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► **To cite this version:**

Ana Pinto, Audrey Bergouignan, Paddy Dempsey, Hamilton Roschel, Neville Owen, et al.. Physiology of sedentary behavior. *Physiological Reviews*, 2023, 103 (4), pp.2561-2622. 10.1152/physrev.00022.2022 . hal-04272960

HAL Id: hal-04272960

<https://hal.science/hal-04272960>

Submitted on 8 Nov 2023

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The Physiology of Sedentary Behavior

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44

45 **ABSTRACT**

46

47 Sedentary behaviors (SB) are characterized by a low energy expenditure while in a
48 sitting or reclining posture. Evidence relevant to understanding the physiology of SB can
49 be derived from studies employing several experimental models: bed rest,
50 immobilization, reduced step count, and reducing/interrupting prolonged SB. We
51 examine the relevant physiological evidence relating to body weight and energy
52 balance, intermediary metabolism, cardiovascular and respiratory systems, the
53 musculoskeletal system, the central nervous system, and immunity and inflammatory
54 responses. Excessive and prolonged SB can lead to insulin resistance, vascular
55 dysfunction, shift in substrate use towards carbohydrate oxidation, shift in muscle fiber
56 from oxidative to glycolytic type, reduced cardiorespiratory fitness, loss of muscle mass
57 and strength, and bone mass, and increased total body fat mass and visceral fat depot,
58 blood lipid concentrations, and inflammation. Despite marked differences across
59 individual studies, longer-term interventions aimed at reducing/interrupting SB have
60 resulted in small, albeit marginally clinically meaningful, benefits on body weight, waist
61 circumference, percent body fat, fasting glucose, insulin, HbA1c and HDL
62 concentrations, systolic blood pressure, and vascular function in adults and older adults.
63 There is more-limited evidence on other health-related outcomes and physiological
64 systems, and for children and adolescents. Future research should focus on the
65 investigation of molecular and cellular mechanisms underpinning adaptations to
66 increasing and reducing/ interrupting SB and the necessary changes in SB and physical
67 activity to impact physiological systems and overall health in diverse population groups.

68 **CLINICAL HIGHLIGHTS**

69

70 1) Sedentary behavior (SB; time spent sitting) occupies a high proportion of adults'
71 waking hours and its effects can be examined distinctly from lack of exercise or
72 physical activity (PA).

73 2) The average energy cost of common SBs ranges between 1.0 and 1.5 METs. Energy
74 expenditure, heart rate, skeletal muscle blood flow and contractile activity are higher
75 during sitting than when reclining, but lower than in a standing position and during PA
76 of any intensity.

77 3) Prolonged and uninterrupted SB leads to insulin resistance, vascular dysfunction,
78 shift in substrate use towards carbohydrate oxidation, shift in muscle fiber from
79 oxidative to glycolytic type, reduced cardiorespiratory fitness, loss of muscle mass
80 and strength, and bone mass, and increased total body fat mass and visceral fat
81 depot, blood lipid concentrations, and inflammation.

82 4) From a physiological perspective, there are impacts of SB on physiological responses
83 that relate to those of physical inactivity, i.e., too little exercise. Even though such
84 effects are similar, high volumes of SB can have adverse physiological impacts even
85 in the presence of large volumes of aerobic and/or resistance exercise.

86 5) Acutely, reducing/interrupting SB improves postprandial glucose and insulin
87 responses, systolic blood pressure, mean arterial pressure, and lower limb vascular
88 function. In the longer-term, there are small improvements on body weight, waist
89 circumference, percent body fat, fasting glucose, HbA1c and HDL concentrations,

90 systolic blood pressure, and vascular function. Evidence is more limited for other
91 health outcomes and physiological systems.

92 6) Reducing/interrupting SB improves body composition, intermediary metabolism, and
93 cardiovascular health outcomes, but effects are small, albeit marginally clinically
94 meaningful. Most studies have been conducted in healthy population groups (i.e.,
95 outcomes within normal ranges) and larger effects may be observed in unhealthy
96 populations.

97 7) The ‘sit less, move more and exercise’ focus of contemporary public health guidelines
98 is a consensus based primarily on epidemiological findings, and further experimental
99 evidence is needed to elucidate the physiological effects of interventions combining
100 exercise and reduction/interruptions to SB. Nevertheless, reducing/interrupting SB is
101 a low-risk strategy of clinical and population health relevance, and can serve as a
102 stepping stone to regular participation in moderate-to-vigorous intensity PA.

103 1. INTRODUCTION

104

105 Sedentary behavior (SB) is defined as any waking behavior characterized by a low
106 energy expenditure (≤ 1.5 metabolic equivalent of task [MET]) while sitting or lying down
107 (1). Driven by environmental, economic, social, and technological changes, SB is now
108 understood to be a major component of the human movement spectrum that can impact
109 health adversely (2, 3). In adults and older adults, time spent sedentary can range from
110 5 to 11.5 h/day (4-7).

111 Particular attention has been given to SB only since the early 2000's when the
112 term "inactivity physiology" (i.e., acute and chronic physiological effects of SB [non-
113 exercise activity deficiency]) was put forward by Hamilton and colleagues as a separate
114 research field from exercise physiology (8-10). This shift was motivated by experimental
115 findings demonstrating key differences in mechanisms driving skeletal muscle
116 lipoprotein lipase (LPL) responses between physical inactivity and exercise compared to
117 normal standing and ambulatory activity in rats (8, 9). The rapid accumulation of
118 experimental evidence on SB over the past 20 years has built upon these early insights,
119 with greater attention being directed at understanding the health consequences of daily
120 hours spent sedentary and the countermeasure strategies aimed at
121 reducing/interrupting time spent sedentary.

122 This review provides a perspective on: (i) how to characterize SB; (ii) the pros
123 and cons of the currently available experimental models employed in the investigation of
124 physiology of SB; (iii) the physiological effects of variations in SB and potential
125 underlying mechanisms; and, (iv) the gaps that currently exist in the scientific

126 understanding of the physiology of SB. For a broad and practically-informed
127 perspective, we address the extent to which the physiological evidence base can help to
128 further focus and sharpen public health and clinical practice guidelines, extending
129 beyond the well-understood and accepted ‘exercise more’ message, towards a more
130 comprehensive ‘sit less, move more and exercise’ message.

131
132 **2. AN OPERATIONAL FRAMEWORK FOR SEDENTARY BEHAVIOR PHYSIOLOGY**
133 **RESEARCH**

134
135 The term SB, derived from the Latin word *sedere* (“to sit”), refers to any waking behavior
136 posture (1) (see TABLE 1 for key terms in SB research). First, we discuss key features
137 and themes of SB research. This operational framework provides guidance for more in-
138 depth considerations on the physiology of SB later in the review.

139
140 ----- INSERT TABLE 1 ABOUT HERE -----

141
142 **2.1. Characteristics of sedentary behavior**

143 SBs are identified based on their physiological and postural characteristics.
144 Physiologically, the average energy cost of common types of SB ranges between 1.0
145 and 1.5 METs in healthy adults (11) during fasting (12-14) and postprandial states (15,
146 16), as measured by indirect calorimetry (12-15) or by whole-room calorimetry (16).
147 Overall, energy expenditure during sitting is higher than reclining (13, 16), but lower

148 than standing (12, 13) and lower than all intensities of physical activity (PA) (15). For
149 heart rate (HR), similar responses are observed (12, 14).

150 In skeletal muscle, increased contractile activity is required to sustain standing
151 and ambulatory activities (17); consequently, blood flow increases and the metabolic
152 demands of the contracting muscles are accommodated (18). In contrast, muscle
153 contractile activity during sitting postures (as measured by electromyographic [EMG]
154 activity) is significantly lower than for standing and ambulatory activities (14, 17, 19).
155 EMG activity in the quadriceps and hamstring muscle groups is ~2.0-2.5, ~7.0-10.5 and
156 ~18.0 times higher during standing, walking and stair climbing in daily living settings,
157 respectively, as compared to sitting (17, 19). To match the reduced metabolic demands
158 of low muscle activity, skeletal muscle blood flow is also significantly lower and less
159 variable during sitting as compared to standing and ambulatory activities (18, 20, 21).

160 Elements pertaining to SB that may explain the differential impacts of SB on
161 health outcomes include frequency, intensity, time, and type, the so-called FITT
162 principle (TABLE 2) (22). Engagement in SB can be accordingly described and
163 monitored, which aligns with and builds on the FITT principle for exercise prescription.
164 These key postural, physiological, and behavioral features are what define/characterize
165 SB and should be considered explicitly in SB research.

166
167 ----- INSERT TABLE 2 ABOUT HERE -----

168

169 **2.2. Sedentary behavior versus physical inactivity**

170 The term 'sedentary' had previously been used interchangeably with physical inactivity
171 to denote insufficient levels of moderate-to-vigorous intensity PA (MVPA), i.e., not
172 achieving the current PA guidelines (1). However, SB and physical inactivity are now
173 viewed as being separate entities on a continuum of human movement and non-
174 movement behaviors (1). According to this definition, a person could be classified as
175 being both highly sedentary and physically active. This points to four distinct
176 classifications: being physically active and highly sedentary, physically active and
177 slightly sedentary, physically inactive and highly sedentary, or physically inactive and
178 slightly sedentary (FIGURE 1). The importance of addressing the behavioral phenotype
179 of both excessive SB and physical inactivity is now embodied in contemporary public
180 health PA guidelines (2, 23).

181

182 ----- INSERT FIGURE 1 ABOUT HERE -----

183

184 From a physiological perspective, differentiating between 'SB' and 'physical
185 inactivity' may initially seem rather semantic. Indeed, reviews have already summarized
186 the evidence to date on numerous physiological responses of increasing SB (24-29)
187 and it is evident that many of these relate to the responses following imposed physical
188 inactivity (30-33). Even though the effects of SB and physical inactivity are generally in
189 a similar direction (deleterious), excessive participation in SB has been shown to result
190 in adverse effects even in the presence of large volumes of aerobic and/or resistance
191 exercise (a sedentary yet physically active condition) (34). Exercising (above current PA
192 guidelines) during bed rest does not necessarily counteract, or only partially

193 counteracts, some of the adverse effects of large volumes of SB in healthy adults (34).
194 These findings point not only to likely independent adverse health effects of SB, but also
195 to the potential benefits of regular non-exercise activity and/or muscle contractions.

196

197 **2.3. Sedentary behavior physiology research: The seminal role of ‘inactivity** 198 **physiology’ and animal studies**

199 The term ‘inactivity physiology’ was first proposed in the early 2000’s as a separate field
200 and distinct from exercise physiology (8, 10, 35). The premise was that excessive SB
201 was not the same as lack of exercise and that SBs have their own unique physiological
202 consequences.

203 Research in the ‘inactivity physiology’ context has been examined primarily using
204 hindlimb unloading and wheel lock methodologies in rats. The key objective of these
205 studies was to better understand how increasing SB and imposing physical inactivity (or
206 rather hypodynamia and hypokinesia) may trigger maladaptations linked to chronic
207 diseases. Here, key findings pertinent to SB physiology are summarized.

208 Hindlimb unloading models involve suspending rodents by their tail, thereby
209 preventing any weight-bearing activities of the lower limbs. Hindlimb unloading studies
210 have reported rapid development of insulin resistance (increased glucose-insulin index)
211 after one day of limb suspension (36). Notably, glucose transport activity and
212 intramuscular triglycerides were significantly lower in soleus muscle, but not in extensor
213 digitorum longus after one day of limb suspension, which was driven by increases in
214 p38 mitogen-activated protein kinase expression (MAPK), known to negatively interact
215 with insulin signaling cascade (36). This suggests that those muscles which

216 predominantly contain type 1 fibers are more susceptible to maladaptations related to
217 increasing SB than muscles composed of type 2 fibers predominantly. This has also
218 been shown in humans following periods of bed rest. For example, plantar flexor and
219 monoarticular knee extensor muscles were found to be more affected than hip
220 extensor/adductor muscles after head-down bed rest (HDBR) and horizontal bed rest
221 (37-40). In contrast, the biarticular knee extensor and hip flexor rectus femoris, other
222 anteromedial hip muscles and short head of biceps femoris were found to be
223 comparatively less affected by horizontal bed rest (37). Notably, faster rates of muscle
224 atrophy were observed in anti-gravity muscles and those that are more intensively
225 required for standing and walking (37). Similarly, myosin heavy chain (MHC) distribution
226 in the skeletal muscle shifted from slow-twitch (MHC I) towards hybrid (I/IIa and IIa/IIx)
227 and fast-twitch (IIa and IIx) fiber types in vastus lateralis following 35-84 days of HDBR
228 and horizontal bed rest (41-43). Similar alterations in slow and hybrid fibers, but not fast
229 fibers, were observed in soleus muscle after 84 days of HDBR (42).

230 Hamilton and colleagues showed that distinctive physiological pathways are
231 activated with hindlimb unloading, particularly LPL activity, which seems to remain
232 largely unaffected by MVPA (44). Rat skeletal muscle triglyceride uptake was reduced
233 by 75% and LPL protein expression and enzymatic activity were rapidly suppressed
234 during acute (1-18 h) and chronic (~10 h/day over 11 days) periods of hindlimb
235 unloading (44). Alterations in heparin-released and intracellular LPL activity decreased
236 mono-exponentially in both the soleus (type 1) and red quadriceps (predominantly type
237 2) muscles after 12 hours of limb unloading. These alterations were rapidly reversed
238 with light-intensity contractile activity in both soleus and quadriceps muscles (9, 44, 45).

239 Interestingly, MVPA/exercise training did not enhance LPL regulation in type 1 muscles
240 and type 2 muscles that were not recruited during running (8, 46). In type 1 muscles
241 recruited during running, there was an increase in heparin-released LPL activity, LPL
242 mRNA level, and LPL protein mass (8, 46). Additionally, heparin-released LPL activity
243 was ten-fold less in the soleus and quadriceps muscles and two-fold less in the rectus
244 femoris muscle of rats subjected to 12-hour limb unloading as compared with low-
245 intensity ambulatory controls (44). The absence of changes in LPL activity in the heart
246 and diaphragm, both muscles with high oxidative capacity, also suggested loss of
247 muscle LPL activity was constrained to unused muscles (44).

248 Despite changes in LPL activity, no changes were observed in skeletal muscle
249 LPL gene expression following acute (1-18 h) and sustained (~10 h/day over 11 days)
250 periods of hindlimb unloading (45). However, Zderic and Hamilton (47) demonstrated
251 that skeletal muscle differentially expresses at least 17 genes involved in homeostasis
252 in humans and rats. Of particular interest, *Lipid Phosphatase-1 (LPP1/PAP2A)*, a key
253 gene for degradation of prothrombotic and proinflammatory lysophospholipids, was
254 suppressed locally in muscle tissue after 12 hours of hindlimb unloading in rats, and
255 after 12 hours of prolonged sitting in humans (47). Of note, exercise was ineffective at
256 counteracting this decrease in both species (47).

257 Wheel lock models involve periods of habitual or voluntary activity (3-6 weeks;
258 typically, 5-10 km/day of running) which is suddenly restricted (i.e., running wheel
259 locked) to permit only minimal movement within the cage for up to 7 days. While daily
260 wheel running increased insulin-dependent glucose uptake in isolated skeletal muscle,
261 a rapid decrease in insulin sensitivity was reported following only 2 days of wheel lock in

262 rats (48). This reduction in insulin-dependent glucose transport was associated with
263 reduced activation of the insulin-signaling pathway and glucose transporter 4 (GLUT-4)
264 protein content. Pronounced gains in intra-abdominal fat mass (25 to 48%) were also
265 reported following 1 week of wheel lock (49, 50). Interestingly, lowering food intake
266 during wheel lock did not significantly change fat mass increase compared to the rats
267 that were fed *ad libitum*, indicating that fat storage was the result of SB and physical
268 inactivity *per se*, rather than positive energy balance (49). Despite providing important
269 initial insights for SB research, wheel lock models are considered to be extreme models
270 of inactivity in which animals transit from very high daily amounts of exercise to
271 sedentariness/inactivity. Therefore, it may be more a model of detraining from exercise
272 rather than a model to study adaptations to increasing SB.

273 Evidence from ‘inactivity physiology’ studies using hindlimb unloading, and wheel
274 lock methodologies have been instrumental in laying the foundation for experimental
275 studies related to SB and physical inactivity physiology in humans by providing initial
276 evidence on the potential adverse effects and underpinning mechanisms associated
277 with these behaviors as compared to habitual activity and exercise.

278

279 **2.4. Role of sedentary behavior in health and disease**

280 Extensive epidemiological evidence has highlighted that excessive daily time in SB is
281 associated with increased risk of early mortality and chronic diseases, including obesity,
282 type 2 diabetes, cardiovascular disease, metabolic syndrome, certain type of cancers,
283 and others (4, 51-59). Yet mechanisms involved in this increased risk are poorly
284 understood.

285 A systematic review synthesized current knowledge of the associations of SB
286 with gene expression and epigenetic modifications in children and adolescents. Overall,
287 evidence is still limited, but some studies suggest candidate genes and non-coding
288 ribonucleic acids (RNAs) that are linked to/regulated by SB, including higher miRNA-
289 222 and miRNA-146a levels (related to angiogenesis and inflammation), and
290 methylation at HSD11B2 promoter (related to stress/cortisol metabolism) (60).
291 Additionally, screen time was a significant moderator in the association of the
292 rs9939609 single nucleotide polymorphism (SNP) located on the fat mass and obesity-
293 associated gene (FTO) with metabolic syndrome clustered cardiometabolic risk score in
294 children and adolescents of low cardiorespiratory fitness (61). In adults, FTO SNP
295 rs9939609 was significantly associated with self-reported time spent in SB, and
296 sedentary time partially mediated the association between FTO and body mass index
297 (BMI) (62). In contrast, another study demonstrated that the association between
298 objectively measured SB and FTO SNP rs17817449 was fully attenuated by BMI in
299 adults, suggesting the association between SB and FTO was explained by adiposity
300 (63). Although the mechanisms through which FTO increases BMI and adiposity have
301 not been elucidated, knockout mice models suggest that FTO may be involved in
302 energy homeostasis via regulation of energy expenditure (64).

303 Ascribing causality from observational evidence is difficult. To overcome this
304 limitation some studies have used Mendelian randomization, which is a well-established
305 tool that employs genetic variants as instrumental variables for exposures (e.g., SB and
306 PA). Since the genetic variants are randomly assigned during meiosis, Mendelian
307 randomization can minimize confounding and reverse causation, potentially providing

308 stronger evidence for causal inference (65). Overall, findings from Mendelian
309 randomization studies are aligned with those from observational studies.

310 Totals of 136, 43 and 5 genetic SNPs have been found to be associated with
311 leisure-time TV watching, computer use, and driving, respectively. Genetically predicted
312 duration of TV watching was positively associated with risk of myocardial infarction,
313 heart failure and atrial fibrillation, which remained significant after adjustments for
314 genetically predicted PA. Associations between computer time use and driving and
315 cardiovascular diseases were inconsistent and non-significant (66). Similarly, another
316 study identified a total of 89 genetic SNPs that were associated with TV watching.
317 Genetically predicted duration of TV watching, but not computer use and driving, was
318 positively associated with risk of developing type 2 diabetes (67). A study using
319 individual-level data from 130,957 females identified 6 SNPs as predictors of
320 participation in SB. Females with genetic variants predisposing them to a higher time in
321 SB had a higher risk of hormone-receptor-negative and *in situ* breast cancer. Sub-
322 analysis suggested that SB and PA independently influence risk of breast cancer (68).
323 Finally, a genome-wide association study (GWAS) of PA and SB provided some
324 insights into underlying mechanism and roles in disease prevention (69). Eighty-eight
325 loci (89 independent SNPs) were associated with leisure screen time, 8 loci for SB at
326 work, and none for SB during commuting. Authors observed significant genetic
327 correlation between high leisure screen time and higher adiposity-related traits,
328 particularly fat percentage, and poor cardiometabolic status, including higher
329 triglycerides, cholesterol, fasting glucose and insulin concentrations, and odds of type 2
330 diabetes, coronary artery disease, cancer, worsened mental health outcomes, and

331 decreased longevity. Additionally, Mendelian randomization has consistently shown that
332 leisure screen time and BMI causally influence each other, with the causal role of
333 leisure screen time in BMI being two-to-threefold larger than the effect of BMI on leisure
334 screen time. In this same study, tissue and cell-type enrichment analysis has also
335 suggested a role for visual information processing and the reward system in leisure
336 screen time. Leisure screen time-associated loci were mildly enriched for genes whose
337 expression in skeletal muscle is altered by resistance exercise training. Forty-six
338 candidate genes pointed to pathways related to endocytosis, locomotion, and
339 myopathy, but in vivo models are required to confirm or refuse a role in SB. Overall,
340 such causal inferences can support public health message that increasing MVPA and
341 reducing SB mitigate risk of multiple chronic diseases (69).

342 The summary of available literature highlights the small evidence base with
343 respect to the mechanisms behind the relationship between SB, health, and disease.
344 Very few genes and genetic regions have been studied in SB research. Despite the
345 limited evidence, Mendelian randomization studies provide genetic support for a causal
346 relationship between SB and risk of chronic diseases. There is a need for larger cohort
347 studies and randomized controlled trials using 'omics' approaches (e.g., genomics,
348 epigenomics, transcriptomics, proteomics, and metabolomics) to better understand the
349 molecular mechanisms underlying the effects of SB on health and disease.

350

351 **2.5. The influence of sedentary behaviors on the relationship between** 352 **exercise and health outcomes**

353 Recent experimental evidence indicates excessive sedentary time also affects the
354 relationship between participation in MVPA/exercise and health benefits (70-74).

355 Coyle and colleagues have demonstrated adverse effects of excessive
356 participation in SB on postprandial metabolic responses and metabolic benefits of acute
357 exercise (72-74). Two or four days of prolonged sitting (>14 h/day and ~1,650 steps)
358 resulted in increased postprandial plasma triglyceride responses regardless of energy
359 intake, compared to 4 days of standing/walking (~8.4 h/day of SB and ~17,000
360 steps/day) in healthy, physically active males. Importantly, this altered response was not
361 attenuated by a subsequent acute 1-hour bout of MVPA (~67% VO_{2max}) performed at
362 1700h of day 4 (72). Using a similar study design, Akins and colleagues (73)
363 demonstrated that acute exposure to prolonged sitting (~13.5 h/day of sitting and
364 <4,000 steps/day) not only prevented the traditional exercise-related benefits in
365 postprandial triglycerides responses, but also improvements in postprandial plasma
366 glucose and insulin responses in healthy adults. Furthermore, Burton & Coyle (74)
367 compared postprandial plasma triglycerides responses to an acute exercise bout after 2
368 days of low (~2,500 steps/day), limited (~5,000 steps/day), or normal (~8,500 steps/day)
369 daily step count to determine the range of step counts that elicited this blunted
370 postprandial metabolic response to acute exercise. Following low and limited step
371 counts, postprandial triglyceride responses were elevated by 22-23% and whole-body
372 fat oxidation was reduced by 14-19% as compared to normal step count in healthy
373 adults (74). This finding indicates that altered metabolic responses to acute exercise
374 can occur in those taking ~5,000 steps/day or lower.

375 These studies provide initial insights into a unique perspective that excessive SB
376 might be a health hazard, not only via the physiological maladaptations that occur
377 during sitting, but also by impacting the health benefits provided by MVPA/exercise.
378 Collectively, these findings highlight the need for: (i) addressing large amounts of time
379 spent in SB to minimize/counteract its adverse effects; (ii) examining the physiological
380 responses and adaptations within and across each of these distinct behavioral
381 constructs, as there may be differential, additive and/or interacting physiological effects
382 to consider.

383 These are research questions that arise from SB physiology that have not been
384 pursued by exercise and physical inactivity physiology. A key feature in SB research
385 has been to focus on shifting the balance of participation in SB towards LPA, rather than
386 solely focusing on MVPA. This has also been included in the development of
387 countermeasures to specifically address SB, with a growing body of experimental
388 studies aiming to reduce and interrupt prolonged SB with various types of PA (75-79).
389 The understanding of the physiological impact of the interdependent relationships
390 between SB, LPA and MVPA is a more recent focus and available evidence has been
391 limited to the abovementioned studies. In the next subsection, the pros and cons of the
392 relevant experimental models will be discussed in the context of their potential
393 implications for investigations of the physiology of SB.

394

395 **2.6. Experimental models with relevance for sedentary behavior research**

396 SB-induced physiological changes in humans can be inferred from a variety of
397 experimental models – bed rest, limb immobilization/casting, reduced daily step count,

398 reducing/interrupting prolonged sitting, and others (e.g., detraining, confinement, and
399 natural experiments) – that collectively induce variations in time spent in SB (FIGURE
400 2). Each of these approaches can provide complementary information related to the
401 impacts of SB on health outcomes. Identifying and understanding the different goals,
402 methodologies and assumptions that can be made under these models is fundamental
403 when attempting to generalize their findings to SB physiology research.

404

405 ----- INSERT FIGURE 2 ABOUT HERE -----

406

407 *2.6.1. Bed rest*

408 Bed rest is a common practice within medical treatment for selected conditions. In
409 research, bed rest was initially developed in the context of space exploration as a
410 ground-based model used to mimic on Earth the physiological effects of microgravity.
411 The bed rest model is characterized by a postural change (lying down or lying down
412 combined with head tilt [i.e., HDBR]) and lack of muscle contraction for extended
413 periods of time. In the quest of mitigating the adverse health effects of microgravity on
414 the body, space agencies have developed and tested countermeasures during bed rest
415 studies including different exercise training protocols (30, 34, 80-85).

416 Beyond the interest in space science, bed rest models have implications for SB
417 and physical inactivity research. Bed rest has the advantage of taking place in a highly
418 controlled environment, which allows accurate monitoring of the activity performed and
419 of food intake. The degree of physical inactivity observed during bed rest may be seen
420 as too extreme compared with that seen in the general population, who spend more

421 time sitting with some level of body movement rather than strictly lying down. However,
422 because of upper body movements and fidgeting movements in the bed, the PA level
423 (i.e., the ratio between total and resting energy expenditures) measured during bed rest
424 studies is similar to that of sedentary individuals (i.e., 1.4 to 1.5) (86, 87). In contrast,
425 strict bed rest involves prolonged exposures to the lying down position, which distinctly
426 affects organs and physiological systems as compared to the free-living context, where
427 sitting is the most predominant type of SB. For example, lower-limb muscles and
428 weight-bearing muscles are more affected than upper limbs and non-weight-bearing
429 muscles during bed rest (37, 88, 89). Shift of fluid from the lower to upper part of the
430 body is a unique adaptation to the lying down position, particularly in HDBR (90), which
431 may distinctly affect cardiovascular and central nervous systems which is in contrast to
432 how SB manifests in the free-living context.

433

434 *2.6.2. Immobilization*

435 Limb immobilization/casting models are characterized by periods during which a limb is
436 physically immobilized (91, 92). In the case of lower-limb immobilization, participants
437 are commonly supported by crutches and asked to refrain from weight-bearing activity
438 on the immobilized leg. Consequently, there is an extensive restriction of motion for the
439 target limb with a reduction in habitual ambulatory activity, which ultimately results in
440 local muscle disuse (91). The main focus of contemporary studies has been to
441 investigate mechanisms underlying muscle disuse atrophy (92, 93), particularly related
442 to aging (94-97), and identifying potential countermeasures (97-99).

