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Executive function mediates prospective relationships between sleep duration and sedentary behavior in children

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Abstract

Childhood sedentary behavior has been linked to increased obesity risk. Prior work has identified associations between sedentary behavior, executive function (EF), and sleep. This study tested the hypothesis that reduced sleep duration may adversely impact EF and lead to increased childhood sedentary behavior. Southern California schoolchildren participating in the school-based health promotion program *Pathways to Health* ($N = 709$) were assessed annually from 4th through 6th grades (2010–2013) on self-report measures of sedentary behavior, sleep duration, and executive function. A series of path models were specified treating average nightly sleep duration and weekend wake/bed-time shift at 4th grade as predictors of 6th grade sedentary behavior. Four EF subdomains were tested as potential mediators of longitudinal associations at 5th grade. Significant associations between average nightly sleep duration, EF and sedentary behavior were identified ($p < 0.05$), adjusting for participant gender, physical activity, SES, ethnicity, program group assignment, and the presence/absence of parental screen time rules. Fifth grade overall EF $(p < 0.05)$ —and in particular the subdomains of inhibitory control $(p < 0.05)$ and organization of materials ($p < 0.01$)—significantly mediated the relationship between 4th grade sleep duration and 6th grade sedentary behavior ($p < 0.05$). Furthermore, delay of weekend bed- or wake-times relative to weekdays was prospectively associated with decreased overall EF ($p < 0.05$), but not increased sedentary behavior ($p = 0.35$ for bed-time delay; $p = 0.64$ for wake-time delay), irrespective of average nightly sleep duration. Findings suggest that sleep promotion efforts may reduce children's sedentary behavior both directly and indirectly through changes in EF.

Keywords

Sedentary behavior; Screen time; Executive function; Neurocognition; Inhibitory control; Sleep; Obesity prevention

Transparency document

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Conflict of interest

The authors declare that there are no conflicts of interest.

The Transparency document associated with this article can be found, in online version.

1. Background

Compared to previous generations, today's youth increasingly engage in sedentary activities such as computer use, video games and viewing other electronic media—frequently characterized as screen time (Rideout et al., 2010). For example from 2000 to 2010 the average US child's screen time-including television, movies, videogames and computer use —increased by more than 2.5 h/day (Rideout et al., 2010). Data from the CDC's Youth Risk Behavior Survey (Eaton et al., 2012; Kann et al., 2014) suggest that video gaming and nonschool-related computer use have increased particularly sharply in recent years. Moreover, growing use of internet-enabled mobile phones and tablets is likely to result in further screen time increases as these devices become increasingly incorporated into children's lives.

Sedentary behavior has been defined as "any waking activity characterized by an energy expenditure $\langle 1.5 \rangle$ metabolic equivalents and a sitting or reclining posture" (Cart, 2012), and is associated with increased risk of childhood obesity (Wong and Leatherdale, 2009; Anderson et al., 2008), hypertension (Pardee et al., 2007) and numerous psychosocial outcomes (Strasburger et al., 2010; Russ et al., 2009). A recent Danish study found that long-term sedentary behavior was related to greater reduction of quality adjusted life years (7 years) than either obesity (3–6 years) or high rates of alcohol consumption (3–5 years) (Bronnum-Hansen et al., 2007). This suggests that sedentary behavior constitutes an important target for health promotion interventions, independent of physical activity. Within the home environment, previous work has found that the establishment of parental rules governing screen time within the household can lead to significantly reduced sedentary behavior in children (Ramirez et al., 2011).

One behavior which has been found to be inversely associated with sedentary behavior is sleep (Cain and Gradisar, 2010; Costigan et al., 2013). A longitudinal British study found screen time to be associated with increased sleep problems (e.g. daytime fatigue) though nightly sleep duration was not assessed (Viner and Cole, 2006). Two cross-sectional studies found high levels of internet use and computer game playing to be positively associated with self-reported insufficient sleep (Van den Bulck, 2004; Belanger et al., 2011). Furthermore, a recent longitudinal birth cohort study of television viewing and sleep in children found that not only did children who watched more television sleep less, but that changes in TV viewing patterns were negatively associated with changes in sleep duration across time (Marinelli et al., 2014). While each study suggested that efforts to cap children's screen time would lead to improved sleep, none explored the possibility that sleeping less might increase sedentary behavior.

