Sedentary Behavior and Physical Activity in Youth With Recent Onset of Type 2 Diabetes

OBJECTIVE: With the rise of type 2 diabetes in youth, it is critical to investigate factors such as physical activity (PA) and time spent sedentary that may be contributing to this public health problem. This article describes PA and sedentary time in a large cohort of youth with type 2 diabetes and compares these levels with other large-scale investigations.

METHODS: The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) trial is a study in 699 youth, recruited from 15 US clinical centers, aged 10 to 17 years with <2 years of type 2 diabetes and a BMI ≥85th percentile.

RESULTS: In comparison with the subset of the NHANES cohort who were obese (BMI ≥95th percentile), TODAY youth spent significantly more time being sedentary (difference averaging 56 minutes per day; \( P < .001 \)) as assessed by accelerometry. Although moderate to vigorous activity levels in both obese cohorts for all age groups were exceptionally low, younger TODAY boys were still significantly less active than similarly aged NHANES youth. Comparisons between the TODAY girls and other investigations suggest that the TODAY girls also had relatively lower PA and fitness levels.

CONCLUSIONS: Adolescents with type 2 diabetes from the large TODAY cohort appear to be less physically active and tend to spend more time being sedentary than similarly aged youth without diabetes identified from other large national investigations. Treatment efforts in adolescents with type 2 diabetes should include decreasing sitting along with efforts to increase PA levels. *Pediatrics* 2013;131:e850–e856

WHAT’S KNOWN ON THIS SUBJECT: The rise in type 2 diabetes in youth is a major public health concern thought to be partially due to decreasing activity levels and increasing obesity. The role of sedentary time as a possible contributor also needs to be examined.

WHAT THIS STUDY ADDS: Measured objectively, obese youth, with or without type 2 diabetes, spend little time in moderate to vigorous physical activity. Those with type 2 diabetes, however, were significantly more sedentary than their obese counterparts, identifying an important area for future intervention efforts.
Obesity in youth is a critical public health problem, with estimates for the number of youth 10 to 17 years of age who are overweight or obese approaching 30%.1 This alarming prevalence of obesity is thought to be driving the increasing rates of type 2 diabetes in youth.2

One of the factors implicated in this epidemic of both obesity and type 2 diabetes in youth is physical inactivity. In an investigation involving a representative multiracial sample of US adolescents aged 12 to 19 years without diabetes, physical activity (PA) and cardiovascular fitness were found to be positively associated with insulin sensitivity in boys and, to a lesser extent, in girls.3 However, whether youth with type 2 diabetes are less active and spend more time sitting than do nondiabetic youth, particularly obese nondiabetic youth, has not been examined.

A National Institute of Diabetes and Digestive and Kidney Diseases–funded randomized clinical trial was conducted to examine various treatment therapies for youth with type 2 diabetes. The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study represents the largest ethnically and geographically diverse group of pediatric patients with type 2 diabetes ever assembled. This article describes the PA/fitness levels of the youth in this cohort. It is hypothesized that these youth with type 2 diabetes spend more time sitting and less time in moderate to vigorous PA than do similarly aged obese youth from a national cohort and other large-scale investigations of adolescents.

METHODS

The TODAY trial was a randomized, double-blind, parallel-group clinical trial designed to evaluate the relative efficacy and safety of 3 treatments for type 2 diabetes in youth: metformin alone, metformin plus rosiglitazone, and metformin plus an intensive lifestyle program.4 The primary objective of the trial was to compare the 3 treatment arms on time to treatment failure, defined as loss of glycemic control.

Participants were recruited from 15 clinical centers as previously described4 (see the study group listing in the Supplemental Information). Enrollment ended in February 2009 with 699 youth enlisted. Entry criteria for participants were as follows: aged 10 to 17 years, with <2 years of type 2 diabetes; a BMI ≥85th percentile at time of diagnosis or at screening; availability and agreement of an adult caregiver to support participation; negative for pancreatic autoimmunity (both glutamic acid decarboxylase and anti-tyrosine phosphatase antibodies); and fasting C-peptide >0.6 ng/mL. Participants with hemoglobinopathies were excluded.