443 Lower-limb immobilization models can be useful to investigate local muscle
444 disuse since movements in the casted leg are tightly controlled during the protocol. Of
445 importance for SB research, the lower-limb immobilization protocol likely induces
446 increases in sedentary time. This can be presumed from free-living studies showing a
447 higher sedentary time and lower PA during casting due to lower- or upper-limb fractures
448 compared to healthy peers (100) or previous PA level (101). Similar to bed rest studies,
449 immobilization protocols impose extreme changes in PA and SB levels. Additionally, the
450 absence of control for PA level requires cautious interpretation of findings on systemic
451 alterations in organs and systems other than the impacted limb.

452

453 *2.6.3. Reduced daily step count*

454 Reduced step count models have a focus on addressing how physical inactivity
455 (reduced daily movement) is manifested in the daily lives of the majority of the
456 population. For reference, median daily steps count in adult and older adult populations
457 is typically around 5,000 (102). However, it is common that people may intermittently
458 transition to lower daily step count. Reduced steps models aim to mimic such transitions
459 by reducing participants' daily ambulatory activity from normal to sub-normal levels
460 (103-106). Most protocols aim to reduce the total amount of daily steps to ~1,500
461 steps/day (range: 750 to 5,000) (105, 107-113). While the subsequent reduction in PA
462 does not constitute complete disuse, it is plausible to assume that even short-term
463 exposure to such periods of physical inactivity may have profound physiological
464 consequences (109).

465 The main strength of reduced steps models is that they induce changes that are
466 more similar to typical reductions in ambulatory activity that can occur in daily living due
467 to hospitalization (740 to 2620 steps/day) (114, 115) as compared to strict bed rest, but
468 it is less severe than those observed due to sickness (i.e., influenza-like symptoms;
469 average of 924 steps/day) (116). The measure of PA level used in these studies has
470 almost exclusively been step count. SB is not typically the focus and only a few have
471 accurately reported increases in sedentary time during the step reduction protocols
472 (106, 112, 113, 117), with inferences made about changes in sedentary time as a
473 consequence of reductions seen in time spent in ambulatory activities.

474

475 2.6.4. *Reducing and interrupting prolonged sedentary behavior*

476 Experimental models aimed at reducing and/or interrupting sitting time in laboratory-
477 based and free-living settings are a relatively new approach. A key distinction here from
478 the experimental models described above is the 'solution focused' treatment paradigm
479 whereby typically physically inactive/sedentary individuals modify their typical low PA
480 level to a higher PA level (118). Most reducing/interrupting prolonged SB models are
481 acute in nature (most lasting >2 hours but <24 hours) (75-77, 119, 120). There is a
482 growing number of multi-days (≤ 4 days) (121-124) to longer-term clinical trials (2 weeks
483 to 36 months) (78, 79).

484 Acute and multi-day protocols have utilized a control condition of imposed
485 prolonged sitting and one or more experimental conditions involving, for example, a
486 single continuous bout of activity and/or frequent, short bouts of activity (often referred
487 to as 'breaks') (75-77). These experimental conditions may vary in terms of frequency,

488 intensity, duration, and type of activity used to interrupt sitting (20, 75-77). Acute models
489 targeting reducing/interrupting prolonged SB can provide insights on the physiological
490 effects and underlying mechanisms of such strategies. These studies have been
491 typically conducted in highly controlled research environments, which allows accurate
492 monitoring of potential confounders, including PA level. Consequently, the control
493 condition (prolonged sitting) and experimental conditions (activity protocols) are typically
494 unrepresentative of daily living activity patterns (125). To date, it is still uncertain
495 whether some of the acute adaptations observed within this experimental model can be
496 sustained over time (78) and whether maladaptations to sitting are an impairment to, or
497 a sign of plasticity of, the physiological systems; e.g., health being defined as the ability
498 of our body to cope with daily-life challenges (i.e., phenotypic flexibility) (126).

499 Longer-term clinical trials have generally incorporated a control condition, in
500 which sedentary/physically inactive participants are instructed to maintain their lifestyle,
501 or have received usual care, and an experimental group, in which participants undergo
502 an intervention to reduce/interrupt SB (78, 79). Longer-term randomized clinical trials
503 can provide useful information on the longer-term dose-response effects of
504 reducing/interrupting on health outcomes. Due to the nature of these studies,
505 effectiveness of interventions at reducing/interrupting sedentary time, adherence to
506 intervention, and duration, frequency and intensity of interruptions to SB must be closely
507 monitored, as these factors likely affects the effects of such interventions on health
508 outcomes.

509

510 **2.7. Semantic considerations for interpreting the evidence that can be**
511 **pertinent to sedentary behavior research**

512 In this review, SB is defined based on both physiological and postural features (see
513 subsection 2.1) and is being considered as a distinct behavior that coexists with
514 physical inactivity in daily living. In studies where SB cannot be separated from physical
515 inactivity due to methodological limitations, the evidence that we will now consider
516 below will be discussed in light of study limitations and noted as a consequence of both
517 behaviors. Given the interdependent nature of SB, LPA and MVPA and the lack of
518 studies focusing on this interrelationship, changes in SB and PA level will be reported
519 whenever original studies or meta-analyses reported such changes. This information
520 will be particularly useful to triangulate available evidence to better understand potential
521 differential, additive and/or interacting effects of behaviors on physiological outcomes.
522 The duration of SB interventions will also be reported as we address the relevant
523 findings. The terms ‘acute’ and ‘multi-days’ will be used for studies lasting hours to ≤14
524 days. The term ‘longer-term’ will be used for studies lasting >2 weeks to years.

525 Adaptations to physical inactivity are not the opposite of adaptations to
526 exercise/PA (8-10, 127). Accordingly, evidence from studies using models for increasing
527 SB will be discussed separately from those of models aimed at reducing/interrupting
528 SB. The consequences of increasing SB will be discussed using evidence from bed
529 rest, bed rest combined with exercise, immobilization/casting, reduced daily steps, and
530 acute studies that included a condition imposing prolonged SB. In contrast, the effects
531 of reducing/interrupting SB will be discussed using evidence from acute and longer-term
532 studies that include at least one condition aimed at reducing/interrupting SB with

533 multiple active bouts. Because of the limitations associated with bed rest models,
534 evidence from bed rest related to whole-body outcomes will not be discussed herein.
535 However, given the absence of evidence from other human models of increased SB,
536 bed rest data will be used to inform the potential mechanistic underpinnings of
537 excessive and prolonged SB. Evidence from detraining, confinement, and natural
538 experiments have not been used either, as study findings are constrained by limitations
539 related to study design, targeted population (e.g., athletes, those highly physically
540 active), and the lack of control for sedentary time.

541 Finally, SB research studies to date have included a variety of population groups.
542 Therefore, the use of some key terms has been standardized over the next sections to
543 facilitate the discussion of available evidence. Adult and older adult groups have been
544 defined according to age cut points used by the original studies ('adults': 18-59 or 18-
545 64; 'older adults': ≥ 60 or ≥ 65 years old). The term 'children' has been used for 5-12
546 years old and 'adolescents' for 13-17 years old. With respect to health status, the term
547 'healthy' will be used to refer to population groups without any existing medical
548 condition. Otherwise, health status or condition (e.g., overweight, obesity, type 2
549 diabetes) will be reported along with study findings. The sex of participants will be
550 reported for studies that included females or males only. Sex-neutral terms will be used
551 in studies that included participants from both sexes.

552 The considerations outlined above in Section 2 provide perspectives and caveats
553 of relevance to the evidence we address below for each of the relevant major bodily
554 systems and processes. Accordingly, in the following sections (3 to 8), we examine the
555 relevant physiological evidence relating to: body weight and energy balance;

556 intermediary metabolism; cardiovascular and respiratory systems; the musculoskeletal
557 system; the central nervous system; and immunity and inflammatory responses.

558

559 **3. BODY WEIGHT AND ENERGY BALANCE**

560

561 **3.1. Body mass and composition**

562

563 *3.1.1. Increasing sedentary behavior*

564

565 *3.1.1.1. Evidence from longer-term studies*

566 Fourteen days of reduced step count (from 10,501 to 1,344 steps/day) reduced leg lean
567 mass (~0.5 kg) and increased intra-abdominal fat mass (7%), but not total fat mass in
568 healthy male adults (108, 109). Other studies (14 to 20 days; from ~11,500 to 2,000
569 steps/day) have shown similar alterations in healthy male adults, but also revealed
570 increases in total and percent body fat (~3 to 14%) (106, 107, 128). Interestingly,
571 alterations in body composition observed after 14 days of reduced step count (81%
572 reduction from baseline plus a 3.7 h/day increase in sedentary time) returned to
573 baseline levels after resuming habitual PA for 14 days in healthy adults with or without a
574 first-degree relative with type 2 diabetes (112). Although inconsistent across studies,
575 most step reduction protocols were detrimental for at least one body composition-
576 related outcome in healthy older adults (105, 110, 129, 130). Fourteen days of reduced
577 step count (from ~9,000 to 3,000 steps/day) also resulted in intramuscular
578 (nuclear/myofibrillar fraction) ceramides accumulation (~20%) in healthy older adults
579 (130).

580

581 3.1.1.2. Clinical significance

582 Increases in body fat mass induced by models involving increased SB are likely
583 clinically relevant. Specifically, increases in body fat mass reported in reduced step
584 studies (3 to 14%) are considerably more pronounced than longitudinal changes
585 observed in the general population. A populational cohort study showed an ~1% (0.7
586 kg) increase in body fat mass over the course of 12 years (131). Of concern, measures
587 of adiposity (BMI, visceral fat mass or central adiposity and body fat percentage) are
588 positively associated with increased risk of all-cause, cardiovascular disease and
589 cancer mortality (132), and 21 major chronic diseases (133).

590

591 3.1.2. *Reducing and interrupting sedentary behavior*

592

593 3.1.2.1. Evidence from longer-term studies

594 A meta-analysis has analyzed data from longer-term studies investigating the effects of
595 SB interventions conducted in free-living settings on adiposity outcomes. Intervention
596 duration ranged between 2 weeks and 36 months, and the average change in total
597 sedentary time was -28.6 min/day (78). There were small significant reductions in body
598 weight (-0.6 kg), waist circumference (-0.7 cm) and percent body fat (-0.3%) in adults
599 and older adults, but no changes have been reported in BMI, total body fat and total fat-
600 free mass (78). Another meta-analysis investigating the effects of SB interventions
601 (range: 6 to 24 weeks) on body composition demonstrated significant decreases in total
602 sedentary time (-64 min/day) and increases in walking time (27 min/day), but no

603 significant changes in time spent standing and in MVPA (134). Authors noted small
604 significant decreases in percent body fat (-0.7%) and waist circumference (-1.5 cm)
605 following SB interventions, but no changes in body weight and BMI in clinical population
606 groups (those with overweight, obesity, type 2 diabetes, cardiovascular,
607 neurological/cognitive, and musculoskeletal diseases) (134).

608 A meta-analysis of studies aiming to replace SB with standing time (mean follow-
609 up: 3.8 months) demonstrated a significant increase in total standing time (1.3 h/day) in
610 adults. This was associated with a significant decrease in total body fat mass (-0.75 kg),
611 but no changes in body weight and waist circumference (135). Finally, a systematic
612 review of studies implementing workplace SB interventions in apparently healthy and
613 overweight/obese desk-based office workers demonstrated that the effects of workplace
614 interventions on body composition have been inconsistent across studies. Only 11 out
615 of 29 studies reported improvements in measures of adiposity, with most studies
616 showing no changes following SB interventions (136).

617 In children, an 8-month, school-based intervention using height-adjustable desks
618 in the classroom was ineffective at reducing classroom and total daily SB (137).
619 Consequently, no significant changes were observed on BMI z-score and waist
620 circumference (137). However, other longitudinal studies demonstrated that when
621 coupled with increases in PA, reducing SB prevents unhealthy weight gain. The socio-
622 ecological French ICAPS (Intervention Centered on Adolescents' Physical activity and
623 Sedentary behavior) study (138) concomitantly targeted PA and SB through a 4-year
624 multi-level intervention that focused on the school and family of the children and the
625 children themselves. Compared to the controls (no intervention), pupils who received

626 the intervention were more active, less sedentary and gained less weight throughout the
627 4 year-study (138). Importantly, these changes in physical behavior and the prevention
628 of weight gain were maintained 2.6 years after the end of the intervention with the
629 highest efficacy in the most sedentary adolescents (139).

630 In summary, it is evident that findings are inconsistent across studies and meta-
631 analyses. It is not clear whether type, intensity, and frequency of interruptions to sitting
632 differentially affect body composition outcomes, nor if there are specific factors that
633 mediate adaptations in body composition following SB interventions (e.g., age, sex,
634 BMI, population group).

635 *Reducing/interrupting SB vs continuous MVPA/exercise.* A small-scale, 12-week,
636 multifactorial, pilot study examined both the independent and the combined effects of
637 exercise training (40-65% heart rate reserve, i.e., moderate to vigorous intensity) and
638 reducing SB (replace SB with standing and LPA plus increasing daily step count by 5-
639 10%) on body composition in adults with overweight/obesity (140). Compared to control,
640 both exercise training and exercise training combined with reducing SB significantly
641 changed SB and PA level (no change in SB + 27 min/day increase in MVPA and -7.3%
642 of daily hours in SB + 45 min/day increase in MVPA, respectively). This resulted in a
643 significant decrease in BMI (-0.5 and -1.1 kg/m², respectively), body weight (-2.3 and -
644 3.4 kg, respectively) and total body fat (-1.0 and -1.4%, respectively). The addition of
645 interruptions to SB did not result in greater improvements in body composition
646 compared with exercise training only (140). No changes in body composition outcomes
647 were observed in the group reducing SB only (140).

648 Another small-scale, 6-month pilot study examined the effects of a stepping
649 protocol during TV commercial (brisk walking around the room during at least 90 min of
650 TV programming at least 5 days/week) versus brisk walking for 30 min/day (at least 5
651 days/week) in adults with overweight/obesity (141). After 6 months of intervention, both
652 protocols increased the number of daily steps and decreased time watching TV (2,994
653 vs 2,956 steps/day and -1.2 vs -1.4 h/day, respectively) as well as TV-related energy
654 intake (-282 vs -517 kcals/day, respectively). Both interventions significantly reduced
655 percent body fat (-1.0 vs -0.9%, respectively), waist circumference (-2.5 vs -1.6 cm,
656 respectively) and hip circumference (-1.9 vs -1.2 cm, respectively) at the 6-month time-
657 point, but neither changed body weight and BMI (141). In contrast, 4 weeks of
658 interventions aimed at reducing SB (-53 min/day in SB with no change in MVPA) or
659 increasing MVPA to at least 30 min day (+16 min/day of MVPA with no change in SB)
660 did not lower BMI and waist circumference in physically inactive adults with obesity
661 (142).

662

663 3.1.2.2. Clinical significance

664 There is some evidence of small to trivial improvements in body mass and composition
665 (body weight [-0.6 kg], waist circumference [-0.7 to -1.5 cm] and percent body fat [-0.3
666 to -0.7%]) associated with reducing/interrupting SB. Mixed results have also been
667 reported across original studies and meta-analyses. Despite intervention effects being
668 small and likely not clinically relevant, it is important to highlight that mean baseline BMI
669 ranged between 25 and 30 kg/m² and study duration varied between 6 to 24 weeks in
670 available meta-analyses (78, 134). It has been suggested that the weight loss induced

671 by 1-year exercise programs is more pronounced in those with existing obesity as
672 compared to individuals with overweight (1.1 to 1.5 kg less than individuals with obesity)
673 (143). It is not clear whether baseline BMI, other measures of adiposity and duration of
674 intervention may have affected responses to reducing/interrupting SB interventions.

675 Reducing SB and increasing LPA without increasing engagement in
676 MVPA/exercise does not seem to be an effective strategy to improve markers of
677 adiposity as compared to traditional, continuous exercise (141, 142). These findings
678 indicate that reducing/interrupting SB with PA in higher intensities might be required to
679 improve markers of adiposity in those with overweight/obesity. As recently reviewed
680 thoroughly (144), reducing/interrupting SB is likely not effective at inducing weight loss,
681 like exercise, but may prevent unhealthy weight gain. Future studies will also need to
682 investigate whether changes in SB/LPA trigger spontaneous behavioral and
683 physiological compensatory responses (e.g., decrease in activity and/or non-activity
684 energy expenditures, increase in appetite and food intake) like those observed following
685 the initiation of exercise training and thought to minimize the effect of exercise on body
686 weight (145, 146), as further discussed in the next topics.

687

688 **3.2. Total energy expenditure**

689

690 *3.2.1. Increasing sedentary behavior*

691

692 3.2.1.1. Evidence from multi-day studies

693 Participation in high levels of SB results in lower energy expenditure and PA levels. For
694 example, 7 days of exposure to a highly sedentary condition in free-living (increased
695 sedentary time and limited participation in PA of any intensity) significantly reduced
696 energy expenditure (-15% in MET-hour/week estimated from a validated accelerometer)
697 (147).

698

699 3.2.1.2. Evidence from longer-term studies

700 In a clinical study requiring physically active, but not trained, lean male adults to refrain
701 from PA for 1 month, total daily energy expenditure decreased by 8% due to a drop in
702 activity-related energy expenditure only (127).

703

704 3.2.2. *Reducing and interrupting sedentary behavior*

705

706 3.2.2.1. Evidence from acute studies

707 Experimental studies have demonstrated that both standing and ambulatory
708 interruptions to prolonged sitting time increased energy expenditure in adults, as a
709 function of the duration, intensity and modality used (148-150). Interestingly, the
710 increased energy utilization in response to frequent 2-minute moderate-intensity walking
711 interruptions to sitting was maintained for ~4 minutes after every walking bout in adults.
712 When repeated throughout the day, performing 28 minutes of interruptions to sitting
713 resulted in ~70 minutes of elevated energy utilization over 7 hours (150).

714

715 3.2.2.2. Evidence from longer-term studies

716 It is still unclear whether SB reducing/interruption interventions may result in
717 compensatory changes in total energy expenditure and/or energy balance over the
718 longer term. A study demonstrating the differential impact of low- and moderate-
719 intensity training provides some relevant insights (151). Moderate-intensity aerobic
720 exercise training (60% $VO_{2\text{reserve}}$) tended to increase total energy expenditure in female
721 adults with overweight/obesity compared to low-intensity training (40% $VO_{2\text{reserve}}$)
722 matched for energy expenditure over 3 months of intervention. Exercise energy
723 expenditure was almost entirely compensated (96%). Interestingly, greater energy
724 compensation was observed in the moderate-intensity group than in the low-intensity
725 group (161% vs 49%) (151). Participants in the low-intensity group spent more time
726 walking and less time lying down compared to the moderate-intensity group. These
727 behavioral and energetic differences translated into about 1 kg weight gain in the
728 moderate-intensity group versus 1 kg weight loss in the low-intensity group (151). The
729 clinical significance of these findings is still unclear.

730

731 **3.3. Energy intake and appetite**

732

733 *3.3.1. Increasing sedentary behavior*

734

735 3.3.1.1. Evidence from acute studies

736 Granados and colleagues (152) showed that 1 day of sitting decreased energy
737 expenditure without a reduction in appetite in adults, suggesting this would favor a
738 positive energy balance. This is consistent with findings that demonstrated no

739 compensatory decline in *ad libitum* food intake in response to large reductions in energy
740 expenditure (~24%) in healthy male adults (153). Another study (154) also
741 demonstrated that energy intake during 1 day of decreased energy expenditure (275
742 steps/day) was comparable to energy intake during higher PA levels (equivalent to ~1.5
743 and 2.1 resting metabolic rate) in healthy male adults.

744

745 3.3.1.2. Evidence from longer-term studies

746 While there is no evidence on the impact of a reduced step count protocol on appetite
747 regulation, 14 days of reduced step count (81% reduction from baseline) did not
748 significantly alter fasting adiponectin and leptin levels in health male adults (108).

749 Acute experimental findings indicate that increasing SB can result in positive
750 energy balance, yet caution is warranted when interpreting the clinical relevance of
751 these findings, as acute changes in appetite may not affect weight control in the longer-
752 term. Longer-term investigation into the effects of increasing SB on energy balance is
753 warranted.

754 *Potential mechanisms.* Several hormones are involved in the regulation of
755 appetite and feeding behavior. A study demonstrated a significant decrease in
756 adiponectin levels (~21%; a hormone associated with increased sensation of hunger) in
757 healthy male adults following 16 days of horizontal bed rest, but no changes in other
758 appetite-regulating hormones (ghrelin, peptide YY [PYY], glucagon-like peptide 1 [GLP-
759 1], and leptin) (155). In a 2-month HDBR study in females fasting leptin was negatively
760 associated with the spontaneous decrease in energy intake, thus suggesting a
761 relationship between PA, leptin and food intake (86).

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3.3.2. Reducing interrupting sedentary behavior

3.3.2.1. Evidence from acute studies

Over a 12-hour period, performing hourly 5-minute vigorous-intensity walking bouts (60-65% VO_{2peak} , total: 60 minutes) resulted in lower perceived hunger (~23%) in the mid-afternoon hours as compared to prolonged sitting and an energy-matched moderate-intensity continuous walking bout (60-65% VO_{2peak} , total: 60 minutes) in adults with obesity, but did not affect PYY levels (156). Changes in perceived hunger were not observed for continuous exercise (60-65% VO_{2peak} , total: 60 minutes) followed by prolonged sitting (156). Another study demonstrated that, while frequent 5-minute moderate-walking interruptions (perceived effort: 12 – 13 “somewhat hard”, total: 30 minutes) did not affect hunger and desire for food consumption, this strategy resulted in lower food cravings (~6%) compared to prolonged sitting in healthy adults (157). This was not observed for continuous exercise (perceived effort: 12 – 13 “somewhat hard”, total: 30 minutes) followed by prolonged sitting (157). Furthermore, while interrupting sitting with 2-minute light or moderate-intensity walking bouts every 20 minutes (perceived effort: 6–9 “very light” and 12–14 “somewhat hard”, respectively; total: 28 minutes) did not alter appetite, it resulted in reduced relative energy intake (39 and 120%, respectively) that was not compensated for in a subsequent meal in healthy adults, which could have important implications for weight management (158). In contrast, other studies showed no alterations in appetite measures, *ad libitum* intake and circulating gut hormone concentrations following interruptions to sitting in healthy

785 adults (158-160). It is not clear whether type, intensity, and frequency of interruptions to
786 sitting can differentially affect energy intake and appetite.

787

788 3.3.2.2. Evidence from longer-term studies

789 Replacing prolonged sitting time with regular standing bouts at the workplace (a 21%
790 reduction of workplace sedentary time) reduced dietary intake (~10%) in sedentary,
791 adult office workers after 4 weeks of intervention (161). It remains unclear the extent to
792 which this reduction in caloric intake impacted measures of adiposity and other
793 cardiometabolic outcomes. Longer-term investigation into the effects of
794 reducing/interrupting SB on appetite and food intake is warranted.

795

796 **4. INTERMEDIARY METABOLISM**

797

798 **4.1. Glucose metabolism**

799

800 *4.1.1. Increasing sedentary behavior*

801

802 4.1.1.1. Evidence from acute and multi-day studies

803 Experimental findings show that in healthy adults as little as 1 day of exposure to SB
804 (~17 h/day of SB) combined with energy surplus reduced whole-body insulin sensitivity
805 (-39%), but did not change fasting glucose and insulin concentrations, as compared to a
806 minimal sitting condition (~6 hours of sitting). Importantly, reducing energy intake to
807 match energy demand during prolonged sitting significantly attenuated, but did not fully

808 mitigate, the decline in insulin action (-18%) (162). This finding indicates that excessive
809 sitting might be detrimental for insulin sensitivity irrespective of energy balance. Seven
810 days of exposure to a highly sedentary condition (increased sedentary time and limited
811 participation in PA of any intensity) did not alter fasting glucose and insulin
812 concentrations in healthy-lean adults (147). Increasing SB significantly increased 2-hour
813 post-load insulin concentration ($38.8 \text{ uIU}\cdot\text{mL}^{-1}$) and reduced insulin sensitivity (-17.2%),
814 as assessed by a composite insulin sensitivity index. Changes in time spent in
815 prolonged sedentary bouts (>30 and 60 continuous minutes), but not in LPA and MVPA,
816 were positively associated with 2-hour post-load insulin concentrations (147).

817 In a crossover randomized trial, healthy adults performed 10 days of reduced
818 steps (from 12,154 to 4,275 steps/day, with a 10% increase in SB) while consuming a
819 control diet (16% protein, 64% carbohydrate, 20% fat; 80% of daily energy need) or a
820 high-protein diet (30% protein, 50% carbohydrate, 20% fat; 80% of daily energy need)
821 (163). Independent of diet, there were no changes in fasting glucose and insulin
822 concentrations, and post-load glucose and insulin responses in healthy adults following
823 step reduction. However, in another study 10 days of step reduction resulted in reduced
824 insulin sensitivity along with increases in carbohydrate oxidation measured in response
825 to an OGTT (73). Combining step reduction with overfeeding also increased fasting
826 glucose and insulin concentrations, post-load 2-hour glucose concentrations, and post-
827 load glucose responses in healthy male adults (163), thus suggesting that energy
828 surplus exacerbates the metabolic deteriorations triggered by exposures to both SB and
829 physical inactivity.

830

831 4.1.1.2. Evidence from longer-term studies

832 Fourteen days of reduced step count (from 10,501 to 1,344 steps/day) resulted in rapid
833 decreases in whole-body (~58%) and peripheral insulin sensitivity (~17%) in healthy
834 male adults (108, 109), which was accompanied by a significant reduction in insulin-
835 stimulated pAkt^{thr308}/total Akt protein expression (108). Similar alterations have been
836 observed in healthy adults with/without a first-degree relative with type 2 diabetes (112)
837 and older adults (130, 164). Reducing daily step count (3 days, from 12,956 to 4,319
838 steps/day) increased postprandial (30 to 90 minutes after a meal) glucose responses (6
839 to 9%) and glycemic variability (33 to 97%) in healthy adults as assessed by continuous
840 glucose monitors, despite the absence of changes in post-load glucose responses
841 following an oral glucose tolerance test (117). Twenty days of reduced steps (from
842 14,000 to 3,000 steps/day) increased total glucose oxidation in healthy physically active
843 male adults, which was associated with a significant decrease in nonprotein respiratory
844 quotient during an oral glucose tolerance test (106), which indicates the development of
845 metabolic inflexibility (i.e., the inability of the body to adjust substrate use to changes in
846 substrate availability). While no changes have been reported for fasting glucose
847 concentrations following step reduction protocols in healthy adults (106-108, 117), some
848 studies demonstrated increases in fasting insulin concentrations (106, 107, 117). The
849 absence of changes in glycemia may reflect compensatory increased insulin levels in
850 response to reduced step count. Resuming habitual daily activities was sufficient to
851 restore whole-body insulin sensitivity to baseline levels in healthy adults and older
852 adults (107, 112, 165).

