effects						
var (morning)	0.175	**<.001***	0.117	**.002***	0.232	**.001***
var (afternoon)	0.279	**<.001***	0.203	**<.001***	0.357	**<.001***
var (night)	0.456	**<.001***	0.522	**<.001***	0.390	**<.001***
Intercept	0.098	**.001***	0.050	*.079†*	0.138	**.008***

Note: DS = Down syndrome; TD = typical development; BF% = body fat percentage; MVPA = moderate to vigorous physical activity (minutes per day); Sex = reference is female; -- Not included in model;

[a] Unable to calculate due to no observations ($n = 0$)

* $p < .05$, **bolded**

† $p < .10$, *italicized*

Figure 2.1   Diurnal cortisol pattern in adolescents with and without Down syndrome
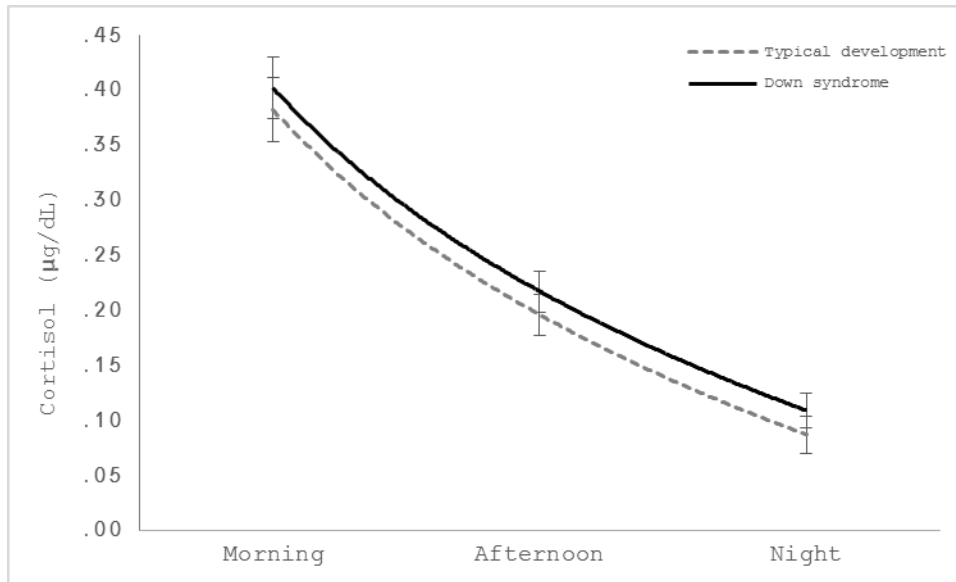
Cortisol (µg/dL) across time points. Analyses are based on log-transformed cortisol. Post-hoc linear independent pairwise corrections with Bonferroni corrections based on estimated marginal means controlling for sex and hours since waking. (*) Differences in cortisol between adolescents with Down syndrome and typical development, $p < .05$ (*no differences observed*)

Figure 2.2   Association between diurnal cortisol pattern, adiposity, and physical activity in adolescents with typical development
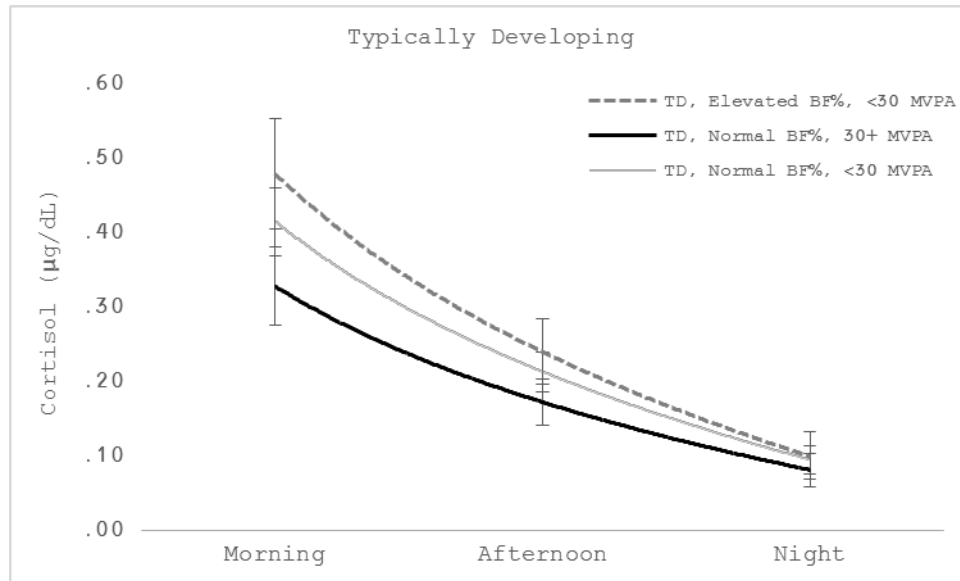
Cortisol (μg/dL) across time points. Analyses used log-transformed cortisol. Pairwise comparisons with Bonferroni corrections on estimated marginal means controlling for sex and hours since waking. TD = typical development, DS = Down syndrome, BF% = body fat percentage (elevated or normal), MVPA = moderate to vigorous physical activity (< 30 minutes or ≥ 30 minutes/day).
* Differences in cortisol across MVPA (30 min/day) groups, among adolescents with **elevated** body fat percentage, $p < .05$.
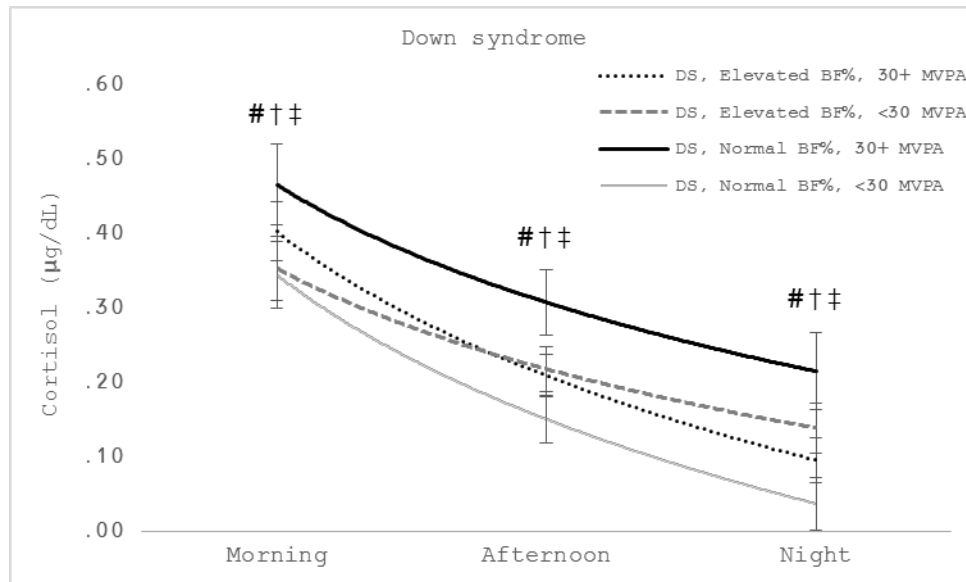\# Differences in cortisol across MVPA (30 min/day) groups, among adolescents with **normal** body fat percentage, $p < .05$.
† Differences in cortisol across body fat percentage groups, among adolescents engaging in $\geq$ **30 minutes** of MVPA per day, $p < .05$.
‡ Differences in cortisol across body fat percentage groups, among adolescents engaging in **< 30 minutes** of MVPA per day, $p < .05$.
Note: no differences observed.

Figure 2.3. Association between diurnal cortisol pattern, adiposity, and physical activity in adolescents with Down syndrome

Cortisol (µg/dL) across time points. Analyses used log-transformed cortisol. Pairwise comparisons with Bonferroni corrections on estimated marginal means controlling for sex and hours since waking. DS = Down syndrome, BF% = body fat percentage (elevated or normal), MVPA = moderate to vigorous physical activity (< 30 minutes or ≥ 30 minutes/day).
* Differences in cortisol across MVPA (30 min/day) groups, among adolescents with **elevated** body fat percentage, $p < .05$ (*no differences observed*; *trending toward significance, p < .10: Afternoon = .091, Night = .064*).
# Differences in cortisol across MVPA (30 min/day) groups, among adolescents with **normal** body fat percentage, $p < .05$.
† Differences in cortisol across body fat percentage groups, among adolescents engaging in **≥ 30 minutes** of MVPA per day, $p < .05$.
‡ Differences in cortisol across body fat percentage groups, among adolescents engaging in **< 30 minutes** of MVPA per day, $p < .05$.

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CHAPTER 3

Salivary cortisol response to acute exercise in adolescents with and without Down syndrome

Introduction

The public health crisis related to the high rates of childhood obesity is well documented (Ogden, Carroll, Kit & Flegal, 2014; Speiser et al., 2005; World Health Organization, 2012). However, less attention has been cast on the obesity-related health disparities among individuals with intellectual disabilities (Rimmer, Wang, Yamaki & Davis, 2009; Rimmer & Yamaki, 2006). Down syndrome, the most common genetic form of intellectual disability (Parker et al., 2010), is associated with increased risk for obesity. Prevalence rates of obesity among children and adolescents with Down syndrome exceed 30% (Rimmer, Yamaki, Lowry, Wang & Vogel, 2010), which is nearly twice the obesity rate among the general population (16.9%; Ogden et al., 2014). This health disparity represents a critical barrier to quality of life. A common recommendation to address the excess obesity in both general and disability populations is to increase levels of physical activity among youth (U.S. Department of Health and Human Services, 2002, 2005, 2008; World Health Organization, 2012). However, the physiological responses to exercise, particularly with regards to obesity, in youth with Down syndrome are not clearly understood and are in need of further examination.

Due to the phenotypic characteristics of Down syndrome, there exist multiple physiological differences that impact physical fitness and responses to acute exercise in this population (Baynard, Pitetti, Guerra, Unnithan & Fernhall, 2008; Mendonca, Pereira & Fernhall, 2010b; Pitetti, Baynard & Agiovlasitis, 2013). Most notably, peak aerobic capacity (VO_{2peak})

(Fernhall et al., 1996; Mendonca, Pereira & Fernhall, 2010a; Mendonca, Pereira, et al., 2010b; Mendonca, Pereira, Morato & Fernhall, 2010) and peak heart rate (Baynard, Pitetti, Guerra & Fernhall, 2004; Fernhall et al., 2001; Fernhall et al., 1996; Figueroa et al., 2005; Guerra, Llorens & Fernhall, 2003; Mendonca, Pereira, et al., 2010a; Mendonca, Pereira, Morato, et al., 2010) are significantly lower among youth and adults with Down syndrome compared to both peers with typical development or non-genetic forms of intellectual disabilities. A recent study (Wee et al., 2015) found that aerobic capacity, but not peak heart rate, was associated with obesity in adults with Down syndrome. However, significant associations were not observed among youth with Down syndrome for either variable, suggesting that the relationship between aerobic capacity and obesity may be age-dependent in this population. Baynard et al. (2008) found significant differences in absolute and relative VO_2 between individuals with and without Down syndrome across ages, with the largest differences observed among children. The majority of studies on aerobic capacity have been conducted in adults with Down syndrome, leaving children and adolescents with Down syndrome as underrepresented sub-populations in the research literature. Given the effect of age on lower aerobic capacity and the high rates of pediatric obesity in Down syndrome, this relationship needs to be further examined.

While obesity may have a small effect on aerobic capacity (Wee et al., 2015), the primary causes of low aerobic capacity in Down syndrome are blunted catecholamine responses, altered autonomic responses, and chronotropic incompetence (Fernhall et al., 2009; Fernhall, Mendonca & Baynard, 2013; Mendonca, Pereira, et al., 2010b). Catecholamines (e.g. epinephrine, norepinephrine) in adults with Down syndrome appear to be less responsive to maximal and submaximal exercise compared to typically developing controls (Bricout et al., 2008; Fernhall et al., 2009). The lack of exercise response is critical as catecholamines drive heart rate response

above the anaerobic threshold (Fernhall et al., 2009). In addition to measuring catecholamines, Bricout et al. (2008) attempted to further explain reduced aerobic capacity in young adults with Down syndrome through a variety of hormonal responses to exercise. A novel finding from this study was that cortisol levels also differed in young adults with Down syndrome, including lower resting cortisol levels and a blunted response to submaximal exercise (Bricout et al., 2008). This may be an indication of dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis.

