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Biomarkers associated with sedentary behaviour in older adults: a systematic review

Running title: Sedentary behaviour and biomarkers

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Highlights of "Biomarkers associated with sedentary behaviour in older adults: a systematic review"

- Focus on the relationship of sedentary behaviour and biomarkers especially in older people aged 60 or above.
- Intensive summary of all available information not only biochemical biomarkers but also performance biomarkers etc.
- Clear separation between sedentary behaviour and simple lack of physical activity.
- Objective assessment of results by categorisation with CADTH tool*
- Results almost exclusively cardio-metabolic biomarkers; only few inflammation and performance biomarkers were evaluated too

*for more details please see: https://www.cadth.ca/interventions-directed-professionals

Abstract (word count 247/250)

Objective: Pathomechanisms of sedentary behavior (SB) are unclear. We conducted a systematic review to investigate the associations between SB and various biomarkers in older adults.

Methods: Electronic databases were searched (MEDLINE, EMBASE, CINAHL, AMED) up to July 2015 to identify studies with objective or subjective measures of SB, sample size ≥ 50, mean age ≥ 60 years and accelerometer wear time ≥3 days. Methodological quality was appraised with the CASP tool. The protocol was pre-specified (PROSPERO CRD42015023731).

Results: 12701 abstracts were retrieved, 275 full text articles further explored, from which 249 were excluded. In the final sample (26 articles) a total of 63 biomarkers were detected. Most investigated markers were: body mass index (BMI, n=15), waist circumference (WC, n=15), blood pressure (n=11), triglycerides (n=12) and high density lipoprotein (HDL, n=15). Some inflammation markers were identified such as interleukin-6, C-reactive protein or tumor

necrosis factor alpha. There was a lack of renal, muscle or bone biomarkers. Randomized controlled trials found a positive correlation for SB with BMI, neck circumference, fat mass, HbA1C, cholesterol and insulin levels, cohort studies additionally for WC, leptin, C-peptide, ApoA1 and Low density lipoprotein and a negative correlation for HDL.

Conclusion: Most studied biomarkers associated with SB were of cardiovascular or metabolic origin. There is a suggestion of a negative impact of SB on biomarkers but still a paucity of high quality investigations exist. Longitudinal studies with objectively measured SB are needed to further elucidate the pathophysiological pathways and possible associations of unexplored biomarkers.

Key words: older adults, sedentary behaviour, biomarker

Introduction

According to the National Institute of Health (NIH) Biomarkers Definitions Working Group[4], a biomarker is a characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or a pharmacological response to a therapeutic intervention. Therefore, biomarkers can be very helpful as surrogate markers for diseases or pathophysiological links between exposure and disease; or as intermediate measures of the effectiveness of interventions on disease processes. Within the past few decades, a considerable amount of literature has clearly demonstrated that physical activity (PA) has a range of benefits on the health[1,2] and wellbeing of older adults[3]. Recently, there has been an interest in understanding the biomarkers underlying the response to PA. For example, in a cohort of community dwelling older adults, levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) and high-sensitive troponin T have been associated with objectively monitored PA and showed a more beneficial profile with increasing PA, suggesting a dose response relationship[7,8]. To date, most of the PA biomarker research has focussed on cardiovascular risk factors[5,6,7], but there are many other biological systems with associated biomarkers which may be affected by PA or especially SB. Recent examples include β-amyloid burden and glucose metabolism as markers of neurodegeneration[18], interleukin-6 (IL-6) and C-reactive protein (CRP) as markers for systemic inflammation[19] or DNA-repair as a marker for cell homeostasis[20].

An emerging evidence base has started to demonstrate that sedentary behaviour (SB), over and above time spent in PA, is independently associated with several important detrimental health outcomes, including endpoints such as mortality, frailty, sarcopenia, dementia, and cardiovascular diseases[9]. According to the Sedentary Behaviour Research Network, SB is defined as any waking behaviour characterised by an energy expenditure ≤ 1.5 metabolic equivalents (METs) whilst in a sitting or reclining posture[10]. The emerging research highlighting the deleterious impact of SB on health is of particular concerns as adults spend on average 5 hours of their time in sedentary behaviour[11]. Indeed, some studies have demonstrated that on the population-level sedentary time (ST) increased over the decades from 1960-2010[12]. Especially older people spent most of their time in SB. A recent meta-analysis illustrated that older people were sedentary for 65-80% of their waking time[13], other sources mentioned ST with an average of 9 hours[14] to 13.8 hours per day[15]. Older people are seen as the age group engaging in the highest level of SB[13] and thus could benefit most from changing their daily habits. The developing evidence on the harms associated with SB has illustrated that it is not only the absence of daily or weekly moderate-to-vigorous physical activity (MVPA), but rather, SB is a separate category of behaviour with unique determinants, consequences and sequences for possible intervention[16].

Considering the physiological changes occurring with age in several organ systems[17], results from middle-aged adults can't be simply transferred to older adults. Therefor the EU study SITLESS investigates how SB can be reduced sustainably and how sedentariness effects biomarkers especially in older adults. In this framework the interest on outcomes of studies performed in elderly, assessing SB and its impact on biomarkers was the focus. In addition, biomarker studies are important to further understand the link between SB, PA and adverse health outcomes like total mortality and harmful phenotypes like Metabolic Syndrome (MES)[21], frailty or sarcopenia. Perhaps it can help to understand the role of biomarkers as possible mediators of the association between SB and adverse phenotypes or aging-related diseases. Therefore, the aims of this systematic review were to provide a comprehensive overview of aging-related biomarkers associated with SB and report on the strength of the observed associations in community-dwelling older adults.

Methods

Study design

This systematic review adhered to the PRISMA guidelines[22] and followed a predetermined published protocol (PROSPERO No. CRD42015023731)[23].

Condition or domain being studied

SB, as defined by the Sedentary Behaviour Research Network[10] (waking behaviour with an energy expenditure ≤ 1.5 METs whilst in a sitting or reclining posture), represented our exposure of interest. We also considered studies which did not fully comply with this definition (e.g. television watching time, SB identified by other questionnaires or accelerometer data that do not allow for disentangling posture issues or clearly indicate METs) but are highly relevant to SB.

With respect to the biomarkers we were interested in any inflammatory, renal and cardiac biomarkers, lipids and metabolic markers, genetic and metabolomics markers, endocrine markers and markers of muscle strength, body composition, as well as of specific physical performance measures (e.g. gait speed and balance).

Information sources and searches

Two authors (KW, BS) searched the electronic databases: MEDLINE (PubMed), EMBASE, CINAHL (via EBSCO), AMED (via Ovid/EBSCO) from inception to 15 July 2015. We used search terms described in appendix 1. Appropriate search strategies and MESH-terms were selected (see appendix 1).

Study selection and eligibility criteria

Studies meeting the following criteria were included:

1) Explicitly measured SB using objective (accelerometer wear time ≥ 3 days (to follow the recommendations of good clinical practice[24]) or self-report instruments. Studies defining SB purely as a lack of PA were excluded.

2) Including community dwelling, older adults (mean age of sample ≥ 60 years).

3) Sample size of n ≥ 50 participants, to ensure adequate power.

4) Quantitative study design including randomized controlled trials (RCTs), controlled clinical trials (CCTs), pre- and post-intervention measurement studies, prospective observational studies (POS), or

studies (only prospective trials) that examined an association of any biomarker with SB. We also considered cross-sectional studies (CSS) but present them separately because of their descriptive nature due to the inability to clearly establish the temporal sequence between SB and biomarkers.

Participants and population

We selected studies, with the above mentioned characteristics that included older adults (mean age ≥ 60 years) conducted in the community.

When we encountered studies with a large age range and a mean age below 60 years, indicating the study included some older adults (> 60 years), we attempted to contact the authors to acquire the variables of interest for all participants with an age of 60 years and older. Populations with specific co-morbidity (e.g. diabetes mellitus type 2 (DM-II), peripheral artery disease (PAD), chronic obstructive pulmonary disease (COPD) were included, but critically evaluated and highlighted as such.

Data extraction

All results of the searches were inserted in a bibliographic database. A data extraction form was created and amended to the requirements of the review. Two authors piloted (KW; SB) the data extraction form in a random sample of 3 studies that employ different study designs. This ensured that the relevant information was selected to assess the effectiveness and study quality.

All data were extracted by these two reviewers. Data extraction included: first author, country, setting, population, aims of the study, type of the study (RCT, POS or CSS), number of studies and participants included in the article, details of the intervention (including duration), inclusion criteria, type of recruitment, type and definition of SB or PA used, biomarkers analysed and results, details of control condition, overall study quality (internal risk of bias), association statistics, acknowledged limitations by authors, the authors' conclusions and other notes.

Any disagreements in data extraction were resolved through discussion between the reviewers.

Risk of bias and quality assessment

Assessment of studies followed the PRISMA[22] guidelines. Two authors conducted the methodological quality appraisal of all included studies using a modified Critical Appraisal Skills Programme (CASP) tool, adapted for each study design[25]:

- RCTs (max. CASP score = 6) were assessed for risk of bias in the following domains: clearly focused issue, randomization, performance (blinding, personnel), comparability (treatment, groups at baseline) and attrition (participants accounted for at its conclusion).
- POS (max. CASP score = 8) were assessed for risk of bias in the following domains: clearly focused issue, selection and recruitment (random approach or representative for a defined population, accuracy of measurement (exposure, outcome), identification of important confounding factors, adjustment for confounding factors and follow-up (period, completion).
- CCS (max. CASP score = 6) were assessed for risk of bias in: clearly focused issue, selection and recruitment (random approach or representative for defined population), accuracy of measurement (exposure, outcome), identification of important confounding factors and adjustment for confounding factors.

In an attempt to assess the potential effect and direction of the effect of SB on specific biomarkers, additional information related to statistical evidence of an association, as adapted from the Canadian Agency for Drugs and Technology in Health (CADTH)[26], was included in Table 2 for the high quality studies. The following decision rules as suggested by CADTH[26], were used for standardized statements about the statistical significance:

- 0% of studies showed statistically significant results = no evidence for any association
- 1% to 33% of studies showed statistically significant results = generally no evidence for any association.
- 34% to 66% of studies showed statistically significant results = mixed evidence for association
- 67% or more studies showed statistically significant results = generally evidence for association

Due to the few studies of high quality, we decided to apply this method of categorisation, although often less than 5 studies with statistically significant results were found. To ensure a minimum level of validity, we applied this tool in all biomarkers measured in ≥ 3 studies (RCT and/or POS).

Strategy for data synthesis and subgroup analysis

We tabulated the single study results and grouped them according to comparable biomarkers. All results were stratified with appropriate subgroup analyses, for instance according to exposure type (SB and PA separately), type of SB/PA assessment (questionnaire- versus sensor-based), biomarker type and study design (RCT and CCT separately). We anticipated conducting a meta-analysis if sufficient homogeneity was evident across the study types and outcomes of interest and enough studies could be identified in comparable areas.