Before randomization, a run-in period was performed to ensure that participants were able to tolerate therapy with metformin, accomplish mastery of a standard diabetes education curriculum, and demonstrate the ability to adhere to study requirements for pill taking and visit attendance.4 The youth who successfully completed the 2- to 6-month run-in period were found to decrease their weight by a mean of 0.68 kg compared with a 0.71 kg weight gain in those who failed the run-in period.

The youth who successfully completed the run-in period were randomly assigned into the main clinical trial and completed baseline measures.5 This article describes the PA and cardiorespiratory fitness levels of the youth who mastered the run-in period and entered the formal trial. During the baseline visit of the clinical trial, an objective and a subjective measure of PA were performed along with measurement of cardiorespiratory fitness.

Anthropometric Measures

All anthropometric measures were conducted with youth wearing lightweight clothing and without shoes by trained and certified research staff. Height was measured to the nearest 0.1 cm, and weight was measured to the nearest 0.1 kg. BMI was calculated as kilograms/meters squared, and BMI z score was derived from the gender- and age-specific standards published by the National Center for Health Statistics.6

Accelerometry

Objective PA data were collected by using the ActiGraph AM7164 accelerometer (ActiGraph, Pensacola, FL), which was worn at the waist and measured vertical acceleration. Previous studies have revealed that the ActiGraph accelerometer is an accurate measure of PA in children and adolescents.7

Participants received an accelerometer before their clinic visit and wore the accelerometer for a period of 7 days. Participants were asked to record in a diary the time at which they put on the monitors in the morning and the time they took off the monitors at night. At the end of the 7-day period, the participant returned the accelerometer and diary at his or her clinic visit. Data from the accelerometer were downloaded, processed, and screened for wear time by using a modified version of previously reported methods (http://riskfactor.cancer.gov/tools/nhanes). Average total activity counts per day were calculated by using summed daily counts detected over wear periods. Time in minutes spent in different activity intensities was calculated by using age-specific formulas for count cutoffs (metabolic equivalents [METS] = 2.757 + [0.0015 × cpm] − [0.0896 × age (years)] − [0.000058 × cpm × age]) corresponding to sedentary, light (1–3.99 METs), moderate (4–6.99 METs), and vigorous intensity (≥7 METs). (A MET is an estimate of relative intensity such that 1 MET is equal to 35 mL/kg/min basal oxygen consumption.)
MET represents the energy expenditure for an individual at rest, whereas a 10-MET activity requires 10 times that amount. These intensity levels were derived from a published age-specific energy expenditure prediction equation. To be included in these analyses, participants must have had accelerometer data that included 10 hours of wear time on ≥3 valid days. Non–wear time was defined as intervals of ≥60 consecutive minutes of zero counts with allowance for up to 2 minutes of observations of 1 to 100 cpm. Wear time was determined by subtracting non–wear time from the total observation time for that day. Time spent in sedentary behavior was defined as the amount of time accumulated in counts that were <100 cpm.

**NHANES Accelerometry**

During NHANES 2005–2006, accelerometry measures were collected from a nationally representative sample of US civilian noninstitutionalized adults and youth (N = 7086) by using the same accelerometer model (ActiGraph) and methodology as was used in TODAY. NHANES participants were asked to wear the device during their waking hours and to remove the monitor for water-related activities. For the data presented here, only participants aged 10 to 17 years old with accelerometer data that included ≥10 hours of monitor wear time on ≥3 valid days were included. PA variables for either TODAY or NHANES participants with 3 days of accelerometer data were not significantly different than those for participants with ≥5 days. Pregnant girls and/or children with missing BMI information were also excluded. The weighted characteristics for this sample were as follows: 51.5% boys, 62.4% non-Hispanic white, 14.6% non-Hispanic black, 15.5% Hispanic, 7.5% other race/ethnicity, and 16.3% obese. For the current comparison, only obese NHANES youth (BMI ≥95th percentile) were included (n = 312; 177 youth aged 10–14 years and 135 youth aged 15–17 years).