The HPA axis is responsible for regulating energy storage and energy expenditure (Adam & Epel, 2007; Bjorntorp, 2001). Cortisol, a glucocorticoid, is released from the adrenal gland (Adam & Epel, 2007; Bjorntorp, 1996, 2001; Bjorntorp & Rosmond, 2000; De Vriendt, Moreno & De Henauw, 2009) and responds directly to stressors. Cortisol is strongly associated with adiposity in the abdominal region, likely due to increased levels of glucocorticoid receptors in this type of tissue (Bjorntorp & Rosmond, 2000; De Vriendt et al., 2009; Rodriguez et al., 2015). Cortisol dysregulation in children and adolescents is associated with greater body mass index (BMI) or BMI z-score (Hill, Eisenmann, Gentile, Holmes & Walsh, 2011; Misra et al., 2008; Ondrak, McMurray, Hackney & Harrell, 2011; Reinehr et al., 2014; Rosmalen et al., 2005; Ruttle et al., 2013), waist circumference (Guzzetti, Pilia, Ibba & Loche, 2014; Hill et al., 2011; Reinehr et al., 2014), and abdominal fat mass (Barat et al., 2007; Misra et al., 2008; Weigensberg, Toledo-Corral & Goran, 2008). Similar relationships have consistently been shown in adults (Adam & Epel, 2007; Anagnostis, Athyros, Tziomalos, Karagiannis & Mikhailidis, 2009; Bjorntorp, 1996, 2001; De Vriendt et al., 2009; Foss & Dyrstad, 2011). The majority of this research shows that higher cortisol levels are associated with greater adiposity, however, other studies have shown the opposite direction of association (Ondrak et al., 2011; Ruttle et al., 2013).

Physical exercise, considered a positive stressor, can activate HPA axis activity, including cortisol release. During and after exercise, cortisol helps mediate physiological mechanisms that maximize exercise capacity and recovery. In adults, exercise of sufficient intensity and/or duration can significantly increase cortisol levels (Hackney, 2006; Hill et al., 2008; Jacks, Sowash, Anning, McGloughlin & Andres, 2002; Rudolph & McAuley, 1998; Viru & Viru, 2004). However, cortisol responses to exercise in youth are highly variable (Rubin, Tufano & McMurray, 2013). Multiple studies have shown significant increases in cortisol in children (Aucouturier et al., 2013; del Corral, Mahon, Duncan, Howe & Craig, 1994) and adolescents (Hackney et al., 2011; Viru, Laaneots, Karelson, Smirnova & Viru, 1998) in response to exercise; while others found no significant changes (Eliakim et al., 2006; Galassetti et al., 2006). Pubertal stage may be an important factor to explain the variability in cortisol response (Hackney et al., 2011; Viru et al., 1998). The cortisol response to exercise may also be affected by obesity in children. A recent study compared cortisol levels at rest and in response to exercise between lean and obese children (Aucouturier et al., 2013). Resting cortisol levels were similar between lean and obese children, but the cortisol response to exercise was significantly lower in obese children. Conversely, Eliakim et al. (2006) did not find a significant cortisol response to exercise in either lean or obese youth. Despite non-significance, the direction of change favored a cortisol increase among lean youth and a blunted cortisol response among obese youth.

As noted previously, evidence on cortisol function in Down syndrome is very limited. Previous studies in persons with Down syndrome have examined cortisol in single measure (Anneren, Sara, Hall & Tuvemo, 1986; Arnell, Gustafsson, Ivarsson & Anneren, 1996; Hestnes et al., 1991; Murdoch, Giles, Grant & Ratcliffe, 1979), during stress tests (Murdoch et al., 1979),

and during periods of rest and exercise (Bricout et al., 2008). Most studies have found cortisol levels to be within normal limits, but these are also limited by measuring at only a single time point (Anneren et al., 1986; Arnell et al., 1996; Hestnes et al., 1991; Murdoch et al., 1979). Bricout et al. (2008) reported that young adult males with Down syndrome exhibited lower cortisol levels at rest and significantly lower responses to physical exercise across multiple time points. Similarly stunted cortisol responses have been reported during stress tests in adults with Down syndrome (Murdoch et al., 1979). While Bricout et al. (2008) had differences in fat mass between subjects with and without Down syndrome, results comparing cortisol and adiposity were not examined.

Due to the association between cortisol function and fat mass, an examination of this hormone in adolescents with Down syndrome was warranted. It is currently unclear if the cortisol responses to exercise observed in adults Down syndrome are similar in adolescents or if this hormonal response is associated with adiposity within a population at risk for obesity. Adolescence is a developmental period of particular interest due to changes in adrenal function during puberty (Hackney et al., 2011; Viru et al., 1998), and unique growth trajectories in Down syndrome (Cronk et al., 1988; Van Gameren-Oosterom et al., 2012); both of these factors may influence the development of obesity. Exercise should promote energy substrate metabolism, but blunted cortisol responses coupled with the established limits in aerobic capacity may limit the potential for metabolic health benefits from physical activity among adolescents with Down syndrome.

The purpose of this study was to examine differences in cortisol concentration during periods of rest, a bout of moderate-intensity exercise, and recovery from exercise between adolescents with and without Down syndrome. Furthermore, the study sought to examine the

association between cortisol patterns and adiposity. Cortisol response in this study was examined within the context of the established limitations in aerobic capacity and heart rate response among individuals with Down syndrome.

Methods

Participants

All methods and procedures for the study were approved by the Institutional Review Board of the University of Michigan Medical School. All parents signed written informed consent documents while participants completed written or verbal assessment prior to initiating the study. Adult participants (e.g. 18 years old) were allowed to independently provide written informed consent, however parents of adult participants with Down syndrome also provided written consent. Participants were recruited through Down syndrome parent support groups in Michigan and northern Ohio. Typically developing adolescents were recruited through family referrals, local school districts, and from previous study samples. Inclusion criteria for participation included ages 12 to 18 years old and pubertal Tanner stages III-V (Marshall & Tanner, 1969, 1970). Exclusion criteria included: a) dual disability diagnosis (e.g. autism); b) comorbid disease (e.g. diabetes); c) contraindication limiting ability to safely perform physical exercise (e.g. cardiac insufficiency); d) use of medication that could alter metabolic functions (e.g. prednisone, central nervous systems stimulants, growth hormone, thyroid hormone); e) documented history of hormonal insufficiency (e.g. hypothyroid); and f) severe behaviors that would prevent safe performance of the exercise protocol. Distributions of sex, age, and Tanner pubertal stage were similar based on comparison of group means.

Clinical Visit

Data collection was completed during a single day for all participants. Dual-energy X-ray absorptiometry (DXA) scans were completed at the Michigan Clinical Research Unit within the University of Michigan Hospital. All other procedures including the exercise protocol were conducted in the Childhood Disparities Research Lab within the University of Michigan School of Public Health. All exercise trials took place in the morning (M = 10:52 AM, SD = 1 hour, 10 minutes). Most participants (65%) completed the DXA scan before starting the exercise trial (M = 10:17 AM, SD = 2 hours, 43 minutes). [*Note:* No differences were observed in cortisol levels at any point during the protocol between participants that completed the DXA scan before or after the exercise trial, $p > .20$]

Questionnaires

Parents completed a series of questionnaires. The accompanying parent was used as a proxy-reporter to ensure consistency between participants with and without Down syndrome.

Sociodemographic Survey. A basic survey was used to document the participant's age, race/ethnicity, current medication use, comorbidities, and relevant behaviors.

Pubertal Staging: Tanner pubertal staging was measured via parental report using line drawings (Morris & Udry, 1980). Parental reports can provide an acceptable and reliable report of pubertal stage (Brooks-Gunn, Warren, Rosso & Gargiulo, 1987; Coleman & Coleman, 2002; Dorn, Susman, Nottelmann, Inoffgermain & Chrousos, 1990; Petersen, Crockett & Richards, 1988; Shirtcliff, Dahl & Pollak, 2009), and are less invasive and stressful than a physical assessment of Tanner stage (Marshall & Tanner, 1969, 1970). Stress directly influences cortisol levels (Bjorntorp, 2001; Chrousos & Gold, 1992; De Vriendt et al., 2009; Tsigos & Chrousos, 2002), which could skew study outcomes. Participants were limited to adolescents in Tanner

stages III to V, operationalized as the average of two reported stages for each participant (i.e.

breast and pubic hair development in females or genital and pubic hair development in males).

Anthropometry

All anthropometric measurements were conducted according to guidelines from Lohman

(Lohman, Roche & Martorell, 1988). Height and weight were measured to the nearest 0.1

centimeter (SECA S-214 stadiometer) and nearest 0.01 kilogram (Health O Meter H-349KL

digital scale), respectively. Each measurement was taken in duplicate; if measures differed by

greater than 0.5 cm or 0.5 kg, respectively, a third trial was taken. Data reported are the average

of all trials. BMI (kg/m^2) and BMI percentile from the CDC growth reference (Kuczmarski et al.,

2000) were then calculated to classify body composition into overweight and obese categories.

Dual-Energy X-Ray Absorptiometry

Each participant completed one DXA scan (GE Lunar Prodigy Advance [DPX-IQ 240]

densitometer; Lunar Radiation Corp., Madison, WI) at the MCRU. DXA scans with the Lunar

Prodigy are highly correlated with advanced imaging technologies (e.g. computed tomography

and magnetic resonance imaging), reliable in pediatric populations, and recommended for use in

epidemiological obesity research (Kendler et al., 2013; Margulies et al., 2005). DXA scans

involve a low radiation dose (0.37 μSv), equivalent to the radiation absorbed during a typical day

(Albanese, Diessel & Genant, 2003); this radiation dose is considered medically negligible.

All participants in the current study completed a valid scan. The Lunar Prodigy was

calibrated daily according to manufacturer instructions using a standardized block. Participants

wore light clothing and were positioned in a supine position with hands by the sides in a neutral

position. Pediatric DXA software (EnCore v.14.10) calculated body fat mass and total body fat

percentage. Obesity was classified based on age- and sex-specific cut-points for elevated body fat percentage (Freedman et al., 2009).

Exercise Protocol

Figure 1 depicts the protocol each participant completed. Participants began by sitting (T_0) for a period of 20 minutes to allow heart rate, metabolic rate, breathing rate, and cortisol to reach resting levels (T_1). Following the rest period, each participant completed a graded submaximal bout of exercise culminating at 70% predicted maximal HR (HR_{max}) by walking on a motorized treadmill. The protocol was modified from VO_{2peak} testing protocols designed for adults with Down syndrome (Fernhall et al., 2001; Fernhall, Millar, Tymeson & Burkett, 1990; Mendonca, Pereira & Fernhall, 2009, 2011) and children with intellectual disabilities (Vashdi, Hutzler & Roth, 2008). The exercise bout consisted of two 10 minute phases, totaling 20 minutes of exercise. Phase 1 included incremental graded walking to gradually increase heart rate. Participants began at an easy walking speed (1.5 mph, 0% grade) for acclimation. After 2 minutes, the treadmill speed was increased to 2.5 mph. The incline of the treadmill was then increased by 2.5% every 2 minutes until the target heart rate ranged was reached. If necessary, speed was incrementally increased (0.6 mph/increase) once the treadmill was at 9% grade, until reaching the target heart rate. Phase 2 was intended to be 10 minutes of continuous steady-state exercise within the target heart rate range ($70 \pm 5\%$) of predicted HR_{max}. Equations specific to persons with intellectual disabilities (Fernhall et al., 2001) were used for adolescents with Down syndrome (**Equation 3.1**) while traditional age-predicted HR_{max} equations were used for typically developing adolescents (**Equation 3.2**). Following the exercise segment (T_2), the participant was monitored during seated resting recovery for a total of 40 minutes in two 20-minute periods (T_3 and T_4).