Results

Results of the literature search

Our initial searches identified 12,701 hits. After the exclusion at title level, removing of duplicates and the matching of results from the two independent reviewers (including removing duplicates), a final list of 275 full-text articles was scrutinised. 235 articles were subsequently excluded according to our in- and exclusion criteria (full details in **figure 1**). 3 studies included people with a large age range in their sample, yet a mean age below 60 years. Upon 3 attempts to contact the authors, 1 group (Aadahl and colleagues[27]) provided additional data, whilst 2 authors did not respond and were subsequently excluded due to age < 60 years (Knight et al.[28] and Mohri et al.[29]).

After exclusions, 40 studies were considered eligible, however after further revision and evaluation, another 14 articles (1 POS, 13 CSS) were excluded (for more details see "risk of bias (quality) appraisal"), thus leading to a total of 26 articles (4 RCT, 2 POS and 21 CSS). The study from Cooper at al.[30] was included as a POS and CSS due to longitudinal and cross-sectional analysis of the data reported by the study authors.

Definition of Sedentary Behaviour

We found a highly heterogeneous definition of SB, which was often misclassified as simply the absence of PA and therefor 134 papers were excluded. The most frequent definition of SB was total time spent at less than 100 counts per minute using data from an accelerometer[5,30–38]. Henson et al.[39] defined SB in a similar way but with smaller epochs of less than 25 counts per 15 seconds. Other authors used the same definition of SB as used in this review with less than 1.5 MET[31,40,41]. Some studies did not define SB at all[27,42].

A total of 14 studies (3 RCT, 1 POS, 11 CSS, whereas Cooper et al.[30] were included as POS and CSS) measured SB with sensors or accelerometers, another 2 CSS[31,38] with both, accelerometer and questionnaire, while 10 studies measured SB by questionnaires only. Of these 10 questionnaires, 6 enquired about TV watching time (1 POS, 5 CSS), whilst the remainders included more detailed questions about SB (1 RCT, 3 CSS).

Characteristics of included studies

Randomized controlled trials (RCTs)

An overview of the RCTs is listed in **Table 1a**. Overall, a total of 397 participants in the RCTs were represented (Intervention Group, (IG): 245; Control Group, (CG): 152). Although SB was evaluated, the primary aims of 2 RCTs were to increase PA but not reduce SB. 3 RCTs[27,41,43] captured SB objectively with

an accelerometer (ActiGraph), whereas Kallings et al.[44] evaluated SB with the International Physical Activity Questionnaire (IPAQ), which consists of 2 questions on the amount of sitting time in the last 7 days; one for average weekday and one for average weekend day.

Intervention was mainly focused on increasing habitual PA. This was triggered through different processes: an intervention with pedometer-use plus a weekly visit on an interactive website with the aim of increasing PA level by 10 % each week up to 10,000 steps/day[41]; written PA prescription with the aim of increasing PA level to 30 min MVPA per day[44]; a written PA pack with a self-instructional workbook based on a trans-theoretical model of behaviour change[42]. Only 1 study focused on decreasing SB with 4 main aims, such as decreasing daily TV viewing time, substitute sitting with standing, break up prolonged sitting time and a maximum of 30 minutes of sitting per episode[27].

Prospective observational studies (POS)

An overview of the POS is listed in **Table 1a**. In the 2 cohort studies[30,45] a total of 846 participants were represented. Fung et al.[45] used a questionnaire focusing on the number of hours of television watching to measure SB in men, whereas Cooper et al.[30] used objectively measured SB time by accelerometer measurements (ActiGraph).

Cross-sectional studies (CSS)

A total of 41,816 participants were included across the 21 CSS. The characteristics of these CCS are listed in **Table 1b**. Most of the studies focused on SB and its association to biomarkers (16/21 studies), from which 5 focused on TV watching time. 2 other studies investigated both SB and PA as exposure[34,36], 1 study[46] calculated sitting time, whereas 2 studies evaluated SB as secondary outcome[5,6].

The majority of the studies used objectively measured time of SB by accelerometer (11/21 studies). 2 studies evaluated both objectively measured SB and SB measured by questionnaire[31,38]. 5 studies focused on time spent with TV watching and 3 used the following questionnaires: Reaven et al.[6] adapted a questionnaire from the Health Interview Survey, which measured 17 different leisure time activities in the last 2 weeks; Larsen et al.[46] measured daily SB by asking about time spent being sedentary on a typical weekday; Allison et al.[47] evaluated the time spent being sedentary by using the "Typical Week Physical Activity Survey" which measures SB in the last 7 days.

Sedentary Behaviour and biomarkers

Overview of biomarkers explored in the literature

Table 2 provides an overview of the associations between SB and each biomarker system including: anthropometric parameters, systemic parameters, blood lipids, glycaemic parameters, performance biomarkers, inflammatory biomarkers and others. A total of 63 biomarkers were evaluated (counting ratios of different biomarkers separately). **Table 3** considers the specific biomarker results within each study design. There was insufficient homogenous data to perform a meta-analysis. Therefore, we describe the number of studies that explored each biomarker and the summary statistics reporting the overall proportion of these studies that found a statistical association. Only the statistically significant results from the multivariable analyses are shown. If significant, biomarkers showed evidence for an unfavourable association with higher ST. Body mass index (BMI, 9 of 15 of the studies significant), waist circumference (WC, more than 8 of 15 of the studies significant), insulin (4 of 8 studies significant) and high density lipoprotein (HDL, 6 of 15 studies significant) were examined in a lot of studies and demonstrated the most reliable results. For a more detailed description see **Table 2 and 3**.

We identified 4 "risk population" studies. 1 POS of Cooper et al.[30], performed in diabetes type 2 patients, showed a (statistically significant) positive correlation for SB with WC, HDL, insulin and HOMA-IR. The CSS from Cooper et al.[40], also performed in diabetes type 2 patients, revealed a positive association for SB with WC, too. The study from Lee et al.[33], performed in the high risk osteoarthritis population showed lower gait speed and lower chair stand rate associated with higher levels of SB. There were no statistically significant results for the study of Lynch et al.[34] investigating the association between SB with BMI, WC and insulin in a breast cancer survivor cohort.

Risk of bias (quality) appraisal

After revising the 40 articles by CASP criteria[25] and general quality criteria (correctness of data illustration, selection or reporting bias, misclassification etc.) we excluded 13 CSS for the following quality linked issues [CASP score] and 1 POS:

1) SB was not sufficiently measured: Ewald et al.[43] [3 of 6] and Bianchi et al.[48] [2 of 6] evaluated time spent being sedentary with the Physical Activity Scale for the Elderly (PASE) and Kaino et al.[49] [2 of 6] used the Japan Arteriosclerosis Longitudinal Study Physical Activity Questionnaire (JALSPAQ) which are good instruments to measure PA but weak in calculating SB; Calderon-Garcia et al.[50] [4 of 6] calculated ST by asking "How much do you exercise or strain yourself physically in your leisure time?'', which had poor validity.
2) Missing information about recruitment or cohort characterstics[51];

3) SB defined as simply being the opposite of PA, as in Gaba et al.[52] [3 of 6] or unclear definition of sedentariness (Elkan et al.[53] [1 of 6], Belza et al.[54] [3 of 6]);

4) Poor quality of exposure or outcome assessment; e.g. Inoue et al.[55] [3 of 6] calculated BMI by self- reported weight and height, among other issues;

5) Missing evaluation or lack of adjustment for important confounding factors (comorbidities, medication status etc.), e.g. Li et al.[57] [2 of 6], Babaroutsi et al.[58] [3 of 6] and others[51,53–55];

6) Evidence of selection bias – like in Knight et al.[59] [2 of 6]) who compared a cohort from Day Care Centre to a cohort from a bowling club;

7) Implausible or irreproducible data – e.g. implausible data of sample size and sample origin (Azzabou et al.[51] [3 of 6]);

8) Biomarker calculated by self-report, like BMI from self-reported weight and height or SB and biomarkers weren't measured at the same point in time (Scott et al. 2015[60]);

9) We excluded the POS from Wijndaele et al.[56] [3 of 8] because BMI was calculated by self-report weight.

After the exclusion of these studies only 2 articles[6,61] with a low CASP-score ≤ 3 remained. The mean CASP score of RCTs was 5 out of 6, for cohort studies 5 out of 8 and for CSS 4.5 out of 6.

Relation of SB and biomarkers

Sedentary behaviour and anthropometric and systemic biomarkers

Of the 15 studies exploring this biomarker, 9 demonstrated a positive association, including 1 RCT[44] and 1 POS[45] study (**Table 2, 3**), whereas 2 RCTs[41,42] didn't show statistically significance, thus there is mixed evidence for the association of SB to BMI. WC was also positively associated with SB in 1 POS[30] and 7 CSS, but were not statistically significant in 4 RCTs. Relationships between SB and both systolic BP (3 of 11 studies reporting this biomarker found positive association) and diastolic BP (1 out of 10 studies found a positive associations) were found, whereas the majority showed non-significant results. Neck circumference and fat mass were positively correlated to SB but were investigated in only one RCT. There was only limited or no evidence for the other anthropometric biomarkers (see **Table 2 and 3**).

Sedentary behaviour and blood lipids

Total cholesterol, HDL, low density lipoprotein (LDL) and triglycerides were the main focus in the investigated studies. If statistically significant association was prevalent, it was in an unfavourable direction. For total cholesterol positive association was found in 1 RCT[44], whereas the 3 RCTs[27,41,42] and 1 POS[45] didn't show any statistically significant association. HDL was statistically significant negatively associated with SB in 2 POS[30,45] but results in 4 RCTs[27,41,42,44] were statistically not significant. Similar results were detected for the other blood lipids (see **Table 2** and **3**). Most RCTs didn't show statistically significant results, hence there is generally no evidence for an association of SB and blood lipids. Results linking SB and blood lipids mostly derived from CSS studies and thus should be interpreted accordingly.

Sedentary behaviour and glycaemic biomarkers

There was some indication found of an unfavourable impact of SB on fasting insulin levels, with statistically significant associations in 1 RCT[27] and 1 POS.[30] However 1 RCT[41] and 1 POS[45] didn't show any association, which lead to mixed evidence for a possible impact of SB on insulin levels. For HbA_1c, only 1 RCT[44] was statistically significant. HOMAR-IR[30] and C-peptide[45] were positively correlated to SB in 1 POS. Glucose levels did not appear to be related to SB in 3 RCTs[27,41,44]. Initially equivocal results in 2 CSS, with 1 positive[32] and 1 negative association[62] were clarified by contacting the author. In both studies SB was associated with higher blood glucose levels. The impact of SB on glycaemic biomarkers was limited and largely restricted to CSS (**Table 2 and 3**), precluding definitive conclusion.

SB and muscle or physical performance biomarkers

Muscle tissue, performance, strength or other performance components were measured in 5 CSS (Bann et al.[31], Santos et al.[36], Sardinha et al.[37], Lee et al.[33], Larsen et al.[46]). 4 CSS also evaluated the association of SB and some performance biomarkers. Lee et al.[33] found a statistically significant negative correlation for SB with gait speed and chair stand rate. Santos et al.[36] constructed a composite Z-score of 6 performance biomarkers (6 minute walk test, 8 foot up and go, arm curl, chair stand rate, chair sit and reach or back scratch) which association with SB was significant negative, but he did not list the results separately. Bann et al.[31] and Sardinha et al.[37] did not find a significant correlation for SB and performance biomarkers.

