**Introduction** Increasingly, physical activity (PA) and sedentary behaviour (SED) are independently being associated with physical, mental and emotional well-being. The heightened research interest in this area has resulted in an upsurge of accelerometer usage to objectively quantify PA and SED. However, these epidemiological investigations consistently ignore systematic variation in the number of hours participants wear accelerometers each day (i.e., systematic accelerometer wear-time variation), and this has a direct impact on measured activity.

**Objectives** Using three different cohorts (3–5 years – N=83; 10-14 years – N=455; and 18 years and older – N=21), this study aims to validate a standardization methodology to minimize measurement bias due to accelerometer wear-time variation.

**Methods** In epidemiological studies, accelerometry is generally conducted over seven consecutive days, and participants’ data are considered ‘valid’ only if wear-time is at least 10 hours/day. However, there could be systematic wear-time variation even within ‘valid’ data. To explore this variation, accelerometer data from two studies set in Saskatoon, Saskatchewan were analyzed: Smart Cities, Healthy Kids (smartcitieshealthykids.com) and Healthy Start (http://www.canadainmotion.ca/healthy_start/). Subsequently, a standardization method was developed where case-specific observed wear-time was controlled for using an ‘analyst specified’ time period. Next, case-specific accelerometer data were interpolated to this controlled wear-time to produce standardized variables. To understand discrepancies owing to wear-time variation, identical analyses were conducted with data from all three cohorts both pre- and post-standardization. The results of these identical analyses were compared to objectively validate the standardization methodology.

**Results** In all three cohorts, descriptive analyses revealed systematic wear-time variation between participants. Again, in all three cohorts, pre- and post-standardized analyses of the three outcome variables (SED, light physical activity and moderate vigorous physical activity) that cover the whole range of human activities revealed an identical and often significant trend of wear-time’s influence on activity. For instance, SED which was consistently higher during weekdays pre-standardization, proved to be higher during weekends post-standardization.

**Conclusions** Analyzing accelerometer data without standardizing wear-time will cause biased results and erroneous conclusions. Standardizing accelerometer data using the above mentioned methodology produces stable variables and a uniform platform to compare results between studies, irrespective of the studies’ sample size or age characteristics.