Participants were familiarized and oriented to treadmill exercise prior to data collection. Familiarization procedures allowed each participant to practice walking on the treadmill and breathing with the facemask. This practice occurred on the same day as the data collection; usually prior to the DXA scan appointment. Numerous researchers using treadmill protocols in persons with intellectual disabilities have cited familiarization as an important aspect of the measurement protocol (Balic, Mateos, Blasco & Fernhall, 2000; Fernhall & Tymeson, 1987; Pitetti, Rimmer & Fernhall, 1993; Vashdi et al., 2008). Participants were encouraged throughout the exercise and provided positive reinforcement to increase compliance (Vashdi et al., 2008).

Exercise Measurement

Saliva Sampling. Saliva sampling was conducted with an oral swab (Salimetrics, State College, PA). Participants placed the swab underneath the tongue to absorb saliva. Each sample took approximately 3 minutes to complete and produced approximately 1 mL of saliva for analysis. Samples will be taken at four time points (T_1, T_2, T_3, T_4) during the protocol. Each saliva sample was stored in an individually numbered polypropylene vial and frozen (-20 °C) before extraction and analysis. Samples were analyzed in duplicate for levels of cortisol concentration (µg/dL), using enzyme-linked immunosorbent assay (ELISA) techniques with the Expanded High Range Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics, State College, PA). The assay has a lower limit of sensitivity (minimum detectable concentration) of 0.007 µg/dL (Shirtcliff, Granger, Schwartz & Curran, 2001). Inter-assay and intra-assay coefficients of variation (%CV) were 5.62% and 5.27%, consistent with recommendations (Reed, Lynn & Meade, 2002).

Cortisol concentration from saliva samples was operationalized using two techniques. First, group means and standard deviations were calculated across each cortisol measurement

(T_0, T_2, T_3, T_4) for analyses of group differences. Second, trapezoidal area under the curve (AUC) controlling for time between measurements was used to capture cortisol output during the protocol. AUC equations were modified from Pruessner et al. (2003) to match the parameters of the present study (**Equations 3.3 and 3.4**). AUC_g represents the total cortisol output during a given period. AUC_Δ represents the change in cortisol output during a given period, accounting for initial cortisol concentration. AUC was calculated for the full protocol (T_1:T_4), the exercise response (T_1:T_2), and the recovery response (T_2:T_4).

Indirect calorimetry. Gas exchange was measured breath-by-breath continuously throughout the laboratory protocol (T_0:T_4), except when completing saliva samples, using the Oxycon mobile ergospirometry system (Yorba Linda, CA). Participants wore a flexible silicone facemask (covering the mouth and nose) and the lightweight Oxycon system attached to their back with a harness. Before each test, the Oxycon was calibrated for flow control and gas calibration (4% CO_2 and 16% O_2) using automated calibration functions according the manufacturer's instructions. The Oxycon system has been shown to provide valid and reliable measures of respiratory gas exchange in comparison to traditional methodology and across a variety of settings and exercise intensities (Hannink et al., 2010; Rosdahl, Gullstrand, Salier-Eriksson, Johansson & Schantz, 2010; Salier-Eriksson, Rosdahl & Schantz, 2012). Respiratory gas exchange values from final five minutes of each segment were used for analyses (T_1, T_2, T_3, T_4). Physiological parameters included absolute gas exchange (oxygen consumption, VO_2; carbon dioxide production, VCO_2; mL·min^{-1}), relative gas exchange (VO_2, VCO_2; mL·kg^{-1}·min^{-1}), respiratory exchange ratio (RER; VCO_2/VO_2), and minute ventilation (VE; L·min^{-1}), calculated in 1-minute averages. Heart rate was measured continuously throughout the laboratory protocol (T_0:T_4) using a Polar Vantage (Polar Electro, Woodbury, NY) heart rate monitor. Heart

rate (bpm) was calculated in 1-minute averages and presented as the percentage of predicted HR_{max}. Predicted VO_2 was also calculated (**Equations 3.5 and 3.6**) based on the speed, grade and exercise mode (walking or running) during the final five minutes of the exercise bout (American College of Sports Medicine, 2013) and compared with measured VO_2 during the same phase to calculate cardiorespiratory efficiency (**Equation 3.7**). Work rate (watts) was also calculated based on the participant weight, treadmill speed, and treadmill slope (**Equation 3.8**).

Statistical Analyses

Data analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY) with an a priori α of 0.05. Demographics of the sample were described using descriptive statistics, independent t-tests, and Pearson's Chi-square (X^2) tests.

A two-way (group (2) x time (4)) repeated measures analysis of covariance (RMANCOVA) was employed to examine differences in cortisol and other physiological measures between groups with Down syndrome and typical development across the four time points of the protocol. Planned comparisons were conducted with Bonferroni corrections. Comparisons examined group differences at each time point, differences between each time point and baseline, and between each time point and the preceding time point. Cohen's *d* effect size was also calculated for unadjusted group differences. A follow-up two-way (group (4) x time (4)) RMANCOVA were then conducted to examine an interaction between disability groups and elevated body fatness. All analyses controlled for age, sex, and Tanner pubertal stage. Results are reported as estimated marginal means and standard errors.

Results

A total of 26 participants (12 with Down syndrome, 14 with typical development) completed the exercise protocol. All participants (100%) who began the session were able to

successfully complete the 80 minute protocol including 20 minutes of sustained exercise.

Descriptive statistics and demographic information for the participants are presented in **Table 3.1.** No differences were observed between groups on age, sex, or Tanner stage ($p > .70$). Adolescents with Down syndrome were significantly shorter in stature than adolescents with typical development ($p < .001$); but were similar in weight ($p = .682$). All indices of body composition show that the adolescents with Down syndrome were significantly more overweight than typically developing peers ($p < .05$). DXA scan data identified an elevated body fat percentage in 67% ($n = 8$) of adolescents with Down syndrome and 21% ($n = 3$) of adolescents with typical development.

The exercise protocol was designed to reach and maintain 70% ($\pm 5\%$) of predicted HR_{max} during the second 10 minutes of the bout. Due to the differences in heart rate response to exercise between groups, absolute exercise levels were higher among adolescents with typical development. **Figure 3.2** shows the differences in work rate during the exercise bout. Both groups reached the desired HR_{max} range during the exercise. Adolescents with Down syndrome averaged 76.3% of predicted HR_{max} while typically developing adolescents averaged 73.9% of predicted HR_{max} during the final five minutes of exercise. The typically developing group exercised at a faster walking speed (3.81 mph) and with greater incline (8.54% grade) than the group with Down syndrome (2.26 mph, 3.97% grade; $p < .001$). This resulted in significant differences in work rate (watts, W) during the exercise trial. The ability to describe work rate, however, is limited in the group with Down syndrome as four participants completed the exercise phase without any incline. This resulted in an estimated work rate of 0 W. To account for this issue, results are presented with the full estimated work rate ($n = 12$) and with 0 W treated as missing data ($n = 8$). Adolescents with typical development exercised at a significantly

higher work-rate (83.3 W) than adolescents with Down syndrome (22.5 W and 33.8 W) regardless of descriptive statistics approach ($p < .001$). Despite matching groups on relative exercise intensity (% of predicted HR_{max}), there were large differences in the absolute workload that could affect cardiometabolic and physiological responses to the exercise bout.

Cardiopulmonary data across the four stages of the protocol are shown in **Table 3.2**. Systematic errors in the measurement of VCO_2 resulted in unusable data; thus neither VCO_2 nor RER (a function of the relationship between VO_2 and VCO_2) could be interpreted (data not shown). A significant multivariate effect of disability group by time was observed, $F(24, 145.62) = 17.53$, $p < .001$, Wilk's $\lambda = 0.02$. Significant univariate effects of the two-way disability by time interaction included relative heart rate ($p = .05$), absolute heart rate ($p < .001$), absolute VO_2 ($p < .001$), relative VO_2 ($p < .001$), and ventilatory exchange ($p = .01$). The analysis was repeated to examine the three-way interaction of disability and body fatness groupings across time (**Table 3.3**). A significant multivariate effect of disability group by fatness by time was also observed, $F(24, 145.62) = 2.39$, $p < .001$, Wilk's $\lambda = 0.38$, but only relative VO_2 ($p = .01$) and ventilatory exchange ($p = .03$) had a significant univariate effects. All analyses account for sphericity through Greenhouse-Geisser corrections ($\varepsilon < 0.66$).

To better understand the differences in cardiopulmonary variables, most notably VO_2, cardiorespiratory efficiency during the exercise phase was examined. **Figure 3.3** shows the differences in estimated relative VO_2 (given the speed, incline, and exercise mode during the final five minutes of exercise), actual relative VO_2 (during the final five minutes of the exercise), and efficiency (estimated/actual VO_2). Calculated efficiency was significantly lower among adolescents with Down syndrome (71.8%) compared to typically developing adolescents

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(108.6%). This suggests that adolescents with Down syndrome had measured energy expenditure values that were almost 30% greater than expected for a given work rate.

Figures 3.4 through 3.8 show the cardiopulmonary data across the protocol to examine differences between adolescents with and without Down syndrome (left side of figures) and differences across disability and elevated fatness groupings (right side of figures). Significant group differences between adolescents with Down syndrome and typical development were observed across all time points for relative heart rate ($p < .03$) and during the exercise bout for absolute heart rate ($p < .001$), absolute VO_2 ($p < .001$), relative VO_2 ($p = .001$), and ventilatory exchange ($p = .001$). No differences were observed at any time points in adolescents with Down syndrome between elevated and normal body fatness ($p > .05$). However, differences by body fat groupings in typically developing adolescents were significant during exercise for relative VO_2 ($p = .002$) and ventilatory exchange ($p = .03$). Lastly, significant differences between adolescents with and without Down syndrome within a body fatness group were also observed during exercise for absolute heart rate ($p < .001$), absolute VO2 ($p < .001$), relative VO2 ($p = .001$), and ventilatory exchange ($p < .01$) and across all three resting phases (T_1, T_3, T_4) for relative heart rate ($p < .03$).

As expected, a significantly lower heart rate response ($p < .001$) and aerobic response ($p < .001$) were observed during exercise for adolescents with Down syndrome compared to typically developing adolescents. However, a novel finding is that relative VO_2 (controlling for body mass) was not different between typically developing adolescents with elevated body fat and adolescents with Down syndrome ($p > .60$).

Finally, cortisol concentration across the protocol is presented in **Table 3.4** and **Figure 3.9**. There were no significant univariate effects on cortisol for two-way disability by time

interaction, $F(1.77) = 0.77$, $p = .455$, nor the three-way interaction of disability and body fatness groupings across time, $F(1.82) = 1.63$, $p = .213$. No significant differences were observed in planned comparisons between any possible groupings nor between time points ($p < .05$). Due to small sample size, Cohen's d examined group differences independent of sample size. Large effect sizes were observed at baseline for cortisol differences by body fatness within typically developing adolescents ($d = 1.03$) and within adolescents with Down syndrome ($d = 1.09$), as well as differences between Down syndrome and typical development among adolescents with elevated body fatness ($d = 0.88$). During exercise, large effect sizes were once again observed between body fatness groupings within typical development ($d = 0.87$) and Down syndrome ($d = 1.01$). During the first recovery phase, a large effect size was only observed between body fatness groupings among adolescents with Down syndrome ($d = 0.82$). No substantial effect sizes were observed during the second recovery phase. These effect sizes that suggest that while significant differences cannot be determined due to lack of statistical power, there may still be meaningful differences in cortisol concentration between groups.

Area under the curve was also examined for total cortisol output and change in output across time points (**Table 3.5**). Significant differences in the AUC_Δ during the recovery phase (T_2:T_4) were observed between disability groups ($p = .048$) and between body fatness groupings within typically developing adolescents ($p = .049$). AUC_g during the exercise phase (T_1:T_2) also trended toward significant between body fat groupings within typically developing adolescents ($p = .085$). Despite the limited number of significant differences, multiple large effect sizes were observed in both AUC_Δ and AUC_g.