SB and inflammatory biomarkers

There was a relative paucity of studies investigating inflammatory biomarkers and SB. CRP was investigated most frequently, although restricted to 4 CSS studies and 1 RCT, with only 2 CSS studies demonstrating that SB was positively associated with CRP. Only 2 CSS studies investigated IL-6 and SB, with 1 CSS finding a positive association. Given the limited number of studies and over reliance on CSS, the evidence base is inconclusive concerning the relationship between SB and inflammatory markers.

SB and other biomarkers

There was a distinct lack of studies investigating renal or bone biomarkers and SB. Only 1 study measured Vitamin D status[60], but it was considered as too low in quality (see 9) in "Risk of bias appraisal"), because different points of time exposure and outcome were measured.

Leptin, which can be seen as adiposity-associated inflammation marker or regulation marker of hunger and fat metabolism, was higher with a higher amount of time spent sedentary (2 of 4 studies significant, 1 POS).

We could not identify any study investigating renal, cellular, respiratory, signal transduction or genetic biomarkers and SB meeting our inclusion criteria. None of the included studies evaluated the impact of SB on biomarkers of the gastrointestinal or peripheral/central nervous system, neither focused on steroid or hormone biomarkers.

Discussion

Within our comprehensive systematic review, findings from high quality papers showed mixed evidence for the association of SB and biomarkers. When statistically significant results were prominent, SB was associated in an unfavourable direction, especially in anthropometric (BMI, WC, neck circumference, fat mass), blood lipid (cholesterol, HDL, LDL), glycaemic (HbA_1c, insulin, HOMA-IR, C-peptide) and hormonal (leptin) biomarkers. However several statistically non-significant study results were detected, many of which were of high quality. Some results of lower quality studies may be incidental findings or point to the existence of additional confounders, which are unaccounted so far.

Despite the relative paucity and equivocal nature of SB and biomarkers in older age, studies performed in younger cohorts strengthen the hypothesis that SB has harmful effects on biomarker levels. For instance, Healy et al.[67,68] found an inverse relation of breaks in ST and BMI[67] and WC[67,68] or Zhou[69] revealed an increased risk for developing Metabolic Syndrome (MES) with higher ST support those findings. Fasting insulin levels, another MES risk factor, improved with reducing ST[27,30]. Similar results for glycaemic biomarkers, such as postprandial glucose and insulin levels were detected in other RCTs[70,71] or CSS[72] performed in younger cohorts. Considering results from Krogh-Madsen[73], showing a decrease in insulin-stimulated muscle activity phosphorylation and decreased peripheral insulin sensitivity by reducing daily activity for only 2 weeks, there appears to be a strong connection between SB and impaired glucose and insulin metabolism in younger age.

Our review identified some studies that evaluated the association between change in ST and systemic parameters, including blood pressure[5,6,63], or heart rate[6]. Surprisingly and contrary to our expectation we identified no association between change of SB with blood pressure in 3 included RCTs[41,42,44]. Investigations in younger cohorts demonstrated a clear trend of significantly improving BP levels by reducing ST[74] or by breaking up prolonged sitting periods[75]. Already the advice of increasing PA levels seems to have a positive effect coming along with lower BP levels[76]. Possible explanations for no effects in older cohorts could be confounding by antihypertensive medication, increased arterial stiffness or reduced heart rate variability[77] in older age. Similar effects were detected for blood lipids, with better profiles associated to less ST[78]. As underlying mechanism Hamilton et al.[79,80] suggested a poor lipid metabolism with inactivity by suppression of skeletal muscle lipoprotein lipase activity. SB has also been associated with chronic low-grade inflammation in younger cohorts[72,81]. When looking in elderly people we only identified few, mainly CSS[32,39,45,47,66], showing higher levels of CRP, IL-6 and leptin in those with less physical activity. Besides missing longitudinal data a higher low-grade inflammation[82] in older age could distort or reduce the effect size of these outcomes.

Over and above preserving autonomy in older age is important in order to maintain independence and quality of life. Dunlop et al.[14] reported a 46% greater odds of ADL disability for each hour spent sedentary. Muscle function[83] also appears to be negatively affected by SB suggesting that macroscopic/performance[15] and microscopic/biochemical parameters[83] would change depending on ST. The results found for our systematic review were few. Results from Santos et al.[36] , who constructed a composite Z-score out of different performance biomarkers, suggests a negative association for performance biomarkers with SB, but no longitudinal data of performance or muscle biomarkers is available and thus drawing of causal conclusions is not possible. Given this, future prospective studies should prioritise functional assessments like the short physical performance battery (SPPB), grip strength and dynamic muscle function. Such measures are easy to ascertain with an evaluated predictive profile and can serve as modifiable surrogates of autonomy in later life.

The highlighted results of the four "risk population" studies showed associations for SB with biomarkers in the same direction as the studies performed in non-risk populations. The results from Lee et al.[33], performed in the high risk osteoarthritis population with lower gait speed and lower chair stand rate associated with higher levels of SB can be argued over. This is the only study, which showed (remaining) statistically significant results for performance parameters. Even if SB measurements were adjusted for osteoarthritis pain index, osteoarthritis symptoms and other comorbidity indices, there could be still another unknown confounder, related to osteoarthritis triggering this biomarker outcome.

Surprisingly, there was an absence of studies (meeting our inclusion criteria) investigating SB and its possible impact on renal, muscle or bone biomarkers performed in the elderly. There is however good reason to believe that especially bone and muscle metabolism is influenced from SB due to multifactorial processes. Prioreschi et al.[84] results from a smaller cohort revealed low bone mass for higher levels of SB and a possible protective effect for bone mineral density with breaking up ST more frequently. Even in younger cohorts, ST has been implicated as being negatively related to changes in whole-body bone mineral density, lumbar spine bone mineral content, lumbar spine bone area and femoral neck[85].

A large number of studies were excluded from our review because they specifically measured PA rather than focus on the distinct construct of SB. For instance, several studies focussed on a lack of PA rather than SB[42,44]. Recently there is a rising interest of SB consequences and the idea of clearly differentiating between the distinct behaviours of SB and PA. In this direction, Gibbs et al.[86] demonstrated a higher effectiveness for improving the SPPB score by reducing SB compared to increasing moderate to vigorous PA. For that reason, biomarkers should be evaluated for both, PA and SB. Former investigations have shown that SB effects on biomarkers are independently of MVPA levels[40,68,72]. Additionally reducing inactivity often has a higher effectiveness on the biomarker level, than the amount of physical activity

itself[70,71]. For that reason new studies should investigate biomarkers and health outcomes with focusing on reducing SB.

Whilst our comprehensive review provides novel insights, some limitations should be mentioned. First, we identified relatively few high quality or longitudinal studies investigating SB and biomarkers specifically in older adults. Therefore, we were not able to conduct a meta-analysis as we anticipated. Additionally the CADTH tool[26], used for standardized statements about the statistical significance, was adapted, so we were able to apply it to fewer studies available. This should be considered, when rating the state of evidence. Second, there were no stratified analyses assessing the question if age or gender is a possible effect modifier. Both, age and gender were often added into the analysis as confounders, but there is still the necessity to evaluate the possible presence of interaction in the association between sedentary behaviour and different biomarkers. Third there was considerable heterogeneity in the definitions of SB and the high diversity of reported outcome-parameters, again a pertinent factor making meta-analysis impossible. SB was often misclassified as simply a lack of PA. With respect to the performed analyses some studies measured the mean change[31,44], others calculated odds ratios[64] or Pearson correlation coefficients[5], whereas others calculated a linear or multiple regression coefficient[39,40,47]. Strict definitions focussing specifically on SB are necessary to allow comparison of results from different studies. There are currently several initiatives attempting to harmonize these approaches such as the standardised definition of SB published in 2012[10], the 2011 launched online "Sedentary Behaviour Research Network" (SBRN)[87] or the SIT project from Skelton and Chastin[88]. There are some initiatives aimed tackling SB. A large Canadian organization called ParticipACTION[89] is trying to help Canadians to sit less by offering age adjusted activity programs. Similar intentions are given in the multi-centre EU study SITLESS[90] with the aim of reducing SB in elderly by a PA intervention enhanced by self-management-strategies. Objectively measured SB will be correlated with several biomarkers and muscle biopsy results to further elucidate the biochemical influence of SB on health outcomes.

Currently, we have limited understanding of the impact of SB on different biomarker systems in older age. The current knowledge base in this regard is overwhelmingly based upon CSS. Given our findings, there is an urgent need for adequately representative, prospective cohort and randomized controlled studies to investigate the impact of SB on various biomarkers in order to ascertain a better understanding of the pathophysiological and also to test the hypothesis for causality. Besides the majority of studies were of moderate to high quality, the presence of reporting bias should still be considered. Some effects of selection bias could be present as well, regarding that some studies focused on participants of a high risk population, such as diabetes mellitus patients[30,40] or breast cancer survivors[34]. Additionally 17 of our 26 studies calculated SB by subjective methods, which are less accurate than objective methods, since people tend to underestimate their time spend in SB, due to

simple uncertainty or social desirability[91]. In future research objectively measured SB should be preferred to better calculate the real time spent sedentary.

Conclusion

There is a paucity of studies investigating the impact of sedentariness in older people. Currently there is mixed evidence for the impact of SB and biomarkers. When statistically significant results were found, SB was associated in an unfavourable way to biomarkers, but results were mostly derived from cross sectional studies and thus should be interpreted accordingly. Due to a broad definition and misclassification of sedentary behaviour as simple lack of physical activity there is still a deficiency of evident, causal relations. There is a need for high quality studies to better understand the underlying pathophysiological pathways and finally the burden between sedentary behaviour and the biomarkers implicated. Broad investigations are necessary to evaluate possible impact of sedentary behaviour on biomarkers, including those with an absence of data such as bone and muscles biomarkers. Future research should utilise an official definition of sedentary behaviour, clearly disentangle the relationships between each biomarker and sedentary behaviour and physical activity and use objective or at the least use standardised self-report measures for assessing sedentary time.