**Physical Work Capacity**

Cardiorespiratory fitness was assessed by using a submaximal test that determines the physical work capacity (PWC) of an individual by predicting the workload (kg*m) at a heart rate of 170 beats per minute with a Monark 818E cycle ergometer (Quinton Monark, Seattle, WA). The heart rate recorded from the last 5 seconds of each stage was used to extrapolate a PWC at a heart rate of 170 beats per minute (PWC-170). A best-fit line was used to approximate the workload of the bike at a heart rate of 170 beats per minute, resulting in an estimate of the PWC-170 expressed in kilogram*metres per minute. The raw measurement of PWC was divided by the participant’s weight in kilograms to provide a weight-adjusted estimate. Participants were required to complete the test within 24 hours of when their accelerometer data were downloaded, but not within 48 hours of an oral glucose tolerance test, because the test was to be performed nonfasting. Participants who weighed ≥350 lb were excluded from the PWC-170 assessment due to bike weight limits. In addition, the test was discontinued for individuals who were unable to maintain a minimal speed during the warm-up period. Individuals who failed to complete the test due to leg pain or lack of motivation or whose heart rate exceeded 170 beats per minute during the initial stage were also excluded.

**Trial of Activity for Adolescent Girls and Lifestyle Education for Activity Program PWC-170 Protocol**

The Trial of Activity for Adolescent Girls (TAAG) was a randomized controlled trial examining the effect of an intervention to reduce the decline of PA levels in middle-school–aged girls and was composed of 45% non-Hispanic white, 21% Hispanic, and 21% non-Hispanic black girls. The Lifestyle Education for Activity Program (LEAP) was an activity intervention that began in eighth-grade girls who were then followed through to high school and was composed of 51% non-Hispanic white and 49% non-Hispanic black girls. Both TAAG and LEAP used the PWC-170 to determine cardiorespiratory fitness with a similar protocol as that used in TODAY. Both TAAG and LEAP conducted the test with 2-minute stages; however, neither study provides information regarding exclusion criteria on the basis of participant weight. In addition, TAAG imputed values for participants who maintained proper cadence, but who completed only 2 stages. Assessments in TAAG and LEAP were conducted in girls aged 13 to 14 years.

Comparisons of baseline fitness and activity levels (3-day physical activity recall [3DPAR] below) between the TODAY girls and those from TAAG and LEAP were performed, although there were too few obese girls in the later 2 trials to limit those cohorts to obese girls as was done above with the NHANES data set.

**3DPAR**

Subjective PA data were measured by using the 3DPAR, which is a self-administered questionnaire of PA for the previous 3 days. The 3DPAR was administered during the clinic visit, after the participant’s use of the accelerometer, and asked about the recall of activity over the time period that coincided with the final 3 days of accelerometer monitoring. Trained interviewers guided the process of completing the questionnaire.

On the basis of a list of leisure, occupation, and daily living activities, participants were asked to record the main activity that they participated in during each 30-minute time increment over the past 3 days. They were also instructed to
estimate the intensity level of the activity (light, moderate, hard, or very hard). Intensity values (METs) were assigned to each of the activities and their 4 intensity levels listed on the questionnaire.\cite{16} PA from the 3DPAR was analyzed in terms of total hours of PA and total daily MET hours (the total being the sum of the various intensity levels).

**Statistical Methods**

All statistical analyses were conducted in SAS versions 9.1 and 9.2 (SAS Institute, Cary, NC). $\chi^2$ tests were used to compare the percentages of gender, age, race, and BMI categories among all study participants with those individuals who had complete data for the 3DPAR, the accelerometer, and/or the PWC-170 fitness test. Means, SDs, and SEs for TODAY were calculated by gender, age group (10–14 and 15–17 years), and race/ethnic group. Means and SEs for NHANES used weighting procedures, on the basis of the sampling strategy, to ensure that the results were representative of the US population. Independent sample $t$ tests were used to test the differences between groups, and $t$ tests for comparisons between NHANES and TODAY trial participants used the weighted SEs for the NHANES data.

**RESULTS**

A total of 699 adolescents participated in the TODAY trial. The number of youth who successfully completed each PA/fitness measure at baseline was 672 for the 3DPAR, 242 for accelerometry, and 527 for the PWC-170. The relatively smaller number of youth (34% of the entire cohort) with usable accelerometry data was due to both a computer error that accidentally erased the data was due to both a computer error and a computer error (Table 1). For all age and gender groups, the rates of obesity were high. In comparisons between both gender and age groups, the BMI $z$ scores were similar.