A recent systematic review examining relationships between sleep and sedentary behavior suggested that—beyond the direct metabolic effects of sleep—there are two indirect pathways through which sleeping less may lead to increased sedentary behavior (Must and Parisi, 2009). The first is that sleep deprivation may lead to fatigue, rendering children less likely to engage in physical activity and more likely to engage in sedentary activities. The second is that time children spend asleep may simply displace time that would otherwise be spent in obesogenic environments, which promote sedentary behavior. While these pathways are plausible, a novel third, cognitive pathway may provide a useful framework to link sleep

and sedentary behavior. In this latter framework, it may be that children with the cognitive capacity to better plan their behavior in a goal-directed manner, organize their environments, and inhibit the tendency to engage in default, obesogenic behaviors (e.g. TV watching), will be less sedentary than children lacking these complex cognitive skills. In support of this possibility, a recent study utilizing polysomnography found that television viewing and computer game playing adversely impacted both children's sleep efficiency and their verbal cognitive performance (Dworak et al., 2007)—implying a link between sleep, cognition and sedentary behaviors in school-aged children.

An important set of cognitive processes that may relate to both sedentary behavior and sleep is executive function (EF). EF refers to a cluster of cognitive processes such as inhibitory control and working memory, which play a significant role in the planning and execution of decision-making, self-regulatory, and goal-directed behaviors (Diamond, 2013). Deficits in EF have been found to predict sedentary behavior in elementary schoolchildren (Riggs et al., 2012a) and have been associated with decreased sleep duration among adolescents (Anderson et al., 2009). Although EF has been found to be relatively stable during late childhood-early adolescence (Harms et al., 2014), studies have identified links between insufficient sleep and EF impairment (Chuah et al., 2006; Durmer and Dinges, 2005; Killgore, 2010). Such work has found acute sleep deprivation to substantially impair inhibitory control (Chuah et al., 2006; Drummond et al., 2006), working memory (Lim and Dinges, 2010), and integration of emotional cues into complex decision-making (Killgore et al., 2006).

Research has also identified associations between delaying weekend wake-times relative to school nights and multiple health risk behaviors (O'Brien and Mindell, 2005). Such changes in sleep patterns may be particularly detrimental during adolescence since circadian rhythms governing sleep during this developmental period adapt more readily to delayed bedtimes than advanced wake-times (Dahl and Lewin, 2002). However, little is known about interrelationships between sleep, EF, and sedentary behavior. The present study tested the longitudinal impact of total weekly sleep duration, as well as delay of weekend wake- or bed-times on sedentary behavior among elementary schoolchildren, hypothesizing that EF would mediate these prospective relationships.

2. Methods

2.1. Background

Data were taken from the 4th (2010 baseline), 5th, and 6th grade assessment waves of Pathways to Health, a randomized controlled trial for prevention of obesity and substance use in children in 24 Southern California elementary schools (Sakuma et al., 2012). This program translated evidence-based social-emotional learning and substance use interventions (Riggs et al., 2006a) into a health promotion curriculum focused on improving health behavior decision-making.

2.2. Participants

Participants were 1005 4th grade assented students with full active parent consent to participate in the study, and who constituted a panel that was followed from grades four through six. Of these, 709 had complete data for all three years and constituted the analytic sample. Of the 296 participants who were not retained, participant attrition was due to the following: 185 (63%) to families moving, 18 (6%) to students, parents and/or administrators declining participation, 29 (10%) to student absences on days of assessment, and 64 (22%) to school closures. When compared to the 709 study participants the 296 participants lost to follow-up were more likely to be Hispanic (33% vs. 26%; $p < 0.05$) and low socioeconomic status (SES) (35% vs. 21%; $p < 0.001$). The final analytic sample was 50% female, 26% Hispanic and 21% low SES. These students represent 75 classrooms and 24 Southern California schools. Human subjects procedures were approved by the relevant Institutional Review Board.

2.3. Measures

Data for the study were drawn from a self-report survey administered in one 45-min classroom period. The survey was administered verbally by a trained data collector.