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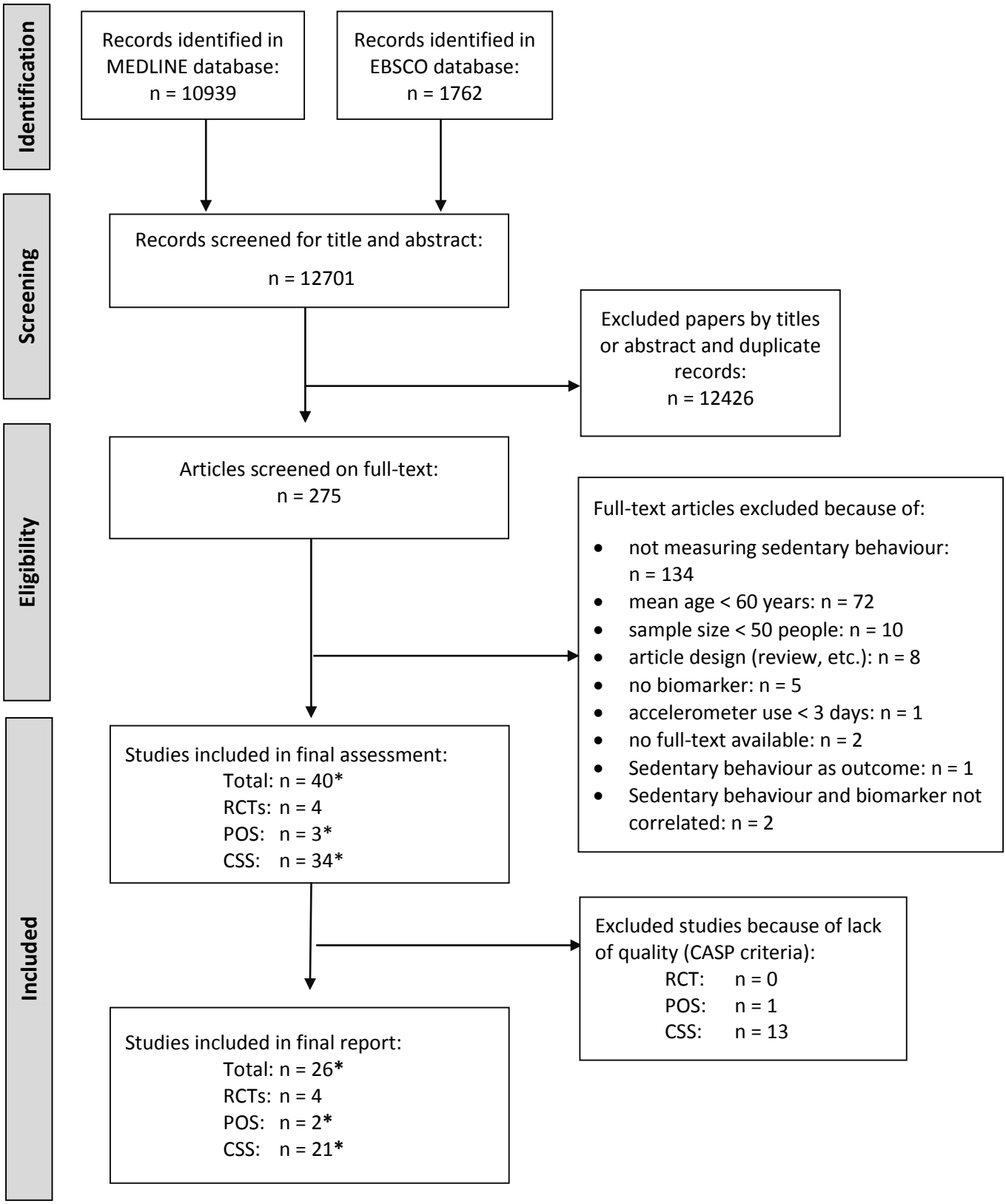
Figure 1 - Flow chart for article selection of randomized controlled trials (RCT), prospective observational studies (POS) and cross-sectional studies (CSS)



*****Remark: One study of Cooper et al.[30] was a cohort study with prospective data as well as cross sectional data and thus counted as POS and CS*

Table 1a - Descriptive overview of randomized controlled trials (RCTs) and prospective observational studies (POS)

AD = abdominal diameter; Apo = Apo lipoprotein; BF% = percent of body fat; BMI= body mass index ; BP = blood pressure; CD = community-dwelling; CG = control group; CMRF = cardio-metabolic risk factors;

	Author, Year, Country	Setting, country, study	Follow-up	No. of participants	male	Age, mean ±SD [years]	sedentary behavior assessment (method: measure)	measured biomarkers	CASP score	remarks
RCTs	**Kallings et al.**[44] **(2009), Sweden**	CD, "Move study", efficacy of PA on prescription to reduce CMRF	6 months	CG: 54 IG: 47	43% 43%	all 68 years	IPAQ questionnaire: total sitting time in hours/day	BMI, WC, AD, NC, BF%, fat mass, BF% in trunk, fat mass in trunk, glucose, HbA_1c, s/d BP, cholesterol, HDL, LDL, LDL/HDL, triglycerides, ApoA1, ApoB, ApoB/ApoA1	5 of 6	
	Kirk et al.[42] **(2009), UK**	CD + RP,"Time2Act study" in DM-II patients, compare 2 methods of PA promotion to standard care	6 & 12 months	CG: 35 IG1: 47 IG2: 52	51% 53% 42%	59.2±10.4 60.9±9.6 63.2±10.6	ActiGraph GT1M (waist) for 7 days (≥10 h/d and ≥4days), SB not defined	BMI, WC, s/d BP, cholesterol, HDL, HbA_1c	4 of 6	Groups were not equally balanced
	Suboc et al.[41] **(2014), USA**	CD, investigate if reduction of SB improves vascular endothelial function and specific biomarkers	12 weeks	CG: 35 IG1: 32 IG2: 29	76% 61% 60%	62±7 64±7 63±8	ActiGraph GT3X for 7 days, (≥600 min/d and ≥4 days): SB defined as ≤ 1.5 METS or <100 counts/min	BMI, WC, glucose, insulin, QUICKI, HOMA-IR, cholesterol, HDL, LDL, triglycerides, CRP, s/d BP, HR, brachial artery diameter, peak shear, hyperemic peak shear, Nitroglycerin mediated dilation, carotid-femoral pulse wave velocity, augmentation index, aortic s/d BP	6 of 6	
	Aadahl et al.[27] **(2014), Denmark**	CD, Health2010 study, effect of motivational counseling at reducing sitting time	6 months	total: 66 CG: 28 IG: 38	nr	63 IG: 64.1 CG: 63.8	ActivPAL 3TM (triaxial, right thigh) for 7 days (at least 2 days for analysis), SB not defined	WC, BF%, glucose, insulin, HbA_1c, cholesterol, HDL, LDL, triglycerides	5 of 6	Only 2 days of accelerometer but 94 % of total group n=166 had 5days or more; special sub-analysis for us with people > 60 years;
POS	**Fung et al.**[45] **(2000), USA**	CD, "Health Professionals Follow-up Study", television watching and biomarkers	1986-1994	466	100%	60	Questionnaire: hours of television watching/week	BMI, cholesterol, HDL, LDL, triglycerides, ApoA1, Lp(a), Leptin Fibrinogen, Insulin, C-peptide, HbA_1c	5 of 8	Biomarker only at follow-up → no change available
	Cooper et al.[30] **(2012), UK**	CD + RP, "Early ACTivity in Diabetes study", with DM-II patients, RCT but results treated as a cohort	6 months	528 cross sectional; 380 longitudinal data	65%	59.8±10.0	ActiGraph GT1M (waist) for 7 days (≥600 min/d and ≥ 3 valid days) removed for sleeping; non-wear time ≥ 20 min with 0 counts, SB (h/day) defined as < 100 counts/minute	WC, HbA_1c, HDL, glucose, insulin, HOMA-IR	5 of 8	

CRP = C-reactive protein; DM-II = diabetes mellitus type 2; HbA_1c = glycated hemoglobin; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; HOMA%B = homeostatic model assessment of B-cell function; HR = heart rate; IG = intervention group; MET = metabolic equivalent; LDL = low density lipoprotein; Lp(a) = lipoprotein a; NC = neck circumference; nr = not reported; s/d BP = systolic/diastolic blood pressure; PAS = physical activity scale; POS = prospective observational studies; QUICKI = quantitative insulin sensitivity check index; RCT = randomized controlled trials; RP = risk population; WC= waist circumference

Table 1b - Descriptive overview of 21 cross-sectional studies (CSS)

Author, year, country	Setting, study, aim	No. of participants	Male (%)	Age, mean (±SD/range) [years]	Sedentary behavior (SB) assessment (method: measure)	Analyzed biomarkers	CASP score	Remarks
Allison et al.[47] (2012), USA	CD, Multi-Ethnic Study of Atherosclerosis (MESA), association of SB with adiposity associated measures of inflammation	1543	49.8	64.3 (9.6) (45-84)	Questionnaire "Typical Week Physical Activity Survey" (TWPAS), which measures also SB (TV, computer, reading) (min/week: continuous and in tertiles) during a typical week	adiponectin, leptin, TNF-a, resistin, adiponectin/leptin	4 of 6	Multivariate adjusted means and coefficients of multivariate linear regression models, three models with different level of adjustment;
Anuradha et al.[65] (2011), USA[3]	CD, Multi-Ethnic Study of Atherosclerosis (MESA), association of TV watching time and retinal vascular caliber	5893	48	63.1 (9.9) (45-84)	Questionnaire about TV watching time (quartiles: hours/week) during a typical week	central retinal artery equivalent, central retinal vein equivalent	4 of 6	Least square means of multivariate linear regression models, two models with different level of adjustment
Bankoski et al.[62] (2011), USA	CD, National Examination Survey (NHANES); association between SB and MES	1367	51.8	71 (7.8) (≥60)	ActiGraph AM-7164 (uniaxial, waist) for 7 days (≥ 4 valid days) removed for bathing and sleeping; non-wear time > 60 min with 0 counts; SB (hours/day) defined as < 100 counts/minute	dichotomized: WC, HDL, triglycerides, glucose, BP	5 of 6	Means adjusted for age and sex
Bann et al.[31] (2015), USA	CD, Lifestyle Interventions and Independence for Elders (LIFE) Study, association of SB with BMI and grip strength	1130	33	NR (70-89) m: 79.3 (5.3) w: 78.5 (5.3)	ActiGraph GT3X (triaxial, waist) for 7 days (≥600 min/d and ≥3 valid days) removed for bathing and sleeping; non-wear time ≥90 min with 0 counts; SB (min/day) defined as < 100 counts/minute CHAMPS questionnaire about a typical week; SB (min/day) was defined as time ≤1.5 METs	BMI, grip strength	4 of 6	Coefficients of multivariate linear regression models, two models with different level of adjustment; no numbers regarding men and women although all analyses were stratified according to sex
Cooper et al.[30] (2012), UK	RP (type 2 diabetes), Early Activity in Diabetes (Early-ACTID), association between SB & CMRF	528 m: 344 w: 184	65	59.8 (10) (30-80) m: 60.7 (9.7) w: 58.1 (10.4)	ActiGraph GT1M (waist) for 7 days (≥600 min/d and ≥ 3 valid days) removed for bathing and sleeping; non-wear time ≥ 20 min with 0 counts, SB (h/day) defined as < 100 counts/minute	WC, HDL, insulin, HOMA-IR	5 of 6	Subsample in POS table Coefficients of multivariate linear regressions
Cooper et al.[40] (2014), UK	RP (type 2 diabetes), ADDITION-Plus Study, associations of SB and PA with metabolic risk	394 m: 250 w: 144	63	60.3 (7.4) m: 60.2 (7.4) w: 60.5 (7.4)	Actiheart for 4 days; SB was defined as activity < 1.5 MET	WC, systolic BP, HbA_1c, triglycerides, HDL	6 of 6	Coefficients of multivariate linear regression models, three models with different level of adjustment; adjusted to possible confounders, limitations mentioned; difficult to distinguish between sitting and standing by using Actiheart