Discussion

This study sought to elicit a cortisol response through a bout of moderate-intensity exercise and compare differences in this response between adolescents with and without Down syndrome and with and without elevated fatness. No changes in salivary cortisol were observed across any of the phases of the protocol. However, there were multiple differences in cortisol concentration between adolescents with high and normal body fatness as observed through large effect sizes. As expected, there were also pervasive differences in the heart rate and aerobic responses in adolescents with Down syndrome compared to peers with typical development during the same relative exercise intensity.

Cortisol levels were unresponsive to the protocol in both groups. The measurement of salivary cortisol instead of blood (plasma or serum) or urine cortisol may be partially responsible for this lack of response. While cortisol levels in saliva can respond quickly to a stimulus (e.g. exercise, stress), there may also be a delay in peak cortisol response. Thus, it is possible that the anticipated responses occurred in between the four measurement time points. However, the consistently flat nature of cortisol concentration over the protocol and low levels of variance between measurements would suggest this is unlikely. While a blunted cortisol response in the Down syndrome group is consistent with previous studies (Bricout et al., 2008), the lack of response among typically developing adolescents make any group comparisons difficult to interpret.

Bricout et al. (2008) found significantly lower cortisol levels across a 2.5-hour period of rest and a blunted response to exercise in young adult males with Down syndrome compared to controls. No differences were observed between time points of either the rest or exercise protocols among these young adults with Down syndrome, while significant changes occurred in

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the control group. At each time point presented, young adults with Down syndrome exhibited lower cortisol levels than controls. The current study is consistent with these findings as no changes in cortisol were observed in adolescents with Down syndrome between rest, exercise, and extended recovery. We also found that adolescents with Down syndrome exhibited lower levels of cortisol across the protocol; however this difference was neither statistically significant nor of a large effect size. The levels of salivary cortisol observed in the current study of adolescents with Down syndrome reflect very similar blood cortisol levels to those previously reported in young adults with Down syndrome (Bricout et al., 2008). This would further support the initial hypothesis that cortisol response to exercise would be blunted in adolescents with Down syndrome.

However, as noted previously, the control group did not respond as expected which limits the inferences that can be made from the study results. Exercise in adults consistently induces an increase in cortisol, provided the exercise bout is performed at a sufficient intensity and duration (Hackney, 2006; Hill et al., 2008; Jacks et al., 2002; Rudolph & McAuley, 1998; Viru & Viru, 2004). The increase in cortisol observed among the Bricout et al. (2008) control group during 40 minutes of progressive exercise culminating at 75% of VO_{2max} was representative of the expected response. Cortisol reactivity in adolescents is highly variable as both significant increases in cortisol (Hackney et al., 2011; Viru et al., 1998) and no changes (Eliakim et al., 2006; Galassetti et al., 2006) have been reported in response to exercise. Pubertal stage may be an important factor to explain the variability in cortisol response (Hackney et al., 2011; Viru et al., 1998). Thus, the lack of response may be due to having more typically developing adolescents at Tanner stage III than were included in studies that found significant changes. It is also possible that progressive exercise for only 20 minutes culminating at 70% of predicted

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HR$_{max}$ in the current study was not of sufficient intensity or duration to elicit the expected response.

A novel finding of this study was that differences began to emerge when groups were further divided into normal and elevated fatness groupings based on percent body fat (Freedman et al., 2009). The difference in total cortisol output across the protocol among adolescents with Down syndrome was substantially higher with elevated fatness compared to normal fatness. These differences were observed through large effect sizes, but did not reach statistical significance. The magnitude of differences in total cortisol output across the protocol was not as large between typically developing adolescents with and without elevated fatness; however, the difference in incremental cortisol output during recovery was significantly different in those with elevated body fat levels ($p = .049$). Typically developing adolescents with elevated fatness also had higher cortisol output during the exercise phase that trended toward statistical significance ($p = .085$). The cortisol patterns represented in **Figure 3.9** also show that within each disability group, adolescents with elevated fat mass had higher levels of cortisol production throughout the protocol.

These findings are consistent with much of the research literature that has found positive associations between cortisol levels and adiposity (Barat et al., 2007; Guzzetti et al., 2014; Hill et al., 2011; Misra et al., 2008; Reinehr et al., 2014; Rosmalen et al., 2005; Weigensberg et al., 2008). Within exercise studies, Eliakim et al. (2006) did not find a difference in cortisol level between normal and obese children and adolescents during pre-exercise, peak exercise, or 120-minute post-exercise measurements. Aucouturier et al. (2013) found a significantly blunted cortisol response to exercise in obese pre-pubescent children compared to lean controls, but cortisol levels outside of the exercise bout were similar. Given that no exercise response was

observed in the current study, it is difficult to infer whether differences in overall cortisol between groupings of fatness are related to exercise in any way.

Despite the lack of a true cortisol response to exercise, the protocol was effective in producing the desired cardiovascular exercise response. Lower heart rate response and aerobic capacity to exercise in Down syndrome have been well described in the literature (Fernhall et al., 2013; Fernhall & Pitetti, 2001; Mendonca, Pereira, et al., 2010b). To account for these differences, a relative exercise intensity (70% predicted $HR_{max} \pm 5\%$) was used to standardize the protocol between groups. Each group averaged approximately 75% of their respective HR_{max} during the final five minutes of the exercise bout as intended. During exercise at the same relative intensity, adolescents with Down syndrome had significantly lower absolute heart rates and significantly lower absolute and relative (normalized to body mass) VO_2. Efficiency during the final phase of exercise was also significantly lower among adolescents with Down syndrome.

The heart rate and aerobic responses observed are consistent with the exercise physiology literature on adults with Down syndrome (Baynard et al., 2008; Fernhall et al., 2001; Guerra et al., 2003; Mendonca, Pereira, et al., 2010a; Wee et al., 2015). Previous studies have also consistently shown an increased energetic cost and lower economy during exercise in adults with Down syndrome (Agiovlasitis, McCubbin, Yun, Pavol & Widrick, 2009; Agiovlasitis, Motl, et al., 2011a, 2011b; Mendonca, Pereira, Morato, et al., 2010). These differences have clinical relevance as they directly impact physical fitness and likely contribute to decreased motivation to participate in physical activity. Furthermore, the altered metabolic rate has implications for the validity of a variety of physical activity measurement tools used in persons with Down syndrome, including pedometers (Agiovlasitis, Beets, Motl & Fernhall, 2012), accelerometers

(Agiovlasitis, Motl, Fahs, et al., 2011; Agiovlasitis, Motl, Foley & Fernhall, 2012), and heart rate index (Agiovlasitis et al., 2014).

The present study has a number of limitations that warrant serious consideration when interpreting these findings. First, the current sample is small in size, thus limiting statistical power in analyses. Many comparisons did not have statistically significant differences, yet very large effect sizes were observed. Repeating this study with a sample size consistent with statistical power is warranted, but would require substantial effort. For example, a power analysis on the difference in cortisol response during exercise between adolescents with Down syndrome with and without elevated fatness ($d = 1.07$) would have adequate statistical power (1-β = 80%, α = 5%) with 21 participants; 9 more participants with Down syndrome than are currently in the study. Second, recruiting for this study was very difficult due to timing required for the DXA scan and the inclusion of the treadmill exercise. Thus, the convenience sample of participants may not represent adolescents with Down syndrome. Third, the procedures and study design may have limited the ability to detect a cortisol response for exercise. While many studies use saliva sampling for cortisol measurement, most of the exercise-based studies discussed used blood samples. It is also possible that the employed exercise bout was not intense or long enough to produce the desired cortisol response. However, the heart rate and metabolic data do reflect that both participants with and without Down syndrome engaged in moderate-intensity exercise. Fourth, the measurement error regarding VCO_2 is concerning. While the intention was always to focus on aerobic output through VO_2 measurement, the lack of valid VCO_2 meant that a valid RER could not be calculated. RER would have provided another index of exercise intensity as well as allowed for inferences regarding substrate utilization during the exercise bout. Although

the VO_2 data appear to be appropriate both at rest and during exercise, these measurements from the Oxycon device must also be questioned in light of the errors in VCO_2 measurement.

Despite these limitations, this is the first study to attempt to elicit a cortisol response in adolescents with Down syndrome through exercise. The study is strengthened by the use of DXA to measure adiposity and classify groups. The finding of differences, as observed through effect size, based on having elevated or normal fatness is novel. Differences in the hormonal and aerobic responses to exercise could be related to the development of fat mass and high prevalence of obesity in this population. It is our hope that these findings, while limited, may encourage future investigations into the effect of cortisol on obesity in Down syndrome.

Equations

Equation 3.1. HR_{maxDS}: predicted maximal heart rate for DS (Fernhall et al., 2001)

$$HR_{maxDS} = 210 - 0.56(age) - 15.5(DS)$$

Equation 3.2. HR_{maxTD}: traditional predicted maximal heart rate

$$HR_{maxDS} = 220 - (age)$$

Equation 3.3. AUC_g: Area under the curve with respect to ground (total cortisol output)

$$AUC_g = \sum_{i=1}^{n-1} \left(\frac{(m_{(i+1)} + m_i)t_i}{2} \right)$$

Equation 3.4. AUC_Δ: Area under the curve with respect to increase (change within phase)

$$AUC_\Delta = \sum_{i=1}^{n-1} \left(\frac{(m_{(i+1)} + m_i)}{2} \right) - m_1(n-1)$$

Notation: time (t), measurements (m), total number of measurements (n)

Both AUC equations are adapted from Pruessner et al. (2003)

Equation 3.5. Estimated VO_2 for walking $(mL \bullet kg^{-1} \bullet min^{-1})$

$$VO_2 = [0.1 \text{ x speed } (m \bullet min^{-1})] + [1.8 \text{ x speed } (m \bullet min^{-1}) \text{ x grade}] + 3.5$$

Equation 3.6. Estimated VO_2 for running $(mL \bullet kg^{-1} \bullet min^{-1})$

$$VO_2 = [0.2 \text{ x speed } (m \bullet min^{-1})] + [0.9 \text{ x speed } (m \bullet min^{-1}) \text{ x grade}] + 3.5$$

Both estimated VO_2 equations are from ACSM (2013)

Equation 3.7. Efficiency (%)

$$\textbf{Efficiency = Estimated } VO_2 \textbf{ / Measured } VO_2 \textbf{ x 100}$$

Equation 3.8. Work rate (watts, W)

$$W = \text{body mass (kg) x 9.8 x sin[arctan(grade)] x speed } (m \bullet sec^{-1})$$

Table Captions

Table 3.1.   Characteristics of the sample

Table 3.2.   Cardiopulmonary outcomes across the protocol by group

Table 3.3.   Cardiopulmonary outcomes across the protocol by group and elevated body
                fatness

Table 3.4.   Cortisol concentration across the protocol by group and elevated body fatness

Table 3.5.   Group differences in cortisol area under the curve

Figure Captions

Figure 3.1.   Exercise protocol

Figure 3.2.   Work load of exercise trial

Figure 3.3.   Efficiency during exercise trial

Figure 3.4.   Changes in relative heart rate across protocol

Figure 3.5.   Changes in absolute heart rate across protocol

Figure 3.6.   Changes in absolute oxygen consumption across protocol

Figure 3.7.   Changes in relative oxygen consumption across protocol

Figure 3.8.   Changes in ventilatory exchange across protocol

Figure 3.9.   Change in cortisol across protocol

Table 3.1.   Characteristics of the sample

Demographic characteristic	Typically developing (n=14)	Down syndrome (n=12)	p
Female, n(%)	7 (50.0%)	6 (50.0%)	1.00 [a]
Age (years)	15.47 (1.78)	15.24 (2.01)	.764 [b]
Tanner (III-V)	3.96 (0.82)	3.92 (0.79)	.882 [b]
Caucasian, n(%)	14 (100%)	11 (91.7%)	.271 [a]
Height	167.01 (9.68)	147.86 (8.43)	**<.001** [b]*
Weight	60.27 (15.27)	58.15 (9.61)	.682 [b]
BMI	21.39 (3.88)	26.55 (3.50)	**.002** [b]*
CDC-BMI %ile	55.90 (26.18)	88.50 (12.73)	**.001** [b]*
DS-BMI %ile	68.12 (18.94)	--	**--**
Percent Body Fat	25.81% (7.57%)	32.62% (2.61%)	**.047** [b]*
Elevated Body Fat, n(%)	3 (21.43%)	8 (66.67%)	**.020** [a]*