2.3.1. Sleep—Average nightly sleep duration was calculated using four survey items asking participants: "On an average school day what time do you go to bed/wake up?" and "On an average weekend day what time do you go to bed/wake up?" These items were adapted from the Sleep Habits Survey, which was developed and validated for use in adolescents (Wolfson et al., 2003). Numerous studies have employed similar self-report measures of sleep duration in comparable populations (Nuutinen et al., 2013; Appelhans et al., 2014; Hoedlmoser et al., 2010). An estimate of participants' average nightly sleep hours was calculated as the weighted mean of weekday and weekend sleep duration. To estimate how much participants shifted their wake/bed times on weekends relative to school nights, self-reported weekend wake/bed times were subtracted from self-reported weekday wake/bed times, creating variables indicating the number of hours that children "slept in", and "stayed up" on weekends, respectively.

2.3.2. Executive function—Items from the following four of eight clinical sub-scales of the Behavioral Rating Inventory of Executive Function-Self-Report (BRIEF-SR) were used to assess EF (Guy et al., 2004): inhibitory control, emotional control, working memory and organization of materials. These four scales were selected by investigators to reflect the subdomains of EF hypothesized to be more relevant to the observable and modifiable health behaviors targeted by the Pathways intervention. To abbreviate the scales, factor analyses were conducted on each subscale and items demonstrating factor loadings <0.45 were dropped. Each BRIEF-SR item asks participants: "How often each of the following has been a problem in the last month?" Response choices are: $1 =$ Never, $2 =$ Sometimes, $3 =$ Often. An example item from the inhibitory sub-scale is "I do things without thinking first," and for the working memory sub-scale is "I forget what I'm doing in the middle of things". Subscale means and total mean scores were reverse-coded so higher scores reflect greater EF competency. The BRIEF was designed to be an appropriate and ecologically valid measure of EF skills associated with goals and actions in everyday problem solving situations

encountered by school-age children, rather than an indicator of laboratory or structured task performance (Guy et al., 2004; Toplak et al., 2013). Over 400 peer-reviewed manuscripts report the reliability, validity and clinical utility of the BRIEF (Roth et al., 2015). Our decision to abbreviate the BRIEF-SR measure was motivated by time-constraints imposed by partner schools for in-class survey administration. Previous work found these abbreviated BRIEF-SR scales to perform comparably to full BRIEF-SR scales (Riggs et al., 2010; Pentz and Riggs, 2013). Cronbach's alpha statistics for the four abbreviated subscales were as follows: inhibitory control=0.76 (6/13 items), emotional control=0.71 (6/10 items), working memory = 0.77 (5/12 items) and organization of materials = 0.69 (5/7 items).

2.3.3. Sedentary behavior—Sedentary behavior was assessed in the present study using three items adapted from the School-Based Nutrition Monitoring (SBMN) Student Questionnaire (Hoelscher et al., 2003). These items read "On a regular school day, how many hours per day do you (Rideout et al., 2010) usually watch TV or video movies at home or away from school, (Eaton et al., 2012) spend on a computer at home or away from school and (Kann et al., 2014) play video games that you sit down to play like PlayStation, Xbox, GameBoy, or arcade games". Response options ranged from 0 ("none") to 6 ("6 or more hours"). A summed sedentary behavior score reflecting daily sedentary screen time hours was created. The SBNM, has been found to be valid (Hoelscher et al., 2003) and reproducible (Penkilo et al., 2008) in children as young as 4th grade.

2.3.4. Covariates—Gender, ethnicity, and socioeconomic status (using free lunch eligibility as a proxy) were included as covariates based on previous research linking them to sleep and cognition (Shanahan et al., 2008). Participation in the intervention condition was also included as a covariate since it also may have influenced key study variables. Physical activity was included as a continuous covariate due to past work identifying inverse associations with sedentary behavior (Leech et al., 2014) and a growing literature linking aerobic exercise and fitness to improved EF (Best, 2010; Hillman et al., 2014).

The mean of three survey items from the Physical Activity Questionnaire for Older Children were utilized for assessment of physical activity outside of school during the previous 7 days (Crocker et al., 1997; Kowalski et al., 1997). Finally, presence/absence of parental rules in the home governing screen time was included as a covariate since the imposition of such rules was hypothesized to impact sleep duration and sedentary behavior. These were assessed by the following yes/no survey item adapted from previous research: "Does your parent have rules about how much time each day you can watch TV/listen to music/be on a computer?" (Riggs et al., 2006b).