853 *Potential mechanisms.* Short- to longer-term exposures (3 to 90 days) to
854 horizontal bed rest and HDBR are associated with reduced whole-body insulin
855 sensitivity (166, 167), altered fuel selection towards the use of carbohydrate (166, 168),
856 and metabolic inflexibility that precedes the development of glucose intolerance (168).
857 Some mechanisms have been proposed including changes in body composition, body
858 fat repartition, alterations in lipid content, oxidative stress, and capillary density (169).
859 While some have been confirmed, controversial results have been obtained for others.
860 For example, 60 days of HDBR induced fat accumulation in skeletal muscle (170) and
861 with low-grade inflammation (171). However, Shur and colleagues (172) failed to
862 demonstrate an increase in intramyocellular lipid content following 3 and 56 days of
863 HDBR, which was not confounded by positive energy balance unlike previous studies
864 (173, 174). Similarly, after one week of bed rest, reduced insulin sensitivity was
865 observed along with reduced oxidative capacity but not increases in muscle lipid level or
866 degree of saturation, markers of oxidative stress or reductions in capillary density,
867 suggesting other mechanisms are likely at play (88). Pronounced transcriptomic
868 changes of the skeletal muscle metabolic pathways have also been proposed. Shur and
869 colleagues (172) reported extensive changes in mRNA abundance in gene targets
870 controlling carbohydrate metabolism (40 transcripts) after only 3 days of HDBR. These
871 changes preceded the modifications in whole-body fuel selection and the reduction in
872 muscle glycogen storage. Another study demonstrated similar alterations in the
873 expression of genes associated with insulin resistance and fuel metabolism following 9
874 days of bed rest (175). Additionally, bed rest-induced reduction in insulin sensitivity has
875 been accompanied by reduced skeletal muscle GLUT-4, hexokinase II, protein kinase B

876 (Akt) 1, and Akt2 proteins content, and decreased insulin-stimulated glycogen synthase
877 (GS) activity and Akt signaling (176). These findings suggest that both decreased
878 glucose transport and decreased nonoxidative glucose metabolism in skeletal muscle
879 contribute to changes in carbohydrate metabolism (FIGURE 3).

880

881 ----- INSERT FIGURE 3 ABOUT HERE -----

882

883 4.1.1.3. Clinical significance

884 Excessive SB has been positively associated with increased 2-hour post-load glucose
885 and fasting insulin concentrations in the general population, independent of participation
886 in MVPA (177, 178). Experimental models imposing periods of increased sedentary
887 time in both adults and older adults consistently induced alterations in whole-body and
888 peripheral insulin sensitivity (-17 to -58%), but not in fasting glucose concentrations. The
889 clinical significance of these findings is unclear. However, the dramatic reduction in
890 insulin action within days of increased prolonged SB is relevant given that SB is the
891 most prevalent behavior (8.3 to 11.5 h/day) (4) and alterations in markers of glucose
892 control are associated with greater risk for cardiovascular disease and events even in
893 those without diabetes (179, 180).

894

895 4.1.2. *Reducing and interrupting sedentary behavior*

896

897 4.1.2.1. Evidence from acute and multi-day studies

898 Acutely, interrupting sitting with frequent, short bouts of LPA improved postprandial
899 glucose responses by 17.5% and insulin responses by 25.1% compared to prolonged,
900 uninterrupted sitting in both healthy and metabolically impaired adults and older adults,
901 as evidenced by meta-analysis (75). These results have been corroborated by other
902 meta-analyses that have included studies investigating other types (e.g., standing still,
903 walking, and simple resistance activities) and intensities (e.g., light, moderate and
904 vigorous intensity) of interruptions to sitting (Cohen's d for glucose and insulin: -0.26 to -
905 0.83 favoring intervention) (76, 77, 181). However, it should be noted that some original
906 studies have not demonstrated changes in postprandial glucose and/or insulin
907 responses following at least one of the interruptions to SB protocols (standing, simple
908 resistance activities, LPA to VPA walking) compared to prolonged sitting (21, 123, 124,
909 182-196).

910 Reducing/interrupting sitting for 3 to 4 days reduced postprandial glucose (121,
911 197) and insulin responses (122-124, 197) in adults and older adults with
912 overweight/obesity and type 2 diabetes (PA intensity and duration: 3.2 km/h, 2.8 METs,
913 total: 34 min/day (121); perceived effort 13 "somewhat hard", total: 45 min/day (124);
914 93-95 steps/min, total: 3.1-4.1 h/day of standing and 3.1-4.9 h/day of stepping (122,
915 123, 197)). However, results have been more inconsistent for postprandial glucose
916 responses following interruptions to sitting with standing/LPA (122, 123) and moderate
917 PA (MPA) (124). No changes were observed in fasting glucose concentrations following
918 3 days of interrupting SB with standing or LPA (121-123, 197) and MPA (124), but
919 reductions in fasting insulin concentration were reported in adults with overweight (-1.8
920 mIU/L) (122) and type 2 diabetes (-13 pmol/L) (197). Despite these inconsistencies,

921 insulin sensitivity improved after 3 days of interrupting SB (12-29%) (122-124, 197).
922 Interestingly, 3 days of interrupting prolonged sitting with regular 2-minute bouts of light-
923 intensity walking (pace: 3.2 km/h, 2.8 METs, perceived effort: 6–11 “fairly light”; total: 28
924 minutes) sustained, but did not further enhance, improvements in postprandial glucose
925 (-4%; estimated average concentration: 103 mg/dL following interruptions vs 107 mg/dL
926 following prolonged sitting) and insulin responses (-12%; estimated average
927 concentration: 262 pmol/L following interruptions vs 297 pmol/L following prolonged
928 sitting) observed on the first day of intervention (121).

929 Both light- and moderate-intensity bouts improved postprandial glucose and
930 insulin responses, while standing interruptions did not significantly affect these
931 responses (76, 181, 198, 199). A pooled analysis of three acute laboratory-based trials
932 also showed that the estimated energy cost of interruptions to sitting was associated
933 with lower postprandial glucose and insulin responses in a dose dependent manner in
934 sedentary adults with overweight or obesity. Specifically, light- and moderate-walking
935 interruptions (3.2 and 5.8 km/h; 2.8 and ~4.3 METs, respectively; 2-minute bouts every
936 20 minutes, total: 28 minutes), but not standing still, significantly reduced postprandial
937 responses of both markers compared to prolonged sitting (200). Yet, a meta-analysis
938 (181) and a few original studies (201-203) suggested standing interruptions are effective
939 at improving glucose responses.

940 As for frequency of the active interruptions, results have been inconsistent across
941 studies. Interrupting prolonged sitting with 6-minute bouts of simple resistance activities
942 every 60 minutes (squatting, calf raises; total: 36 minutes) was more effective at
943 decreasing postprandial glucose responses (-21%, estimated average concentration:

944 193 mg/dL following interruptions vs 203 mg/dL following prolonged sitting) in adults
945 and older adults with type 2 diabetes compared to a higher frequency of interruptions
946 (i.e., 3-minute bouts every 30 minutes, total: 39 minutes) and 8 hours of prolonged
947 sitting (186), for which no differences were observed. Additionally, less-frequent
948 interruptions to sitting acutely improved glycemic control in the 4-hour period following
949 lunch, while more-frequent interruptions were likely more beneficial for nocturnal
950 glucose control, as assessed by continuous glucose monitors over 22 hours (204). No
951 differences were noted for mean glucose and other markers of glucose variability
952 between interruptions and prolonged sitting (204). Other studies also showed similar
953 inconsistencies for glucose and insulin responses across different frequencies of
954 interruptions to SB compared to prolonged sitting (188, 192, 205). In addition, Duran
955 and colleagues (206) suggested higher frequency and higher duration of light-walking
956 interruptions to sitting (3.2 km/h, 2.8 METs; 5 minutes every 30 minutes, total: 70
957 minutes) should be considered when targeting improvements in glycemic responses in
958 healthy adults and older adults. This strategy significantly attenuated postprandial
959 glucose responses as compared to sedentary control (total: 8 hours), and conditions
960 with lower frequency (every 60 minutes, total: 35 minutes) and lower duration (1 minute,
961 total: ~7 or 14 minutes) of interruptions to sitting, for which no changes were observed
962 (206). To date, available evidence is not sufficient to draw clear-cut conclusions on the
963 dose-response relationship between the frequency of the active bouts to interrupt
964 prolonged sitting and post-prandial glycemia and insulinemia, and further studies are
965 needed.

966 Subgroup analyses in original studies showed that attenuations in the magnitude
967 of reductions in postprandial glucose (122, 199, 207) and/or insulin (199) responses are
968 more pronounced in females than in males following interruptions to sitting as compared
969 to prolonged sitting, which was corroborated by meta-analytic evidence (77).
970 Additionally, meta-regressions revealed that higher BMI is significantly associated with
971 greater reductions in postprandial glucose and insulin responses following interruptions
972 to sitting as compared to prolonged sitting (77). Pooled data from three randomized
973 crossover trials showed that those with higher underlying levels of fasting insulin and
974 insulin resistance may derive greater reductions in postprandial insulin responses from
975 regularly interrupting prolonged sitting than their healthier counterparts. Similarly, those
976 with poorer fasting glucose and β -cell function may derive greater reductions in
977 postprandial glucose responses from performing walking interruptions to sitting (208).
978 These findings have been corroborated by subgroup analyses in two meta-analyses
979 indicating that improvements on glycemia were more prominent in metabolically
980 impaired adults and older adults compared to healthy counterparts (75, 77). Moreover,
981 McCarthy and colleagues (209) demonstrated that reductions in postprandial glucose
982 responses were more pronounced in those with lower cardiorespiratory fitness (25th and
983 50th centiles) following light-intensity walking interruptions to sitting (3.0 km/h, 2.0
984 METs, 5-minute bouts every 30 minutes, total: 1 hour) (209). Altogether these findings
985 show that females, those with higher BMI, higher insulin resistance and lower
986 cardiorespiratory fitness may derive greater benefit from interrupting sitting with respect
987 to glucose metabolism.

988 Results have been mixed in children and adolescents. Interrupting sitting with 3-
989 minute bouts of moderate-intensity walking (heart rate at 80% of ventilatory threshold,
990 total: 18 minutes) significantly reduced postprandial insulin responses (-21%; estimated
991 average concentration: 92 mIU/dL following interruptions vs 117 mIU/dL following
992 prolonged sitting), but not glucose responses, in children with overweight/obesity
993 compared to 3 hours of prolonged sitting (210). Insulin sensitivity, as assessed by the
994 Matsuda index, was also greater during the interruption protocol (17%) (210). In
995 healthy-weight children, this same interruption protocol significantly reduced
996 postprandial glucose (-7%; estimated average concentration: 105 mg/dL following
997 interruptions vs 112 mg/dL following prolonged sitting) and insulin (-32%; estimated
998 average concentration: 30 uU/mL following interruptions vs 45 uU/mL following
999 prolonged sitting) responses compared to 3 hours of prolonged sitting (211).
1000 Furthermore, in healthy children and adolescents, both light-intensity walking
1001 interruptions to sitting (30% $\dot{V}O_{2peak}$, 2-minute every 20 minutes, total: 42 minutes) and
1002 interruptions combined with two 20-minute bouts of moderate-intensity walking did not
1003 affect postprandial glucose and insulin responses compared to 8 hours of prolonged
1004 sitting (212).

1005 *Reducing/interrupting SB vs continuous MVPA/exercise. Acute (8 to 9 hours)*
1006 reductions in postprandial glucose responses were more pronounced following frequent
1007 light-walking interruptions to sitting (total: 30 – 42 minutes) compared to a continuous
1008 bout of activity (total: 30 minutes) in healthy and at-risk adults and older adults (213-
1009 215). However, other studies did not observe such differences between patterns of
1010 activity (216-219). Frequent moderate-intensity interruptions were more effective at

1011 improving postprandial glucose and insulin responses than a single, continuous bout of
1012 moderate-intensity activity when compared to prolonged sitting (Cohen's d: -0.69 and -
1013 0.47 versus -0.16 and -0.22, respectively), as evidenced by meta-analysis (198).
1014 Another meta-analysis demonstrated similar results for postprandial glucose responses,
1015 but no differences were observed for insulin responses (77). In multi-day studies (2 to 4
1016 days), reducing/interrupting SB was shown to be more effective at reducing fasting
1017 insulin, 24-hour glucose responses and duration of hyperglycemia episodes, but not
1018 other markers of glucose metabolism (e.g., fasting glucose), compared to continuous
1019 exercise in adults and older adults with type 2 diabetes (93 steps/min, total: 4.1 h/day of
1020 standing and 3.1 h/day of stepping vs 50-60% W_{max} , total: 1 h/day) (197). In healthy
1021 adults and adults with overweight/obesity, there were no differences between patterns
1022 of activity in markers of glucose metabolism (124, 220).

1023 *Potential mechanisms.* The effects of interrupting sedentary time on glucose
1024 control are likely related to a greater reliance upon carbohydrate oxidation as fuel. Acute
1025 studies (6 to 9 hours) in healthy adults (214, 218) showed that the lower glucose
1026 response following light- (25% VO_{2max} , total: 3.5 hours) and moderate- (~46% VO_{2max} ,
1027 total: 30 minutes) walking interruptions to sitting compared to prolonged sitting (214,
1028 218) was associated greater total carbohydrate oxidation. De Jong and colleagues
1029 (124) also demonstrated in adults with overweight or obesity that hourly 5-minute bouts
1030 of moderate-walking interruptions during sitting (perceived effort 13 "somewhat hard",
1031 total: 45 min/day) primarily relies upon carbohydrate as fuel over 24 h after 4 days of
1032 intervention as compared to prolonged sitting and a time-matched continuous bout of
1033 brisk walking. This effect does not appear to be related to energy expenditure and

1034 balance, but rather to increasing the frequency of muscle contractions spread across
1035 the day. However, these differences in substrate use were not accompanied by
1036 changes in fasting and postprandial glucose responses (124).

1037 The skeletal muscle is the largest glucose consuming organ of the body (221)
1038 and the largest lean tissue mass in adults without obesity (222). Lack of muscle
1039 contractions have been one of the proposed mechanisms for SB-related impairments in
1040 glucose metabolism. During periods of SB, skeletal muscle accounts for only 15% of
1041 whole-body glucose (223), whereas it accounts for more than 80% of the insulin-
1042 stimulated glucose disposal and is quantitatively the most dominant tissue during
1043 exercise (221). Hamilton and colleagues (224) developed a physiological method of
1044 muscle contractile activity to magnify and sustain soleus oxidative metabolism (~88%
1045 type I slow-twitch fibers) through performing 'soleus push ups' (~1.3 and 1.7 METs, 50
1046 to 100 contractions/min). Sustained continuous soleus contractile activity improved
1047 systemic metabolic regulation, by reducing 3-hour post-load glucose and insulin
1048 responses (-39-52% and -41-60%, respectively) and 2-hour post-load glucose
1049 concentration (-29-46 mg/dL) in adults and older adults (BMI: 20-43 kg/m²). Sustained
1050 contractions also increased energy demand (91 kcal/h above sedentary control) and
1051 local carbohydrate oxidation (100-200 mg/min above sedentary control) (224). These
1052 findings indicate that increasing local contractile activity in small oxidative muscles can
1053 be a potent strategy for improving systemic metabolic regulation.

1054 In the skeletal muscle, 5 hours of frequent, walking interruptions during sitting
1055 (3.2 km/h, 2.8 METs, 2-minute bouts every 20 minutes, total: 28 minutes) altered
1056 expression of 10 genes involved in carbohydrate metabolism, including increased gene

1057 expression of *dynein light chain (DYNLL1)*, which may regulate translocation of the
1058 GLUT-4, and *pyruvate dehydrogenase kinase 4 (PDK-4)*, which inhibits the pyruvate
1059 dehydrogenase complex and increases glucose utilization in adults with
1060 overweight/obesity (225). Five hours of interrupting prolonged sitting with light- and
1061 moderate-intensity walking (3.2 km/h, 2.8 METs and 5.8 km/h, ~4.3 METs, respectively;
1062 2-minute bouts of walking every 20 minutes, total: 28 minutes) further resulted in an
1063 upregulation of the contraction-stimulated glucose uptake pathway (i.e., adenosine
1064 monophosphate-activated protein kinase (AMPK)-mediated), while three consecutive
1065 days of interrupting sitting resulted in a transition towards upregulation of the insulin-
1066 mediated glucose uptake pathway (i.e., Akt-mediated) along with greater capacity for
1067 glycogen synthesis (i.e., increase in total GSK3 β protein expression) in the skeletal
1068 muscle of adults with overweight/obesity (226). In contrast, there were no differences in
1069 pAS160^{Thr642}/AS160 ratio and GLUT4 protein expression in the skeletal muscle of post-
1070 menopausal females with rheumatoid arthritis following light-intensity walking
1071 interruptions to sitting (~25% heart rate reserve, 3-minute bouts every 30 minutes, total:
1072 42 minutes) compared to 8 hours of prolonged sitting (213). Finally, although acute
1073 exposure to moderate-intensity active interruptions in adults with overweight/obesity
1074 was associated with increased gene expression of complex V of the electron transport
1075 chain indicating greater capacity for ATP production (226), four days of moderate-
1076 walking interruptions to sitting (perceived effort: 12–13 “somewhat hard”, 5-minute bouts
1077 every hour, total: 45 minutes/day) did not elicit changes in mitochondrial respiration in
1078 presence of carbohydrates (227); of note this later measurement was performed in
1079 fasting state and >12 hours after the last active interruption. Notably, pathways

1080 associated with muscle contraction transcription signaling, namely oxidative
1081 phosphorylation and sirtuin signaling expression, were enhanced in the skeletal muscle
1082 of these same participants when compared to a highly sedentary condition, as indicated
1083 by pathway enrichment analysis with RNA sequencing data (227).

1084 In the subcutaneous abdominal adipose tissue, interrupting prolonged sitting with
1085 light-intensity walking (3.2 km/h, 2.8 METs, 2-minute bouts of walking every 20 minutes,
1086 total: 28 minutes) led to a downregulation of pathways linked to carbohydrate oxidation
1087 and upregulation of pathways linked to lipid oxidation in adults with overweight/obesity
1088 as compared to prolonged sitting and moderate-intensity walking interruptions. In
1089 contrast, genes associated with glucose oxidation were upregulated in the moderate-
1090 intensity walking condition (5.8 km/h, ~4.3 METs, 2-minute bouts of every 20 minutes,
1091 total: 28 minutes) (228).

1092 These studies provided the first insights into the muscle regulatory systems and
1093 molecular processes underlying the effects of interrupting prolonged sitting on glucose
1094 metabolism (FIGURE 4).

1095

1096 ----- INSERT FIGURE 4 ABOUT HERE -----

1097

1098 4.1.2.2. Evidence from longer-term studies

1099 A meta-analysis examined findings from longer-term studies investigating the effects of
1100 SB interventions conducted in free-living settings on glucose/insulin outcomes.
1101 Intervention duration ranged between 2 weeks and 36 months, and the average change
1102 in total sedentary time was -28.6 min/day (78). There was a small significant decrease

1103 in fasting insulin concentration (-1.42 pM), but no changes in fasting glucose
1104 concentration and HbA1c (78). Another meta-analysis investigating the effects of SB
1105 interventions (range: 6 to 24 weeks) on markers of glucose metabolism demonstrated
1106 significant decreases in total sedentary time (-64 min/day) and increases in walking time
1107 (27 min/day), but no significant changes in time spent standing and in MVPA (134).
1108 Authors noted a small significant decrease in HbA1c (-0.2%), but no change in fasting
1109 glucose concentration in individuals with overweight, obesity, type 2 diabetes,
1110 cardiovascular, neurological/cognitive, or musculoskeletal diseases (134). A meta-
1111 analysis of studies aiming to replace SB with standing time (mean follow-up: 3.8
1112 months) demonstrated a significant increase in total standing time (1.3 h/day) in adults
1113 along with significant decreases in fasting glucose (-2.53 mg/dL) and insulin (-2.30
1114 mg/dL) concentrations (135). A systematic review of studies implementing workplace
1115 SB interventions in apparently healthy and overweight/obese desk-based office workers
1116 found that the effects of workplace interventions on markers of glucose metabolism
1117 have been inconsistent across studies. Only 6 out of 15 studies reported improvements
1118 in at least one glucose metabolism-related outcomes, while most studies showed no
1119 changes (136). Furthermore, three weeks of frequent active interruptions to sitting
1120 (increase of 744 steps/day from baseline, with no changes reported for SB) reduced
1121 mean fasting glucose levels (-0.34 mmol/L) and glucose variability (-2%) in highly
1122 sedentary adults with obesity, but did not improve glucose tolerance, insulin sensitivity,
1123 post-load glucose responses, and average interstitial glucose concentrations (229).

1124 Altogether, it becomes evident that results on the effects of reducing/interrupting
1125 SB on markers of glucose metabolism are mostly inconsistent across studies and meta-

1126 analyses. It is not clear whether type, intensity, and frequency of interruptions to sitting,
1127 characteristics of population groups and/or duration of intervention affect responses to
1128 reducing/interrupting SB interventions.

1129 *Reducing/interrupting SB vs continuous MVPA/exercise.* A small-scale, 12-week,
1130 multifactorial, pilot-study examined both the independent and combined effects of
1131 exercise training (40-65% hear rate reserve, i.e., moderate to vigorous intensity) and
1132 reducing SB (replace SB with standing and LPA plus increasing daily step count by 5-
1133 10%) on markers of insulin action in adults with overweight/obesity (140). Exercise
1134 training combined with reducing SB (-7.3% of daily hours in SB and + 45 min/day
1135 increase in MVPA) was the most effective intervention to improve markers of insulin
1136 action. This approach improved insulin sensitivity (17.8%), as assessed by a composite
1137 insulin-sensitivity index, and decreased 2-hour insulin concentration (-30.5 uIU·mL⁻¹)
1138 and post-load glucose responses (-19.4%). Reducing SB alone (-4.8% of daily hours in
1139 SB, +28 min/day in MVPA) reduced fasting insulin concentrations (-3.6 uIU·mL⁻¹) but,
1140 surprisingly, concomitantly increased 2-hour glucose (+26.5 mg/dL). In contrast,
1141 exercise training alone (no change in SB + 27 min/day increase in MVPA) did not
1142 improve any marker of insulin action. In addition, exercise and SB interventions, when
1143 performed independently, did not affect fasting and post-load glycemia (140). Similarly,
1144 4 weeks of interventions aiming at reducing SB (-53 min/day in SB with no change in
1145 MVPA) or increasing MVPA to at least 30 min/day (+16 min/day of MVPA with no
1146 change in SB) did not change fasting glucose and HbA1c concentrations in physically
1147 inactive adults with obesity (142).

1148

1149 4.1.2.3. Clinical significance

1150 There is some evidence of small improvements on markers of glucose control (fasting
1151 insulin [-1.42 pM], glucose [-2.53 mg/dL] and HbA1c [-0.2%] concentrations) associated
1152 with reducing/interrupting SB. However, mixed results have also been reported across
1153 original studies and meta-analyses. It is important to highlight that mean baseline levels
1154 of fasting glucose, insulin and HbA1c were mostly within normal ranges in available
1155 meta-analyses (78, 134, 135), which may partly explain the small magnitude of reported
1156 changes following SB interventions. In fact, these small changes are comparable to the
1157 findings that have been observed with continuous exercise in people without glycemic
1158 dysfunction (230).

1159 The most commonly used marker of glucose control in clinical practice is HbA1c.
1160 A reduction of 0.5 to 1.0% is generally used as a cut point for clinically meaningful
1161 change, which associates with significant reductions in risk of all-cause mortality,
1162 myocardial infarction, stroke, and heart failure in patients with type 2 diabetes (231).
1163 One meta-analysis of SB interventions demonstrated a 0.2% reduction in HbA1c (mean
1164 baseline level was 6.4%) after 6 to 24 weeks of follow up (134), which is not clinically
1165 meaningful. Interestingly, a 3-year intervention (1-month exercise counseling every
1166 year; SB: -0.8 h/day, LPA: 0.7 h/day, and MVPA: 3 min/day) significantly reduced
1167 HbA1c values (-0.5%) in those with type 2 diabetes compared to standard care (i.e.,
1168 general physician recommendations for increasing daily PA and decreasing SB) (232).
1169 The highest quartile for changes in SB (-1.53 h/day and +10.5 min/day of MVPA)
1170 significantly reduced HbA1c values (-0.85%) as compared to other quartiles, with SB
1171 being an independent predictor of changes in HbA1c (233). Another randomized

1172 controlled trial aiming at increasing participation in standing and LPA (SB: -39 min/day,
1173 standing: 39 min/day) performed a subgroup analysis in the participants who had high
1174 fasting glucose concentrations at baseline and found effects sizes to be larger for
1175 fasting glucose and HbA1c (-7.2 mg/dL and -0.3%, respectively) (234). Altogether,
1176 these findings indicate that reducing/interrupting SB has the potential to improve
1177 glucose control in those with dysglycemia and type 2 diabetes.

1178

1179 **4.2. Lipid metabolism**

1180

1181 *4.2.1. Increasing sedentary behavior*

1182

1183 4.2.1.1. Evidence from acute and multi-day studies

1184 Four days of exposure to a highly sedentary regime (14 hours sitting/day) did not alter
1185 fasting blood lipids concentrations (i.e., total cholesterol, HDL, LDL, and triglycerides) in
1186 healthy sedentary adults (123). Similar results were observed in those with overweight
1187 and obesity (122) and type 2 diabetes (197). Seven days of exposure to a highly
1188 sedentary condition (increased sedentary time and limited participation in PA of any
1189 intensity) did not alter fasting blood lipids concentrations in healthy-lean adults (147).

1190

1191 4.2.1.2. Evidence from longer-term studies

1192 Fourteen days of reduced step count (from 10,501 to 1,344 steps/day) led to increased
1193 triglyceride responses (~21%) to an oral fat tolerance test in healthy male adults (109).
1194 A 14-day period of reduced steps (by 81%, and a 3.7 h/day increase in sedentary time)

1195 significantly increased fasting total cholesterol, LDL and triglycerides (by 0.5, 0.3 and
1196 0.5 mmol/L, respectively) in healthy adults with/without a first-degree relative diagnosed
1197 with type 2 diabetes. These changes returned to baseline levels after resuming habitual
1198 PA for 14 days (112). Twenty days of reduced steps (from 14,000 to 3,000 steps/day)
1199 not only worsened fasting HDL concentration but decreased total lipid oxidation and
1200 increased fasting triglycerides concentration (0.3 mmol/L) along with *de novo*
1201 lipogenesis in healthy physically active male adults (106). Periods of reduced step count
1202 (14 days; from 9,008 to 2,994 steps/day) also resulted in increased serum and
1203 intramuscular ceramides in healthy older adults (130). Other studies demonstrated
1204 deleterious effects of reduced steps on blood lipids in healthy male adults (128, 235);
1205 however, results have also been inconsistent, with some studies demonstrating no
1206 effects (107, 108, 236).

1207 *Potential mechanisms.* Results from bed rest studies have been inconsistent for
1208 blood lipids concentrations in healthy adults and older adults, with some reporting
1209 hypertriglyceridemia and hypercholesterolemia and others no effect (34, 166, 237). Bed
1210 rest also impairs lipid oxidation in both fasting and postprandial states, which was
1211 observed to be independent of detectable changes in energy balance (166) (FIGURE
1212 3). This reduced fatty acid oxidation does not seem to be due to an impaired trafficking
1213 towards peripheral tissue but rather to structural, metabolic, and molecular changes in
1214 the skeletal muscle (170). For example, physical inactivity coupled with sedentariness
1215 has been shown to reduce content of slow oxidative muscle fibers (238-240), reduce
1216 mitochondrial density and oxidative capacity (241), and decrease the expression of
1217 genes involved in mitochondrial function (242). Gene expression and activity of

1218 enzymes coupled with oxidative metabolism, such as skeletal muscle *LPL*, fatty acid
1219 transporter into the myocyte (*CD36*) and into the mitochondria (*carnitine*
1220 *palmitoyltransferase I* [*CPT1b*]) (127, 170), are also decreased (170, 242, 243). These
1221 changes are particularly relevant following meal ingestion since they lead to decreased
1222 clearance of dietary lipids, which can contribute to hyperlipidemia. In this line,
1223 decreases in the oxidation of dietary saturated, but not monounsaturated, fatty acids
1224 were observed (127, 170, 244). The changes in the oxidative rate of dietary saturated
1225 fatty acid have been associated with lower clearance and uptake by peripheral tissues
1226 and decreases in the gene expression of *CD36*, *fatty acid binding protein* [*FABPpm*],
1227 *CPT1* and *acyl-CoA synthetase long chain family member 1* [*ACSL1*]. Despite a
1228 reduction in adipose tissue lipolysis (245, 246), excess of plasma lipids has been shown
1229 to enhance fat accumulation in the visceral adipose depot (247) and ectopic fat storage
1230 in muscle, liver and bone (170, 248-250). Fat accumulation is known to impair the
1231 function of the tissues. In this line, a recent study in non-human primates (Macaques)
1232 showed that 42 days of HDBR altered transcriptome signatures with up-regulation of
1233 genes in lipid metabolisms in liver samples, revealing mildly disturbed fatty acid
1234 metabolism (251). In humans, indices of fatty liver such as high levels of plasma
1235 transaminases (252) have been associated with increased de novo lipogenesis (106),
1236 an index of hepatic insulin resistance.