Note: Values are Mean (Standard Deviation) unless otherwise noted; Abbreviations: n = frequency, % = proportion of sample, BMI = body mass index (kg/m^2), CDC-BMI = Centers for Disease Control and Prevention growth chart (Kuczmarski et al., 2000), DS-BMI = Down syndrome specific growth chart (Zemel et al., 2015); %ile = BMI percentile.
[a] Pearson's chi-square test (X^2)
[b] Independent samples t-test (t)
* $p < .05$, **bolded**

Table 3.2.   Cardiopulmonary outcomes across the protocol by group

Variables	T1 Baseline		T2 Exercise		T3 Recovery A		T4 Recovery B	
	TD	DS	TD	DS	TD	DS	TD	DS
Relative Heart Rate	34.50	43.10 *	73.90 †‡	76.30 *†‡	39.80 †‡	47.30 *†‡	38.30 †	46.60 *†
($\%HR_{max}$)	(1.46)	(1.58)	(0.68)	(0.74)	(1.38)	(1.49)	(1.53)	(1.65)
Absolute Heart Rate	70.62	73.42	151.16 †‡	129.96 *†‡	81.36 †‡	80.54 †‡	78.44 †‡	79.39 †
(bpm)	(2.85)	(3.07)	(1.30)	(1.41)	(2.61)	(2.82)	(2.86)	(3.09)
Absolute VO_2	253.37	245.20	1653.82 †‡	1111.36 *†‡	270.50 ‡	268.92 ‡	271.60	270.49
($mL{\bullet}min^{-1}$)	(20.65)	(22.31)	(73.60)	(79.51)	(14.16)	(15.29)	(14.54)	(16.71)
Relative VO_2	4.36	4.20	28.16 †‡	18.65 *†‡	4.65 ‡	4.63 ‡	4.66	4.62
($mL{\bullet}kg^{-1}{\bullet}min^{-1}$)	(0.24)	(0.29)	(1.34)	(1.60)	(0.22)	(0.26)	(0.25)	(0.29)
Ventilatory Exchange	8.26	7.64	42.96 †‡	29.73 *†‡	8.83 ‡	8.14 ‡	8.58	7.93
($L{\bullet}min^{-1}$)	(0.63)	(0.68)	(2.25)	(2.43)	(0.46)	(0.50)	(0.49)	(0.53)

Estimated Marginal Means (Standard Error) controlling for sex, age, and Tanner stage.
Abbreviations: TD = typically developing, DS = Down syndrome; $\%HR_{max}$ = percent of predicted maximal heart rate; VO_2 = oxygen consumption.
Pairwise comparisons with Bonferroni adjustments examined group differences.
* Differences between TD and DS, $p < .05$
† Differences between Time 1 (baseline) and each time point, $p < .05$
‡ Differences between each time point and the preceding time point ($t_i - 1$), $p < .05$

Table 3.3.   Cardiopulmonary outcomes across the protocol by group and elevated body fatness

Variables	T1 Baseline		T2 Exercise		T3 Recovery A		T4 Recovery B	
Typically Developing	Normal BF	Elevated BF	Normal BF	Elevated BF	Normal BF	Elevated BF	Normal BF	Elevated BF
Relative Heart Rate	32.80	41.00 *	73.50 †‡	75.30 †‡	39.00 †‡	42.00 ‡	37.30 †‡	42.00
($\%HR_{max}$)	(1.50)	(2.90)	(0.80)	(1.50)	(1.60)	(3.10)	(1.70)	(3.40)
Absolute Heart Rate	67.04	83.75 *	150.41 †‡	153.91 †‡	79.91 †‡	86.69 ‡	76.43 †‡	85.82
(bpm)	(2.88)	(5.56)	(1.50)	(2.90)	(3.01)	(5.82)	(3.25)	(6.29)
Absolute VO_2	231.03	335.49 *	1680.35 †‡	1555.49 †‡	260.83 ‡	306.03 ‡	261.15	310.38
($mL•min^{-1}$)	(21.96)	(42.42)	(86.27)	(166.59)	(16.15)	(31.18)	(16.23)	(31.34)
Relative VO_2	4.37	4.30	30.05 †‡	19.73 *†‡	4.80 ‡	4.03 ‡	4.80	4.03
($mL•kg^{-1}•min^{-1}$)	(0.27)	(0.59)	(1.25)	(2.71)	(0.24)	(0.52)	(0.26)	(0.56)
Ventilatory	7.96	9.38	45.51 †‡	33.60 *†‡	8.74 ‡	9.15 ‡	8.52	8.84
Exchange ($L•min^{-1}$)	(0.74)	(01.42)	(2.36)	(4.55)	(0.55)	(1.06)	(0.56)	(1.08)
Down syndrome								
Relative Heart Rate	42.00 #	43.50	76.3 †‡	76.30 †‡	46.40 #‡	47.70 †‡	46.20 #	46.80
($\%HR_{max}$)	(2.50)	(1.80)	(1.30)	(0.90)	(2.70)	(1.90)	(2.90)	(2.10)
Absolute Heart Rate	71.86	74.20	130.22 #†‡	129.82 #†‡	79.32 ‡	81.16 †‡	78.91	79.63
(bpm)	(4.52)	(3.40)	(2.51)	(1.78)	(5.05)	(3.57)	(5.45)	(3.85)
Absolute VO_2	247.43	244.00	1070.26 †‡	1132.31 #†‡	268.20 ‡	269.25 ‡	288.78	261.17
($mL•min^{-1}$)	(36.76)	(25.98)	(144.40)	(102.05)	(27.03)	(19.10)	(27.16)	(19.20)
Relative VO_2	4.67	3.97	19.79 #†‡	17.93 †‡	5.02 ‡	4.42 ‡	5.37	4.24
($mL•kg^{-1}•min^{-1}$)	(0.51)	(0.36)	(2.34)	(1.64)	(0.44)	(0.31)	(0.48)	(0.34)
Ventilatory	7.98	7.47	29.00 #†‡	30.11 †‡	8.52 ‡	7.95 ‡	8.89	7.45
Exchange ($L•min^{-1}$)	(1.23)	(0.87)	(3.95)	(2.79)	(0.92)	(0.65)	(0.94)	(0.66)

Estimated Marginal Means (Standard Error) controlling for sex, age, and Tanner stage. Pairwise comparisons with Bonferroni adjustments examined group differences.

Abbreviations: $\%HR_{max}$ = percent of predicted maximal heart rate; VO_2 = oxygen consumption.

* Within disability grouping, differences between normal and elevated fatness, $p < .05$

Within body fatness grouping, differences between TD and DS, $p < .05$

† Differences between Time 1 (baseline) and each time point, $p < .05$

‡ Differences between each time point and the preceding time point ($t_i - 1$), $p < .05$

Table 3.4. Cortisol concentration across the protocol by group and elevated body fatness

Cortisol (µg/dL)	T1 Baseline	T2 Exercise	T3 Recovery A	T4 Recovery B
Typically Developing	0.22 (0.03)	0.21 (0.03)	0.20 (0.03)	0.20 (0.03)
Typically Developing – Normal BF	0.19 (0.03)	0.18 (0.04)	0.19 (0.03)	0.19 (0.04)
Typically Developing – Elevated BF	0.32 (0.06)	0.30 (0.07)	0.22 (0.06)	0.23 (0.07)
Down syndrome	0.18 (0.03)	0.20 (0.04)	0.20 (0.03)	0.18 (0.04)
Down syndrome – Normal BF	0.13 (0.05)	0.14 (0.06)	0.14 (0.05)	0.14 (0.06)
Down syndrome – Elevated BF	0.21 (0.04)	0.23 (0.04)	0.23 (0.04)	0.20 (0.04)
Effect size (Cohen's *d*)				
Between disability groups	.351	.077	.057	.176
Within TD, difference by BF	**1.03**	**.875**	.385	.370
Within DS, difference by BF	**1.09**	**1.01**	**.824**	.536
Within NBF, difference by DS	.651	.461	.391	.352
Within EBF, difference by DS	**.882**	.435	.035	.314

Estimated Marginal Means (Standard Error) controlling for sex, age, and Tanner stage.
Abbreviations: BF = body fatness, TD = typically developing, DS = Down syndrome, NBF = normal body fatness, EBF = elevated body fatness
Pairwise comparisons with Bonferroni adjustments and Cohen's *d* effect size examined group differences.
* $p < .05$ (no observations)
d > .80, bolded

Table 3.5.   Group differences in cortisol area under the curve

	AUC_g T1:T4 Full	AUC_g T1:T2 Exercise	AUC_g T2:T4 Recover	AUC_Δ T1:T4 Full	AUC_Δ T1:T2 Exercise	AUC_Δ T2:T4 Recover
Group Differences						
Typically Developing	15.16 (2.21)	6.64 (0.88)	8.52 (1.41)	-1.84 (1.23)	-0.21 (0.29)	0.47 (0.23)
Down syndrome	12.08 (2.07)	4.76 (0.82)	7.31 (1.32)	0.71 (1.16)	0.18 (0.28)	-0.19 (0.21)
Typically Developing – Normal BF	12.47 (2.04)	5.04 (0.81)	7.43 (1.30)	-0.27 (1.40)	-0.15 (0.27)	-0.01 (0.21)
Typically Developing – Elevated BF	17.86 (3.94)	8.25 (1.56)	9.61 (2.51)	-3.40 (2.20)	-0.26 (0.53)	0.95 (0.40)
Down syndrome – Normal BF	9.31 (3.41)	3.75 (1.36)	5.56 (2.18)	0.31 (1.91)	0.04 (0.46)	-0.11 (0.35)
Down syndrome – Elevated BF	14.85 (2.41)	5.78 (0.96)	9.06 (1.54)	1.10 (1.35)	0.32 (0.32)	-0.26 (0.25)
Effect Size (Cohen's *d*)						
Between disability groups	.112	.246	.015	.476	.457	.511
Within TD, difference by BF	.778	**.994**	.587	.706	.021	**1.338 ***
Within DS, difference by BF	**1.011**	**1.068**	**.867**	.042	.255	.069
Within NBF, difference by DS	.468	.544	.388	.533	.567	.355
Within EBF, difference by DS	.432	.728	.228	**.800**	.339	**1.044 †**

Estimated Marginal Means (Standard Error) controlling for sex, age, and Tanner stage.
Abbreviations: BF = body fatness, TD = typically developing, DS = Down syndrome, NBF = normal body fatness, EBF = elevated body fatness
Pairwise comparisons with Bonferroni adjustments and Cohen's *d* effect size examined group differences.
* $p < .05$
† $p < .10$ (trending toward significance)
$d > .80$, bolded

Figure 3.1.   Exercise protocol
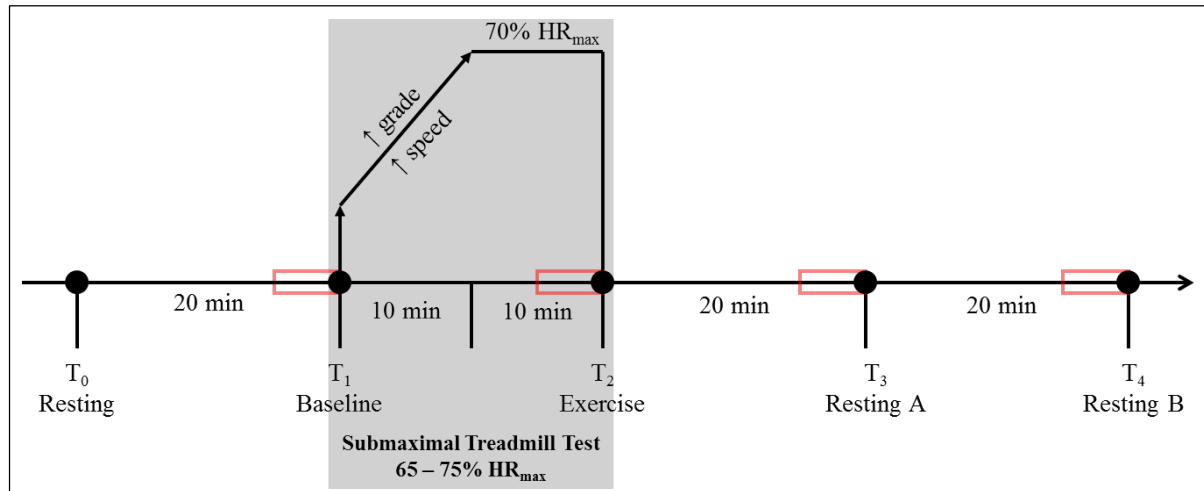
Protocol for laboratory visit to measure respiratory exchange and cortisol before, during and after submaximal graded exercise. Black dots indicate time points with salivary cortisol samples. Red boxes indicate periods for respiratory exchange analysis.