2.4. Analysis plan

Hypothesis testing was conducted via path models specified by the Stata 14 SEM command, using maximum likelihood estimation. Rates of missing data not due to attrition were low, thus, analyses were conducted on the full panel sample $(N = 709)$. Indirect and total effects were calculated using the delta method (MacKinnon, 2008). Results are reported as standardized beta coefficients, hypothesis tests are two-tailed, and significance was set at $p <$ 0.05.

Sleep duration at 4th grade was treated as the primary predictor of sedentary behavior at 6th grade and EF at 5th grade was examined for its direct and mediational relationships to subsequent sedentary behavior. First a basic path model was specified with a path leading directly from average nightly sleep duration to sedentary behavior. The overall EF construct and each of the four EF subdomains were then entered as putative mediators of this relationship in separate models. Covariate paths were then specified for each model, first adding each of the following covariates separately to test their independent relations and then adding all paths simultaneously to examine unique associations after adjusting for covariance among all included variables. Covariates remained in each model if they contributed to the overall fit ($p < 0.10$). These covariates included: gender (male/female), free lunch eligibility (yes/no), group assignment (intervention vs. control), parental screen time rules (yes/no), physical activity outside of school, BMI and BMI z-score. Participant ethnicity was entered as a dichotomous covariate two ways (Hispanic vs. non-Hispanic and White vs. non-White). Final models adjusted for White vs. non-White as it explained a greater proportion of the overall variance. Two models investigating the impact of weekend shifts in sleep-wake patterns substituted weekend bed/wake-time delay for nightly sleep hours as the primary predictor and adjusted for nightly sleep hours in addition to the above covariates. All models demonstrated excellent fit $(X^2 p > 0.05$; CFI >0.95; RMSEA <0.05). Finally, robust standard errors clustered by 6th grade classroom were applied for reporting parameter estimates. These estimators address both the observed multivariate non-normality of the residual variance in sedentary behavior, and the potential lack of independence among children sharing the same classroom environment.

3. Results

Table 1 provides baseline (4th grade) means and standard deviations for all scales and levels of included covariates. In a longitudinal path model [Fig. 1] where 6th grade sedentary behavior was predicted by 4th grade weekly sleep duration, both a direct path ($\beta = -0.10$; p < 0.05) and an indirect path through changes in 5th grade EF ($\beta = -0.014$; $p < 0.05$) were significant, as were total effects ($\beta = -0.12$; $p < 0.05$). Fifth grade EF was significantly associated with 6th grade sedentary behavior (β = -0.16; $p < 0.001$). The presence of parental rules governing screen time ($r = 0.13$; $p < 0.01$) and white ethnicity ($r = 0.08$; $p <$ 0.05) were significantly associated with increased sleep at 4th grade, whereas increased physical activity ($r = -0.10$; $p < 0.05$) was associated with decreased sleep at 4th grade and increased EF at 5th grade (β = 0.12; $p < 0.01$). Male gender (β= 0.20; $p < 0.001$), low SES $(\beta = 0.14; p<0.001)$, and non-white ethnicity $(\beta = -0.10; p < 0.05)$ were each associated with increased sedentary behavior at 6th grade.

When the EF subdomains of inhibitory control, organization of materials, emotional control and working memory were individually entered into the aforementioned longitudinal path model in lieu of the composite measure of EF [Fig. 2], inhibitory control ($\beta = -0.01$; p < 0.05) and organization of materials ($\beta = -0.02$; $p < 0.05$) were significant mediators of the relationship between sleep and sedentary behavior, whereas emotional control (β = -0.004 ; $p = 33$) and working memory ($\beta = -0.004$; $p = 0.36$) were not. Total effects of sleep duration on sedentary behavior were significant regardless of the EF subdomain tested as a mediator

(βinhibitory control = -0.11, $p < 0.05$; βemotional control = -0.11, $p < 0.05$; β_{working memory} = $-0.11, p < 0.05; \beta_{\text{organization}} = -0.12; p < 0.05$.

A longitudinal path model investigating the impact of weekend wake-time delay found weekend wake-time delay at 4th grade to be negatively associated with EF at 5th grade (β = -0.10 ; $p < 0.05$), which in turn was negatively associated with sedentary behavior at 6th grade ($\beta = -0.17$; $p < 0.001$) [Fig. 3]. However, there was neither a significant direct ($\beta =$ 0.000; $p = 0.99$) nor total effect ($\beta = 0.02$; $p = 0.64$) of 4th grade weekend wake-time delay on 6th grade sedentary behavior. Similar patterns were identified for weekend bed-time delay [Fig. 3].