1237

1238 4.2.1.3. Clinical significance

1239 Excessive SB has been positively associated with increased fasting triglyceride
1240 concentrations and decreased fasting HDL concentrations in the general population,

1241 independent of participation in MVPA (253-255). Some detrimental changes in blood
1242 lipids have been reported in reduced step count studies in both adults and older adults,
1243 but results have been inconsistent. The clinical significance of these findings is unclear.

1244

1245 *4.2.2. Reducing and interrupting sedentary behavior*

1246

1247 *4.2.2.1. Evidence from acute and multi-day studies*

1248 Findings from single and multi-day experimental studies examining the effects of
1249 interrupting prolonged sitting on fasting (122, 123, 147, 197) and postprandial lipid
1250 concentrations (75-77) have been less consistent than those observed for glucose and
1251 insulin responses. Some original studies failed to show improvements in triglycerides
1252 following at least one of the interruptions to SB compared to prolonged sitting (121, 124,
1253 186, 188, 190, 191, 201, 207, 213, 214, 256). Two meta-analyses (75, 76) indicated no
1254 effect of standing, light- and moderate-intensity activity interruptions to sitting on
1255 postprandial triglycerides responses in healthy and metabolically impaired adults and
1256 older adults compared to prolonged, uninterrupted sitting. In contrast, one meta-analysis
1257 (77) indicated a small significant effect (Cohen's d : -0.26 favoring intervention) of
1258 interruptions to sitting on postprandial triglycerides in healthy and metabolically impaired
1259 adults and older adults compared to prolonged, uninterrupted sitting. This was mainly
1260 driven by the evidence from multi-day studies, which may suggest an additive effect of
1261 interruptions to sitting on blood lipids (77).

1262 The magnitude of changes in triglyceride responses following interruptions to
1263 sitting have been reported to be independent of the intensity of PA interruptions

1264 (standing vs light- vs moderate-intensity interruptions), as indicated by subgroup
1265 analysis (76). For frequency of interruptions to sitting, there were no differences in
1266 postprandial triglycerides responses following light-intensity walking interruptions to
1267 sitting every 20, 60 and 120 minutes (30% VO_{2max} , total: 48 minutes) in male adults with
1268 central obesity (188). Similarly, there were no differences between low- and high-
1269 frequency simple resistance activities interruptions to sitting (squatting, calf raises, total:
1270 36 minutes; or moderate-intensity walking, i.e., 65% VO_{2peak} , total: 30 minutes) on
1271 postprandial triglycerides responses in adults and older adults with type 2 diabetes
1272 (186) and in healthy female adults (192). It is unclear whether the type of interruptions
1273 to sitting differentially affect markers of lipid metabolism.

1274 The magnitude of changes in triglyceride responses following interruptions to
1275 sitting were not affected by meal composition, BMI, and population characteristics, as
1276 indicated by subgroup analysis and meta-regressions (76, 77). Interestingly, fasting
1277 triglycerides had a negative quadratic association with postprandial triglyceride
1278 responses to a single, moderate-walking bout followed by intermittent light-walking
1279 interruptions compared to prolonged sitting in healthy older adults. This may imply that
1280 those with high triglyceride levels were more resistant to intervention-induced reductions
1281 in triglycerides responses (378).

1282 In children with normal weight or overweight/obesity, interrupting sitting with 3-
1283 minute bouts of moderate-intensity walking (heart rate at 80% of ventilatory threshold,
1284 total: 18 minutes) did not affect postprandial triglycerides and free fatty acids responses
1285 compared to 3 hours of prolonged sitting (210, 211). Similarly, light-intensity walking
1286 interruptions to sitting (30% VO_{2peak} , 2-minute every 20 minutes, total: 42 minutes) did

1287 not affect postprandial triglyceride, HDL and LDL responses in healthy children and
1288 adolescents compared to 8 hours of prolonged sitting (212).

1289 Regarding other markers of lipid metabolism, acute and multi-day studies (6
1290 hours to up to 4 days) indicate no changes in postprandial concentrations of free fatty
1291 acids (124, 215, 217, 218, 220, 257), 3-hydroxybutyrate, apolipoproteins C-II and C-III
1292 (257), apolipoproteins B-48 and B-100, total ketone bodies, and acetoacetic acid (215)
1293 following interruptions to sitting (standing and moderate-intensity walking) in healthy
1294 adults, adults with overweight/obesity and postmenopausal females.

1295 *Reducing/interrupting SB vs continuous MVPA/exercise.* Acutely (7 to 9 hours), a
1296 continuous bout of PA (moderate intensity, total: 30 minutes) in the morning was more
1297 effective at attenuating postprandial triglyceride responses than frequent standing (45-
1298 minute interruptions every 15 minutes, total: 4.5 hours) (257), moderate-walking (46%
1299 VO_{2max} , 1.6-minute interruptions every 28 minutes, total: 30 min) (214) and vigorous-
1300 cycling (70% VO_{2max} , 6-minute interruptions every 40 minutes, total: 30 minutes) (258)
1301 interruptions to sitting in healthy adults. In contrast, other studies did not report such
1302 differences between patterns of activity (213, 215, 216, 218) or showed a superiority of
1303 interruptions to sitting at decreasing postprandial triglyceride responses in healthy and
1304 at-risk adults and older adults (217, 219). A meta-analysis summarizing acute evidence
1305 demonstrated no differences between interruptions to sitting and a continuous bout of
1306 activity on postprandial triglycerides responses (77), but activity protocols were not
1307 always matched for duration and energy expenditure. In multi-day studies (2 to 4 days),
1308 reducing/interrupting SB was shown to be as effective as continuous exercise in
1309 reducing fasting triglyceride concentrations in adults and older adults with type 2

1310 diabetes (197). In healthy adults and adults with overweight and obesity, there were no
1311 differences between interruptions to sitting and continuous activity in postprandial
1312 triglycerides and free fatty acids responses (124, 220).

1313 *Potential mechanisms.* As expected, a single continuous bout of PA leads to
1314 increases in total fat oxidation compared to a sedentary control, while greater reliance
1315 upon carbohydrate was shown following interruptions to sitting in healthy adults and
1316 adults with overweight/obesity (124, 214). Peddie and colleagues (214) observed a
1317 significant reduction in postprandial triglyceride responses following a single moderate-
1318 intensity walking bout (~46% VO_{2max} , total: 30 minutes) compared to prolonged sitting
1319 and interruptions to sitting (214, 218). Despite changes in substrate use, De Jong and
1320 colleagues (124) failed to detect reductions in fasting and postprandial triglycerides
1321 responses following a continuous bout of activity (perceived effort 13 “somewhat hard”,
1322 total: 45 min/day). It is likely that glycogen storage was partly depleted when performing
1323 continuous PA, thus allowing lipids to be oxidized for energy expenditure and glycogen
1324 pools to be refilled, which likely did not occur during brief interruptions to sitting as
1325 glucose was constantly available and competing against lipids. Additionally,
1326 postprandial responses of markers for hepatic fatty acid oxidation (total ketone bodies
1327 and acetoacetic acid) were elevated following continuous exercise, but not interruptions
1328 to sitting. This indicates increased hepatic fatty acid oxidation and reduced availability of
1329 triglycerides for incorporation into VLDL with exercise (215).

1330 It is difficult to explain the inconsistencies in the acute effects of
1331 reducing/interrupting SB on postprandial triglyceride responses. Based on findings from
1332 animal models, it has been hypothesized that the lack of muscle contraction during

1333 exposures to increased SB reduces skeletal muscle LPL mass and activity and alters
1334 lipid metabolism (8, 44). Of note, these defects were reversible only with light-intensity
1335 contractile activity but not MVPA/exercise (9, 44). No change was observed in serum
1336 inactive monomeric LPL protein concentrations following standing interruptions to sitting
1337 in healthy males (257) and in plasma pre-heparin LPL concentrations following walking
1338 interruptions to sitting in postmenopausal females (215). Similarly, no changes in LPL
1339 gene expression were reported following frequent light- to moderate-intensity walking
1340 interruptions to sitting (over 5 to 8 hours, 2-3-minute bouts every 20-30 minutes) in the
1341 skeletal muscle of adults with overweight/obesity (225) and postmenopausal females
1342 with rheumatoid arthritis (213). One possibility is that the pattern and frequency of
1343 activity used in published studies were insufficient to elicit changes in LPL enzymatic
1344 activity.

1345

1346 4.2.2.2. Evidence from longer-term studies

1347 A meta-analysis examined data from longer-term studies of the effects of SB
1348 interventions conducted in free-living settings on blood-lipid outcomes. Intervention
1349 duration ranged between 2 weeks and 36 months, and the average change in total
1350 sedentary time was -28.6 min/day (78). There was a small significant increase in HDL
1351 (0.04 mM), but no change in total cholesterol, LDL and triglycerides, in response to
1352 interrupting/reducing SB interventions (78). Another meta-analysis investigating the
1353 effects of SB interventions (range: 6 to 24 weeks) showed that lipid profile was not
1354 ameliorated despite significant decreases in total sedentary time (-64 min/day) and
1355 increases in walking time (27 min/day) in individuals with overweight, obesity, type 2

1356 diabetes, cardiovascular, neurological/cognitive, or musculoskeletal diseases (134). A
1357 meta-analysis of studies aiming at replacing SB with standing time (mean follow-up: 3.8
1358 months) showed that significant increases in total standing time (1.3 h/day) in adults
1359 had no effect on lipid profile (135). Another systematic review of studies also concluded
1360 that SB interventions did not improve blood lipids in most published studies (13 out of
1361 18 studies) (136). Furthermore, skeletal muscle lipidome was largely unaffected after
1362 three weeks of an intervention consisting of frequent active interruptions to sitting
1363 (increase of 744 steps/day from baseline, no change in SB) in adults with central
1364 obesity (229).

1365 Taken these data together, it is evident that results on the effects of
1366 reducing/interrupting SB on markers of lipid metabolism are inconsistent across studies
1367 and meta-analysis. It is not clear whether type, intensity, and frequency of interruptions
1368 to sitting, characteristics of population groups and/or duration of intervention influence
1369 the responses to reducing/interrupting SB interventions.

1370 *Reducing/interrupting SB vs continuous MVPA/exercise.* A small-scale, 12-week,
1371 multifactorial, pilot-study examined both the independent and the combined effects of
1372 exercise training (40-65% hear rate reserve, i.e., moderate to vigorous intensity) and
1373 reducing SB (replace SB with standing and LPA plus increasing daily step count by 5-
1374 10%) on blood lipids in adults with overweight/obesity (140). Reducing SB with non-
1375 exercise PA (-4.8% of daily hours in SB, +28 min/day in MVPA) and exercise training
1376 combined with reducing SB (-7.3% of daily hours in SB + 45 min/day increase in MVPA)
1377 did not change fasting total cholesterol, HDL, and triglycerides concentrations. In
1378 contrast, exercise training only (no change in SB, + 27 min/day increase in MVPA)

1379 significantly reduced triglyceride concentrations ($-0.4 \text{ mmol}\cdot\text{L}^{-1}$) (140). Four weeks of
1380 intervention aiming at reducing SB (-53 min/day in SB with no change in MVPA) or
1381 increasing MVPA to at least 30 min/day ($+16 \text{ min/day}$ of MVPA with no change in SB)
1382 did not improve HDL, LDL and triglyceride concentrations in physically inactive adults
1383 with obesity (142).

1384

1385 4.2.2.3. Clinical significance

1386 There is some evidence of small improvements, likely not clinically meaningful, on
1387 fasting HDL (0.04 mM) concentrations associated with reducing/interrupting SB.
1388 However, no consistent improvements have been reported for other blood lipids. It is
1389 important to highlight that those studies within available meta-analyses reported mean
1390 baseline values for all fasting blood lipids within normal ranges (78, 134), which may
1391 contribute to the small magnitude of changes reported following SB interventions.
1392 Putting these results into perspective with the effects of continuous exercise, the small
1393 benefit for HDL is comparable to the increases in HDL associated with supervised
1394 aerobic exercise in adults and older adults with type 2 diabetes (259). A meta-analysis
1395 also demonstrated continuous aerobic exercise decreased fasting triglycerides
1396 concentrations (-6.8 mg/dL), but not total cholesterol, LDL and HDL, in adults and older
1397 adults with overweight/obesity (260). The current evidence indicates that
1398 reducing/interrupting SB has marginal effects on fasting blood lipids. Longer-term
1399 investigations into the effects of reducing/interrupting SB on blood lipids is warranted,
1400 particularly in individuals with dyslipidemia.

1401

1402 **4.3. Protein metabolism**

1403

1404 *4.3.1. Increasing sedentary behavior*

1405

1406 4.3.1.1. Evidence from longer-term studies

1407 Most evidence related to the effects of changing sedentary and physically-active
1408 behaviors on protein metabolism has focused on skeletal muscle. In order to further
1409 understand how sedentary-induced hypokinesia may affect protein metabolism in
1410 skeletal muscle, it is important to acknowledge that muscle mass is regulated by an
1411 intricate and coordinated balance between daily fluctuations in muscle protein synthesis
1412 and breakdown (i.e., muscle protein balance) (261, 262). While breakdown is
1413 considered somewhat stable in non-pathological conditions, muscle protein synthesis is
1414 modulated by anabolic stimuli such as PA and nutrition (261, 262). This indicates that
1415 SB-related mechanical unloading of muscles may lead to muscle wasting (263, 264).

1416 Step reduction (~80% reduction from baseline) for 14 days resulted in a
1417 significant decrease in postprandial myofibrillar protein synthetic rate (-25 to -50%) in
1418 older adults (105, 110). This also seems to be the case in clinical populations, in which
1419 reducing step count (7,362 to 991 steps/day) significantly reduced measures of the 14-
1420 day integrated rates of muscle protein synthesis (~-12%) in older adults with overweight
1421 and prediabetes, which remained lower after resuming habitual activity for 14 days
1422 (164). Using a unilateral leg model, Devries and colleagues (105) demonstrated that the
1423 step reduction-induced decrease in postprandial myofibrillar protein synthetic rate can
1424 be fully mitigated in older adults by performing unilateral resistance exercise training (3

1425 times/week) while performing step reduction. A study employing 14 days of unilateral
1426 immobilization also showed reduced post-absorptive muscle protein synthesis (-27%) in
1427 healthy adults (93).

1428 *Potential mechanisms.* Bed rest studies indicate the loss of body protein with
1429 physical inactivity/SB is predominantly due to a decrease in muscle protein synthesis
1430 (265). This latter was associated with reductions in lean mass (-1.7%) following 14 days
1431 of HDBR (265).

1432

1433 4.3.1.2. Clinical significance

1434 The pronounced reductions in muscle protein synthesis (-12 to 50%) following periods
1435 of increased SB are likely relevant. The physiological and clinical impact of increasing
1436 SB on skeletal muscle will be further discussed in topic 6.1.

1437

1438 4.3.2. Reducing and interrupting sedentary behavior

1439

1440 4.3.2.1. Evidence from acute studies

1441 Acutely, myofibrillar protein synthesis was greater following light-walking (1.9 km/hour,
1442 <2.8 METs, 2 minutes of walking every 30 minutes, total: 30 minutes) and squatting
1443 interruptions to sitting (15 body-weight squats every 30 minutes, total: 225 repetitions)
1444 as compared to 7.5 hours of prolonged sitting in healthy adults (~47% and ~20%,
1445 respectively) (266). The increases in myofibrillar protein synthesis are comparable to
1446 those observed following an acute bout of structured resistance exercise (~40%) in
1447 healthy male adults (267). Additionally, rpS6^{Ser240/244} phosphorylation was greater in

1448 squatting interruptions compared to prolonged sitting, but no differences were observed
1449 for other anabolic signaling protein targets (4E-BP1^{Thr37/46}, eEF2^{Thr56}, mTOR^{Ser2448},
1450 ERK1/2^{Thr202/Tyr204}) (266). This could suggest that the squats interruptions resulted in the
1451 stimulation of translation initiation and myofibrillar protein synthesis. In contrast, walking
1452 had no effect on any anabolic signaling protein targets, which may be related to a lower
1453 activation of the *vastus lateralis* during walking compared to squatting (268). It is not
1454 clear whether type, intensity, and frequency of interruptions to sitting differentially affect
1455 markers of protein metabolism.

1456 As for whole-body nutrient oxidation, De Jong and colleagues (124) showed that
1457 frequent 5-minute bouts of brisk walking at every hour (total: 45 min/day) increased
1458 protein oxidation (11.4%) during the sleeping period (8 hours) as compared to the
1459 sedentary condition (67% of waking hours) in people with overweight/obesity. The
1460 authors suggest that the greater disappearance in protein may reflect the use of protein
1461 for gluconeogenesis to replenish muscle glycogen, as the short bouts of activity likely
1462 triggered the use and replenishment of glycogen stores, thus enhancing glycogen
1463 turnover (124).

1464 It has been proposed that muscle hypertrophy following resistance exercise
1465 occurs as a result of summed periods of repeated acute exercise-induced positive
1466 protein balance where muscle protein synthesis exceeds muscle protein breakdown
1467 (269). As such, the practical relevance of acute increases in myofibrillar protein
1468 synthesis (20-47%) seen following aerobic and resistance activity interruptions to sitting
1469 and the extension of skeletal muscle remodeling should be addressed in future longer-
1470 term studies.

1471

1472 **5. CARDIOVASCULAR AND RESPIRATORY SYSTEMS**

1473

1474 **5.1. Hemodynamics**

1475

1476 *5.1.1. Increasing sedentary behavior*

1477

1478 *5.1.1.1. Evidence from acute studies*

1479 A seated posture creates bends in major blood vessels, such as the femoral and
1480 popliteal arteries, which may result in turbulent blood flow patterns (270, 271). Also,
1481 such posture not only results in diminished skeletal muscle contractile activity that aids
1482 in venous return via the muscle pump, but also detrimentally affects blood flow and
1483 vascular shear stress (physiological stressors that may underlie the health benefits of
1484 PA on the endothelium) (24). In healthy adults, shear rate in the lower limbs, but not in
1485 the upper limbs, is reduced after only 30 minutes of uninterrupted sitting (20, 272). After
1486 approximately 2 hours, blood pools in the calf and whole-blood leg viscosity is also
1487 reduced (273). After 3 hours, blood flow in lower limbs is decreased in parallel with a
1488 further reduction in shear rate, as evidenced by meta-analysis (20). Although most of
1489 the evidence suggests that prolonged sitting detrimentally affects peripheral
1490 hemodynamics (274), some studies have demonstrated no alterations in blood flow
1491 (275) and retrograde shear rate (276, 277) in healthy males following 3-5 hours of
1492 uninterrupted sitting.

1493 A meta-analysis reported that exposures to prolonged, uninterrupted sitting
1494 resulted in significant increases in systolic blood pressure (3.2 mmHg) and mean
1495 arterial pressure (3.3 mmHg) among adults and older adults, but diastolic blood
1496 pressure was unaffected (119). In contrast, another meta-analysis demonstrated no
1497 change in mean arterial pressure following exposures to prolonged sitting >3 hours (20).
1498 Some studies also failed to observe sitting-induced alterations in systolic (190, 278-
1499 281), diastolic pressure (195, 278, 282-284), and mean arterial pressure (281, 285-
1500 288). In addition, 7 days of reduced step count (~9,000 to ~6,000 steps/day) did not
1501 modify systolic and diastolic blood pressure and mean arterial pressure in healthy-active
1502 adults (289).

1503 Five days of reduced steps (from ~12,000 to 4,000 steps/day) did not affect
1504 femoral or brachial artery blood flow responses (104), nor did it alter shear rate in
1505 healthy-active male adults (103).

1506 Changes in catecholamines have been inconsistent across studies; some
1507 indicated no changes and others small increases in plasma/serum concentrations in
1508 adults and older adults following 7-8 hours of prolonged sitting (284, 290, 291).
1509 Increased lower leg and foot venous pressure/swelling was also observed after
1510 prolonged sitting, possibly impairing the regulation of capillary fluid filtration and edema
1511 formation in the feet (292).

1512 *Potential mechanisms.* Atherosclerotic plaques have been reported to commonly
1513 manifest near arterial bifurcations, which is at least partially due to an unfavorable local
1514 hemodynamic environment (293). The seated position may mimic a similar environment
1515 to arterial bifurcations due to the 'bent artery' morphology created by 90-degree angles

1516 in the hip and knees. Acute evidence revealed that as little as 3 hours of leg bending,
1517 similar to sitting, produces detrimental hemodynamic changes in the popliteal artery as
1518 compared with a straight limb (294). Therefore, the 'bent artery' position induced by
1519 sitting may be a key contributor of changes in hemodynamics (274).

1520

1521 5.1.1.2. Evidence from longer-term studies

1522 Unilateral leg immobilization (12 days) did not induce changes in blood pressure, mean
1523 blood flow in carotid, femoral and popliteal arteries in the immobilized leg versus the
1524 non-immobilized leg in healthy adults (295). However, mean blood velocity (~22%) and
1525 vessel shear rate (~35%) in femoral artery were increased in the immobilized leg (295).
1526 Hemodynamic adaptations to lower-limb immobilization seem to be constrained to
1527 peripheral arteries, not altering large central arteries (295).

1528 Fourteen days of step reduction (~82% reduction from baseline combined or not
1529 with unilateral low-load resistance exercise training, 3 times/week) did not alter
1530 superficial femoral artery blood flow during both fasted and fed state in healthy older
1531 males (105). Systolic blood pressure increased by 4 mmHg following a 14-day step
1532 reduction protocol (81% reduction from baseline; +3.7 h/day in sedentary time) in
1533 healthy adults with/without a first-degree relative with type 2 diabetes but returned to
1534 baseline after resuming habitual activity for 14 days (112). Diastolic blood pressure
1535 remained unchanged during step reduction and recovery periods (112).

1536

1537 5.1.1.3. Clinical significance

1538 Acute exposures to prolonged sitting resulted in significant increases in systolic blood
1539 pressure (3.2 mmHg) and mean arterial pressure (3.3 mmHg) among adults and older
1540 adults (119). Similar increases (4 mmHg) were reported following 14 days of reduced
1541 step count in healthy adults with/without a first-degree relative with type 2 diabetes
1542 (112). While it is unclear whether these detrimental changes are sustained over time,
1543 the magnitude of these increases in blood pressure are likely to be clinically significant if
1544 maintained. For some perspective, at population level, 1 to 10 mmHg increases in mean
1545 systolic blood pressure are associated with increases in cardiovascular diseases
1546 incidence (296) and mortality (296, 297) and stroke mortality (298).

1547

1548 *5.1.2. Reducing and interrupting sedentary behavior*

1549

1550 *5.1.2.1. Evidence from acute studies*

1551 A meta-analysis of acute randomized crossover trials showed that interruptions to sitting
1552 (aerobic, simple resistance activities and standing) reduced systolic blood pressure by
1553 4.4 mmHg and diastolic blood pressure by 2.4 mmHg versus prolonged sitting (119). A
1554 meta-analysis demonstrated no change in systolic and diastolic blood pressure
1555 following standing interruptions to sitting compared to prolonged sitting in adults and
1556 older adults (181), suggesting interruptions at higher intensities may be necessary to
1557 affect blood pressure. However, mixed results have been reported by original studies. A
1558 systematic review summarized findings from acute randomized crossover studies
1559 investigating blood pressure responses to different intensities of interruptions to sitting
1560 in adults and older adults at risk for type 2 diabetes (299). Five out of ten studies found

1561 significant improvements in blood pressure responses following light-intensity
1562 interruptions, and three out of five studies demonstrated reductions following moderate-
1563 to-vigorous-intensity interruptions (299). In contrast, only one out of six studies found
1564 reductions in blood pressure following standing interruptions to prolonged sitting (299),
1565 indicating that standing interruptions might be less effective at improving blood pressure
1566 responses.

1567 Regarding the type of interruptions to sitting, the decreases in systolic blood
1568 pressure were mainly driven by studies implementing aerobic activity interruption
1569 strategies, as evidenced by a meta-analysis (119). In terms of frequency, interrupting
1570 prolonged sitting with 3-minute or 6-minute bouts of simple resistance activities every 30
1571 or 60 minutes (squatting, calf raises; total: 36-39 minutes) did not elicit changes in blood
1572 pressure responses compared to 8 hours of prolonged sitting in adults and older adults
1573 with type 2 diabetes (283). Similarly, there were no differences in blood pressure
1574 responses following high (2-minute bouts every 20 minutes, total: 30 minutes) and low
1575 frequency (10-minute bouts every 60 minutes, total: 50 minutes) of standing bouts
1576 compared to 6 hours of prolonged sitting in older adults (195). In addition, Duran and
1577 colleagues (206) suggested light-walking interruptions to sitting (3.2 km/h, 2.8 METs) of
1578 high and low frequency (every 30 and 60 minutes, respectively) and high and low
1579 duration (5- and 1-minute interruptions, respectively) can be considered when targeting
1580 improvements in blood pressure responses in adults and older adults. These strategies
1581 significantly attenuated systolic blood pressure responses (-3 to -5 mmHg) as compared
1582 to sedentary control (total: 8 hours), and there were no differences between high/low
1583 frequencies and durations of interruptions to sitting protocols (206). Both high and low

1584 frequencies of single resistance activity interruptions (squatting, calf raises; every 30 vs
1585 60 minutes, total: 36-39 minutes) significantly increased blood flow (125 and 114%,
1586 respectively) and shear rate (87 and 98%, respectively) in adults and older adults with
1587 type 2 diabetes as compared to 8 hours of prolonged sitting (283).

1588 Reductions in systolic and diastolic blood pressure responses were more
1589 pronounced in older females than older males in response to a 30-min bout of exercise
1590 followed by frequent, brief light-intensity walking bouts as compared to 8 hours of
1591 uninterrupted sitting (291). This finding suggests that females might benefit more from
1592 performing exercise plus interruption to sitting strategy.

1593 In preadolescent children, interrupting sitting with light-, moderate- and high-
1594 intensity walking (25, 50 and 75% heart rate reserve, respectively; 2-minute bouts every
1595 20 minutes, total: 40 minutes) did not alter systolic and diastolic blood pressure
1596 responses compared to 8 hours of prolonged sitting (300).

1597 Simple resistance activity interruptions to sitting (squatting, calf raises; 3-minute
1598 bouts every 30 minutes, total: 27 minutes) resulted in increased resting blood flow
1599 (~43%) and shear rate (~98%) in the femoral, but not brachial artery, compared to 5
1600 hours uninterrupted sitting in adults with overweight and obesity (279). Interruptions to
1601 sitting with calisthenic exercises (squats, arm circles, calf raises; 2-minute interruptions
1602 every 20 minutes, total: 6 minutes) increased shear rate in the brachial artery (~16%) in
1603 healthy adults (287). Performing simple leg movements such as fidgeting was also
1604 sufficient to attenuate sitting-induced decreases in popliteal artery blood flow (-19% vs -
1605 29%) and shear rate (-22% vs -43%) in healthy adults (301). Finally, brief walking
1606 interruptions to sitting (3.2 and 5.8 km/h; 2.8 and ~4.3 METs, respectively; 2-minute

1607 bouts every 20 minutes, total: 28 minutes) may also play an important role at improving
1608 concentration of hemostatic and/or pro-coagulant risk factors (e.g., fibrinogen,
1609 hematocrit, and hemoglobin) (302).