Jakes et al.[63] (2003), UK	CD, European Prospective Investigation into Cancer (EPIC) study, association between TV watching and vigorous activity with obesity and CVD risk profile	14189	42	60.3 (45-74) m: 61(9) w: 59.9 (8.9)	Self-reported TV watching time (four groups: hours/day), separated for weekday and weekend day,	BMI, WC, HC, WHR, BF%, s/d BP, HbA_1c, triglycerides, cholesterol, HDL, LDL	4 of 6	Means adjusted for age and multivariate adjusted; rational for grouping of TV watching is unclear, numbers per group are not given
Gabriel et al.[5] (2013), USA	CD, Healthy Women Study (HWS), association of PA with coronary artery calcification progression	148	0	73.2 (1.7)	ActiGraph GT1M (uni-axial, waist) for 7 days for 24h (≥600min/d and ≥4 days); non-wear time ≥ 60 min with 0 counts; SB (min/day) was defined as <100 counts per minute	BMI, WC, s/d BP, cholesterol, LDL, HDL, triglycerides, glucose, insulin	5 of 6	Correlation coefficients between SB and biomarkers; different methods used during different FU state
Gao et al.[64] (2007), USA	CD, association of TV watching time and prevalence of MES	455	40	68.8 (≥60)	Self-reported TV watching time (quartiles: hours/day)	BMI, dichotomized: WC, triglycerides, HDL, BP, glucose, cholesterol/HDL, WHR	5 of 6	Means for BMI, proportions and multivariate adjusted ORs for all dichotomized variables; only Hispanics with Puerto Rican or Dominican origin;
Gardiner et al.[21] (2011), Australia	CD, Australian Diabetes, Obesity and Lifestyle (AusDiab) study, relation between TV watching and sitting time with MES	1958	46	69 (≥60) m: 69.6 w: 69	Questionnaire about TV and sitting time (quartiles: hours/day)	Dichotomized: WC, triglycerides, HDL, BP, glucose	4 of 6	OR from multivariate adjusted models; TV time and sitting time measured separately. Discrepancy in numbers, given in the paper, detected: Females: No MES (n=643) and MES (n=460) -> n=1103 does not match total number of 1062;
Genusso et al.[32] (2013), USA	CD, National Examination Survey (NHANES) subsample, association between SB and CMRF	1914	52	74.6 (6.5) (≥65)	ActiGraph AM-7164 (uniaxial, waist) for 7 days (≥600 min/day and ≥1 valid day) removed for bathing and sleeping; non-wear time ≥ 60 min with 0 counts; SB (quartiles: hours/day) defined as < 100 counts/minute	BMI, WC, s/d BP, cholesterol, HDL, triglycerides, LDL, glucose, HbA_1c, CRP	5 of 6	Least square means multivariate adjusted; 1 day enough for getting included in analysis; analysis of triglycerides, LDL and glucose only on subsample of 809 people – we already excluded papers because of accel. only 1 day
Hamer et al.[66] (2013), UK	CD, English Longitudinal Study of Ageing (ELSA), association between TV watching time. CRP and depressive symptoms	4964	45.1	64.5 (8.9)	Questionnaire about TV watching time on 5 weekdays and weekend separately (4 groups: hours/day)	CRP	4 of 6	Mean change in relation to a reference group, two models with different level of adjustment
Henson et al.[39] (2013), UK	CD, „Walking Away from Type 2 Diabetes study", association between SB and inflammation and adiposity	558	65	63.6 (7.7)	ActiGraph GT3X (tri axial) for 7 days (≥600 min/d and ≥ 4 valid days); non-wear time ≥ 60 min with 0 counts; SB (hours/day)defined as <25 counts/15 sec;	CRP, leptin, IL-6, adiponectin, leptin/adiponectin	5 of 6	Coefficients of multivariate linear regression models, three models with different level of adjustment; not mentioned where accelerometer got attached to;
Larsen et al.[46] (2014), USA	CD, Rancho Bernardo Study (RBS); associations of sitting time with regional fat and abdominal muscle	539 m: 135 w: 404	25	64.6 (7.4) (≥55)	Single item about time spent in leisure time sitting activities on a typical weekday (tertiles: hours/day)	BMI, TNF-a, adiponectin, leptin, IL-6, HDL, LDL, triglycerides; pericardial-, intra-thoracic-,visceral-, intermuscular- and subcutaneous fat, abdominal and psoas muscle	4 of 6	Unadjusted means; low sensitivity with measuring SB by a single self-report item for 1 day

Lee et al.[33] (2015), USA	RP (adults with or at high risk for knee osteoarthritis), osteoarthritis initiative (OAI), association between SB and physical function	1168	45	66 (45-79)	ActiGraph GT1M (uniaxial, waist) for 7 days (≥600 min/d and ≥4 days) removed for bathing and sleeping; non-wear time by ≥ 90 min with 0 counts; SB (quartiles: % of day) was defined as <100 counts per min	BMI (3 categories), gait speed, chair stand rate	5 of 6	Unadjusted means for BMI and multivariate adjusted mean differences between categories of SB; adjusted to confounders but only arthritis patients
Lynch et al.[34] (2010), USA	RP (breast cancer survivors), National Examination Survey (NHANES), association of PA and SB with adiposity	111	0	69.2 (13)	ActiGraph 7164 (uniaxial, waist) for 7 days (≥600 min/d) removed for bathing and sleeping; non-wear time ≥60 min with 0 counts; SB (hours/day) was defined as < 100 counts/min;	BMI, WC, insulin	5 of 6	Coefficients of multivariate linear regression models, three models with different level of adjustment; due to missing values the number of subjects varied (BMI: 106, WC: 100, insulin: 35); not mentioned how many days of accelerometer were necessary to get included in study
Lynch et al.[35] (2011), USA	CD, postmenopausal women of National Examination Survey (NHANES),	1024	0	63.0 (9.4)	ActiGraph 7164 (uniaxial, waist) for 7 days (≥600 min/d) removed for bathing and sleeping; non-wear time ≥60 min with 0 counts; SB (hours/day) was defined as < 100 counts/min;	BMI, WC, CRP, fasting glucose, insulin, HOMA-IR	5 of 6	Coefficients of multivariate linear regression models, three models with different level of adjustment; data not following normal distribution were transformed by natural logarithm
Reaven et al.[6] (1991), USA	CD, relation between leisure time PA and BP	641	0	66.5 (50-89)	Questionnaire adapted from Health Interview Survey with 17 leisure time activities, (2 weeks)	HR, BMI, s/d BP, fasting insulin, 2h insulin	3 of 6	Means adjusted for age (all), means multivariate adjusted (s/d BP)
Santos et al.[36] (2012), Portugal	CD, association of PA and SB with functional fitness	312 m: 117 w: 195	37.5	74.3(6.6) (≥65) m: 74.2 (6.2) w: 74.3(6.9)	ActiGraph, GT1M (waist) for 4 days (2 weekdays and 2 weekend days) (≥ 10h/d and ≥ 3 days with ≥ 1 weekend day); non-wear time ≥ 60 min 0 counts; SB (min/day) was defined as <100 counts per minute	Chair stand repetitions, arm curl, 6MWT, 8 foot up and go, chair sit and reach, back scratch	4 of 6	Coefficients of multivariate linear regression models, four models with different level of adjustment; nothing said about exclusion criteria, not mentioned if it was performed in the same center of same examiners, not medication or comorbidities got evaluated
Sardinha et al.[37] (2015), Portugal	CD, association of SB with physical function	215 m: 87 w: 128	40	73.3 (5.9) (65-94) m: 73.7 (6.2) w: 73.0 (5.7)	ActiGraph, GT1M (waist) for 4 days (2 weekdays and 2 weekend days) (≥ 10h/d and ≥ 3 days with ≥1 weekend day); non-wear time ≥ 60 min 0 counts; SB (min/day) was defined as <100 counts per minute	6MWT, 8 foot up and go, arm curl, chair stand, chair sit and reach, back scratch	6 of 6	Good adjustment for possible confounders
Stamatakis et al.[38] (2011), UK	CD, Health Survey for England (HSE), association between SB and CMRF	2765 with self-report; 649 with accelerometer	45	70 (≥60)	ActiGraph GT1M for 7 days (≥600 min/d and ≥ 1 valid day), non-wear time ≥ 60 min with 0 count, SB (tertiles: min/day) defined as < 100 counts/minute; self-reported leisure-time SB (tertiles: min/day)	BMI, WC, cholesterol, HDL, HbA_1c, cholesterol/HDL ratio	5 of 6	Unadjusted means; 1 valid day included in analysis, but 91% had > 5 days; different sample sizes of accelerometer measured and self-report sample (sensitivity analysis showed that this difference might contribute to differential associations; sample for blood biomarker was considerably smaller (1354/333)

6MWT = 6 meter walk test; ABI = ankle brachial index; AD = abdominal diameter; Apo = Apo lipoprotein; BP = blood pressure; BF% = percent of body fat; BFMI = body fat mass index (kg/m2); BMD = bone mineral density; BMI= body mass index; CCS = cross-sectional study; CD = community-dwelling; CG = control group; CMRF = cardio-metabolic risk factors; CRP = C-reactive protein; CV = cardiovascular; CVBM = cardiovascular biomarker; CVD = cardiovascular disease; DM-II = diabetes mellitus type 2; FEV1 = forced expiratory volume within 1 second; FFMI = fat free mass index (kg/m2); FVC = forced vital capacity; HC = hip circumference ;HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; HOMA%B = homeostatic model assessment of B-cell function; HR = heart rate; IG = intervention group; IL-6 = interleukin 6; LDL = low density lipoprotein; MES = metabolic syndrome; MET = metabolic equivalent; NC = neck circumference; NHANES = National Health and Nutrition Examination Survey; NR = not reported; OR = odds ratio; PA = physical activity; PAD = peripheral artery disease; PAI = plasminogen activator inhibitor 1; PAL = physical activity level; PAS = physical activity scale; PASE = physical activity scale for the elderly; RCT = randomized controlled trials; RP = "risk population" defined as population with specific illness such as diabetes or peripheral artery disease; s/d = systolic/diastolic; SB = sedentary behavior; t-PA = tissue plasminogen activator; TNF-a = tumor necrosis factor a; WC= waist circumference; WHR = waist to hip ratio