4. Discussion

The present study suggests that average nightly sleep duration is significantly associated with sedentary behavior among late-elementary schoolchildren. Importantly, this study is the first to find this relationship to be significantly mediated by a child's EF, which recent work has found to be a key antecedent of multiple health risk behaviors (Riggs et al., 2006a; Pentz et al., 2015). Moreover, we found that delaying either weekend bed- or wake-times relative to weekdays is prospectively associated with decreased EF, but not increased sedentary behavior, irrespective of the number of hours that children sleep. Finally these data suggest that children whose parents have established rules governing screen time within the household generally sleep more, and engage in less sedentary behavior than children whose parents do not impose such rules. These findings have implications for the design of future health promotion interventions and may be useful in guiding parents and school administrators in health promotion efforts.

The identification of EF as a significant mediator of the relationship between sleep and sedentary behavior bridges the gap between prevention science literature linking EF to multiple obesogenic behaviors in school-aged children (Riggs et al., 2012a; Riggs et al., 2012b; Liang et al., 2014) and studies linking inadequate sleep to impaired EF (Killgore, 2010; Bub et al., 2011). Beyond providing the first evidence for a mediational role of overall EF, we found the EF subdomains of inhibitory control and organization of materials to be more adversely impacted by insufficient sleep than working memory, and emotional control. As illustrated by Fig. 2, significant paths were identified between each EF subdomain and sedentary behavior, but sleep duration was significantly associated with inhibitory control and organization, and not associated with emotional control and working memory. Findings in the domains of inhibitory control and organization are consistent with work in adults linking sleep deprivation to impaired performance on common computerized inhibitory control tasks (e.g. Stroop and Go/No-go tasks) (Drummond et al., 2006; Hall et al., 2006) and laboratory paradigms designed to assess more complex organization/ planning skills (e.g. Tower of London task) (Killgore et al., 2009; Horne, 1988). While the non-significant relationship identified here between sleep duration and working memory seemingly contradicts a meta-analysis identifying moderate effects of sleep deprivation on working memory (Lim and Dinges, 2010), more recent work suggests that insufficient sleep may only impact non-executive components of working memory task performance (Tucker et al., 2010). Such non-executive components of working memory task performance (e.g. reaction

time) are unlikely to be reflected by scores on the BRIEF—a self-report behavioral EF measure. Furthermore, given that considerable previous work has linked insufficient sleep to deficits in affective processing among adult participants (Killgore, 2010; Killgore et al., 2007), the lack of an observed relationship between sleep duration and emotional control suggests that these relationships may operate differently in pediatric populations. Future work in children including EF assessment via both behavioral and computerized measures may shed further light on relationships between sleep duration and both working memory and emotional control.

The present study suggests that to reduce sedentary behavior in children it is important to both ensure that they receive adequate sleep, and that EF is maximized. Multiple systematic reviews and meta-analyses of intervention studies aiming to decrease sedentary behavior in children found that a variety of intervention types (e.g. single vs. multi-component) administered in numerous settings (i.e. school-based, home-based, clinic-based) are effective in reducing sedentary behavior (van Grieken et al., 2012; Wahi et al., 2011; Leung et al., 2012). However, a recent review of these reviews found that the impact of such interventions is generally low (Biddle et al., 2014). Despite the diversity of intervention approaches utilized, none to date has incorporated sleep promotion into their intervention curricula nor is statistical adjustment typically made for systematic differences in participants' sleep patterns. Interventions targeting sedentary behavior have also generally failed to take into account growing evidence linking screen time to children's EF. Interventions ranging in format from martial arts and mindfulness meditation training to computerized training and classroom curricula have each been found to promote EF in children (Diamond and Lee, 2011). In light of the present study findings we suggest that future interventions both promote healthy sleep habits and EF among participants as well as statistically adjust for systematic differences in sleep duration and EF among participants, given the independent associations with sedentary behavior observed here.