1610 *Reducing/interrupting SB vs continuous MVPA/exercise.* A 30-minute bout of
1611 moderate-intensity walking (71% maximum heart rate), but not regular 2-minute bouts of
1612 moderate- or vigorous-intensity walking (53 and 79% maximum heart rate; total: 42 and
1613 16 minutes, respectively), significantly reduced ambulatory systolic blood pressure (-3
1614 mmHg) in adults with overweight/obesity compared to 9 hours of prolonged sitting (303).
1615 Similarly, a 30-minute bout of moderate-intensity walking (55% heart rate reserve)
1616 reduced systolic blood pressure responses in the 4-hour period after exercise in
1617 postmenopausal females with rheumatoid arthritis (47% participants had hypertension),
1618 which was not observed following 3-minute bouts of light-intensity walking every 30
1619 minutes (24% heart rate reserve, total: 42 minutes) (213). Interestingly, resting systolic
1620 blood pressure was significantly lower in the morning after performing both a single bout
1621 and multiple 3-minute bouts of brisk walking every 30 minutes (41-42% VO_{2max} , total: 30
1622 minutes) compared to a sedentary condition (-7 and -8 mmHg, respectively) in healthy
1623 male adults (304). Finally, a single 30-minute bout of moderate-intensity walking (65-
1624 75% maximum heart rate) was effective at reducing systolic and diastolic blood
1625 pressure in older adults (-3 and -1 mmHg, respectively). Performing regular 3-minute
1626 light-intensity walking interruptions every 30 minutes (3.2 km/h, 2.8 METs; total: 36
1627 minutes) after exercising further reduced systolic blood pressure (-5 mmHg) but not
1628 diastolic blood pressure (291).

1629

1630 5.1.2.2. Evidence from longer-term studies

1631 A meta-analysis has summarized data from longer-term studies investigating the effects
1632 of SB interventions conducted in free-living (-28.6 min/day of SB). Performing
1633 interruptions to sitting resulted in a small but significant reduction in systolic, but not
1634 diastolic, blood pressure (-1.1 mmHg) compared to control groups in both apparently
1635 healthy and clinical populations (78). Nonetheless, other meta-analyses of studies
1636 involving clinical population groups (134) and focused on reducing/interrupting SB with
1637 standing in adults and older adults (135) showed no change in blood pressure following
1638 intervention. A systematic review of workplace reducing/interrupting sitting interventions
1639 showed that only five out of 22 studies reported reductions in systolic blood pressure
1640 and two observed drops in diastolic blood pressure (136).

1641 In children, an 8-month, school-based intervention using height-adjustable desks
1642 in the classroom was ineffective at reducing classroom and total daily SB (137).
1643 Consequently, no significant changes were observed on systolic and diastolic blood
1644 pressure (137).

1645 Regarding other hemodynamic parameters, the effect sizes for increases in
1646 shear rate in the femoral and brachial arteries following an 8-week intervention to
1647 reduce/interrupt SB at work (-38 min/8-hour workday of sedentary time and +35 min/8-
1648 hour workday of standing time) were considered small (Cohen's *d*: 0.31 and 0.23) (305).
1649 In a 16-week non-randomized trial, an intervention to reduce sitting (-60 min/day of
1650 sedentary, +36 min/day of standing and +30 min/day of walking time) resulted in
1651 increased femoral artery antegrade shear rate (~14%), but not in basal blood flow or

1652 retrograde shear rate, among those (≥ 55 years old) with increased cardiovascular risk
1653 (306).

1654 In summary, the effects of reducing/interrupting SB on hemodynamics are
1655 inconsistent across studies and meta-analysis. It is not clear whether type, intensity,
1656 and frequency of interruptions to sitting, characteristics of population groups and/or
1657 duration of intervention affect responses to reducing/interrupting SB interventions.

1658 *Reducing/interrupting SB vs continuous MVPA/exercise.* A small-scale, 12-week,
1659 multifactorial, pilot-study examined both the independent and the combined effects of
1660 exercise training (40-65% heart rate reserve, i.e., moderate to vigorous intensity) and
1661 reducing SB (replace SB with standing and LPA plus increasing daily step count by 5-
1662 10%) on blood pressure in adults with overweight/obesity (140). Reducing SB with LPA
1663 (-4.8% of daily hours in SB, +28 min/day in MVPA) resulted in significant decreases in
1664 systolic and diastolic blood pressure (-4.7 and -4.0 mmHg, respectively). Interestingly,
1665 exercise training (no change in SB + 27 min/day increase in MVPA) and exercise
1666 training combined with reducing SB (-7.3% of daily hours in SB + 45 min/day increase in
1667 MVPA) significantly reduced systolic (-7.0 and -5.9 mmHg, respectively), but not
1668 diastolic, blood pressure. There were no between-group differences with respect to
1669 improvements in systolic blood pressure (140). In contrast, 4 weeks of multiple
1670 interventions aimed at reducing SB (-53 min/day in SB with no change in MVPA) or
1671 increasing MVPA to at least 30 min/day (+16 min/day of MVPA with no change in SB)
1672 had no effect on blood pressure in physically inactive adults with obesity (142).

1673

1674 5.1.2.3. Clinical significance

1675 Blood pressure is one of the most commonly used hemodynamic parameters in clinical
1676 practice. There is some evidence of small improvements in systolic blood pressure (-1.1
1677 mmHg) associated with reducing/interrupting SB (78). Such benefits were not observed
1678 for diastolic blood pressure. It is important to highlight that mean baseline blood
1679 pressure of the participants were typically within normal ranges in available meta-
1680 analyses (78, 134), which may reflect the small magnitude of reported changes
1681 following SB interventions. Importantly, these small changes in systolic blood pressure
1682 are comparable to those shown to be associated with continuous aerobic exercise and
1683 resistance exercise training in people with normal blood pressure (-0.6 to -0.8 mmHg)
1684 (307). In contrast, exercise training could be more effective at reducing diastolic blood
1685 pressure (-1.1 to -3.3 mmHg) (307). Despite the small effects of reducing/interrupting
1686 SB, even discrete reductions in blood pressure (e.g., 2 mmHg) are relevant at a
1687 population level, as they have been shown to be associated with significant reductions
1688 in the risk of all-cause, coronary diseases and stroke mortality (12,000 lives saved per
1689 year) (308).

1690 Evidence on the effects of reducing/interrupting SB on blood pressure in
1691 individuals with hypertension is still scarce. An analysis of pooled data from 4 acute
1692 randomized crossover trials indicated that blood pressure reductions were of greater
1693 magnitude in adults with overweight/obesity and hypertension (~-10 to -13 vs -2 to -7
1694 mmHg) following intermittent light-walking or simple resistance activity interruptions to
1695 sitting (3.2 km/h, 2.8 METs, or body-weight squatting and calf raises; 3-minute bouts
1696 every 30 minutes, total: 36 minutes) compared to those without hypertension (24).
1697 Additionally, in a 12-month randomized controlled trial, individuals who were

1698 randomized to the group STAND+ (sit to stand desk plus goal of ≥ 30 minutes of
1699 additional LPA) significantly reduced sitting time by -59.2 min/8-hour workday compared
1700 to the group MOVE+ (goal of ≥ 30 minutes of additional LPA). These changes in physical
1701 behavior resulted in non-significant changes in cardiometabolic outcomes (234). A
1702 subgroup analysis in individuals with dysglycemia (baseline blood pressure: 135/83
1703 mmHg) revealed larger reductions in systolic blood pressure (-6.6 mmHg) for
1704 participants randomized to STAND+ compared to MOVE+ (234). Altogether, these
1705 findings indicate that reducing/interrupting SB has the potential to improve blood
1706 pressure to a greater extent in those with hypertension.

1707

1708 **5.2. Cardiovascular function and structure**

1709

1710 *5.2.1. Increasing sedentary behavior*

1711

1712 5.2.1.1. Evidence from acute studies

1713 Prolonged sitting may induce endothelial dysfunction (i.e., the inability of the blood
1714 vessels to dilate appropriately) and oxidative stress (309, 310). Padilla and colleagues
1715 (272) observed that 3 hours of uninterrupted sitting attenuated popliteal artery shear
1716 rate (~75%) in healthy adults, but this reduction was not paralleled by a concomitant
1717 reduction in flow-mediated dilation (FMD). Conversely, Thosar and colleagues (276)
1718 reported a reduction in the superficial femoral artery FMD (~2.5 %FMD) following 3
1719 hours of uninterrupted sitting. Restaino and colleagues (311) also demonstrated that
1720 prolonged sitting for 6 hours impairs lower-limb FMD (~5 %FMD), but not upper-limb.

1721 This is possibly because upper limb movement was not restricted in this study. These
1722 results are corroborated by meta-analytic findings showing that acute exposures to
1723 prolonged sitting result in reduced lower-limb (~2 to 5 %FMD), but not upper-limb,
1724 vascular function in healthy adults (20, 120). Notably, no significant reductions were
1725 observed for exposures that were shorter than 2 hours of uninterrupted sitting (20).
1726 Additionally, some studies reported increases in artery stiffness, as measured by pulse
1727 wave velocity (PWV). Carotid-to-femoral PWV was increased in healthy adults following
1728 3 hours of prolonged sitting (288, 312) and carotid-to-ankle PWV has been also shown
1729 to increase in adults with overweight/obesity and elevated blood pressure following 10
1730 hours of prolonged sitting (313). However, these increases are rather marginal and
1731 likely not clinically relevant (314). Despite this evidence, some studies have not
1732 observed alterations in markers of macrovascular function (e.g., FMD (281, 283) and
1733 PWV (281, 313, 315)).

1734 Some studies demonstrated that microvascular reactivity is blunted following 1.5-
1735 6-hour exposures to prolonged sitting in both upper and lower extremities, as evidenced
1736 by reductions in peak blood flow and blood flow area under the curve (AUC) (282, 288,
1737 311, 316-319). Studies also demonstrated impairments in tissue oxygenation index
1738 recovery rate during reactive hyperemia after ~3 hours of prolonged sitting (281, 315,
1739 320). However, some studies demonstrated alteration in markers of microvascular
1740 function (e.g., shear rate (279) and blood flow area under the curve (AUC) (319)).

1741 Seven days of lower-limb immobilization resulted in reductions in femoral artery
1742 base diameter (~5%) and vascular conductance (~23%), and increases in vascular

1743 resistance (~35%) in healthy male adults, but all parameters returned to baseline levels
1744 after resuming habitual activity for 14 days (321).

1745 Five days of reduced step count (~12,000 to ~3,500 steps/day) reduced popliteal
1746 artery (-3 %FMD), but not brachial artery, FMD in healthy male adults (103).
1747 Conversely, base diameter was decreased in the brachial artery (~5%, suggestive of
1748 inward vascular remodeling), but not in the popliteal artery (103). Additionally,
1749 CD31⁺/CD42b⁻ endothelial microparticles concentration (a marker of endothelial
1750 apoptosis) increased significantly by ~490% after 5 days of reduced activity (103).

1751 *Potential mechanisms.* The seated posture results in greater amount of low and
1752 oscillatory shear rates in the lower extremities conduit arteries (272, 276, 277, 288, 311,
1753 316). Both oscillatory and low shear stress can increase endothelial cell-derived
1754 reactive oxygen species (ROS) and downregulate endothelial nitric oxide synthase
1755 (eNOS) expression and production of NO (a potent dilator released from endothelial
1756 cells) (322, 323). It has also been proposed that increased endothelin-1 concentrations
1757 (ET-1, a potent vasoconstrictor) may upregulate ROS and reduce total plasma nitrate
1758 and nitrite, markers of NO bioavailability (29). Increases in ET-1 concentrations, but not
1759 NO bioavailability, have been shown following exposures to prolonged sitting (279,
1760 315). However, others did not report such increases in ET-1 concentrations (283, 312).

1761

1762 5.2.1.2. Evidence from longer-term studies

1763 Unilateral leg immobilization for 12 days reduced femoral and popliteal arteries mean
1764 diameter (~7% and 14%, respectively) but did not change resting carotid artery diameter

1765 in healthy adults (295). Popliteal artery FMD increased in the immobilized leg (~7
1766 %FMD), but not in the non-immobilized leg (295).

1767

1768 5.2.1.3. Clinical significance

1769 Acute exposures to prolonged sitting resulted in significant decreases in lower-limb (-2
1770 to -5 %FMD), but not upper-limb, in healthy adults (20, 120). Similar decreases in
1771 vascular function (-3 %FMD) have been reported following 5 days of reduced step count
1772 in healthy male adults (103). FMD is a predictor of cardiovascular events in the general
1773 population (324). However, it is unclear whether these detrimental changes are likely to
1774 be sustained over time and whether upper limb FMD might also be impacted by SB.

1775

1776 5.2.2. *Reducing and interrupting sedentary behavior*

1777

1778 5.2.2.1. Evidence from acute studies

1779 Three meta-analyses have summarized the effects of interrupting sitting on FMD (20,
1780 120, 325). Two of those indicated that vascular dysfunction can be mitigated by
1781 interrupting prolonged sitting with aerobic, standing or simple resistance activities when
1782 compared to prolonged sitting (1.5 to 5 hours) (120, 325). These short-term
1783 interventions improved FMD by 1.5 to 1.9 %FMD (120, 325), and increased shear rate
1784 by 12.7 S^{-1} in adults and older adults (325). In contrast, the most recent meta-analysis
1785 demonstrated a small, non-significant effect of PA interruptions to sitting on FMD (20).
1786 Of note, some original studies failed to show improvements in lower- and/or upper-limb

1787 FMD following at least one of the interruptions to SB compared to prolonged sitting (21,
1788 279, 280, 283, 287, 326, 327).

1789 Meta-regressions revealed that higher BMI is significantly associated with greater
1790 reductions in FMD responses following interruptions to sitting as compared to prolonged
1791 sitting (325), indicating that those with higher BMI may benefit more from interrupting
1792 sitting. Subgroup analysis also indicated that aerobic and simple resistance activities
1793 may be more effective than standing interruptions at improving FMD, but this
1794 comparison did not reach statistical significance due to the low number of included
1795 studies (120). Regarding frequency of interruptions to sitting, interrupting prolonged
1796 sitting with 3-minute of simple resistance activities every 30 minutes (squat, calf raises;
1797 total: 39 minutes) was more effective at increasing FMD in the femoral artery in adults
1798 and older adults with type 2 diabetes compared to a lower frequency of interruptions
1799 (i.e., squatting, calf raises; 6-minute bouts every 60 minutes, total: 36 minutes), for
1800 which FMD did not change (283). It is not clear whether intensity of interruptions
1801 differentially affects FMD responses.

1802 *Reducing/interrupting SB vs continuous MVPA/exercise.* Continuous exercise (60
1803 min/day of MVPA), but not substituting sitting with 5-6 h/day of LPA, over 4 days
1804 improved circulating markers of endothelial dysfunction in adults with normal weight,
1805 overweight and type 2 diabetes (328).

1806 *Potential mechanisms.* It has been suggested that changes in vascular function
1807 may be mediated by decreases in plasma ET-1 concentration (279, 315) and increases
1808 in plasma nitrate/nitrite concentration and NO bioavailability/endothelin-1 ratio (315).
1809 Change in these biomarkers, along with increased skeletal muscle activity and other

1810 systemic changes (reduced postprandial glucose responses, blood lipids, inflammatory
1811 markers, and sympathetic nervous system activity) are thought to be the potential
1812 mechanisms underpinning the effects of reducing/interrupting SB on cardiovascular
1813 function (FIGURE 5) (26, 329).

1814

1815 ----- INSERT FIGURE 5 ABOUT HERE -----

1816

1817 5.2.2.2. Evidence from longer-term studies

1818 Only four longer-term trials have investigated the effects of reducing/interrupting SB with
1819 standing, walking and/or other types of PA on vascular function (325). A meta-analysis
1820 summarizing the available evidence showed a significant increase in FMD (0.93 %FMD)
1821 in adults and older adults following 8 to 16 weeks of intervention (325). However, this
1822 beneficial effect was mainly driven by one study (305), with other studies suggesting no
1823 change in lower- and/or upper-limb FMD following reducing/interrupting SB (305, 306,
1824 330).

1825

1826 5.2.2.3. Clinical significance

1827 There is some evidence of improvements on brachial and lower-limb vascular function
1828 (0.93 %FMD) associated with reducing/interrupting SB. This change is likely to be
1829 clinically meaningful, given that a 1% increase in FMD is significantly associated with a
1830 17% decrease in future risk of cardiovascular events (331, 332). However, the effects of
1831 reducing/interrupting SB on vascular function appear to be less pronounced relative to
1832 those observed for aerobic, resistance and combined exercise training (+2.1 to 2.8

1833 %FMD) in adults and older adults (333). Despite this initial evidence indicating a
1834 potential benefit of reducing/interrupting SB, the evidence base is still limited, and future
1835 studies are needed to elucidate the effects of reducing/interrupting SB on vascular
1836 function and the factors that mediate intervention effects.

1837

1838 **5.3. Cardiorespiratory fitness**

1839

1840 *5.3.1. Increasing sedentary behavior*

1841

1842 5.3.1.1. Evidence from longer-term studies

1843 Fourteen days of reduced step count (10,501 to 1,344 steps/day) resulted in a 6.6%
1844 decline in VO_{2max} in healthy male adults, which was significantly correlated with
1845 reductions in daily steps (108). Other studies showed similar results in healthy adults
1846 (107, 112). Importantly, VO_{2max} returned to baseline levels by simply resuming habitual
1847 activity for 14 days (107, 112).

1848

1849 5.3.1.2. Clinical significance

1850 Increasing SB results in profound decreases in VO_{2max} in healthy adults (e.g., -6.6% in
1851 14 days). Changes observed over 14 days of increased SB are dramatically accelerated
1852 compared to age-related decline in VO_{2max} per decade (~10%, regardless of PA level) in
1853 females and males (334). Given low cardiorespiratory fitness is significantly associated
1854 with increased risk for all-cause mortality and cardiovascular events (335, 336), these
1855 findings hold important clinical implications for those who may undergo periods of

1856 reduced activity, bed rest or immobilization. In contrast, it is important to highlight these
1857 alterations can be reverted after resuming habitual daily activities (107, 112).

1858

1859 5.3.2. *Reducing and interrupting sedentary behavior*

1860

1861 5.3.2.1. Evidence from acute studies

1862 Acutely, regular sit-to-stand transitions (stand up and return to a seated position every 1
1863 minute for 10 minutes) significantly increased VO_{2max} by ~32% in healthy adults as
1864 compared to 10 minutes seated in a chair motionless (3.86 vs. 2.93 $ml \cdot kg^{-1} \cdot min^{-1}$) (12).

1865

1866 5.3.2.2. Evidence from longer-term studies

1867 A 3-year randomized controlled trial involving physically inactive and sedentary
1868 individuals with type 2 diabetes compared 1-month exercise counseling every year or
1869 standard care (233). In an ancillary analysis, when participants were divided into
1870 quartiles based on changes in PA and SB irrespective of experimental arm, the highest
1871 quartile for changes in SB (-1.53 h/day) presented a significant increase in VO_{2max} (4.49
1872 $ml \cdot kg^{-1} \cdot min^{-1}$). Importantly, increases in time spent in SB were negatively associated
1873 with changes in VO_{2max} , which was independent from time spent in MVPA (233). This
1874 finding indicates that reducing sedentary time, leading to substantial increases in total
1875 PA may be sufficient to improve cardiorespiratory fitness in a highly physically inactive
1876 and sedentary, at-risk population group.

1877 *Reducing/interrupting SB vs continuous MVPA/exercise.* In a small-scale, 12-
1878 week, multifactorial, pilot-study involving 57 sedentary, overweight/obese males and

1879 females, Keadle and colleagues (140) examined both the independent and the
1880 combined effects of exercise training (40-65% heart rate reserve, i.e., moderate to
1881 vigorous intensity) and reducing SB (replace SB with standing and LPA plus increasing
1882 daily step count by 5-10%) on VO_{2max} . Compared to control, both exercise training (no
1883 change in SB, +27 min/day in MVPA) and exercise training combined with reducing SB
1884 (-7.3% of daily hours in SB, +45 min/day in MVPA) improved VO_{2max} by ~10% (2.5
1885 $ml \cdot kg^{-1} \cdot min^{-1}$ and 2.9 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). Additionally, changes in VO_{2max}
1886 resulting from reducing SB only (-4.8% of daily hours in SB, +28 min/day in MVPA) were
1887 not statistically significant (0.2 $ml \cdot kg^{-1} \cdot min^{-1}$) (140), indicating that improvements in
1888 VO_{2max} may depend on the PA intensity. Four weeks of an intervention to reduce SB (-
1889 53 min/day in SB with no change in MVPA) resulted in significant increases in VO_{2max}
1890 (1.9 $ml \cdot kg^{-1} \cdot min^{-1}$) in physically inactive adults with obesity (142). This improvement in
1891 VO_{2max} was comparable to the effects of an intervention aiming to increase MVPA to at
1892 least 30 min day (2.2 $ml \cdot kg^{-1} \cdot min^{-1}$; +16 min/day of MVPA with no change in SB) (142).

1893

1894 5.3.2.3. Clinical significance

1895 Significant increases in VO_{2max} (1.9 to 4.4 $ml \cdot kg^{-1} \cdot min^{-1}$) have been reported following
1896 interventions to reduce/interrupt SB (-1 to 1.5 h/day) (142, 233). Despite these findings,
1897 evidence is still limited, and future studies are needed to elucidate the effects of
1898 reducing/interrupting SB on cardiorespiratory fitness, the clinical significance of these
1899 improvements, and the mediating factors (e.g., intensity of PA replacing SB).

1900

1901 6. MUSCULOSKELETAL SYSTEM

1902

1903 **6.1. Skeletal muscle**

1904

1905 *6.1.1. Increasing sedentary behavior*

1906

1907 6.1.1.1. Evidence from longer-term studies

1908 Skeletal muscle contractile activity during sitting is significantly lower than for standing
1909 and ambulatory activities (14, 17, 19). As discussed in subtopic 4.3.1, existing studies
1910 are reasonably consistent in showing impaired anabolic responses after different
1911 periods and forms of SB. Significant losses in muscle mass have been consistently
1912 reported after only a few days of exposure to increased SB, regardless of study model.

1913 Computed tomography scans revealed ~2% decrements in quadriceps cross-
1914 sectional area (CSA) after only 5 days of a knee-immobilization protocol in healthy older
1915 males (337). After 14 days of full-leg casting, both young and older males had
1916 experienced significant decreases (~5 to 9%) in quadriceps muscle volume as assessed
1917 by magnetic resonance imaging (MRI) (95). Longer time periods have also been studied
1918 (e.g., 3.5 months), and muscle fiber CSA of the *vastus lateralis* was shown to be
1919 dramatically reduced (-24 to -51% depending on fiber type) in older males submitted to
1920 full-leg immobilization after total knee arthroplasty, when compared to control, physically
1921 inactive individuals (338). Notably, initial quadriceps CSA was positively associated with
1922 the magnitude of muscle atrophy following 14 days of lower limb immobilization in
1923 females, but not in males (339). These findings highlight possible sex-based differences

1924 in skeletal muscle adaptations to immobilization, in which females with higher pre-
1925 immobilization muscle mass are more likely to present with muscle loss.

1926 Several studies have demonstrated that daily step reductions (~67 to 87%
1927 reduction from baseline) resulted in lean mass decrements varying between 0.5 to 4.5%
1928 in healthy male adults (107-109, 235). Fourteen days of reduced daily steps (from 5,962
1929 to 1,413 steps/day) elicited a 4% decrease in lower-limb fat-free mass in healthy older
1930 adults (110). Furthermore, using a unilateral leg model, Devries and colleagues (105)
1931 demonstrated that 14 days of step reduction resulted in an ~1.3% loss of leg fat-free
1932 mass in healthy older males. Performing concomitant unilateral resistance exercise
1933 counteracted maladaptations by increasing fat-free mass (~1.4%) in the exercised leg.
1934 In addition, MHC distribution in the skeletal muscle shifted from slow-twitch (MHC I)
1935 towards hybrid and fast-twitch (IIa) fiber types in vastus lateralis following 20 days of
1936 reduced steps (from 14,000 to 3,000 steps/day) in healthy male adults (106), which has
1937 also been observed following periods of bed rest (41-43).

1938 *Potential mechanisms.* Bed rest studies showed that both decreased muscle
1939 protein synthesis and increased muscle protein breakdown contribute to skeletal muscle
1940 loss in healthy individuals and for those with chronic disease conditions (340, 341).
1941 Skeletal muscle homeostasis is tightly controlled by numerous anabolic and catabolic
1942 pathways, though the precise interconnection and biological actions of these actors still
1943 need to be fully elucidated (342) (FIGURE 6). In muscle atrophy conditions, several
1944 anabolic signaling pathways may be suppressed, including phosphoinositide 3-kinase
1945 (PI3K)-Akt-mechanistic target of rapamycin 1 (mTORC1), β 2-adrenergic, wingless/int1-
1946 frizzled (WNT/FZD), calcineurin, hippo, and/or bone morphogenetic protein (BMP). In

1947 contrast, several catabolic signaling pathways may be overactivated, including
1948 transforming growth factor- β (TGF- β), AMPK, nuclear factor kappa light chain enhancer
1949 of activated β cells (NF- $\kappa\beta$), glucocorticoid receptors, angiotensin, IL-6-janus
1950 kinases/signal transducers and activators of transcription (JAK/STAT), kinin,
1951 sphingolipids, notch, and/or activating transcription factor 4 (ATF4)-endoplasmic
1952 reticulum stress (342-345). Finally, recent a study showed that diminished mitochondrial
1953 energetics, lipid remodeling and increased H₂O₂ emission in hindlimb muscles of mice
1954 were early features preceding loss of muscle function (346). In addition, the shift in
1955 MHC distribution from slow-twitch towards hybrid and fast-twitch following bed rest (41-
1956 43) may contribute to muscle fatigue, dependance upon muscle glycogen as fuel, and
1957 reduced capacity for fat oxidation (347).

1958

1959 ----- INSERT FIGURE 6 ABOUT HERE -----

1960

1961 6.1.1.2. Clinical significance

1962 Increasing SB results in profound decreases (1.3 to 9%) in skeletal muscle or fat-free
1963 mass in adults and older adults. Changes observed over a few days of increased SB
1964 are comparable to muscle mass decreases (~3 to 8%) over a decade after the age of 30
1965 (348, 349). Given that low muscle mass is significantly associated with increased risk
1966 for all-cause mortality (350), these findings could hold important clinical implications for
1967 those who may undergo periods of reduced activity, bed rest or immobilization and for
1968 older adults who present higher rates of muscle mass loss compared to younger
1969 individuals (348).

1970

1971 *6.1.2. Reducing and interrupting sedentary behavior*

1972

1973 *6.1.2.1. Evidence from longer-term studies*

1974 Five studies investigated the longer-term effects (4 to 16 weeks) of reducing/interrupting
1975 SB on fat-free mass. Four of them showed no effect in adult office workers and adults
1976 and older adults with type 2 diabetes (351-354). In contrast, a 3-month cluster
1977 randomized trial conducted in 161 adult office workers significantly reduced sedentary
1978 time by 35 min/8-hour workday compared to control individuals (n=131), which was
1979 primarily driven by an increase in standing time. After intervention, there was an
1980 increase in total fat-free mass (0.5 kg) in the intervention group compared to the control
1981 group (355).