Table 2 – Overview of biomarkers evaluated in the systematic review articles

			Study results			Interpretation of the statistical significance level in high quality papers, adapted from CADTH[26]		
Category of biomarker	Biomarker type	Number of studies by study type (RCT/ POS/CSS)	Statistically not significant studies (n)	p<0.05 in un-adjusted results (n)	p<0.05 fully adjusted (n, study type, direction of association +/-)	High quality studies (n); participants (n)	Results (%)	Interpretation
Anthropo-metric parameters	BMI	3/1/11	5 (2 RCT[42,41], 3 CSS[46,33,64])	10	9 (1 RCT+[44], 1 POS+[45], 7 CSS+[32,63,6,31,5,38,35])	4 studies; 797 participants	50 % significant	Mixed evidence for association
	WC	4/1/10	5 (4 RCT[42,41,27,44], 1 CSS[64])	10	8 (1 POS+[30], 7 CSS+[5, 32, 63,35, 62, 40,38])	5 studies; 777 participants	20 % significant	Generally no evidence for association
	HC	0/0/1	0	1	1 CSS+[63]	Not applicable		
	WHR	0/0/2	0	2	2 CSS+[64,63]	Not applicable		
	neck circumference	1/0/0	0	1	1 RCT+[44]	Not applicable		
	abdominal diameter	1/0/0	1 RCT[44]	0	0	Not applicable		
	BF%	2/0/1	2 RCT[27,44]	1	1 CSS+[63]	Not applicable		
	fat mass	1/0/0	0	1	1 RCT+[44]	Not applicable		
	BF in trunk	1/0/0	1 RCT[44]	0	0	Not applicable		
	fat mass in trunk	1/0/0	1 RCT[44]	0	0	Not applicable		
	pericardial fat	0/0/1	0	1	1 CSS+[46]	Not applicable		
	intra-thoracic fat	0/0/1	0	1	0	Not applicable		
	visceral fat	0/0/1	0	1	0	Not applicable		
	intermuscular fat	0/0/1	0	1	0	Not applicable		
	subcutaneous fat	0/0/1	0	1	0	Not applicable		
	abdominal muscle	0/0/1	1 CSS[46]	0	0	Not applicable		
	psoas muscle	0/0/1	1 CSS[46]	0	0	Not applicable		
Systemic parameters	systolic BP	3/0/8	7 (3 RCT[44,42,41], 4 CSS[32, 40,62,21])	4	3 CSS+[63,6,5]	3 studies; 331 participants	0 % significant	No evidence for association
	diastolic BP	3/0/7	7 (3 RCT[44,42,41], 4 CSS[32,5,62,21])	3	1 CSS+[63]	3 studies; 331 participants	0 % significant	No evidence for association

	HR	1/0/1	1 RCT[41]	1	1 CSS+[6]	Not applicable		
	brachial artery diameter	1/0/0	1 RCT[41]	0	0	Not applicable		
	peak shear	1/0/0	1 RCT[41]	0	0	Not applicable		
	hyperemic peak shear	1/0/0	1 RCT[41]	0	0	Not applicable		
	nitroglycerin mediated dilation	1/0/0	1 RCT[41]	0	0	Not applicable		
	carotid-femoral pulse wave velocity	1/0/0	1 RCT[41]	0	0	Not applicable		
	augmentation index	1/0/0	1 RCT[41]	0	0	Not applicable		
	aortic s/d BP	1/0/0	1 RCT[41]	0	0	Not applicable		
	central retinal artery equivalent	0/0/1	1 CSS[65]	0	0	Not applicable		
	central retinal vein equivalent	0/0/1	0	1	1 CSS+[65]	Not applicable		
Blood lipids	total cholesterol	4/1/4	7 (3 RCT[27,42,41], 1 POS[45], 3 CSS [32,38,5])	2	2 (1 RCT+[44], 1 CSS+[63])	Not applicable		
	HDL	4/2/9	7 (4 RCT[27,42,41,44], 3 CSS[32,46,5])	8	6 (2 POS-[30,45], 4 CSS-[63, 38, 62, 64])	6 studies; 1243 participants	33 % significant	Generally no evidence for association
	LDL	3/1/4	6 (3 RCT[27,41,44], 3 CSS[32,46,5])	2	2 (1 POS+[45], 1 CSS+[63])	4 studies; 729 participants	25 % significant	Generally no evidence for association
	LDL/HDL	1/0/0	1 RCT[44]	0	0	Not applicable		
	cholesterol/ HDL	0/0/2	0	2	2 CSS+[64,38]	Not applicable		
	triglycerides	3/1/8	8 (3 RCT[27,41,44], 1 POS[45], 4 CSS[32,46,5,64])	4	2 CSS+[62,63]	4 studies; 729 participants	0 % significant	No evidence for association
	ApoA1	1/1/0	1 RCT[44]	1	1 POS-[45]	Not applicable		
	ApoB	1/0/0	1 RCT[44]	0	0	Not applicable		
	ApoB/ApoA1	1/0/0	1 RCT[44]	0	0	Not applicable		
	Lp(a)	0/1/0	1 POS[45]	0	0	Not applicable		
Glycemic parameters	HbA_1c	3/1/4	5 (2 RCT[27,42], 1 POS[45], 2 CSS[32,40])	3	2 (1 RCT+[44], 1 CSS+[38])	4 studies; 767 participants	25 % significant	Generally no evidence for association
	glucose	3/0/6	6 (3 RCT[27,41,44], 3 CSS[35,5,64])	3	2 CSS+[32,62]	3 studies; 263 participants	0 % significant	No evidence for significant association
	insulin (fasting)	2/2/4	2 (1 RCT[41], 1 POS[45])	6	4 (1 RCT+[27], 1 POS+[30], 2 CSS+[5,6])	4 studies; 1008 participants	50 %	Mixed evidence for association
	insulin (after 2h)	0/0/1	0	1	1 CSS-[6]	Not applicable		

	HOMA-IR	1/1/1	1 RCT[41]	2	1 POS+[30]	Not applicable
	QUICKI	1/0/0	1 RCT[41]	0	0	Not applicable
	C-peptide	0/1/0	0	1	1 POS+[45]	Not applicable
Performance biomarkers	6MWT	0/0/2	2 (1 CSS[37], NR*[36])	0	0	Not applicable
	8 foot up and go	0/0/2	2 (1 CSS[37], NR*[36])	0	0	Not applicable
	grip strength	0/0/1	1 CSS[31]	0	0	Not applicable
	gait speed	0/0/1	0	1	1 CSS-[33]	Not applicable
	arm curl	0/0/2	NR*[36]	1	0	Not applicable
	Chair stand rate	0/0/3	NR*[36]	2	1 CSS-[33]	Not applicable
	Chair sit and reach	0/0/2	NR*[36]	1	0	Not applicable
	Back scratch	0/0/2	1 (CSS[37], NR*[36])	0	0	Not applicable
Inflammatory biomarkers	CRP	1/0/4	1 RCT[41]	4	2 CSS+[32,66]	Not applicable
	Fibrinogen	0/1/0	1 POS[45]	0	0	Not applicable
	IL-6	0/0/2	1 CSS[46]	1	1 CSS+[39]	Not applicable
Others	Leptin	0/1/3	1 CSS[46]	3	2 (1 POS+[45], 1 CSS+[47])	Not applicable
	Adiponectin	0/0/3	2 CSS[47,39]	1	1 CSS+[46]	Not applicable
	leptin / adiponectin ratio	0/0/1	0	1	0	Not applicable
	adiponectin/ leptin ratio	0/0/1	0	1	1 CSS-[47]	Not applicable
	TNF-a	0/0/2	1 CSS[46]	1	1 CSS+[47]	Not applicable
	Resistin	0/0/1	1 CSS[47]	0	0	Not applicable

*Remark: Results from Cooper et al.[20] are only listed in POS results, not additionally in CSS column; *Santos et al.[36] calculated a composite Z-score, but didn't report separate associations for each biomarker with SB*

6MWT = 6 meter walk test; adj. = adjusted; Apo = Apo lipoprotein; BF% = percent of body fat; BMI = body mass index (kg/m2); ; BP = blood pressure; CSS = cross-sectional study; CRP = C-reactive protein; FFMI = fat free mass index (kg/m2); HbA_1c = specific glycated hemoglobin; HC = hip circumference; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; HR = heart rate; IL = interleukin; LDL = low density lipoprotein; Lp(a) = lipoprotein a; NR = not reported; PA = physical activity; POS= prospective observational studies; QUICKI = quantitative insulin sensitivity check test; RCT = randomized controlled trials; reg. coeff. = regression coefficient; s/d = systolic/diastolic; sig. = significant; SB = sedentary behaviour; ST = sedentary time; TNF-a = tumor necrosis factor a; unadj. = unadjusted; WC= waist circumference; WHR = waist to hip ratio

Table 3 - Details of randomized controlled trials (RCTs), prospective observational studies (POS) and cross-sectional studies (CSS) including significant associations of biomarkers with Sedentary Behaviour

	Author, Year	No. of participants	What was analyzed? What was measured?	Measured biomarkers	Main results (95%CI) or [SD] or {SE}	P-value	CASP score	Remarks
RCTs	**Kallings et al.[44] (2009)**	CG: 54 IG: 47	Significant differences between IG and CG in mean change (MC) of biomarker from baseline (B) to follow up (FU); reducing SB was measured, thus changes are negative	BMI (kg/m^2)	MC IG: -0.6 (-0.9 to -0.3) vs. MC CG: -0.2 (-0.4 to 0.0)	0.02	5/6	MC from B to FU in sitting time (hours/day) in CG with -1h/d (p <.001) and IG with -2h/d (p <.0.01)
				NC (cm)	MC IG: -1.2 (-1.6 to -0.8) vs. MC CG: -0.6 (-1.0 to -0.2)	0.01		
				Fat mass (kg)	MC IG: -1.7 (-2.5 to -0.9) vs. MC CG: -0.6 (-1.2 to -0.1)	0.03		
				HbA_{1c} (%)	MC IG: -0.1 (-0.2 to 0.0) vs. MC CG: 0.2 (0.1 to 0.3)	0.001		
				Cholesterol (mmol/l)	MC IG: -0.3 (-0.6 to 0.0) vs. MC CG: 0.1 (-0.1 to 0.1)	0.04		
	Aadahl et al.[27] (2014)	Total: 66 IG: 38 CG: 28	Mean difference (MD) in change of fasting serum insulin from baseline (B) to follow up (FU) between IG and CG for **reducing SB**	Fasting insulin (pmol/l)	-0.51 (-0,01 to -1.00)	0.04	5/6	CG means [SD] of sitting time in B = 9.8 [2.0] and FU = 10.2 [1.9]; IG means [SD] of sitting time in B = 9.27 [1.9] and FU = 8.7 [1.5]
POS	**Fung et al.[45] (2000)**	466	Pearson correlation coefficient (PCC) of television hours and biomarker; linear regression coefficient (lrc) for 1994 TV hours[1] or average TV hours in 1988-1994[2]	BMI (kg/m^2)	PCC: 0.13	<0.01	5 of 8	Lrc calculated for increment of 14 hours television watching per week
				Leptin (ng/ml)	PCC: 0.15 lrc: 1.3 {0.5}[2], adj. to BMI 0.8 {0.4}[2]	<0.01; <0.01, <0.05		
				C-peptide (ng/dl)	PCC: 0.12	<0.05		
				ApoA1 (mg/dl)	lrc: -5.3 {2.0}[1] adj. to BMI -4.9 {2.0}[1]	<0.05 <0.05		
				HDL (mg/dl)	lrc: -3.9 {1.2}[1] adj. to BMI -3.4 {1.2}[1]	<0.01 <0.01		
				LDL (mg/dl)	lrc: 6.1 {2.9}[1] adj. to BMI 6.1 {2.9}[1]	<0.05 <0.05		
	Cooper et al.[30] (2012)	528/380	Mean change (MC) in biomarker from baseline (B) to follow-up; cross-sectional regression coefficient (csrc) for baseline sample (bs) and longitudinal sample (ls) or longitudinal linear regression coefficient (llrc); additionally adj. to WC[3]	WC (cm)	MC: -1.9 (-2.3 to -1.4) B csrc: 1.8 (0.9 to 2.8) ls csrc: 1.8 (0.6 to 2.9)	<0.001 <0.001 0.002	5 of 8	Csrc and llrc calculated for ST in hours/day
				HDL (mmol/l)	bs B csrc: -0.03 (-0.06 to -0.01) bs B csrc[3]: -0.03 (-0.05 to -0.004) ls B csrc: -0.04 (-0.076 to -0.01) ls B csrc[3]: -0.03 (-0.07 to -0.00)	0.005 0.02 0.006 0.01		