**Accelerometry**

The mean minutes per day spent in moderate to vigorous activity, light activity, total activity (moderate/vigorous plus light), and sedentary time as

| TABLE 1 Baseline Descriptive Statistics for the TODAY Study Cohort |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Boys 10–14 y | Boys 15–18 y | Girls 10–14 y | Girls 15–18 y |
| Anthropomorphic measures | | | | |
| $n$ | 114 | 133 | 279 | 173 |
| Weight, kg | 85.7 ± 27.8 | 112.6 ± 25.8 | 84.9 ± 19.8 | 100.1 ± 22.6 |
| Height, cm | 168.5 ± 10.6 | 174.4 ± 7.4 | 160.9 ± 7.4 | 163.5 ± 7.4 |
| BMI, kg/m² | 34.1 ± 8.4 | 37.0 ± 8.1 | 32.8 ± 6.5 | 37.3 ± 7.3 |
| BMI $z$ score | 2.3 ± 0.5 | 2.4 ± 0.5 | 2.2 ± 0.4 | 2.1 ± 0.4 |
| Accelerometry | | | | |
| $n$ | 45 | 50 | 98 | 51 |
| Sedentary, min/d | 495.3 ± 144.4 | 526.6 ± 143.4 | 479.0 ± 141.0 | 546.5 ± 143.0 |
| Light PA, min/d | 338.3 ± 80.9 | 350.1 ± 71.5 | 348.6 ± 75.4 | 344.9 ± 101.1 |
| Moderate–vigorous PA, min/d | 35.0 ± 26.0 | 26.3 ± 24.1 | 26.6 ± 18.3 | 8.2 ± 8.9 |
| Total activity* min/d | 373.3 ± 89.5 | 356.4 ± 76.9 | 375.2 ± 80.4 | 353.1 ± 104.2 |
| Physical fitness | | | | |
| $n$ | 81 | 102 | 209 | 135 |
| PWC-170, kg.m - min⁻¹ | 799.9 ± 261.1 | 909.4 ± 287.5 | 628.7 ± 201.7 | 649.2 ± 196.0 |
| PWC-170/kg | 8.8 ± 2.8 | 8.6 ± 3.2 | 7.61 ± 2.7 | 6.7 ± 2.0 |
| 3DPAR | | | | |
| $n$ | 108 | 131 | 271 | 162 |
| Sedentary, h/d | 8.0 ± 2.8 | 5.9 ± 2.9 | 5.6 ± 2.8 | 5.7 ± 3.0 |
| Light, h/d | 5.5 ± 2.2 | 6.5 ± 2.8 | 6.3 ± 2.3 | 7.1 ± 2.3 |
| Moderate–vigorous, h/d | 1.9 ± 1.8 | 1.3 ± 1.4 | 1.4 ± 1.3 | 1.1 ± 1.4 |
| Total activity* MET-h/d | 64.3 ± 18.9 | 58.9 ± 14.5 | 59.9 ± 15.1 | 58.3 ± 12.3 |

Data are presented as means ± SD unless otherwise indicated. $P$ values from $t$ tests represent age group comparisons within each gender, and gender comparisons within each age group.

*Total = light + moderate–vigorous PA.
* $P$ value for weight (in kg).
determined by accelerometry are presented in Fig 1 for both TODAY youth and the obese NHANES youth (BMI ≥95th percentile). These age-adjusted data are presented by gender and age group (10–14 and 15–17 years of age). The obese NHANES 10- to 14-year-old boys were more active than similarly aged TODAY boys for both total (P = .05) and light (P = .06) PA. The NHANES younger boys had higher moderate to vigorous activity levels than the TODAY boys (P = .01), although the mean time spent in moderate to vigorous activity for all age/gender groups was exceptionally low.

In comparisons of these 2 groups of obese youth, the most striking finding was the higher amount of sedentary time, averaging 56 minutes per day (P < .001), spent in the TODAY youth relative to the obese NHANES youth. Stratifying by gender, these results reached statistical significance in boys but not in girls.