1982 Overall, longer-term findings related to the effects of reducing/interrupting
1983 prolonged sitting on skeletal muscle mass have been inconsistent. The extent to which
1984 reducing/interrupting SB increases muscle mass remains unclear. Future experimental
1985 studies are warranted to investigate whether reducing/interrupting SB and which type
1986 and intensity of PA can meaningfully impact skeletal muscle mass.

1987

1988 **6.2. Muscle strength and functioning**

1989

1990 *6.2.1. Increasing sedentary behavior*

1991

1992 *6.2.1.1. Evidence from longer-term studies*

1993 Two weeks of unilateral whole leg casting significantly impaired muscle function by ~16-
1994 31% in both adults and older male adults, as measured by maximal voluntary
1995 contraction, peak torque, specific force, and rate of force development (95).
1996 Interestingly, four weeks of rehabilitation (unilateral resistance training, 3 times/week)
1997 after a 2-week immobilization restored muscle function in both age groups (95). Another
1998 study found that as little as 5 days of knee immobilization resulted in an 8-9% reduction
1999 in muscle strength in healthy older males (337). Four weeks of unilateral lower limb
2000 suspension (99% reduction in daily step count) reduced isometric force during plantar
2001 flexion and knee extension tasks by ~15-25% and increased isometric fluctuations for
2002 both tasks by ~12-22%. In contrast, no change was observed in EMG activity for soleus
2003 and gastrocnemius muscles (356).

2004 Reduction in daily steps (75% reduction from baseline) for 14 days impaired
2005 muscle strength by ~8% in healthy older adults. In contrast, a rehabilitation program of
2006 the same length (i.e., 14 days) was shown to be ineffective in restoring physical function
2007 (130), indicating that this population may be at higher risk for accelerated age-related
2008 loss in muscle mass/function after transitions to reduced activity levels. Nonetheless,
2009 the literature is inconsistent, with other studies having failed to show muscle strength
2010 decrements in response to step reduction in older adults (105, 110, 164).

2011

2012 6.2.1.2. Clinical significance

2013 Increasing SB results in significant decreases in skeletal muscle strength (8 to 25%) in
2014 both adults and older adults. Changes observed with increased SB are comparable to
2015 annual decreases in grip and knee extension/flexion (2.2 to 3.1% and 3.6 to 5.0%,

2016 respectively) observed in older adults (357, 358). Given that low muscle strength is
2017 independently associated with increased risk for all-cause mortality regardless of
2018 muscle mass and participation in SB and leisure time PA (350), these findings could
2019 hold important clinical implications for those who may be exposed to periods of reduced
2020 activity, bed rest or immobilization, and for older adults who may be more susceptible
2021 than younger adults to muscle loss after increased SB (359).

2022

2023 *6.2.2. Reducing and interrupting sedentary behavior*

2024

2025 6.2.2.1. Evidence from longer-term studies

2026 A previous described, a 3-year randomized controlled trial involving physically inactive
2027 and sedentary individuals with type 2 diabetes compared 1-month exercise counseling
2028 every year to standard care. Participants in the intervention group experienced a
2029 significant reduction in sedentary time (-0.9 h/day) and increase in LPA (0.8 h/day) and
2030 MVPA (8 min/day), which resulted in improved lower body strength (~19%) as
2031 compared to the control group (232). Additionally, participants were divided into
2032 quartiles based on changes in PA and SB irrespective of experimental arm. The highest
2033 quartile for changes in SB (-1.53 h/day and +10.5 min/day of MVPA) did not have
2034 different isometric muscle strength in the upper body (shoulder press) but did have
2035 significantly greater lower body strength (leg extension) as compared to other quartiles,
2036 which may have been driven by the higher participation in PA of any intensity observed
2037 in this quartile (233). Interestingly, age was negatively and positively associated with
2038 intervention-induced changes in upper and lower body strength, respectively (233).

2039 In relation to physical functioning, a Cochrane systematic review and meta-
2040 analysis of interventions aiming at reducing SB (1 week to 1 year) in community-
2041 dwelling older adults showed low-certainty evidence related to reductions in sedentary
2042 time (-45 min/day versus control group, which was not significant) and improvements in
2043 physical function following intervention (2 studies; changes in gait speed and physical
2044 function as assessed by a physical performance battery were not significant) (360).
2045 Another meta-analysis, including interventions aimed at increasing participation in PA
2046 (52 min/week) and reducing SB (-58 min/day) among older adults showed a small,
2047 significant increase in physical functioning (standard mean difference: 0.21) compared
2048 to the control group (361). In patients with rheumatoid arthritis, a 4-month motivational
2049 intervention aimed at reducing SB (-1.6 h/day of sedentary, 1.3 h/day of standing and
2050 +0.5 h/day of stepping time) resulted in increased physical functioning (31%) as
2051 assessed by a disease-specific questionnaire (362). Notably, this effect was sustained
2052 up to 18 months after the end of the intervention (363).

2053 Only a few studies have investigated the effects of reducing/interrupting prolonged
2054 sitting on muscle function. Given the limited number of studies, the extent to which
2055 reducing/interrupting SB increases muscle strength and functioning and the associated
2056 clinical significance remain unclear.

2057

2058 **6.3. Bone**

2059

2060 *6.3.1. Increasing sedentary behavior*

2061

2062 6.3.1.1. Evidence from longer-term studies

2063 Increased resorption and decreased formation are considered to be the primary
2064 drivers of immobilization-induced bone loss in weight-bearing bones (364).
2065 Experimental studies have consistently shown significant alterations in bone parameters
2066 when exposed to periods of increased SB. Lower-limb suspension has been used as a
2067 model for such investigations, and 24 days of unilateral lower-limb suspension induced
2068 losses in bone (tibia: 0.3 to 0.9%) of healthy male adults comparable to those seen after
2069 bed rest (365).

2070 *Potential mechanisms.* Markers of bone resorption (urine: hydroxyproline,
2071 deoxypyridinoline, and N-telopeptide of type I collagen; serum: type I collagen
2072 carboxytelepeptide) were significantly increased during bed rest, returning to baseline
2073 levels after resuming ambulation (366).

2074 The effects of bone loss and recovery during/following disuse are unclear in older
2075 adults and individuals with osteopenia/osteoporosis. This should be addressed by future
2076 studies, particularly given the known negative effect of aging on cellular and molecular
2077 processes throughout the different stages of bone fracture healing (367).

2078

2079 6.3.1.2. Clinical significance

2080 Limb immobilization results in profound decreases in bone mineral density (~1%),
2081 primarily in weight-bearing bones in adults. These alterations are likely to be clinically
2082 significant given that the changes observed over 24 days of immobilization are
2083 comparable to annual bone loss (~1%) in older adults (357). These findings hold
2084 important clinical implications for those who may undergo bed rest and immobilization

2085 periods due to injury and for older adults, particularly postmenopausal females who
2086 present higher rates of bone loss compared to males of similar age (357).

2087

2088 *6.3.2. Reducing and interrupting sedentary behavior*

2089 To our knowledge, no experimental study has investigated the effects of
2090 reducing/interrupting SB on bone metabolism, which is a promising area to be
2091 addressed in future studies.

2092

2093 **7. CENTRAL NERVOUS SYSTEM**

2094

2095 **7.1. Central and peripheral neural effects**

2096

2097 *7.1.1. Increasing sedentary behavior*

2098

2099 *7.1.1.1. Evidence from acute studies*

2100 Four to six hours of exposure to prolonged, uninterrupted sitting decreased middle
2101 cerebral artery blood flow velocity and cerebrovascular conductance (~3 to 6%) in
2102 healthy adults with desk-based job as compared to baseline levels, indicating impaired
2103 dynamic cerebral autoregulation (368, 369). Three hours of exposure to prolonged,
2104 uninterrupted sitting resulted in reductions in cerebrovascular conductance index (~8%)
2105 in individuals (≥ 55 years old) with increased cardiovascular risk (306). In contrast, 3
2106 hours of uninterrupted sitting (with low or high mental activity) did not change cerebral
2107 blood flow in older adults, but increased blood pressure (mean arterial pressure: 8.6

2108 mmHg) and cerebrovascular resistance (~13%), which are known to negatively impact
2109 brain health in the long-term (370). Additionally, 3 hours of prolonged, uninterrupted
2110 sitting did not affect corticospinal excitability in adult office workers (371) nor cerebral
2111 vasomotor reactivity in individuals with increased cardiovascular risk (306).

2112 Exposure to prolonged sitting did not change plasma concentrations of brain-
2113 derived neurotrophic factor (BDNF), catecholamines and related precursors or
2114 metabolites (norepinephrine, epinephrine, dopamine, DOPA, and dihydroxyphenylglycol
2115 [DHPG]) (284). Interestingly, changes in total fatigue levels were significantly associated
2116 with increased DHPG and decreased DOPA concentrations over 4 hours of prolonged
2117 sitting, which may reflect alterations in the sympathetic nervous system in response to
2118 prolonged exposures to sitting (284).

2119 *Potential mechanisms.* It has been proposed that there may be involvement of
2120 altered cerebral glucose utilization due to increased postprandial glucose responses
2121 (28, 75-77); altered cortical perfusion and oxygen delivery due to alterations in cerebral
2122 (306, 368, 369) and peripheral vascular function (20, 120, 325), and in the supply of
2123 BDNF (372); and increased levels of inflammatory markers and reactive oxygen species
2124 (310, 373-375). Importantly, all these factors are recognized contributors to cognitive
2125 decline and dementia (28, 376, 377) (FIGURE 7). With respect to glucose utilization,
2126 altered cerebral glucose utilization seems to be a response to increased circulating
2127 glucose concentration. Acute hyperglycemia leads to a reduction in regional cerebral
2128 blood flow and a spike in insulin levels to facilitate glucose clearance. Together, these
2129 two factors favor a glucose nadir. The glucose nadir can impair endocrine counter-

2130 regulation to a subsequent dip in glucose, thus exaggerating the hypoglycemic episode
2131 (28).

2132 SB and physical inactivity have also been shown to alter hippocampal
2133 mitochondrial and synaptic function in rats. Using selective breeding for physical
2134 inactivity, male and female sedentary Low Voluntary Runners, wild type, and High
2135 Voluntary Runner rats underwent cognitive behavioral testing, analysis of hippocampal
2136 neurogenesis and mitochondrial respiration, and molecular analysis of the dentate
2137 gyrus. Preference for physical inactivity (i.e., Low Voluntary Runners) resulted in major
2138 detriments to cognition (spatial learning and memory), brain mitochondrial respiration
2139 (coupled and uncoupled respiration), and neurogenesis (reduced AMPA receptor
2140 subunit GluA1 protein levels, which is suggested to be an initiator of Alzheimer's
2141 disease pathogenesis) compared to wild type and High Voluntary Runner rats. Of note,
2142 a significant sex-effect was noted - these differences were essentially noted in females
2143 with males being more comparable to the wild type rats (378). These findings provide
2144 evidence that selective breeding for physical inactivity has a heritable and detrimental
2145 effect on brain health and females appear to be more susceptible.

2146

2147 ----- INSERT FIGURE 7 ABOUT HERE -----

2148

2149 Future studies are required to better understand longer-term adaptations
2150 associated to increased SB on cerebrovascular function.

2151

2152 *7.1.2. Reducing and interrupting sedentary behavior*

2153

2154 7.1.2.1. Evidence from acute studies

2155 Mixed findings have been reported related to alterations in cerebral blood flow
2156 responses when interrupting prolonged sitting. Carter and colleagues (368) showed that
2157 2-minute walking interruptions every 30 minutes during sitting, but not 8-minute
2158 interruptions every 120 minutes (3.6 km/h, ~2.8-3.0 METs; total: 16 minutes), increased
2159 middle cerebral artery blood flow velocity in healthy-sedentary adults when compared to
2160 prolonged sitting over 4 hours (0.6 vs -1.2 vs -3.2 cm/s, respectively) (368). In contrast,
2161 while a single 30-minute exercise bout (65-75% maximum heart rate) increased middle
2162 cerebral artery blood flow velocity (2 cm/s) in healthy older adults compared to
2163 prolonged sitting, adding frequent light-intensity walking interruptions (3.2 km/h, 2.8
2164 METs; 3-minute bouts every 30 minutes, total: 36 minutes) following the exercise bout
2165 did not counteract the sitting-induced decrease in mean middle cerebral artery blood
2166 flow velocity over the 6.5-hour period following exercise (379). Three hours of frequent
2167 walking interruptions (self-selected pace; 2-minute bouts every 30 minutes, total: 12
2168 minutes) during sitting with low or high mental activity (watch TV vs cognitive puzzles)
2169 did not change cerebral blood flow in older adults compared to prolonged, uninterrupted
2170 sitting (370). It also did not counteract the sitting-induced increase in cerebrovascular
2171 resistance. Frequent, 2-minute light-intensity walking interruptions every 30 minutes of
2172 sitting (self-selected pace, total: 10 minutes) prevented sitting-induced reductions in
2173 cerebral blood flow velocity and cerebrovascular conductance index in individuals (≥ 55
2174 years old) with increased cardiovascular risk as compared to 3 hours of prolonged

2175 sitting (306). Other markers of cerebrovascular flow and function were not affected by
2176 interruptions to sitting (306).

2177 Bojsen- Møller and colleagues (371) showed that 3-minute simple resistance
2178 activities interruptions every 30 minutes of sitting (body-weight squatting, calf raises;
2179 total: ~15 minutes) increased corticospinal excitability in adult office workers compared
2180 to 3 hours of prolonged sitting, suggesting that interruptions to sitting may promote
2181 corticospinal neuroplasticity. In contrast, short interval intracortical inhibition was
2182 unchanged following interruptions to sitting (371).

2183 Interrupting prolonged sitting with light-intensity walking (3.2 km/h, 2.8 METs; 3-
2184 minute bouts every 30 minutes, total: 30 minutes) did not change plasma concentration
2185 of BDNF and, catecholamines and its precursors or metabolites as compared to 7 hours
2186 of prolonged, uninterrupted sitting in overweight and obese adults (284). In contrast,
2187 performing a single 30-minute exercise bout (65-75% maximum heart rate) with or
2188 without subsequent light-walking interruptions to sitting (3.2 km/h, 2.8 METs; 3-minute
2189 bouts every 30 minutes, total: 36 minutes) increased serum BDNF responses (by 160
2190 and 175%, respectively) over 8 hours in older adults, relative to prolonged sitting (372).
2191 In contrast, no differences were observed for salivary cortisol levels between sitting
2192 interrupted with a 6-minute single bout of high-intensity interval training (~84%
2193 maximum heart rate) and uninterrupted sitting in healthy male adults (380). Similarly, no
2194 differences were reported for urinary catecholamines and cortisol concentrations
2195 between six hourly 5-min bouts of moderate-walking interruptions to sitting (perceived
2196 effort: 12 – 13 “somewhat hard”, total: 30 minutes) and uninterrupted sitting in healthy-

2197 sedentary adults (157). It is not clear whether type, intensity, and frequency of
2198 interruptions to sitting differentially affect central and peripheral neural effects.

2199

2200 7.1.2.2. Evidence from longer-term studies

2201 The effect size for decreasing sedentary time can be considered large following an 8-
2202 week e-health intervention to reduce/interrupt SB at the workplace versus a control
2203 condition (-38 min/8-hour workday of sedentary time and +35 min/8-hour workday of
2204 standing time) (305). As for changes in cerebrovascular function, large effects were
2205 observed for the change in gain (Cohen's d : 1.25) and normalized gain (Cohen's d :
2206 0.91). Effect sizes were considered small to moderate for all other outcomes (Cohen's
2207 d : 0.00 to 0.74), indicating little to no improvements in cerebrovascular function
2208 following intervention among healthy adult office workers (305). In a 16-week non-
2209 randomized trial, an intervention to reduce sitting (-60 min/day of sedentary, +36
2210 min/day of standing and +30 min/day of walking time) resulted in increased resting
2211 cerebral blood flow velocity (~6%) and cerebrovascular conductance index (4%) among
2212 individuals (≥ 55 years old) with increased cardiovascular risk (306); no alterations in
2213 cerebral autoregulation, cerebral vasomotor reactivity and cardiac baroreflex sensitivity
2214 were observed. These findings indicate an overall increase in cerebral perfusion after
2215 SB reduction intervention.

2216

2217 7.1.2.3. Clinical significance

2218 Small increases in cerebral blood flow, but not cerebrovascular function, have been
2219 reported following 16 weeks of reducing/interrupting SB (306). It is important to highlight

2220 that these improvements are in line with those observed following exercise programs in
2221 older adults (381, 382). It should be noted that even conditions such as Alzheimer’s
2222 disease do not markedly affect cerebrovascular function (383). This highlights the
2223 robustness of the cerebrovascular system to regulate fluctuations in cerebral blood flow
2224 and may justify the small magnitude of changes observed following PA/SB
2225 interventions. Given the limited number of studies, the extent to which
2226 reducing/interrupting SB alters cerebrovascular flow and function, as well as the clinical
2227 significance of these findings remain imprecise. Future studies are required to better
2228 understand adaptations associated to reducing/interrupting SB in the central nervous
2229 system.

2230

2231 **7.2. Cognitive performance**

2232

2233 *7.2.1. Increasing sedentary behavior*

2234

2235 7.2.1.1. Evidence from acute studies

2236 Eight hours of prolonged, uninterrupted sitting decreased working memory, executive
2237 function, and visual learning in healthy older adults as compared to exercise followed by
2238 prolonged sitting and exercise combined with frequent light-walking interruptions to
2239 sitting (372). In contrast, 6 hours of exposure to prolonged, uninterrupted sitting did not
2240 affect cognitive function in healthy, sedentary adults (369). Other acute studies also
2241 indicate that acute exposures to prolonged, uninterrupted sitting (3 to 7 hours) do not
2242 affect cognitive function in adults and older adults (284, 380, 384, 385). For the acute

2243 context, whilst prolonged sitting decreases cerebral blood flow (368, 369), prefrontal
2244 cortex perfusion and oxygen delivery to specific brain regions is maintained (386), which
2245 may preserve cognitive performance. Future studies are required to better understand
2246 longer-term adaptations associated to increased SB on cognitive performance.

2247

2248 *7.2.2. Reducing and interrupting sedentary behavior*

2249

2250 *7.2.2.1. Evidence from acute studies*

2251 Findings on the effects of interrupting prolonged sitting on cognitive performance have
2252 been highly inconsistent. Interrupting sitting with bouts of standing, light- or moderate-
2253 intensity walking improved some cognitive performance components in three studies,
2254 namely attention (387, 388), executive function (387), working memory (372, 388), and
2255 psychomotor function (388) in adults and older adults. In contrast, six hourly 5-min
2256 bouts of moderate-intensity walking (perceived effort: 12 – 13 “somewhat hard”, total: 30
2257 minutes) did not affect cognitive function in healthy-sedentary adults compared to
2258 prolonged sitting and a time-matched continuous bout of moderate-intensity walking
2259 (perceived effort: 12 – 13 “somewhat hard”) (157). No changes in cognitive performance
2260 following interruptions to sitting have been reported in other studies (157, 206, 284, 369,
2261 380, 384, 385, 389, 390). Nonetheless, none of these studies showed detrimental
2262 effects of interruptions to sitting on cognitive performance. It is not clear whether type,
2263 intensity, and frequency of interruptions to sitting differentially affect cognitive
2264 performance.

2265

2266 7.2.2.2. Evidence from longer-term studies

2267 An 8-week e-health intervention to reduce/interrupt SB within the workplace resulted in
2268 large reductions in sedentary time (-38 min/8-hour workday of sedentary time and +35
2269 min/8-hour workday of standing time). Effect sizes were considered small for changes in
2270 work productivity (Cohen's *d*: 0.47) and concentration/focus (Cohen's *d*: 0.00),
2271 indicating little to no improvements following intervention among healthy adult office
2272 workers (305). In adults (≥50 years old) with knee osteoarthritis, an intervention aimed
2273 at increasing MVPA and reducing SB did not significantly increase working memory and
2274 episodic memory (391). Changes in MVPA and SB were not associated with changes in
2275 cognitive function during the study protocol (391).

2276

2277 7.2.2.3. Clinical significance

2278 Acute and longer-term findings related to the effects of interrupting prolonged sitting on
2279 cognitive performance have been highly inconsistent. It remains unclear the extent to
2280 which reducing/interrupting SB alters cognitive function, but studies indicate that this
2281 strategy does not negatively affect cognition. Longer-term investigation into the effects
2282 of reducing/interrupting SB on cognitive performance is warranted.

2283

2284 **8. IMMUNE SYSTEM**

2285

2286 **8.1. Immunity and inflammatory responses**

2287

2288 *8.1.1. Increasing sedentary behavior*

2289

2290 8.1.1.1. Evidence from acute and multi-day studies

2291 Two experimental studies reported that an acute 3-to-5-hour exposure to prolonged
2292 sitting increased plasma IL-6 (by ~38 to 50%) concentrations in healthy adults (392) and
2293 adults with central obesity (393); IL-6 is a pleiotropic cytokine with a broad range of
2294 inflammatory, immune, and hematopoietic effects. However, three other studies showed
2295 no change in this pro-inflammatory marker after 7-8 hours of uninterrupted sitting in
2296 healthy adults (369), adults with overweight or obesity (284), and postmenopausal
2297 females with rheumatoid arthritis (213). Another study reported significant decreases in
2298 high-sensitivity C-reactive protein (~91%) concentration following 6 hours of prolonged,
2299 uninterrupted sitting (369). In the adipose tissue, uninterrupted sitting increased gene
2300 expression of *IL-6* and *Monocyte Chemoattractant Protein-1 (MCP1)*, but not of *Tumor*
2301 *Necrosis Factor-alpha (TNF- α)* and *IL-18* (393).

2302 In a two-day crossover trial, healthy male adults performed prolonged sitting (for
2303 7 hours), standing (6 times, for a 45-minute period each time, for 7 hours) or moderate-
2304 intensity exercise (60% maximum heart rate, total: 30 minutes) on day 1, and remained
2305 seated for 6 hours on day 2 (310). On day 1, plasma superoxide dismutase
2306 concentration increased by ~13% during 7 hours of prolonged sitting as compared to
2307 standing and exercise conditions, but no difference was observed for biological
2308 antioxidant potential and catalase concentrations. Postprandial concentrations of serum
2309 derivatives of reactive oxygen metabolites tended to be higher than in fasting state
2310 following 6 hours of prolonged sitting on day 2 (310).

2311

2312 8.1.1.2. Evidence from longer-term studies

2313 Fourteen days of steps count reduction (81% reduction from baseline) did not alter
2314 fasting plasma TNF, IL-6, IL-15, and adiponectin concentration in healthy male adults
2315 (108). In healthy older adults, a 14-day step reduction protocol (~70 to 76% reduction
2316 from baseline) resulted in increases in plasma C-reactive protein (110, 164), IL-6 (164)
2317 and TNF- α concentrations (110, 164). Surprisingly, cytokine levels remained elevated
2318 throughout a 14-day recovery period (164). In the skeletal muscle, 14 days of step count
2319 reduction (from ~9,000 to 3,000 steps/day) increased expression of key proteins in
2320 inflammatory signaling pathways (i.e., c-Jun N-terminal kinase [JNK], NF- κ B inhibitor- α
2321 [$\text{I}\kappa\text{B}\alpha$] and toll-like receptor 4 [TLR4]) (130) and macrophages infiltration in healthy older
2322 adults, likely due to an increase in transient muscle edema and/or minor myofiber
2323 damage (165).

2324

2325 8.1.1.3. Clinical significance

2326 Exposures to prolonged SB increased some inflammatory markers, including plasma C-
2327 reactive protein, IL-6 and TNF- α concentrations, in both adults and older adults. It is
2328 unclear whether these detrimental changes are clinically meaningful and sustained over
2329 time. Given that low-grade inflammation is important in the pathogenesis of
2330 cardiovascular and other chronic diseases (394, 395), longer-term studies should
2331 further investigate whether the altered inflammatory responses following increased SB
2332 are associated with alterations in other physiological systems.

2333

2334 8.1.2. *Reducing and interrupting sedentary behavior*

2335

2336 8.1.2.1. Evidence from acute and multi-day studies

2337 Little is known about the effects of reducing/interrupting SB on inflammatory markers
2338 and the immune system. For instance, plasma/serum IL-6 responses were not affected
2339 by frequent, short bouts of light- and moderate-intensity walking interruptions to sitting
2340 as compared to 5.5 to 8 hours of prolonged, uninterrupted sitting in adults and older
2341 adults (213, 284, 393). Regarding the other cytokines, frequent, 3-minute light-intensity
2342 walking interruptions to sitting (24% heart rate reserve, total: 42 minutes) decreased
2343 plasma IL-1 β (~21%) and IL-10 (~17%) and increased IL-1ra (~25%) concentrations,
2344 but did not change IL-4, IL-6, IL-8, IL-17, and IFN- γ , compared to 8 hours of prolonged
2345 sitting in post-menopausal females with rheumatoid arthritis (213). These acute
2346 responses were not observed with the traditional single 30-minute bout of moderate-
2347 intensity walking (55% heart rate reserve) performed early in the morning (213). In a
2348 crossover trial, performing multiple standing bouts (6 times, total: 4.5 hours) or a
2349 moderate-intensity exercise bout (60% maximum heart rate, total: 30 minutes) protected
2350 against the increase in plasma superoxide dismutase observed following 7 hours of
2351 prolonged sitting in healthy male adults, with no differences between standing
2352 interruptions and continuous exercise trials (310). It is not clear whether type, intensity,
2353 and frequency of interruptions to sitting differentially affect markers of inflammation and
2354 oxidative stress.

2355 Regular light-intensity and simple resistance activity interruptions to sitting over 7
2356 hours (3.2 km/h, 2.8 METs or body-weight squatting, calf raises; 3-minute bouts every
2357 30 minutes, total: 36 minutes) increased plasma lysoalkylphosphatidylcholine

2358 (associated with anti-inflammatory pathways) and alkenylphosphatidylcholine
2359 (associated with antioxidant capacity), and decreased diacylglycerols and
2360 triacylglycerols (associated with pro-inflammatory pathways) concentrations, but did not
2361 affect other lipid species and subspecies, in adults and older adults with
2362 overweight/obesity and type 2 diabetes (396). In contrast, a 30-minute bout of
2363 moderate-intensity walking (55% heart rate reserve), but not light-intensity walking
2364 interruptions to sitting (24% heart rate reserve, 3-minute bouts every 30 minutes, total:
2365 42 minutes), modified serum concentration of 6 lipid classes and subclasses in a
2366 direction that indicates reduction in inflammation and platelet activation, and increase in
2367 antioxidant capacity in post-menopausal females with rheumatoid arthritis (213).

2368 Moderate-intensity walking interruptions to sitting over 5 hours (5.8 km/h, ~4.3
2369 METs; 2-minute bouts every 20 minutes, total: 28 minutes) resulted in increased
2370 *Nicotamide N-methyltransferase (NNMT*; modulates anti-inflammatory and antioxidative
2371 pathways) gene expression in the skeletal muscle as compared to prolonged,
2372 uninterrupted sitting in adults with overweight/obesity (225). In subcutaneous abdominal
2373 adipose tissue, interrupting sitting with light-intensity walking hours (3.2 km/h, 2.8 METs;
2374 2-minute bouts every 20 minutes, total: 28 minutes) resulted in upregulation of immune
2375 function and downregulation of inflammatory pathways (total of 8 pathways) as
2376 compared to uninterrupted sitting in adults with overweight/obesity (228).