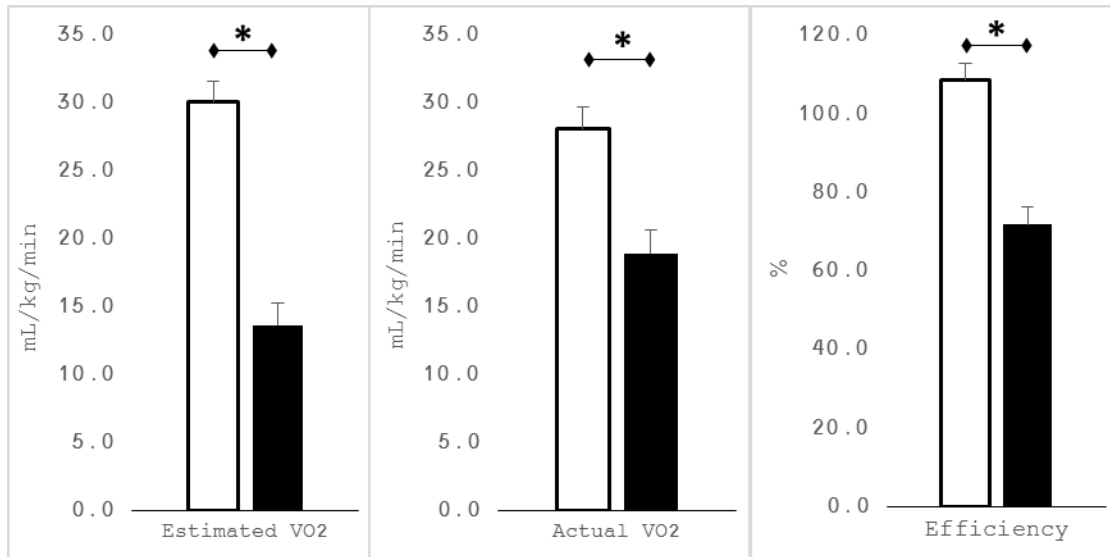Figure 3.2.   Work load of exercise trial
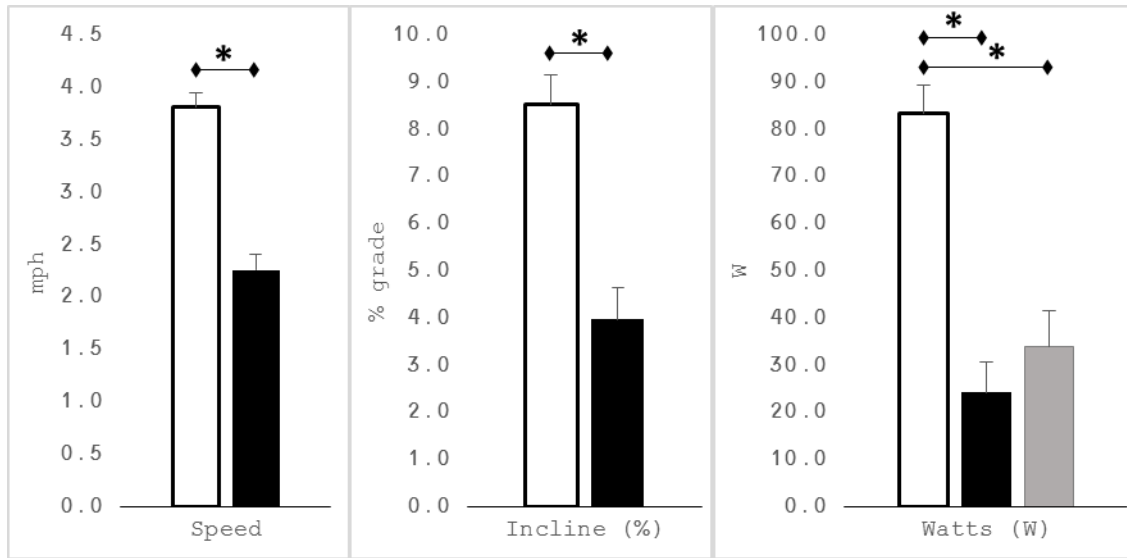
Means ± SE
Typical development (□); Down syndrome (■); Down syndrome (with 0 watts data removed, ▪)
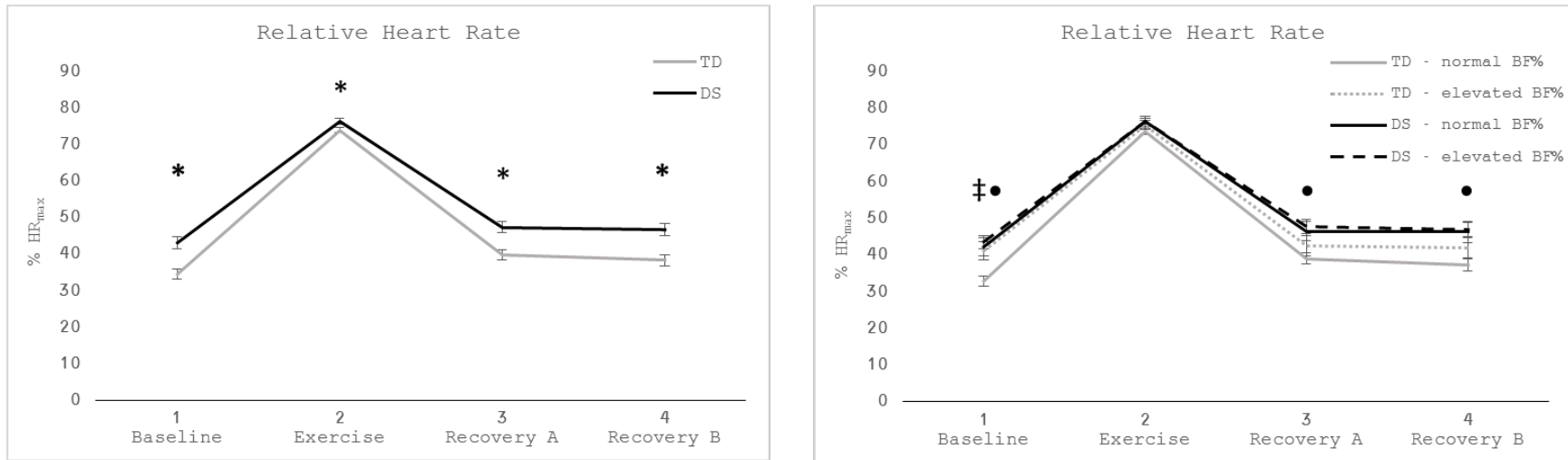* *p* < .05

Figure 3.3.　　　　　　　Efficiency during exercise trial

Means ± SE
Typical development (□); Down syndrome (■)
* *p* < .05

Figure 3.4.   Changes in relative heart rate across protocol

Relative heart rate (% of predicted HR_{max}). Values are means ± SE.
* Significant difference between typically developing (TD) and Down syndrome (DS), $p < .05$
† Significant difference between normal and elevated body fatness in DS, $p < .05$
‡ Significant difference between normal and elevated body fatness in TD, $p < .05$
# Significant difference between TD and DS in adolescents with elevated body fatness, $p < .05$
• Significant difference between TD and DS in adolescents with normal body fatness, $p < .05$
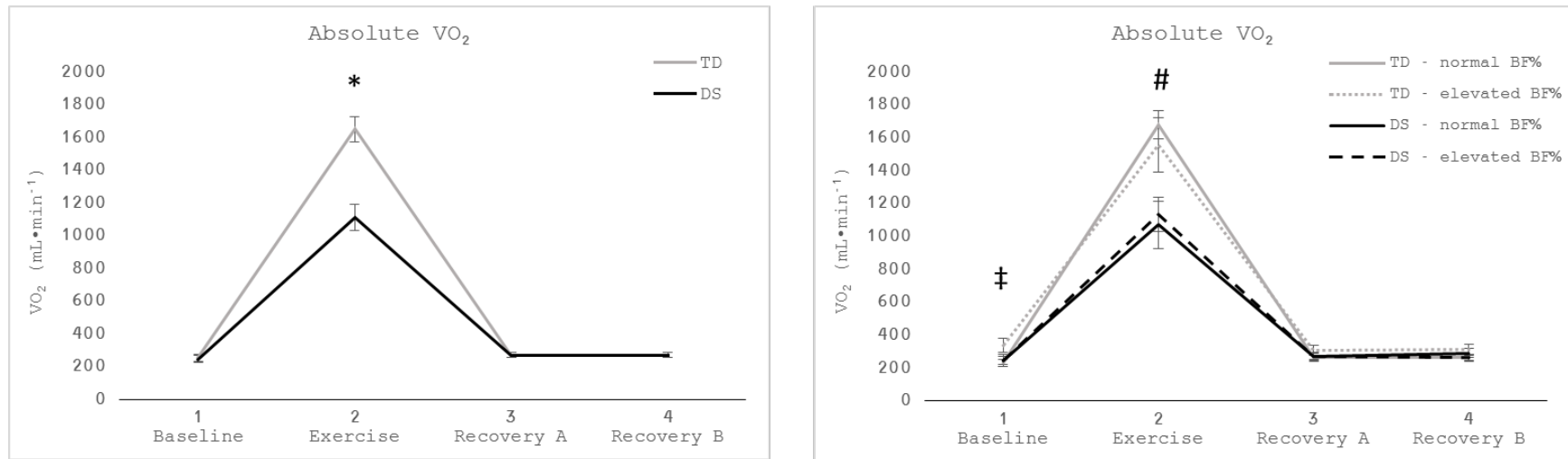
Figure 3.5.   Changes in absolute heart rate across protocol
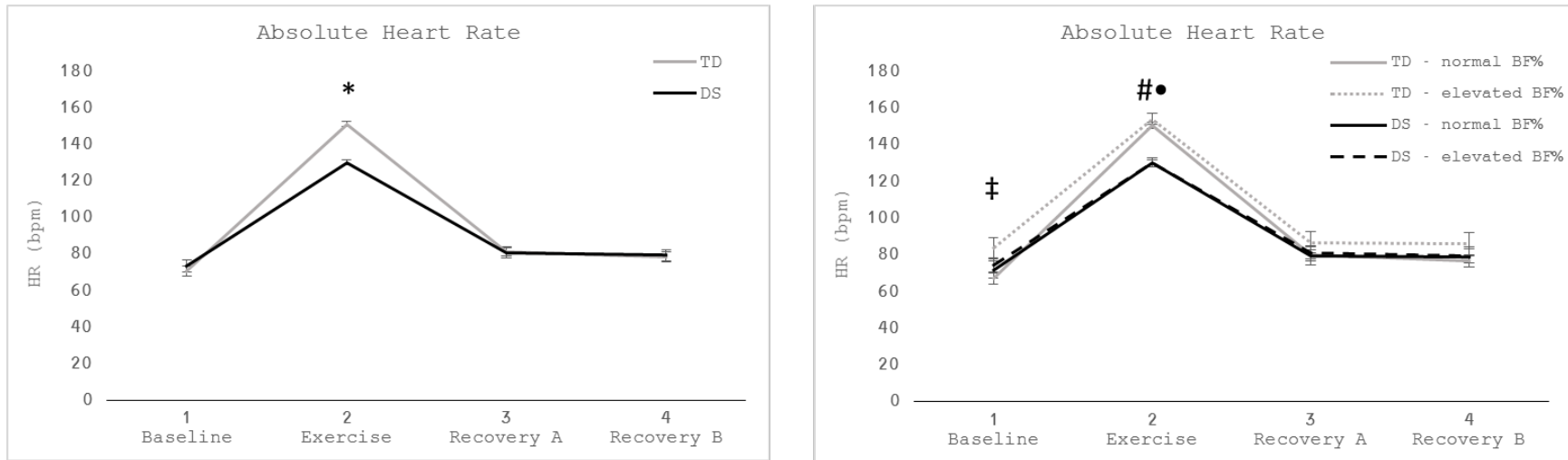
Absolute heart rate (beats per minute). Values are means ± SE.
* Significant difference between typically developing (TD) and Down syndrome (DS), *p* < .05
† Significant difference between normal and elevated body fatness in DS, *p* < .05
‡ Significant difference between normal and elevated body fatness in TD, *p* < .05
# Significant difference between TD and DS in adolescents with elevated body fatness, *p* < .05
• Significant difference between TD and DS in adolescents with normal body fatness, *p* < .05
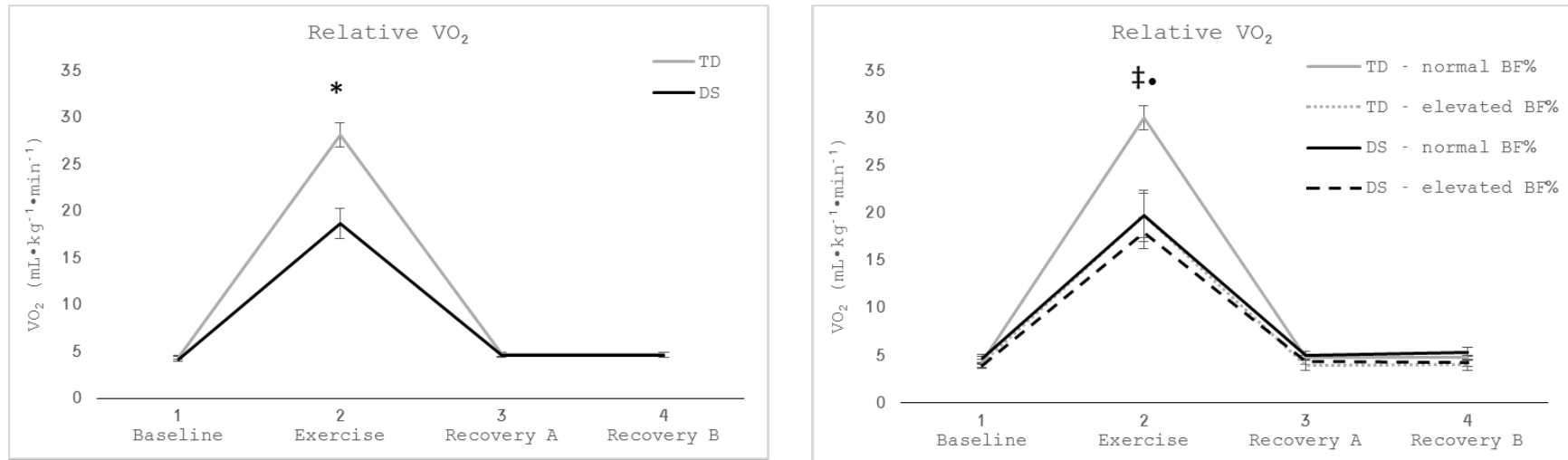
Figure 3.6.   Changes in absolute oxygen consumption across protocol