Maximizing EF through sleep promotion may be particularly important for avoiding sedentary behavior due to the pervasiveness of screens in today's society and cultural norms promoting screen time. However, sleep promotion efforts are further complicated by the growing ubiquity of screens in children's bedrooms. A recent study of media use among US children found that rates of internet access in children's bedrooms have tripled in the last decade (Rideout et al., 2010). Furthermore, nearly 75% of children have a television in their bedroom, and half have a videogame console. In this screen media-rich bedroom environment, it is unsurprising that children whose parents set rules governing home media use are exposed to roughly three hours less media/day than children whose parents lack such rules. In this same study, the presence of a TV in a child's bedroom predicted a fourhour/day increase in media exposure, most of which is screen-based. Given that increases in bedroom screen time are likely to result in reduced sleep duration, we recommend that parents set rules governing home media use. In the present study, the mere presence of any household rules governing screen time was associated with a two-hour decrease in children's sedentary behavior as well as significant increases in nightly sleep duration, irrespective of children's EF.

5. Limitations

The present manuscript has several limitations. First, time and resource restrictions associated with school-based data collection limited the present data to self-report. Second, this study only measured sleep duration. Other important sleep parameters include sleep quality and latency—the amount of time it takes to fall asleep (Roeser et al., 2012). In addition, the present study did not assess the proximity of screen time to sleep, nor the presence of screens in a child's bedroom. Future research should consider more detailed examination of childhood sleep patterns, including use of accelerometry to more accurately assess sleep and physical activity behaviors as well as more objective assessment of participant screen-time. Beyond television and computer use, researchers should consider assessing the degree to which electronic devices like tablets and smart-phones are used proximal to bedtime. The short-wavelength light emitted from such devices has been found to adversely impact both cognition and sleep physiology (Chang et al., 2015).

It is likely that the relationships observed here are at least to some degree bidirectional. As such, studies assessing key study variables earlier in childhood and following children further into adolescence may provide greater insight into the causal relationships linking sleep, EF and sedentary behavior across development. Furthermore, since behavioral problems like ADHD and their medications may impact EF performance, sedentary behavior, and sleep patterns, future studies should consider screening participants for relevant behavioral disorders. Finally, our sample had relatively few African-American participants, who differed significantly from the rest of the sample on key study variables. Therefore future work should further explore these relationships within a larger sample of African American participants, as well as improve upon the single dichotomous measure used to adjust for differences in SES.

6. Conclusions

This study found that average nightly sleep duration was significantly associated with sedentary behavior in elementary school students, and that this association was significantly mediated by children's EF. Given the positive short-term contingencies associated with sedentary behavior (e.g. relaxation), our findings suggest that individuals who can mobilize the executive resources to inhibit their prepotent desire to be sedentary and effectively organize their environments to facilitate participation in non-sedentary activities are less likely to fall victim to the 21st century "default" behavior of screen time.

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Abbreviations

BRIEF-SR Behavioral Rating Inventory of Executive Function-Self-Report

SES Socioeconomic status

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Fig. 1.

Longitudinal mediation model for the effect of nightly sleep duration at 4th grade on sedentary behavior at 6th grade through changes in executive function at 5th grade among Southern California schoolchildren. Two-sided $P < 0.1 * P < 0.05 * P < 0.01 * * P < 0.001$; non-significant covariance paths at 4th grade and error terms for endogenous variables were omitted for clarity.

Fig. 2.

Longitudinal mediation models for the effect of nightly sleep duration at 4th grade (2010) on sedentary behavior at 6th grade through changes in individual EF subdomains at 5th grade among Southern California schoolchildren. [Each path model also adjusts for participant gender, physical activity, SES, ethnicity, parental screen-time rules, & control/intervention group in the same manner as Fig. 1] Two-sided $P < 0.1 * P < 0.05 * P < 0.01 * * P < 0.001$.

Fig. 3.

Longitudinal mediation models for the effect of delaying weekend wake-time and bed-time at 4th grade (2010) on sedentary behavior at 6th grade through changes in executive function at 5th grade among Southern California schoolchildren. [Each model also adjusts for participant gender, physical activity, SES, ethnicity, parental screen-time rules, & control/ intervention group in the same manner as Fig. 1, as well as average nightly sleep duration] Two-sided $P < 0.1$ * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

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 $p < 0.01$,

*** $p < 0.001$. (One-way ANOVA using Scheffe post-hoc test for multiple comparisons). (One-way ANOVA using Scheffe post-hoc test for multiple comparisons).

A= subgroups differ significantly $p < 0.01$. B = subgroups differ significantly $p < 0.05$. C= subgroups differ significantly $p < 0.01$. E

 $\overline{}$ = subgroups differ significantly

 $p < 0.05$.

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