2377

2378 8.1.2.2. Clinical significance

2379 Currently, evidence related to the effects of reducing/interrupting sitting is restricted to
2380 acute exposures. Although small improvements were shown in some inflammatory

2381 markers, it remains unclear whether these changes are clinically meaningful and
2382 sustained over time. Longer-term investigation into the effects of reducing/interrupting
2383 SB on inflammatory responses is warranted, particularly in population groups
2384 characterized by a low-grade inflammatory profile and/or high-grade systemic
2385 inflammation.

2386

2387 **9. SUMMARY OF PHYSIOLOGICAL IMPACTS AND FUTURE DIRECTIONS**

2388

2389 The rapid accumulation of epidemiological and experimental evidence on SB over the
2390 past 20 years has provided a foundation for understanding the physiology of SB. To
2391 date, evidence on the physiological effects of exposures to increased SB and the
2392 potential impact of reducing and interrupting SB raise several pertinent questions,
2393 research needs and opportunities. These include: 1) how evidence on physiological
2394 consequences of SB relates to the already vast knowledge base on physical inactivity
2395 (lack of sufficient exercise); 2) what are the effects of reducing/interrupting SB on
2396 acute/chronic physiological processes or health outcomes and the specific mechanisms
2397 involved; and 3) how the evolving knowledge about reducing/interrupting SB can
2398 provide rational mechanistic bases for interventions and future clinical and public health
2399 recommendations. Hereafter, we provide a summary of available evidence and a
2400 perspective on some of the priority areas for future work in SB physiology.

2401

2402 **9.1. What are the effects of sedentary behaviors on physiological systems?**

2403 From a physiological perspective, the evidence to date indicates the numerous
2404 physiological responses resulting from increasing SB (TABLE 3). To summarize,
2405 excessive and prolonged SB leads to insulin resistance, vascular dysfunction, shift in
2406 substrate use towards carbohydrate oxidation, shift in muscle fiber from oxidative to
2407 glycolytic type, reduced cardiorespiratory fitness, loss of muscle mass and strength, and
2408 bone mass, and increased total body fat mass and visceral fat depot, blood lipid
2409 concentrations, and inflammation. These adaptations relate to those reported for
2410 physical inactivity (30-33, 397, 398).

2411

2412 ----- INSERT TABLE 3 ABOUT HERE -----

2413

2414 A standing question is whether SB (too much sitting) and physical inactivity (too little
2415 exercise) lead to similar or distinct adaptations in relation to the number of physiological
2416 systems being impaired, magnitude of changes, and mechanisms. This question is
2417 challenging to address with the current available study models. However, bed rest
2418 studies that tested the effects of exercise training to prevent the physiological
2419 adaptations to bed rest can provide some insights. While individuals in strict bed rest
2420 are deficient in both LPA and MVPA, those who are concomitantly subjected to bed rest
2421 and exercise training are only deficient in LPA. Therefore, bed rest combined with
2422 exercise represents a model to study the adaptations and potential mechanisms of
2423 exposures to a highly sedentary yet physically active condition. Findings from these
2424 studies suggest excessive participation in SB results in adverse effects, at least for
2425 some outcomes, even in the presence of large volumes of aerobic and/or resistance

2426 exercise, i.e., above current guidelines (34) (TABLE 4). This supports that SB and
2427 physical inactivity likely coexist in a continuum related to energy demand and muscle
2428 contraction, and that the magnitude of maladaptations following physical inactivity
2429 seems to be more pronounced than those of SB in most physiological systems (34), but
2430 it is not clear whether these behaviors differentially affect physiological systems or
2431 present distinct underpinning mechanisms. It also highlights the potential benefits of
2432 regular non-exercise activity and/or muscle contractions.

2433

2434 ----- INSERT TABLE 4 ABOUT HERE -----

2435

2436 Our premise in this review is that the adaptations to increasing SB are not the opposite
2437 of adaptations to reducing/interrupting SB (i.e., increasing PA). Accordingly, evidence
2438 from each paradigm was addressed in separate sections. Notably, a few studies in
2439 rodents and humans have shed some light on potential distinct adaptations to
2440 increasing SB vs increasing non-exercise activity (e.g. LPA) vs MVPA/exercise training.
2441 For example, alterations in heparin-released and intracellular LPL activity decreased
2442 mono-exponentially in both type 1 and predominantly type 2 muscles after 12 hours of
2443 limb unloading. These alterations were rapidly reversed with light-intensity contractile
2444 activity in both soleus and quadriceps muscles (9, 44, 45), but MVPA/exercise training
2445 did not enhance LPL regulation in type 1 muscles and type 2 muscles that were not
2446 recruited during running (8, 46). In addition, *LPP1/PAP2A* has been proposed as a
2447 potential gene that modulates maladaptation related to increased SB/inactivity in both
2448 humans and rats, but exercise has been found to be ineffective at counteracting

2449 alterations in LPP1/PAP2A in both species (40). Finally, a clinical study compared the
2450 effects of 2 months of exercise training in sedentary male adults with the effects of 1
2451 month of reducing non-exercise PA (i.e., increased SB) in active male adults. Notably,
2452 the deleterious effect of increased SB was more marked than the beneficial effects of
2453 exercise training following current PA guidelines for dietary fat oxidation, fasting and
2454 postprandial insulin concentration/response, postprandial triglycerides response, and
2455 fat-free and fat mass (127). In contrast, the magnitude of changes in VO_{2max} was more
2456 pronounced following exercise training than increased SB (127). Altogether, these
2457 findings suggest distinct mechanisms may underpin adaptations to increased SB and
2458 exercise training. Further investigations are however clearly required.

2459 It is critical that well-powered, rigorous studies are conducted to examine the
2460 acute and chronic physiological adaptations to imposed SB. Studies aiming to
2461 investigate the physiological consequences of imposed SB ('problem-focused'
2462 approach) should focus on recruiting individuals with low sedentary time who will
2463 experimentally modify their usual activity (low SB and high LPA) to more sedentary
2464 states (higher SB and lower PA of any intensity). This concept is analogous to the
2465 paradigm put forward initially by Booth and Lees (399) stating that appropriate models
2466 of physical inactivity should utilize an approach whereby physically active individuals
2467 become physically inactive (400). To effectively isolate the effects of physical inactivity
2468 from those of SB, studies should focus on recruiting individuals who do not meet current
2469 PA guidelines (150 min/week of MVPA) or perform regular exercise training. Though
2470 bed rest can be a well-controlled and useful model to study SB physiology, studies

2471 should also focus on increased SB with behaviors more commonly seen in free-living
2472 (i.e., sitting not strict lying down).

2473 Future studies should (i) investigate whether differential changes in muscle mass
2474 and fiber type are associated with SB-related metabolic adaptations; (ii) determine the
2475 molecular and cellular mechanisms underpinning adaptations to increasing SB, e.g.,
2476 using ‘omics’ approaches (genomics, epigenomics, transcriptomics, proteomics,
2477 metabolomics, etc.); (iii) implement different volumes and types of PA/exercise to
2478 determine how much PA and/or exercise is needed to offset the adverse effects of
2479 increased SB; (iv) include rigorous measurements and control PA level at baseline and
2480 during the study protocol, preferably via device-based measures, which is a major
2481 limitation of free-living studies to increase SB (i.e., reduced step count and
2482 immobilization). Given most of available evidence is limited to healthy male adults,
2483 future studies should focus on investigating the effects of increasing SB in females,
2484 children, adolescents, older adults, more-diverse population groups and individuals at
2485 risk for or with chronic conditions. This will assist in providing the strong rational
2486 biological bases that are much needed for improving our understanding of the
2487 physiology of SB and its multiple health consequences.

2488

2489 **9.2. What are the beneficial effects of reducing/interrupting sedentary** 2490 **behavior?**

2491 Acutely, reducing/interrupting SB improves postprandial glucose and insulin responses,
2492 systolic and diastolic blood pressure, and lower-limb vascular function in adults and
2493 older adults (TABLE 5) (75-77, 119, 120, 325). In the longer term, reducing/interrupting

2494 SB interventions result in small improvements on body weight (-0.6 kg), waist
2495 circumference (-0.7 to -1.5 cm), percent body fat (-0.3 to -0.7%), fasting glucose (-2.5
2496 mg/dL), insulin (1.4 pM), HbA1c (-0.2%) and HDL (0.04 mM) concentrations, systolic
2497 blood pressure (-1.1 mmHg), and brachial and lower-limb vascular function (0.93
2498 %FMD) in adults and older adults (TABLE 5) (78, 134, 135, 325). There is more-limited
2499 evidence for other health outcomes and physiological systems. Despite this initial
2500 evidence, findings from acute and longer-term studies aimed at reducing/interrupting SB
2501 are inconsistent with other findings of no benefit for some outcomes. Overall, effects of
2502 reducing/interrupting SB are small, and likely not to be clinically and physiologically
2503 meaningful in healthy population groups, but plausibly, the effects are likely to be larger
2504 in less-healthy populations. Future studies need to investigate whether these small
2505 improvements on health outcomes observed with reducing/interrupting sedentary
2506 behavior associate with reduced risk of chronic diseases and early mortality.

2507

2508 ----- INSERT TABLE 5 ABOUT HERE -----

2509

2510 For studies focused on the consequences of reducing/interrupting SB ('solution
2511 focused' treatment paradigm), the emphasis should be directed towards recruiting
2512 physically inactive-sedentary individuals with modification of their typical low PA level
2513 (high SB and low PA) to higher PA levels (low SB and high PA of any intensity). This
2514 includes conducting randomized controlled trials (with appropriate control groups) of
2515 longer duration (>12 weeks), familiarizing/habituating study participants to interventions
2516 and study procedures, continuously monitoring the adherence to interventions,

2517 controlling for potential confounders during the design, conduct and analysis of the trial,
2518 and analyzing experimental data considering both the intention to treat principle and
2519 adherence to the interventions.

2520 Studies should include rigorous measurements of primary, secondary, and
2521 exploratory outcomes, and report all possible outcomes and indexes being measured
2522 (e.g., acute studies measuring glucose concentrations should report not only AUC, but
2523 also mean glucose concentration during the protocol). The monitoring of PA level at
2524 baseline, preferably via device-based measures, to assess eligibility and during study
2525 follow-up can enhance scientific rigor. Longer-term, randomized controlled trials should
2526 also combine device-based measures with self-reported (e.g., questionnaires and
2527 diaries) to gather information on activity type and context of SB and PA (299).

2528 Future studies should focus on reporting data by key factors, including, but not
2529 limited to, age groups (preferably by smaller age groups, such as 5-year increments),
2530 BMI, sex/gender, race/ethnicity, genetic profiles, menopausal and pregnancy status,
2531 medications, dietary habits, cardiorespiratory fitness, baseline SB/PA/exercise levels,
2532 sleep duration and quality, and populations at increased risk of or with chronic diseases,
2533 as indicated by the 2020 World Health Organization Guidelines Development Group
2534 (401, 402). Identifying whether such factors hold significant importance that will help
2535 identify more ‘at risk’ population groups and those who may derive more benefits from
2536 reducing/interrupting SB. Harmonized analysis using individual participant data and
2537 dose-escalation trials could also be implemented to evaluate the effect of different
2538 “doses” of SB reductions on physiological outcomes. More robust evidence on both the
2539 direct and indirect underlying cellular and molecular mechanisms associated with

2540 reducing/interrupting SB is also needed. This may be garnered through the collection of
2541 tissue samples (e.g., muscle, bone, adipose tissue), including more direct and
2542 integrated physiological measurements, rather than surrogate markers. This will assist
2543 in providing the strong rational biological bases that are much needed for improving our
2544 understanding of the physiology of reducing/interrupting SB, and refining intervention
2545 strategies and guidelines to address SB as a clinical and public health problem.

2546

2547 **9.3. What is the ‘optimum’ Frequency, Intensity, Time and Type of activities**
2548 **when reducing/interrupting sedentary behavior?**

2549 Elements pertaining to SB FITT may influence the effects of reducing/interrupting SB on
2550 glucose responses, which is the most-studied outcome. To summarize, it seems that
2551 more frequent (2-6-minute bouts every 20-60 minutes) and higher-intensity (light- to
2552 moderate-intensity) interruptions yields more pronounced improvements in relation to
2553 postprandial glucose responses than less frequent (every 120 minutes or more) and
2554 lower-intensity (i.e., standing still) interruptions to sitting (75-77, 181, 198, 206). Despite
2555 being beneficial, it is still unclear whether there are differences between higher
2556 frequencies of interruptions (e.g., every 20 minutes vs 30 minutes) or between
2557 intensities (i.e., light vs moderate vs vigorous). There are also some inconsistencies
2558 across original studies and meta-analysis on what is the ideal SB FITT. There is more-
2559 limited evidence on the influence of FITT elements of an intervention on changes in
2560 health outcomes other than glycemia.

2561 To summarize, current evidence does not allow us to conclude what is the
2562 desirable FITT to reducing/interrupting SB and significantly affect physiological systems

2563 in healthy adults and older adults, and those at-risk or with chronic diseases. The “ideal”
2564 or "optimal" FITT of SB and PA elements is likely to be based on the requirements,
2565 context, and activity/health status of the subpopulation, rather than a “one size fits all”
2566 approach. However, in terms of potential countermeasures applicable to the population,
2567 it may be that certain minimal combinations or criteria of mode or posture (e.g., active
2568 sitting, fidgeting, acute or extended postural changes, standing, activities involving
2569 resistance, and/or sit-to-stand transitions), volume or intensity (e.g., LPA or MVPA), or
2570 patterning (e.g., activity bout, active around meals, or standing length/accumulation) of
2571 physical movement are all that is required to derive physiological benefit.

2572 Another pertinent question is whether regular interruptions to sitting would be
2573 more beneficial than performing the traditional continuous bout of activity. Acutely,
2574 reductions in postprandial glucose responses are more pronounced following frequent
2575 interruptions to sitting compared to a time-matched continuous bout of activity (77, 197,
2576 198, 213). In contrast, a continuous bout of PA in the morning is more effective at
2577 attenuating postprandial triglycerides responses and systolic blood pressure than
2578 frequent interruptions to sitting (213, 214, 257, 258, 303). Nonetheless, evidence on the
2579 differential effects of frequent interruptions to sitting versus a continuous bout of
2580 PA/exercise is still limited to acute settings, a select number of health outcomes, and
2581 generally healthy population groups. Evidence on the combined effects of exercise and
2582 reducing/ interrupting SB is also very limited. This is a critical gap in the literature, given
2583 the combination of both strategies is what has been currently recommended in public
2584 health guidelines for all age and population groups.

2585 Future studies should address the evidence gap on the 'optimum' FITT of
2586 activities when reducing/interrupting SB, particularly in the longer term. To do so,
2587 studies should include experimental groups with different FITT of interruptions to SB
2588 versus a sedentary (control) group. If possible, groups should be matched for energy
2589 expenditure and FITT elements other than the one being tested should remain
2590 unchanged. For example, when testing the influence of frequency, total duration,
2591 intensity, and type of activity should be the same across experimental groups. Studies
2592 should also (i) include detailed description on how participants were instructed to
2593 reduce/interrupt SB (as per all FITT elements); (ii) provide participants with specific and
2594 measurable goals, so that adherence to FITT prescription can be objectively assessed;
2595 (iii) describe how adherence was assessed and check adherence to the prescribed
2596 intervention throughout the study protocol; (iv) report behavioral outcomes that reflect
2597 changes in the FITT element being tested, e.g., report changes in the number of daily
2598 interruptions to SB and number/duration of prolonged SB bouts when testing the effects
2599 of different frequencies of interruptions to SB; and (v) report adherence to the
2600 prescribed intervention (as per FITT elements being measured/controlled) using
2601 objective assessment (e.g., accelerometers, HR monitors, wearables, etc.) (TABLE 2).
2602 This will improve the study design, data analysis, reporting/data harmonization in future
2603 studies, and hence the robustness of the findings. It will ultimately assist with refining
2604 preventative strategies and guidelines to combat excessive SB.

2605 **10. CONCLUSIONS**

2606

2607 SB is highly prevalent in daily living and most of the population is exposed, to a greater
2608 or lesser extent, to the health risks of too much sitting. Excessive SB negatively impacts
2609 a multitude of physiological systems, leading to insulin resistance, vascular dysfunction,
2610 shift in substrate use towards carbohydrate oxidation, shift in muscle fiber from oxidative
2611 to glycolytic type, reduced cardiorespiratory fitness, loss of muscle mass and strength,
2612 and bone mass, and increased total body fat mass and visceral fat depot, blood lipid
2613 concentrations, and inflammation. From a physiological perspective, exposures to
2614 increased SB result in maladaptations that are similar to those that have been reported
2615 for physical inactivity, but generally lower in terms of magnitude.

2616 Longer-term interventions aimed at reducing/interrupting SB have only resulted in
2617 small improvements on body weight, waist circumference, percent body fat, fasting
2618 glucose, insulin, HbA1c and HDL concentrations, systolic blood pressure, and vascular
2619 function in adults and older adults. Because of inconsistencies in the reported effects of
2620 reducing/interrupting SB, the clinical significance of these findings still remains
2621 somewhat unclear. Although the 'sit less, move more and exercise' message currently
2622 promoted by contemporary public health guidelines has received a clear general
2623 consensus based on a growing body of epidemiological findings, further experimental
2624 studies are needed to elucidate the physiological effects of interventions combining
2625 exercise and reduction/interruptions to sitting. Nonetheless, reducing/interrupting SB is
2626 a low-risk strategy and is likely relevant from a population point of view, particularly
2627 given that it can serve as a stepping stone to increase participation in PA/exercise for
2628 those who do not, or have significant challenges to, achieve the minimum guidelines on
2629 MVPA.

2630

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2633

2634 **Acknowledgements**

2635 We are extremely grateful for the resourcefulness and hard work of our broader network
2636 of collaborators and the research fellows, PhD students, and support staff who have
2637 worked with us in our programs of sedentary behavior research. Figures were created
2638 with Mind the Graph (mindthegraph.com).

2639

2640 **Grants**

2641 A.J.P. and B.G. were supported by Fundação de Amparo à Pesquisa do Estado de São
2642 Paulo (Grants 2015/26937-4 and 2018/19418-9, and 2017/13552-2). A.J.P. received
2643 funding from the Ludeman Family Center for Women's Health Research at the
2644 University of Colorado Anschutz Medical Campus. A.B. were supported by the National
2645 Institutes of Health (NIH NIDDK, Grant R01DK123334). D.W.D., P.C.D. and N.O. were
2646 supported by the National Health and Medical Research Council of Australia (NHMRC)
2647 Centre of Research Excellence (Grants #1057608 and #1142685), by the Victorian
2648 state Government Operational Infrastructure Support scheme, and by the NHMRC
2649 Fellowships scheme.

2650

2651 **Disclosures**

2652 No conflicts of interest, financial or otherwise, are declared by the authors.

2653

2654 **Authors contributions**

2655 A.J.P., B.G., A.B., P.C.D., H.R., N.O., and D.W.D. conceived the review; A.J.P., B.G.,
2656 A.B., P.C.D., H.R., N.O., and D.W.D. designed the review; A.J.P., A.B., N.O., and
2657 D.W.D. prepared figures; A.J.P., B.G., A.B., P.C.D., H.R., N.O., and D.W.D. drafted and
2658 revised the manuscript; A.J.P., B.G., A.B., P.C.D., H.R., N.O., and D.W.D. approved
2659 final version of the manuscript.

2660

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TABLES

Table 1. Key terms and definitions in sedentary behavior and physical inactivity research.

Term	Definition	Refs.
Continuum of human movement and non-movement	Refers to all behaviors, including sleep, sedentary behavior, standing, and physical activity at any intensity, that occurs in the 24-hour interval. Behaviors comprised within the continuum differ in terms of type, posture and physiological state – metabolic cost, oxygen consumption, heart rate, and skeletal muscle activity and blood flow – which may underpin health effects associated with each behavior.	(1)
Non-movement or stationary behaviors	Any walking behavior performed in a sitting, reclining, lying down, or standing position with no ambulation, irrespective of EE.	(1)
Sedentary behavior	Any waking behavior characterized by a low EE (≤ 1.5 METs) while in a sitting, reclined or lying down posture.	(1, 403)
Pattern	The manner in which sedentary behavior is accumulated, for example, timing of the day, duration and frequency of bouts and breaks.	(1)
Standing	The act of one maintaining an upright position while supported by one's feet.	(1)
Passive	Any waking activity characterized by an EE ≤ 2.0 METs while standing without ambulation.	(1)

Active	Any waking activity characterized by an EE > 2.0 METs while standing without ambulation.	(1)
Movement or non-stationary behaviors	Any walking behavior performed in a standing position with ambulation, irrespective of energy expenditure.	(1)
Physical activity	Any bodily movement produced by the skeletal muscles that result in an increase in EE above resting levels.	(404)
Exercise	Refers to a physical activity that is planned, structured, repetitive, and purposeful (i.e., aimed to increase or maintain one or more components of physical fitness). It is considered as a subcategory of physical activity.	(404)
Light-intensity physical activity	Any waking behavior with an EE between $1.6 < 3.0$ METs or the relative intensity is between $20 < 40\%$ $VO_{2max}/\%HRR$ and $40 < 55\%$ HR_{max} .	(405)
Moderate physical activity	Any waking behavior with an EE between $3.0 < 6.0$ METs or the relative intensity is between $40 < 60\%$ $VO_{2max}/\%HRR$ and $55 < 70\%$ HR_{max} .	(405)
Vigorous physical activity	Any waking behavior with an EE between ≥ 6.0 METs or the relative intensity is between $\geq 60\%$ $VO_{2max}/\%HRR$ and $\geq 70\%$ HR_{max} .	(405)
Physical inactivity	Insufficient level of moderate-to-vigorous physical activity to meet the current physical activity recommendations	(1, 403)
Physical activity recommendations	For adults and older adults, at least 150 min/week of moderate-to-vigorous physical activity or 75 min/ week of vigorous physical activity. For children and adolescents, 60 min or more of moderate-to-vigorous physical activity daily.	(2, 23)

Sedentary behavior recommendations	For adults and older adults, limit the amount of time spent sedentary and replace sedentary time with more physical activity of any intensity. For children and adolescents, limit the amount of time spent sedentary particularly recreational screen time.	(2, 23)
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Legend: EE, energy expenditure; HR, heart rate; %HRR, percentage of heart rate reserve; METs, metabolic equivalent.

TABLE 2. Sedentary behavior frequency, intensity, time, and type principle.

Term	Definition	Refs.
Frequency	<p>Refers to the number of SB bouts over a given time frame. The most common SB bout lengths reported in the literature are ≥ 30 minutes, ≥ 60 minutes, and ≥ 120 minutes. Example: Interruptions to sitting lasting 2-3 minutes every 20-30 minutes seems to yield greater benefits for glycemic control compared to prolonged, uninterrupted sitting.</p> <p><i>Study design, data analysis, and reporting:</i> Include experimental groups with different frequency of interruptions to SB versus a more sedentary experimental group (control group). If possible, groups should be matched for duration and/or EE. Include detailed description about how participants were instructed about the frequency of interruptions and how adherence to prescribed frequency was assessed. Report changes in the number of daily interruptions to SB and number and duration of prolonged SB bouts.</p>	(192, 204, 205)
Intensity	<p>Refers to any waking behavior with an energy expenditure ≤ 1.5 MET, while sitting, reclining, or lying down. When focusing at reducing/interrupting sedentary behavior, intensity refers to the physical activity used to replace sedentary behavior. Example: EE during sitting postures is lower than standing and all intensities of physical activity. Similar responses are observed for oxygen consumption and heart rate.</p> <p><i>Study design, data analysis, and reporting:</i> Include experimental groups with different intensity of interruptions to SB versus a more sedentary experimental group (control group). If possible, groups should be matched for EE. Include detailed description about how participants were instructed about the intensity of interruptions and how adherence to prescribed intensity was assessed. Report changes in objectively measured daily time spent in each physical activity intensity.</p>	(11-16)

Time	<p>Refers to the total duration of time spent in SB or time spent in bouts of uninterrupted, prolonged sitting (i.e., consecutive minutes accumulated in SB – usually reported as bouts of ≥ 30 minutes, ≥ 1 hour and ≥ 2 hours of sitting). Example: Increasing sedentary time results in maladaptations in physiological systems. In contrast, reducing/interrupting sedentary time results in small benefits.</p> <p><i>Study design, data analysis, and reporting:</i> Include detailed description about how participants were instructed to reduce/interrupt SB (as per other FITT elements) and how adherence to prescribed intervention was assessed. If possible, provide participants with specific and measurable goals, so adherence can be objectively assessed. Report changes in duration of objectively measured total daily SB and prolonged SB bouts.</p>	<p>(34, 78, 134, 135, 325)</p>
Type	<p>Refers to the main intention of the SB and the context in which it occurs. Example: SB associated with energy surplus is more detrimental than exposures to sedentary behavior in energy balance.</p> <p><i>Study design, data analysis, and reporting:</i> Measure participation in each type of SB using a validated questionnaire or diary. Similarly, record types of physical activity that can be used to reduce/interrupt SB. If appropriate, design the intervention to tackle specific type/context of SB (e.g., work-related, recreational screen time, etc.) and standardize the FITT of physical activities being used to reduce/interrupt SB. Report changes in duration of objectively measured total daily SB and prolonged SB bouts, and self-reported duration in each specific type/context of SB.</p>	<p>(162, 370)</p>

Legend: EE, energy expenditure; FITT, frequency, intensity, time, and type principle; MET, metabolic equivalent; SB, sedentary behavior.

TABLE 3. Summary of acute (hours to <4 days) and longer-term (>2 weeks) effects (experimental/intervention) of increasing sedentary behavior in adults and older adults.

Outcomes	Acute effects		Longer-term effects	
	Mean change	Refs. ^a	Mean change	Refs. ^a
Body mass and composition				
Body weight (kg)	NA	-	-2%	(108)
Total and percentage body fat (%)	NA	-	3 to 14%	(106, 107, 128)
Intra-abdominal fat mass (%)	NA	-	7%	(108, 109)
Glucose metabolism				
Fasting glucose (mg/dL)	NA	-	NS	(108, 147)
2-hour post-load glucose (mg/dL)	?	-	NS	(147)
Postprandial glucose (%) ^b	17.5 (-26.2 to -8.7)	(75)*	6 to 9%	(117)
Glycemic variability	NA	-	33 to 97%	(117)
Whole-body insulin sensitivity (%)	-39 to -18%	(162)	-17%	(147)
Fasting insulin (μU/mL)	NA	-	NS	(108, 147)
2-hour post-load insulin (μU/mL)	?	-	38.8 μU/mL	(147)
Postprandial insulin (%) ^b	25.1 (-31.8 to -18.3)	(75)*	32%	(147)
Lipid metabolism				
Fasting triglycerides (mg/dL)	NA	-	0.3 mmol/L	(106, 112)
Postprandial triglycerides (%) ^b	27%	(72)	21%	(109)
Fasting total cholesterol (mmol/L)	NA	-	0.5 mmol/L	(112)
Fasting LDL (mmol/L)	NA	-	0.3 mmol/L	(112)
Fasting HDL (mmol/L)	NA	-	-0.1 mmol/L	(106)
Hemodynamics				

Systolic blood pressure (mmHg)	3.2 (0.6 to 5.8)	(119)*	4 mmHg	(112)
Diastolic blood pressure (mmHg)	NS	(119)*	NS	(112)
Mean arterial pressure (mmHg)	3.3 (2.2 to 4.4)	(119)*	?	-
Blood flow (mL/min)	-1.0 (-1.6 to -0.4)	(20)*	NS	(105)
Shear rate (SMD)	-0.8 (-1.0 to -0.5)	(20)*	?	-
Cardiovascular function				
Flow-mediated dilation (%FMD)	-1.2 (-1.7 to -0.7)	(20)*	-3 %FMD	(103)
Base diameter (%)	NS	(279)	-5%	(103, 295)
Cardiorespiratory fitness				
VO _{2max} (ml·kg ⁻¹ ·min ⁻¹ or %)	NA	-	-6.6%	(107, 108, 112)
Musculoskeletal system				
Lean mass (%)	NA	-	-9 to -0.5%	(95, 107- 109, 337)
Muscle strength (%)	NA	-	-31 to -8%	(95, 130, 337, 356)
Bone mineral density (%)	NA		-0.3 to -1%	(365)
Central nervous system				
Cerebral artery blood flow (%)	-6 to -3%	(368, 369)	?	-
Cerebrovascular conductance (%)	-8%	(306)	?	-
Cerebrovascular resistance (%)	13%	(370)	?	-
Inflammatory responses				
C-reactive protein (%)	-91%	(369)	25 to 45%	(110, 164)
Interleukin 6 (%)	38 to 50%	(392, 393)	30%	(164)
Tumor necrosis factor α (%)	?	-	12 to 31%	(110, 164)

Legend: *, evidence from meta-analyses; ^a, when meta-analyses are not available, key references are provided – refer to main document for detailed description and detailed information on study models; ^b, as measured by area under the curve; ?, no data available; NA, not applicable; NS, non-significant. Abbreviations: FMD, flow-mediated dilation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SMC, standardized mean change. For meta-analyses, data are presented as mean absolute or percent change (95% confidence interval) from baseline to post exposure to increased sedentary behavior. For original studies, data are presented as mean change or the range of mean change from baseline to post exposure to increased sedentary behavior.