					ls FU csrc: -0.05 (-0.088 to -0.020) ls FU csrc[3]: -0.05 (-0.08 to -0.01) llrc: -0.04 (-0.08 to -0.01)	0.002 0.003 0.007		
				Insulin (pmol/l)	MC: -9.4 (-14.4 to -4.4) bs b csrc: 8.2 (2.8 to 13.6) ls B csrc: 12.0 (5.0 to 19.1) ls B csrc[3]: 8.5 (1.8 to 15.2) llrc: 8.1 (1.5 to 14.7)	<0.001 0.003 0.001 0.01 0.01		
				HOMA-IR	MC: -0.36 (-0.6 to -0.0) bs B csrc: 0.4 (0.1 to 0.7) ls B csrc: 0.6 (0.2 to 0.9) ls B csrc[3]: 0.4 (0.1 to 0.8) ls llrc: 0.4 (0.0 to 0.9)	0.03 0.004 0.001 0.009 0.02		
CSS	**Allison et al.[47] (2012)**	1543	Linear regression coefficient (lrg) calculated for natural logarithm of biomarker and increment of SB (790 MET-minutes/week) adj. for confounders or additionally to BMI and more conf.[4], or add. to WC[5]	Leptin (ng/ml)	0.15 (0.10 to 0.20) 0.07 (0.04 to 0.11)[4] 0.07 (0.03 to 0.10)[5]	<0.05 <0.05 <0.05	4 of 6	
				TNF-a (pg/ml)	0.04 (0.01 to 0.06) 0.03 (0.01 to 0.06)[4] 0.03 (0.00 to 0.06)[5]	<0.05 <0.05 <0.05		
				Adiponectin/ Leptin ratio	-0.17 (-0.23 to -0.11) -0.08 (-0.12 to -0.03)[4] 0.07 (-0.11 to -0.02)[5]	<0.05 <0.05 <0.05		
	Anuradha et al.[65] (2011)	5893	Multivariate-adjusted mean difference for highest compared to lowest quartile of TV viewing time (>3 h/d to <1 h/d)	Central retinal vein equivalent (µm)	1.8 (0.4 to 3.2)	<0.05	4 of 6	
	Bankoski et al.[62] (2011)	1367	Age and sex adjusted means of biomarker and MES risk profile (according to the ATP III guidelines) for duration of ST[6] (in hours/day) and % of ST of total wear time[7]	Large WC*	no risk 9.2 {0.1}[6] to risk 9.6 {0.1}[6] no risk 62.6 {0.4}[7] to risk 66.6 {0.6}[7]	0.04 <0.01	5 of 6	
				Low HDL*	no risk 64.2 {0.5}[7] to risk 67.7 {0.6}[7]	<0.01		
				High Triglycerides*	no risk 9.2 {0.1}[6] to risk 9.6 {0.1}[6] no risk 63.8 {0.5}[7] to risk 67.0 {0.6}[7]	0.05 <0.01		
				High glucose	no risk 63.6 {0.5}[7] to risk 66.0 {0.6}[7]	<0.01		
	Bann et al.[31] (2015)	1130	Mean differences in BMI per hour/day increase in ST measured by accelerometer (ACC)[8]and self-report (SR)[9]	BMI (kg/m²)	ACC minimally adj.: 0.42 (0.13 to 0.71) ACC fully adj.: 0.44 (0.14 to 0.74) SR minimally adj.: 0.37 (0.06 to 0.67) SR fully adj.: 0.51 (0.19 to 0.82)	0.005 0.004 0.01 0.002	4 of 6	

	Cooper et al.[40] (2014)	394 m: 250 w: 144	Linear reg. coeff. for ST and WC, crude, adj. to confounders and add. adj. to PA	WC (cm)	crude: 0.80 (0.31 to 1.29) adj.: 0.97 (0.46 to 1.48) adj. + PA: 0.68 (0.01 to 1.35)	<0.05 <0.05 <0.05	6 of 6	Linear regression analyses performed for ST in hours/day
	Jakes et al.[63] (2003)	14189	Age adjusted mean value of biomarker in relation to television viewing h/d (<2h, 2-2.9, 3-3.9, >4) in men and women, add. adj. to confounders[10]	BMI (kg/m^2)	m: 26.1 [3.1], 26.4 [3.0], 26.7 [3.2], 27.1 [3.2] w: 25.3 [3.9], 26.0 [4.1], 26.2 [4.0], 26.9 [4.4]	<0.001 <0.001	4 of 6	
				WC (cm)	m: 94.0 [9.1], 94.9 [8.8], 95.7 [9.2], 97.0 [9.3] w: 79.6 [9.5], 80.7 [10.0], 81.5 [9.9], 82.9 [10.4]	<0.001 <0.001		
				HC (cm)	m: 102.3 [5.7], 102.8 [5.7], 103.0 [6.2], 103.7 [6.6] w: 102.0 [8.1] 103.2 [8.9], 103.5 [8.4], 104.7 [9.3]	<0.001 <0.001		
				WHR	m: 0.92 [0.06], 0.92 [0.05], 0.93 [0.05], 0.93 [0.05] w: 0.78 [0.05], 0.78 [0.06], 0.79 [0.06], 0.79 [0.06]	<0.001 <0.001		
				BF (%)	m: 22.5 [6.6], 23.4 [6.2], 24.3 [7.5], 25.3 [7.0] w: 37.1 [8.6], 38.9 [8.8], 40.0 [8.6], 41.6 [9.3]	<0.001 <0.001		
				s BP (mmHg)	m: 135.0 [16.8], 136.4 [17.7], 138.1 [17.7], 138.4 [19.0] m[10]: 135.6 [15.1], 136.7 [15.4], 137.7 [14.9], 137.8 [15.6] w: 130.9 [15.1], 132.3 [11.6], 133.4 [11.2], 134.0 [11.7] w[10]: 131.6 [16.8], 132.6 [13.3], 133.3 [13.3], 133.1 [14.3]	<0.001 <0.001 <0.001 <0.01		
				d BP (mmHg)	m: 83.2 [12.6], 83.7 [13.3], 84.9 [13.3], 85.6 [14.3] m[10]: 83.6 [11.3], 83.9 [11.6], 84.6 [11.2], 85.1 [11.7] w: 79.2 [7.5], 80.1 [7.7], 80.7 [7.5], 81.1 [7.8] w[10]: 79.7 [8.4], 80.3 [8.9], 80.7 [8.9], 80.5 [9.5]	<0.001 <0.001 <0.001 <0.01		
				Triglycerides (mmol/l)	m: 1.70 (0.8–3.6), 1.80 (0.8–3.8), 1.82 (0.9–3.8), 1.92 (0.9–4.1) m[10]:1.73 (0.8–3.6), 1.82 (0.9–3.9), 1.80 (0.9–3.7), 1.88 (0.9–4.0) w: 1.38 (0.6–3.1), 1.40 (0.6–3.3), 1.49 (0.6–3.6), 1.54 (0.6–3.9) w[10]:1.42 (0.6–3.2), 1.42 (0.6–3.4), 1.48 (0.6–3.5), 1.49 (0.6–3.8)	<0.001 <0.01 <0.001 <0.001		
				Cholesterol (mmol/l)	m: 5.91 [1.26], 5.93 [1.33], 5.96 [1.33], 6.05 [1.43] m[10]:5.92 [1.13], 5.93 [1.16], 5.96 [1.12], 6.04 [1.17] w: 6.17 [1.13], 6.22 [0.77], 6.27 [0.75], 6.28 [0.78] w[10]: 6.19 [1.26], 6.23 [0.89], 6.27 [0.89], 6.26 [0.95]	<0.001 <0.01 0.001 <0.01		
				HDL (mmol/l)	m: 1.28 (0.6–2.7), 1.23 (0.6–2.6), 1.22 (0.6–2.5), 1.20 (0.6–2.6) m[10]:1.27 (0.6–2.7), 1.22 (0.6–2.6), 1.22 (0.6–2.5), 1.21 (0.6–2.6) w: 1.63 (0.7–3.7), 1.58 (0.7–3.8), 1.57 (0.7–3.7), 1.51 (0.6–3.8) w[10]: 1.60 (0.7–3.6), 1.57 (0.7–3.7), 1.57 (0.7–3.7), 1.54 (0.6–3.9)	<0.001 <0.01 <0.001 <0.001		
				LDL (mmol/l)	m: 3.75 [0.84], 3.77 [0.89], 3.82 [0.89], 3.87 [0.95] m[10]: 3.75 [0.75], 3.77 [0.77], 3.82 [0.75], 3.87 [0.78] w: 3.82 [0.75], 3.90 [0.77], 3.93 [0.75], 3.97 [0.78] w[10]: 3.85 [0.84], 3.91 [0.89], 3.93 [0.89], 3.95 [0.95]	<0.001 <0.01 <0.001 <0.01		
	Gabriel et	148	Pearson cc between biomarker and	BMI (kg/m^2)	PCC: 0.18	<0.05	5 of 6	