Absolute oxygen consumption (VO_2; mL•min^{-1}). Values are means ± SE.
* Significant difference between typically developing (TD) and Down syndrome (DS), $p < .05$
† Significant difference between normal and elevated body fatness in DS, $p < .05$
‡ Significant difference between normal and elevated body fatness in TD, $p < .05$
# Significant difference between TD and DS in adolescents with elevated body fatness, $p < .05$
• Significant difference between TD and DS in adolescents with normal body fatness, $p < .05$

Figure 3.7.   Changes in relative oxygen consumption across protocol

Relative oxygen consumption (VO$_2$; mL•kg^{-1}•min^{-1}). Values are means ± SE.
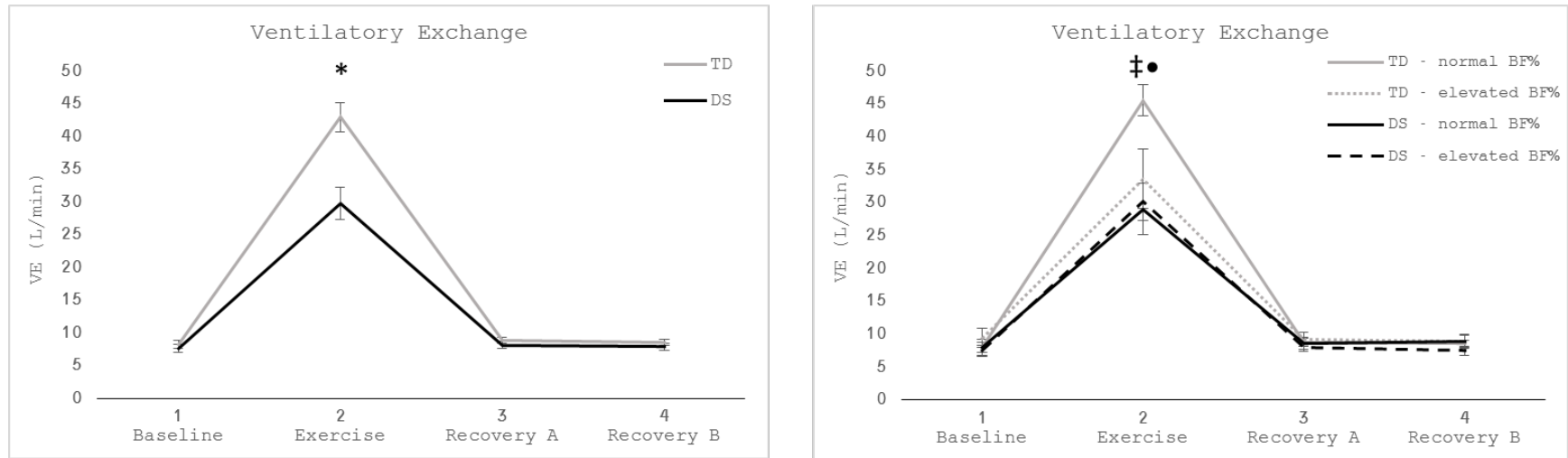* Significant difference between typically developing (TD) and Down syndrome (DS), $p < .05$
† Significant difference between normal and elevated body fatness in DS, $p < .05$
‡ Significant difference between normal and elevated body fatness in TD, $p < .05$
# Significant difference between TD and DS in adolescents with elevated body fatness, $p < .05$
• Significant difference between TD and DS in adolescents with normal body fatness, $p < .05$

Figure 3.8.   Changes in ventilatory exchange across protocol

Ventilatory Exchange (VE, L•min^{-1}). Values are means ± SE.
* Significant difference between typically developing (TD) and Down syndrome (DS), $p < .05$
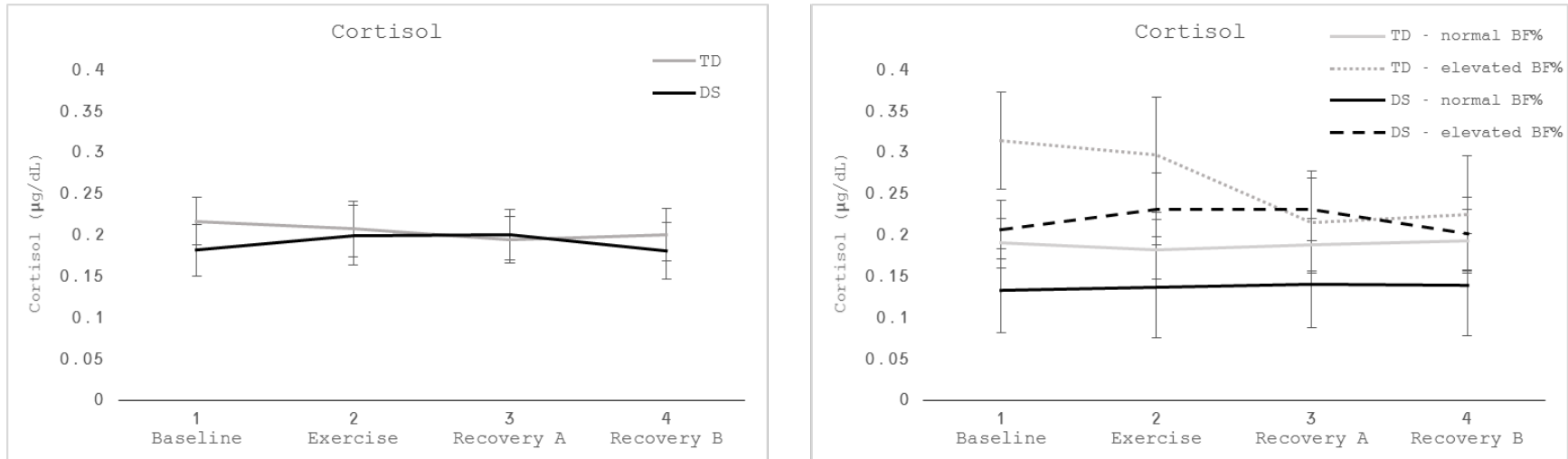† Significant difference between normal and elevated body fatness in DS, $p < .05$
‡ Significant difference between normal and elevated body fatness in TD, $p < .05$
# Significant difference between TD and DS in adolescents with elevated body fatness, $p < .05$
• Significant difference between TD and DS in adolescents with normal body fatness, $p < .05$

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Figure 3.9.   Change in cortisol across protocol

Cortisol concentration (µg/dL). Values are means ± SE.
* Significant difference between typically developing (TD) and Down syndrome (DS), $p < .05$
† Significant difference between normal and elevated body fatness in DS, $p < .05$
‡ Significant difference between normal and elevated body fatness in TD, $p < .05$
# Significant difference between TD and DS in adolescents with elevated body fatness, $p < .05$
• Significant difference between TD and DS in adolescents with normal body fatness, $p < .05$

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CHAPTER 4

Conclusions

High rates of obesity represent a source of health disparity for individuals with Down syndrome. Prior to the current obesity epidemic (Eaton et al., 2008; Ogden, Carroll, Kit & Flegal, 2012; Ogden, Carroll, Kit & Flegal, 2014), researchers were already documenting a greater overweight prevalence among youth with Down syndrome (Chumlea & Cronk, 1981; Cronk, 1978; Cronk, Chumlea & Roche, 1985; Cronk et al., 1988). Recent literature reflects a growing problem of excess weight for height for many youth with Down syndrome. Prevalence estimates have been reported high as 60.9% for overweight status and 31.2% for obesity in youth with Down syndrome (Bandini, Fleming, Scampini, Gleason & Must, 2012; Gonzalez-Aguero, Ara, Moreno, Vicente-Rodriguez & Casajus, 2011; Grammatikopoulou et al., 2008; Krause, Ware, McPherson, Lennox & O'Callaghan, 2015; Myrelid, Gustafsson, Ollars & Anneren, 2002; Rimmer, Yamaki, Lowry, Wang & Vogel, 2010; Styles, Cole, Dennis & Preece, 2002; van Gameren-Oosterom et al., 2012). High rates of overweight and obesity persist and expand throughout adulthood (Bhaumik, Watson, Thorp, Tyrer & McGrother, 2008; Melville, Cooper, McGrother, Thorp & Collacott, 2005; Melville, Hamilton, Hankey, Miller & Boyle, 2008; Rubin, Rimmer, Chicoine, Braddock & McGuire, 1998; Stancliffe et al., 2011). These data suggest the odds of being overweight or obese are over 3 times greater than the general population (Rimmer, Yamaki, et al., 2010) and between 2 and 3 times greater than youth with a non-genetic form of intellectual disability (Bégarie, Maïano, Leconte & Ninot, 2013; Krause et al., 2015; Pan, Davis, Nichols, Hwang & Hsieh, 2016). Thus, the prevention and treatment of

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obesity for children with Down syndrome is considered a priority in both public health and medicine (Murray & Ryan-Krause, 2010).