**PWC-170 Bike Test**

Comparisons for baseline PWC-170 adjusted for weight (kg) between the TODAY girls and those girls who participated in TAAG and LEAP indicate that the girls in TODAY had lower cardiorespiratory fitness. The baseline weight-adjusted PWC of girls in LEAP and TAAG (aged 13–14 years) seemed to be similar to mean values of 11.6 ± 3.5 and 11.68 ± 3.57, respectively. However, the 13- to 14-year-old girls in TODAY had a substantially lower mean value (7.49 ± 2.49). Similar findings were noted for non–weight-adjusted values (not shown here).

**3DPAR**

In comparison with other studies, girls in TODAY had lower reported PA levels on the basis of the 3DPAR. Specifically, girls in TAAG reported a mean of 68 MET-hours/day for sixth graders and 67 MET-hours/day for eighth graders compared with the TODAY girls who reported ∼58 to 60 MET-hours/day for both age groups.

**DISCUSSION**

The TODAY study population is one of the largest cohorts of ethnically and geographically diverse youth with type 2 diabetes ever gathered. This current effort suggests that the cohort tended to be less active, less physically fit, and...
more sedentary when compared with similarly aged youth without diabetes from other large-scale investigations. Cross-sectional examination of PA levels in the TODAY youth with type 2 diabetes compared with similarly aged obese youth from the NHANES data set revealed that the levels of moderate to vigorous activity performed per day in both obese cohorts for all age groups were exceptionally low and that the younger TODAY boys were even less active than similarly aged NHANES youth. Most consistently, TODAY youth, as a whole, spent significantly more time (close to an hour extra per day) being sedentary than the obese NHANES youth, underscoring the need for activity intervention at both ends of the activity spectrum. This difference in sedentary time could not be explained by methodologic issues such as seasonality (as both studies recruited over all seasons) or wear time. Future lifestyle interventions should target the amount of time spent sitting rather than focusing exclusively on increasing the amount of moderate to vigorous activity performed per day as both appear to be a problem in this obese diabetic cohort and both affect energy expenditure.

In comparisons with other large-scale investigations in girls, TODAY girls appeared to have a relatively lower cardiorespiratory fitness than similarly aged girls who participated in TAAG and LEAP.13,14 Also, girls in TODAY reported lower PA levels on the basis of the 3DPAR than did similarly aged girls from TAAG.17 These findings are consistent with results from the SEARCH case-control study in which youth with type 2 diabetes were less active than nondiabetic controls or youth with type 1 diabetes.18

The current findings need to be considered in light of the fact that the TODAY study participants were more obese than those in the comparison studies such as NHANES. The subset of obese youth (BMI ≥ 95th percentile) participating in NHANES had an average BMI of 29.9 for boys and 30.9 for girls, whereas similarly aged TODAY boys and girls had an average BMI of 34.6 and 34.3, respectively. The fact that this subgroup of youth with type 2 diabetes was more obese than the most obese 5% of the NHANES population is not surprising given the fact that obesity and type 2 diabetes are extremely coupled. This coupling of obesity and diabetes makes the independent effect of either condition relatively impossible to sort out.

It is important to note that youth who participated in TODAY had to undergo an extensive run-in period before random assignment. Those who successfully completed the run-in phase had a decrease in weight relative to the youth who failed the run-in phase. Therefore, it is possible that the youth participating in the TODAY trial and included in the current analyses were healthier, more motivated, and/or more active relative to youth with type 2 diabetes who failed the run-in. The implication of this finding is that the levels of activity and fitness in the general population of type 2 diabetic adolescents may be even lower than presented in this current effort. This interpretation is consistent with previous findings in adults that revealed that individuals with prediabetes who join a lifestyle intervention trial are more active and likely more motivated than those in the community who are also eligible but choose not to participate.19

CONCLUSIONS

In this national study involving the largest cohort of youth with type 2 diabetes to date, the results indicate that youth with type 2 diabetes have poor activity habits, spending little time in light and moderate to vigorous intensity activities and high amounts of time in sedentary activities. In addition, the very low cardiovascular fitness of these youth with type 2 diabetes has important implications for their future cardiovascular health as they enter adulthood. Intervention efforts that focus on increasing PA levels and decreasing time spent in sedentary behavior should be a priority for these youth with type 2 diabetes.

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REFERENCES


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