	al.[5] (2013)		accelerometer measured ST (in min/d)	WC (cm)	PCC: 0.21	<0.01		
				S BP (mmHg)	PCC: 0.17	<0.05		
				Insulin (mU/dl)	PCC: 0.24	<0.01		
	Gao et al.[64] (2007)	455	OR of unfavorable biomarker profile by quartiles of TV viewing time (0-1.5h = reference, 1.6-3.4h, 3.5-5.5h, 5.6-18h), adj. for confounders[11], add. for dietary habits[12], add. for ADL[13]	Low HDL*	1 (ref), 0.9 (0.4–2.0), 1.2 (0.5–2.7), 2.5 (1.0–5.9)	0.02[11], 0.02[12], 0.01[13]	5 of 6	P-values for linear trends
				High cholesterol/ HDL ratio*	1 (ref), 1.2 (0.7–2.1), 1.3 (0.7–2.4), 2.0 (1.1–3.7)	0.01[11], 0.03[12], 0.04[13]		
				High WHR*	1 (ref), 1.6 (0.8–3.1), 2.3 (1.1–4.8), 3.9 (1.8–8.4)	0.0003[11], 0.0008[12], 0.0006[13]		
	Genusso et al.[32] (2013)	1914	Association of least square means of biomarkers with quartiles of sedentary hours (0-7.92, 7.93-8.17, 8.18-10.63, >10.64)	BMI (kg/m^2)	26.6 [0.6], 27.4 [0.5], 27.8 [0.5], 28.8 [0.4]	0.01	5 of 6	P-values for linear trends
				WC (cm)	98.2 [1.6], 100.2 [1.3], 101.9 [1.4], 104.4 [1.0]	<0.01		
				Glucose (mg/dl)	115.0 [1.2], 114.8 [1.2], 119.2 [1.2], 119.8 [1.2]	0.04		
				CRP (mg/dl)	0.24 [1.15], 0.24 [1.12], 0.26 [1.12], 0.34 [1.14]	<0.01		
	Hamer et al.[66] (2013)	4964	Dose-response association for TV viewing (<2=Ref., 2-4, 4-6, >6h/d) and log transformed mean CRP values, adj. for age, sex[14]; further adj. to PA, BMI[15]	CRP (log transformed)	Ref., 0.11 (0.04 to 0.18), 0.27 (0.2 to 0.34), 0.29 (0.22 to 0.36)[14] 0.04 (-0.03 to 0.1), 0.12 (0.06 to 0.19), 0.11 (0.04 to 0.17)[15]	<0.001 <0.001	4 of 6	
	Henson et al.[39] (2013)	558	Regression coeff. for ST (in h/day) with biomarker, adj. to confounders, add. to PA[16], add. adj. to BMI and HbA_1c[17]	IL-6 (pg/ml)	0.242 {0.056}, 0.231 {0.073}[16], 0.212 {0.072}[17]	<0.001, 0.002[16], 0.003[17]	5 of 6	
	Larsen et al.[46] (2014)	539 m: 135 w: 404	Variance (V) in mean values of biomarker and ST tertiles or cross-sectional regression coefficient (csrc) of biomarker to ST tertiles (<2.5, 2.5-4, >4 sitting hours/day), unadj., adj. to demographics[18], to CVD RF[19], to BMI[20], to inflammatory markers[21]	Adiponectin (µg/ml)	V: 10.4 [6.0], 9.4 [4.9], 10.8 [6.6], 10.8 [5.9]	0.032	4 of 6	Pos. assoc. for V of intra-thoracic fat (p=0.018), intermuscular fat (p=0.001) and subcutaneous fat (p=0.034) but not sig. in csrc
				Intra-thoracic fat (cm^2)	V: 71.8 [64.1], 61.2 [50.5], 75.8 [70.9], 80.0 [66.1]	0.018		
				Intermuscular fat (cm^2)	V: 21.4 [11.0], 19.4 [8.8], 23.5 [12.1], 21.4 [11.3]	0.001		
				Subcutaneous fat (cm^2)	V: 253.8 [122.7], 243.4 [106.3], 273.2 [131.4], 246.6 [126.2]	0.034		
				Pericardial fat (cm^2)	csrc: 3.19 (0.45 to 5.92) csrc[18]: 3.19 (0.45 to 5.92) csrc[19]: 3.32 (0.84 to 5.81) csrc[20]: 2.39 (0.07 to 4.72) csrc[21]: 2.45 (0.12 to 4.77)	0.022 0.022[18] 0.009[19] 0.044[20] 0.039[21]		

Lee et al.[33] **(2015)**	1168	Unadj.[22] as well as adj.[23] average differences (AD) in function (as biomarker) between SB quartiles (Q2 vs. Q1, Q3 vs. Q1 and Q4 vs. Q1)	Gait speed (feet/s)	AD[22]: 0.35 [0.08], 0.44 [0.08], 0.44 [0.08] AD[23]: 0.20 [0.07], 0.21[0.08], 0.21 [0.08]	<0.001[22] <0.001[23]	5 of 6	
			Chair stand rate (stands/min)	AD[22]: 3.00 [0.95], 3.28 [0.98], 5.30 [0.95] AD[23]: 1.85 [0.90], 1.46 [0.96], 3.43 [0.98]	<0.001[22] 0.0016[23]		
Lynch et al.[35] **(2011)**	1024	Association of SB quartiles (<7.74, 7.74-<8.8, 8.8-<9.84, ≥9.84 h/d), adj. in model 1[24] to age; model 2 for BMI: ethnicity, alcohol intake, age at first birth, age at menarche; model 2[25] for WC: ethnicity, educational attainment, marital status, annual family income, alcohol intake, age at first birth	BMI	model 1[24]: 26.7 (25.9 to 27.5), 27.6 (26.8 to 28.5), 27.6 (26.6 to 28.6), 29.9 (28.6 to 31.2) model 2[25]: 27.2 (26.4 to 27.9), 27.7 (26.9 to 28.6), 27.5 (26.6 to 28.4), 29.3 (28.1 to 30.5)	<0.001[24] 0.02[25]	5 of 6	CRP, insulin, HOMA-IR showed also sig. pos. trend with SB quartiles, sig. after multivariate adj., but ns after adj. to WC; all results as marginal means for each quartile, back-transformed for all log-transformed outcomes
			WC	model 1[24]: 91.9 (89.7 to 94.2), 94.7 (92.9 to 96.5), 95.7 (93.3 to 98.1), 102.1 (99.4 to 104.8) model 2[25]: 93.2 (90.8 to 95.7), 95.1 (93.1 to 97.1), 95.5 (93.2 to 97.9), 100.5 (97.9 to 103.1)	<0.001[24] 0.003[25]		
Reaven et al.[6] **(1991)**	641	Mean values of age adj. biomarker by exercise category (none, light, moderate, heavy); s BP additionally adj. to age[26], age + BMI[27], age + BMI + alcohol + estrogen[28], age+ BMI+ fasting insulin[29],age+BMI+2h insulin[30]	HR (beats/min)	66.5, 64.8, 63.9, 61.4	0.01	3 of 6	P-values for linear trends; values for D BP were ns when unadj. but linear trend adj. to same confounders as S BP were sig (p=0.006[24], p=0.044[25], p=0.049[26], p=0.034[27], p=0.025[28])
			BMI (kg/m^2)	26.3, 24.1, 25.1, 23.4	0.05		
			S BP (mmHg)	143.3, 136.8, 130.3, 122.6 142.1, 135.5, 133.0, 130.3[26] 140.8, 135.6, 132.5, 131.3[27] 140.7 135.6 132.5 131.4[28] 140.7 135.5 132.5 131.4[29] 140.9 134.9 131.0 131.3[30]	<0.001 0.003 0.012 0.013 0.014 0.010		
			Fasting Insulin (µU/ml)	16.9, 13.7, 12.4, 11.2	0.002		
			2h Insulin (µU/ml)	15.0, 88.5, 79.2, 66.2	0.001		
Stamatakis et al.[38] **(2011)**	2765 (SR) 649 (accel.)	Mean values of biomarker and tertiles of self-reported (SR; <291, 291-394, > 394 min/d) or accelerometer measured (accel.; <507, 507-571, > 571 min/d) ST	BMI (kg/m^2)	SR: 27.4 [4.5], 27.9 [4.6], 28.5 [5.1] accel.: 27.1 [4.0], 28.6 [4.9], 28.5 [4.7]	<0.01 <0.01	5 of 6	P-value for one-way ANOVA test; a sig. pos. multivariate reg. coeff. was calculated for SR SB with BMI and HbA_1c and similar for accel. measured SB with cholesterol and HbA_1c, which was ns after further adj.
			WC (cm)	SR: 94.8 [13.1], 96.0 [12.8], 98.3 [13.4] accel.: 93.1 [12.7], 96.5 [13.7], 99.6 [12.8]	<0.01 <0.01		
			HDL (mmol/l)	SR: 1.6 [0.4], 1.6 [0.4], 1.5 [0.4] accel.: 1.7 [0.4], 1.6 [0.4], 1.5 [0.4]	<0.01 <0.01		
			HbA_1c (%)	SR: 5.8 [0.7], 5.8 [0.6], 6.0 [0.9] accel.: 5.8 [0.6], 5.8 [0.6], 6.0 [0.8]	<0.01 0.01		
			Cholesterol / HDL ratio	SR: 3.9 [1.0], 4.0 [1.0], 4.1 [1.2]	0.01		

*according to the definitions of Adult Treatment Panel III (ATP-III); Cholesterol/HDL ratio > 4.5 was considered as high.

accel. = accelerometer; AD = average differences; adj. = adjusted; ADL = activities of daily living; B = baseline; BF% = percent of body fat; BMI= body mass index ; BP = blood pressure; bpm = beats per minute; cc = correlation coefficient; CI = confidence interval; coeff. = coefficient; CG = control group; CMRF = cardio-metabolic risk factors; CRP = C-reactive protein; csrc = cross-sectional regression coefficient; FU = follow up;

HbA1c = glycated hemoglobin; HC = hip circumference; HDL = high density lipoprotein; HOMA-IR = homeostatic model assessment of insulin resistance; HR = heart rate; IG = intervention group; IL = interleukin; LDL = low density lipoprotein; lrc = linear regression coefficient; MC = mean change; MD = mean difference; MES = metabolic syndrome; NC = neck circumference; neg. = negative; NR = not reported; OR = odds ratio; PCC = Pearson correlation coefficient; pos. = positive; POS = prospective observational studies; RCT = randomized controlled trials; ref = reference; S/D = systolic/diastolic; SB = sedentary behaviour; SD = standard deviation; SE = standard error; sig. = significant; SR = self report; ST = sedentary time; TNF-a = tumor necrosis factor alpha; unadj. = unadjusted; V = Variance; WC= waist circumference; WHR = waist to hip ratio

Appendix 1 – Search strategy

concepts	search terms
sedentariness	seden* OR television OR accelerometer OR pedometer
age	age OR aging OR elderly OR older
biomarkers	bone biomarker OR biomarker OR CRP OR interleukin OR endocrine OR diabetes OR insulin OR cardiovascular OR CNS OR central nervous system OR neurological OR hormones OR inflammation OR hematology OR blood OR liquor OR epigenetic OR genetic OR DNA OR RNA or ultrasound OR BIA or bioelectrical OR caliper OR stem cell OR cerebrovascular OR cancer OR cytokine OR mitochondr* OR immune OR protein OR urine OR muscle OR gait OR factor OR transcription OR strength OR handgrip OR oncology OR nephrology OR men health OR women health OR COPD OR pulmonary OR lung OR asthma OR glucose OR GID OR gastrointestinal OR gastric OR lipoprotein OR anabol OR katabol OR thyroid OR steroid OR metabolic OR testosterone OR estrogen

Appendix 2 - Definitions

Accelerometer	An instrument for measuring the acceleration of a moving body
Biomarker	A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention
Moderate-vigorous physical activity	Physical activity performed at intensity ≥3 Metabolic Equivalents (METs)
Sedentary behaviour	Activities with an energy expenditure ≤ 1.5 Metabolic Equivalents (METs) while in a sitting or reclining posture during waking hours; not simply the absence of physical activity