The overall focus of the dissertation was to better understand obesity in adolescents with Down syndrome. This included determining how to properly measure adiposity and examining associations between adiposity, health behaviors, and the adrenal hormone cortisol. There were four specific aims that were addressed through the three dissertations studies. First, I examined differences in body composition between adolescents with and without Down syndrome using metrics of BMI and DXA. The purpose was to examine adiposity between groups and infer on how measurement choices can impact the interpretations of obesity in this population. Second, I described two primary health behaviors, physical activity and diet, and examined the associations of each health behavior with adiposity. Third, I examined cortisol levels during daily life to establish diurnal patterns as well as during exercise to quantify cortisol response. To my knowledge, this research is the first study to examine diurnal cortisol patterns, and only the second study to examine exercise cortisol responses in individuals with Down syndrome. Fourth, I examined group differences in cortisol activity between various sub-groups including adolescents with and without Down syndrome, with and without elevated body fatness, and with and without moderate levels of physical activity. The key findings and implications from these specific aims are discussed here.

Measuring body composition

This study was one of the few to use DXA measurement in persons with Down syndrome (Bandini et al., 2012; Baptista, Varela & Sardinha, 2005; Esco, Nickerson, Bicard, Russell & Bishop, 2016; Gonzalez-Aguero et al., 2011; Guijarro, Valero, Paule, Gonzalez-Macias & Riancho, 2008; Loveday, Thompson & Mitchell, 2012; Nickerson et al., 2015; Wendel et al.,

2016). Body fat percentages as measured by DXA were significantly higher in adolescents with Down syndrome compared to controls, yet the magnitude of the effect sizes were considerably smaller than when differences were examined with BMI. Large differences were also observed in body fat distributions, with greater proportions of fat mass localized to the abdomen among adolescents with Down syndrome. Furthermore, the 2x2 analysis identified substantial misclassification of BMI categories compared to elevated levels of body fat. All of these findings point toward the potential for overestimation of obesity using BMI and reinforce the need for more advanced body composition measurement in Down syndrome. Based on the results of study 1, only DXA-derived adiposity was used in subsequent studies.

All of my results regarding body composition are consistent with literature on adolescents with Down syndrome include reports of high body fat percentage (Bandini et al., 2012; Baptista et al., 2005; Esco et al., 2016; Guijarro et al., 2008; Loveday et al., 2012; Nickerson et al., 2015; Wendel et al., 2016), low efficiency in categorization based on BMI (Bandini et al., 2012), and greater distribution of fat in the abdominal region (Gonzalez-Aguero et al., 2011). However, the body of literature from which these comparisons are drawn remains small.

The strength of this body composition measurement must also be weighed against the considerable cost and effort needed to use DXA. Sample size is a limitation in all three studies of the dissertation. The small sample size is due, at least in part, to difficulties with recruiting participants capable of coming to campus to complete the DXA scan within business hours. While cost was not a restricting factor within this set of studies, at a rate of $103 to $140 per DXA scan, there is considerably more cost to conducting this type of body composition measurement compared to field measures. However, compared to the best available imaging techniques (e.g. computed tomography and magnetic resonance imaging), DXA is more cost-

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effective, time-efficient, and less invasive (Kendler et al., 2013). An area for future research should be to use DXA to validate field-based measures of body composition for persons with Down syndrome (Esco et al., 2016; Nickerson et al., 2015).

Associations between adiposity and health behaviors

Weight gain, by definition, is caused by a positive ratio between calories in and calories out. Thus, dietary intake and physical activity are highly relevant health behaviors to consider within the scope of addressing health disparities in obesity. While the amount of research conducted in these health behaviors for persons with Down syndrome continues to expand, in particular physical activity, we still have insufficient literature to make definitive conclusions.

First, I found that physical activity levels were very low among the sample. This was particularly evident among the adolescents with Down syndrome. Significant differences were observed between groups in light, vigorous, and moderate-to-vigorous physical activity categories. Furthermore, very few participants with or without Down syndrome met recommended physical activity guidelines (U.S. Department of Health and Human Services, 2008; World Health Organization, 2010). Previous studies have also found low levels of physical activity in youth with Down syndrome (Esposito, MacDonald, Hornyak & Ulrich, 2012; Izquierdo-Gomez et al., 2014; Matute-Llorente, González-Agüero, Gómez-Cabello, Vicente-Rodríguez & Casajús, 2013a; Matute-Llorente, González-Agüero, Gómez-Cabello, Vicente-Rodríguez & Casajús, 2013b; Phillips & Holland, 2011; Pitetti, Baynard & Agiovlasitis, 2013; Shields, Dodd & Abblitt, 2009). However, physical activity levels observed in the current studies were even lower than previous reports. What is potentially concerning about this finding is that in our convenience sample, many of the families have participated in previous studies

including motor skill and physical activity interventions. Thus, a broader sample with parents that are less engaged in promoting physical activity may result in even lower levels.

The potentially novel finding of this study was that when using percent body fat from DXA instead of BMI to represent obesity, the association with physical activity was stronger. Furthermore, in the linear regression analysis, the effect of physical activity on percent body fat was trending toward significance. This findings represents the largest reported association between these variables within persons with Down syndrome. Previously reported associations have been consistently small and non-significant (Esposito et al., 2012; Izquierdo-Gomez et al., 2015; Nordstrom, Hansen, Paus & Kolset, 2013; Shields et al., 2009; Shields, Hussey, Murphy, Gormley & Hoey, 2015). While the current findings do not reach statistical significance, the larger magnitude of association is promising and suggests statistical significance could be possible with a larger sample.

Second, we found significant differences in the unadjusted caloric and macronutrient intake between adolescents with and without Down syndrome. However, when caloric intake was normalized to lean body mass and macronutrient levels were normalized to total caloric intake, these differences were no longer significant nor meaningful. All of the reported dietary variables also appear to be consistent with recommended daily allowances (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). There is a very limited body of literature on diet in youth with Down syndrome, but the current findings appear to be consistent with the caloric and macronutrient intake reported in previous studies (Braunschweig et al., 2004; Grammatikopoulou et al., 2008; Hopman et al., 1998; Luke, Sutton, Schoeller & Roizen, 1996; Samarkandy, Mohamed & Al-Hamdan, 2012; Soler Marin & Xandri Graupera,

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2011). It remains unclear if the dietary intake patterns reported are consistent with excess

calories relative to energy expenditure, as resting metabolic rate was not measured.

Cortisol function

Cortisol was selected as a hormone of interest due to the potential to promote metabolic

dysregulation and contribute to excess body fat, particularly in the abdomen (Adam & Epel,

2007; Bjorntorp, 1996, 2001; De Vriendt, Moreno & De Henauw, 2009; Rodriguez et al., 2015).

In typically developing children and adolescents, associations suggesting that cortisol levels are

higher in individuals with greater adiposity have been reported (Barat et al., 2007; Guzzetti,

Pilia, Ibba & Loche, 2014; Hill, Eisenmann, Gentile, Holmes & Walsh, 2011; Misra et al., 2008;

Reinehr et al., 2014; Rosmalen et al., 2005; Weigensberg, Toledo-Corral & Goran, 2008).

Evidence of cortisol function in Down syndrome is very limited, but recent data suggest that

there may be differences in cortisol levels between persons with and without Down syndrome

(Bricout et al., 2008). To better understand how cortisol operates in adolescents with Down

syndrome, I studied the hormone activity with regards to diurnal patterns across the day as well

as the exercise response within a controlled protocol.

First, I found that cortisol followed the expected diurnal pattern for both adolescents with

and without Down syndrome. Although there were no significant differences, cortisol levels

were higher when directly comparing adolescents with and without Down syndrome. Significant

differences began to appear in the diurnal cortisol patterns when participations were further

divided by elevated body fatness and by meeting physical activity thresholds. Multiple

comparisons within adolescents with Down syndrome were statistically significant. Differences

did not reach significance among adolescents with typical development, but followed a similar

pattern. Although there are issues with group composition, the findings in study 2 are consistent

with a positive association between cortisol and adiposity (Barat et al., 2007; Guzzetti et al., 2014; Hill et al., 2011; Misra et al., 2008; Reinehr et al., 2014; Rosmalen et al., 2005; Weigensberg et al., 2008) and negative association between cortisol and physical activity (Martikainen et al., 2014).

Second, I found that cortisol did not significantly (or meaningfully) respond to the exercise bout prescribed for either adolescents with Down syndrome or with typical development. An exercise response was expected, especially for typically developing adolescents. However, other studies in adolescents have not observed a cortisol response to exercise either (Eliakim et al., 2006; Galassetti et al., 2006). Similar to study 2, direct differences between groups with and without Down syndrome were not significant. However, average cortisol levels were lower among adolescents with Down syndrome. This is the opposite direction as study 2, but similar to the pattern observed previously in young adults males with Down syndrome (Bricout et al., 2008). When stratified by body fatness, cortisol levels were moderately higher in adolescents with greater adiposity. This was observed in both adolescents with and without Down syndrome. While no significant differences were observed, large effect sizes were observed in multiple comparisons. In general, the pattern of difference showed associations of higher cortisol in adolescents with greater body fatness, consistent with many previous studies (Barat et al., 2007; Guzzetti et al., 2014; Hill et al., 2011; Misra et al., 2008; Ondrak, McMurray, Hackney & Harrell, 2011; Reinehr et al., 2014; Rosmalen et al., 2005; Ruttle et al., 2013; Weigensberg et al., 2008).

The question remains, what do these two studies tell us about cortisol differences between adolescents with and without Down syndrome? During the exercise trial (study 3), cortisol levels were slightly *lower* in adolescents with Down syndrome compared to adolescents

with typical development. These differences became wider and exhibited large effect sizes when stratified by body fatness. For example, adolescents with Down syndrome who had elevated body fatness exhibited *lower* cortisol levels at baseline compared to adolescents with typical development who also had elevated body fatness ($d = .88$, Figure 3.9). In the daily diurnal patterns (study 2), adolescents with Down syndrome had slightly *higher* cortisol levels across the day. However, if the patterns are compared between disability groups within body fatness and physical activity interactions, the cortisol levels appear to be *lower*. For example, adolescents with Down syndrome who had normal body fatness and engaged in less than 30 minutes of physical activity per day exhibited *lower* levels of cortisol across the day compared to typically developing adolescents who also had normal body fatness and engaged in less than 30 minutes of physical activity ($p = .05$; Figures 2.2 and 2.3). This relationship is difficult to interpret, particularly given the sub-group with Down syndrome (with normal body fatness and met the threshold of 30 minutes of physical activity) had oddly high diurnal cortisol. Lower levels of cortisol would be consistent with the resting and exercise levels observed by (Bricout et al., 2008).

Moving forward

Due to the high levels of obesity and low levels of physical activity observed in adolescents with Down syndrome, directed health promotion interventions are of great need in this population. The number of physical activity interventions targeting populations with intellectual disabilities, including Down syndrome, remain limited compared to the general population (Rimmer, Chen, McCubbin, Drum & Peterson, 2010). It is suggested that future research address this paucity through rigorous randomized-control designs when feasible, with tight sample composition to limit potential confounders, and through demonstrating replicability

of positive interventions (Rimmer et al., 2010). Furthermore, I am in favor of intervention approaches following guidelines proposed by Drum and colleagues (2009) for translational research. These guideline encourage 1) a focus on community-based programing, 2) using theoretical frameworks, process evaluations, and appropriate measures to evaluate outcomes; 3) involve individuals and families, while considering the unique beliefs, practices, and values of participants; and 4) being socially, programmatically, and environmentally accessible. This dissertation reinforces the need to increase physical activity in adolescents with Down syndrome and presents a potential covariate (cortisol) that may assist with understanding within-group differences in obesity. It is critical, however, to focus attention on creating these interventions and translational programs that can provide direct benefits to participants with Down syndrome.

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