TABLE 4. Summary of effects of increasing sedentary behavior through strict bed rest with or without exercise training in healthy adults.

Outcomes	Strict bed rest	Bed rest with exercise*	Refs.
Fat mass and repartition			
Intra-abdominal fat mass	increases	counteracted	(34, 247)
Liver fat accumulation	increases	attenuated	(34)
Glucose metabolism			
Whole-body insulin sensitivity	decreases	attenuated to counteracted	(34, 170, 244)
Fasting insulin	increases	no effect to counteracted	(34, 170, 244)
Fasting carbohydrate oxidation	increases	no effect to attenuated	(34)
Postprandial carbohydrate oxidation	increases	no effect to counteracted	(34, 244)
Lipid metabolism			
Fasting triglycerides	increases	no effect	(34)
Fasting HDL	decreases	no effect	(34)
Fasting lipid oxidation	decreases	no effect to attenuated	(34, 170, 244)
Postprandial lipid oxidation	decreases	no effect to counteracted	(34, 244)
Cardiorespiratory fitness			
VO _{2max}	decreases	attenuated to counteracted	(34, 406)
Musculoskeletal system			
Muscle mass	decreases	attenuated to counteracted	(34)
Muscle fiber type toward glycolytic	increases	attenuated	(34)
Mitochondrial oxidative capacity	decreases	attenuated	(34)
Muscle strength	decreases	counteracted	(42, 43, 407)
Muscle fat storage	increases	no effect	(170)
Bone mineral density	decreases	attenuated or counteracted	(408, 409)
Bone fat storage	increases	no effect to counteracted	(34)
Inflammatory responses			

Pro-inflammatory markers increases counteracted (34, 252, 410)

Legend: *, protocols involved resistance exercise alone or resistance and aerobic exercise training. In general, combined exercise training was more effective at attenuating or counteracting adverse effects related to bed rest than resistance exercise training alone.

TABLE 5. Summary of acute (<24 hours) and longer-term (>2 weeks) beneficial effects (experimental/intervention) of reducing/interrupting sedentary behavior in adults and older adults, as evidenced by meta-analyses.

Outcomes	Acute effects		Longer-term effects	
	Mean difference	Refs.	Mean difference	Refs.
Body mass and composition				
Body weight (kg)	NA	-	-0.6 (-0.9 to -0.2)	(78)
			NS	(134, 135)
Body mass index (kg/m ²)	NA	-	NS	(78, 134)
Waist circumference (cm)	NA	-	-0.7 (-1.2 to -0.2)	(78)
			-1.5 (-2.8 to -0.2)	(134)
			NS	(135)
Body fat percentage (%)	NA	-	-0.3 (-0.5 to -0.0)	(78)
			-0.7 (-1.3 to -0.1)	(134)
Fat mass (kg)	NA	-	-0.8 (-0.9 to -0.6)	(135)
			NS	(78)
Fat-free mass (kg)	NA	-	NS	(78)
Glucose metabolism				
Fasting glucose (mg/dL)	NA	-	-2.5 (-4.3 to -0.8)	(135)
			NS	(78, 134)
Postprandial glucose (% or SMD) ^a	-17.5 (-26.2 to -8.7)	(75)	?	-
			-0.4 (-0.5 to -0.2)	(76)
			-0.5 (-0.7 to -0.4)	(77)
			-0.3 (-0.6 to -0.03)	(181)
			-0.7 (-1.0 to -0.4)	(181)
Fasting insulin (pM)	NA	-	-1.4 (-2.8 to -0.0)	(78)
			-2.3 (-4.4 to -0.3)	(135)
Postprandial insulin (% or SMD) ^a	-25.1 (-31.8 to -18.3)	(75)	?	-
			-0.4 (-0.5 to -0.2)	(76)
			-0.6 (-0.7 to -0.4)	(77)

	-0.8 (-1.2 to -0.5)	(181)		
	NS	(181)		
HbA1c (%)	NA	-	-0.2 (-0.3 to -0.04)	(134)
			NS	(78)
Lipid metabolism				
Fasting triglycerides (mg/dL)	NA	-	NS	(78, 134, 135)
Postprandial triglycerides (SMD) ^a	-0.3 (-0.4 to -0.1)	(77)	?	-
	NS	(75, 76)		
Fasting total cholesterol (mg/dL)	NA	-	NS	(78, 134, 135)
Fasting LDL (mg/dL)	NA	-	NS	(78, 134, 135)
Fasting HDL (mM)	NA	-	0.04 (0.02 to 0.07)	(78)
			NS	(134, 135)
Cardiorespiratory system				
Systolic blood pressure (mmHg)	-4.4 (-7.4 to -1.5)	(119)	-1.1 (-2.1 to -0.0)	(78)
	NS	(181)	NS	(135)
Diastolic blood pressure (mmHg)	-2.4 (-4.5 to -0.3)	(119)	NS	(78, 134, 135)
Flow-mediated dilation (%FMD)	1.5 (1.0 to 2.0)	(325)	0.9 (0.3 to 1.6)	(325)
	1.9 (0.4 to 3.4)	(120)		
	NS	(20)		
Pulse wave velocity (m/s)	0.02 (-0.27 to 0.32)	(325)	0.27 (-0.32 to 0.87)	(325)
Shear rate (S ⁻¹)	12.7 (7.9 to 17.5)	(325)	?	-

Legend: ^a, as measured by area under the curve; ?, no data available; NA, not applicable; NS, non-significant. Abbreviations: FMD, flow-mediated dilation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SMD, standard mean difference. Data presented as mean difference (95% confidence interval) between reducing/interrupting sedentary behavior and prolonged sedentary behavior/control group.

FIGURE LEGENDS

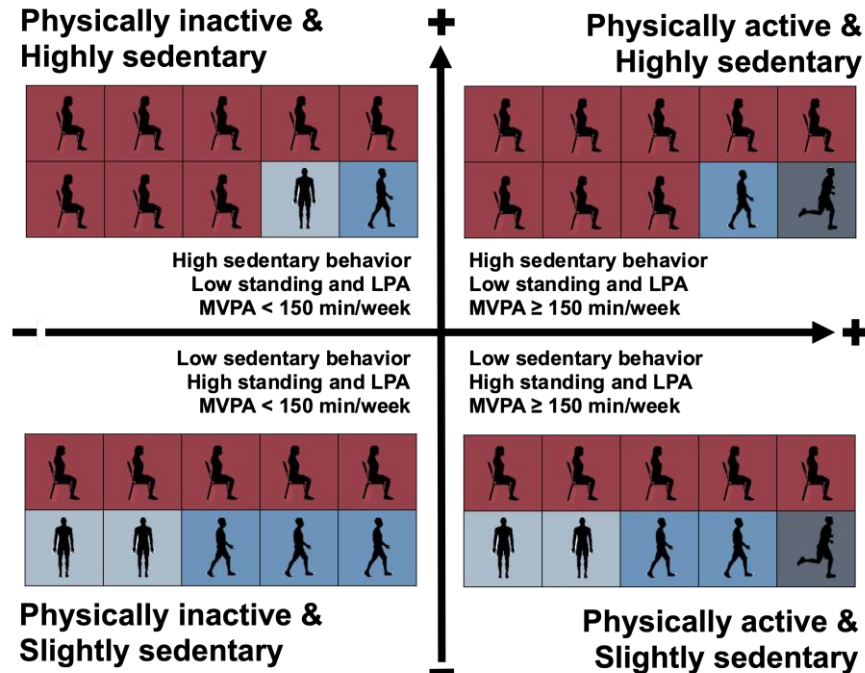


FIGURE 1. Sedentary behavior and physical inactivity operationalized as distinct behaviors identifies four key classifications. A person is classified as physically inactive if he/she engages in <150 min/week of moderate-to-vigorous intensity physical activity or <75 min/week of vigorous-intensity physical activity (1). Despite absence of consensus on the cut point to classify ‘highly’ sedentary; epidemiological evidence suggests a higher risk for premature mortality from ≥9.5 h/day for objectively measured sedentary time (4). Red boxes represent time spent in sedentary behavior, light blue represents time spent standing and in light-intensity physical activity, and dark blue represents time spent in moderate-to-vigorous intensity physical activity. The vertical arrow represents time spent in sedentary behavior; the horizontal arrow represents time

spent in moderate-to-vigorous intensity physical activity. Abbreviations: LPA, light-intensity physical activity; MVPA, moderate-to-vigorous intensity physical activity.

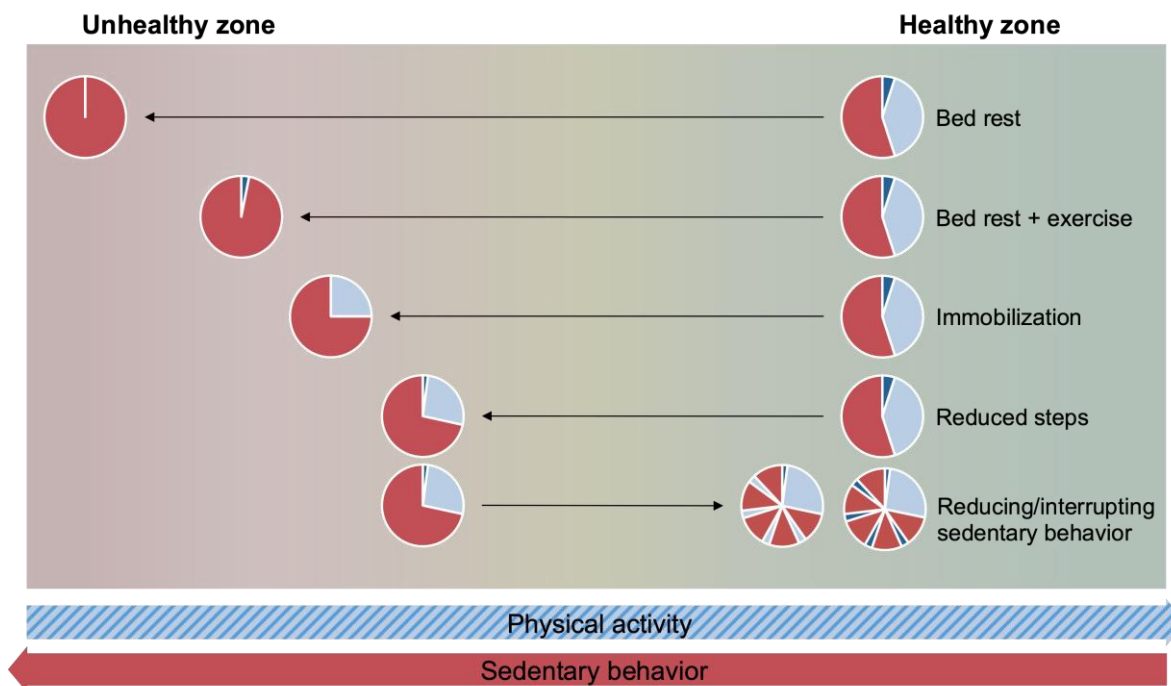


FIGURE 2. Hypothetical representation of imposed changes in physical activity level by experimental models that can induce changes in time spent in sedentary behavior. Fluctuations in physical activity levels, such as those imposed by these experimental models, may result in increased or decreased risk of adverse health outcomes. For the pie charts, red represents sedentary behavior, light blue represents standing and light-intensity physical activity, and dark blue represents moderate-to-vigorous intensity physical activity. Horizontal arrows schematically represent the direction of changes in physical activity and sedentary behavior. Abbreviation: MET, metabolic equivalent of task.

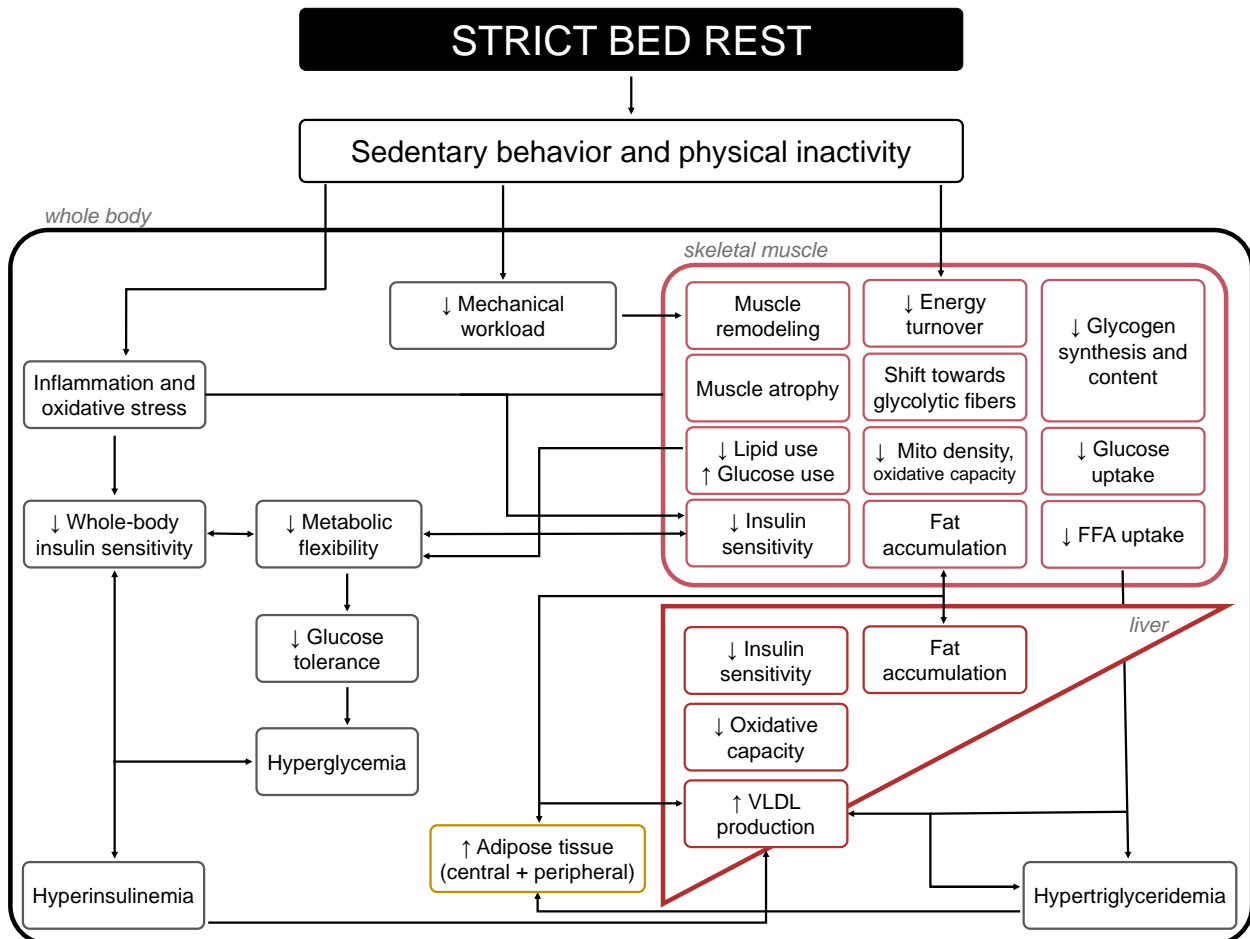


FIGURE 3. Metabolic cascade induced by exposures to sustained periods of strict bed rest. This cascade ultimately results in a plethora of adverse events, including development of whole-body insulin resistance, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, metabolic inflexibility (i.e., the inability of the body to adjust substrate use to changes in substrate availability), and alterations on body composition (increased fat mass and muscle atrophy) (166). Abbreviation: FFA, free-fatty acids; VLDL, very low-density lipoprotein.

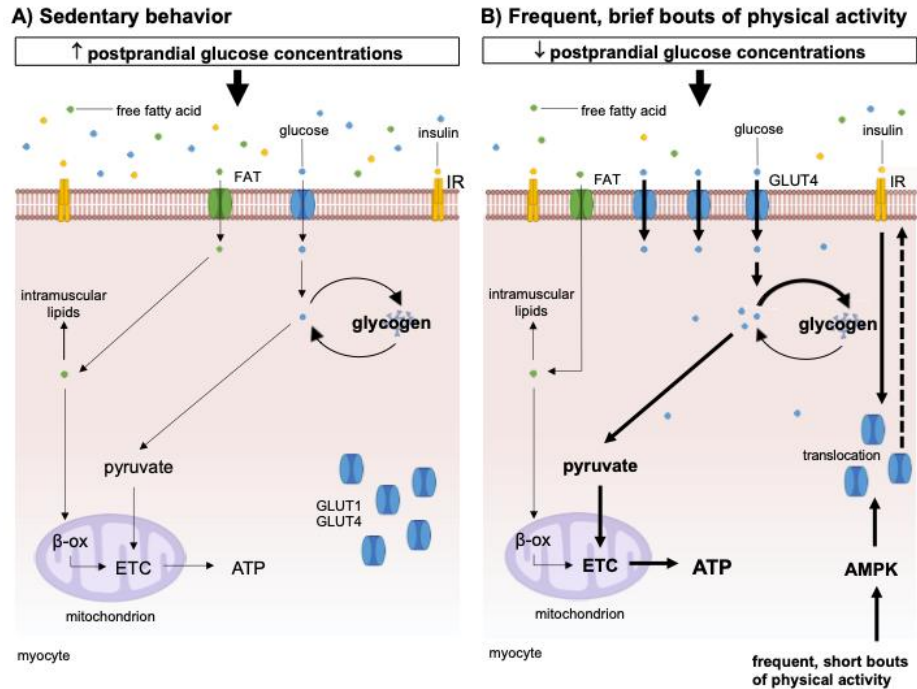


FIGURE 4. Mechanisms underlying sedentary behavior- and interruptions to sitting-induced adaptations on carbohydrate metabolism in skeletal muscle. A) During rest (i.e., prolonged sitting), requirements for glucose and free fatty acids are minimal; therefore, delivery to skeletal muscle is low. Any glucose that does enter the myocyte is stored as glycogen or metabolized to ATP via glycolysis. Any free fatty acid is stored as intramuscular lipid or metabolized to ATP via β -oxidation. Acute exposures to prolonged sitting have been shown to subsequently increase postprandial glycemia (411). B) During short, interruptions to sitting with physical activity, muscle uses glycogen and the glucose available in the blood stream as the main sources of glucose for the generation of ATP. Acutely, frequent muscle contractions increase AMPK levels in the myocytes, which results in the translocation of GLUT4 to the membrane facilitating glucose uptake. Interruptions to sitting also increase the capacity for ATP production and glycogen synthesis. With glucose constantly available and competing against lipids, free-fatty acid oxidation is not required to be increased for ATP production. Ultimately, interruptions

over three days induce a transition to modulation of the insulin-dependent signaling pathway (226). Performing frequent, short bouts of physical activity has been shown to reduce postprandial glycemia (411). Legend: Differences in signaling pathways in Panels B are relative to Panel A, with bolded arrows representing pathways that are upregulated following interruptions to sitting. Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; β -oxi, beta-oxidation; ETC, electron transport chain; FAT, fatty-acid transporter; GLUT1, glucose transporter type 1; GLUT4, glucose transporter type 4; IR, insulin receptor.

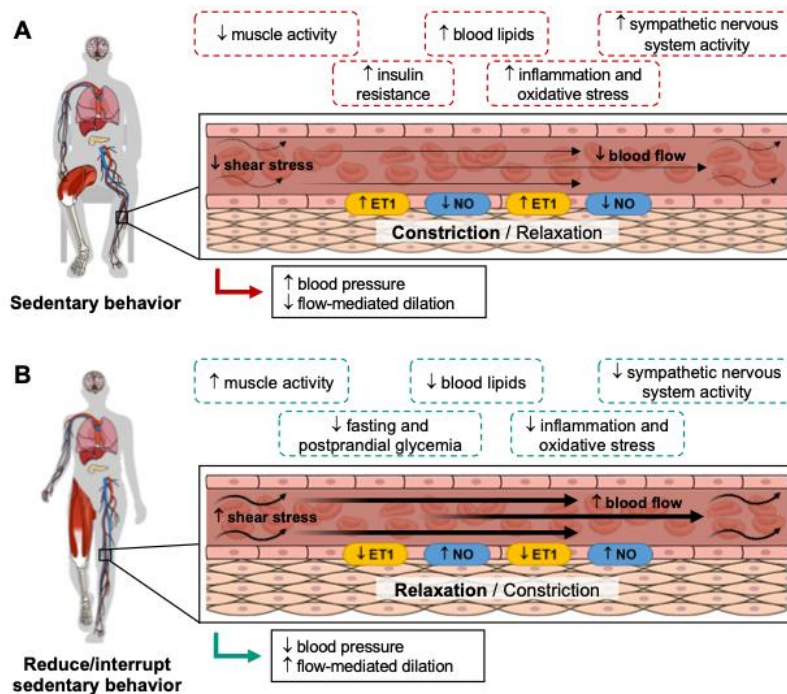


FIGURE 5. Vascular responses following exposures to prolonged sedentary behavior and to reducing/interrupting sedentary behavior. A) Prolonged exposures to sedentary behavior reduce blood flow and shear stress, increasing endothelin-1 and attenuating nitric oxide, subsequently leading to reduced flow-mediated dilation, particularly in the lower limbs, and increased blood pressure. In addition, muscle inactivity, insulin resistance, hyperlipemia, inflammation, oxidative stress, and increased sympathetic nervous system activity may further augment the consequences of sedentary behavior on vascular health. B) Reducing/interrupting sedentary behavior may be a potential strategy to improve cardiovascular health by attenuating sedentary behavior-induced maladaptations in cardiovascular and metabolic health (26, 329). Abbreviations: ET1, endothelin-1; NO, nitric oxide.

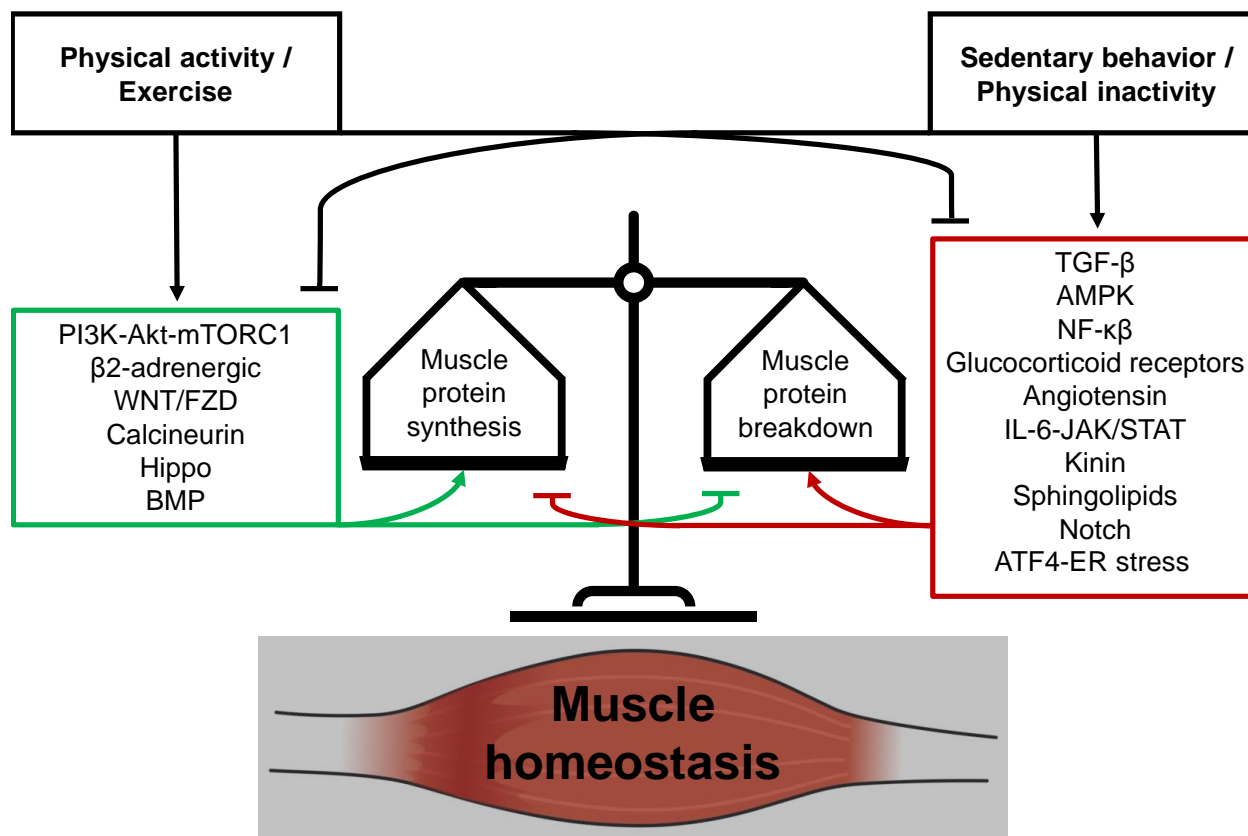


FIGURE 6. Key anabolic and catabolic pathways in muscle atrophy conditions. Several anabolic pathways signaling might be suppressed in muscle atrophy conditions, including PI3K-Akt-mTORC1, β 2-adrenergic, WNT/FZD, calcineurin, hippo, and/or BMP. In contrast, several catabolic pathways signaling might be overactivated, including TGF- β , AMPK, NF- κ β , glucocorticoid receptors, angiotensin, IL-6-JAK/STAT, kinin, sphingolipids, notch, and/or ATF4-ER stress. Physical (in)activity and sedentary behavior also influence muscle protein synthesis and breakdown(342-345). Abbreviations: Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; ATF4, activating transcription factor 4; BMP, bone morphogenetic protein; ER, endoplasmic reticulum; IL-6, interleukin 6; JAK, janus kinases; mTORC1, mechanistic target of rapamycin 1; NF- κ β nuclear factor kappa light chain enhancer of

activated β cells; PI3K, phosphoinositide 3-kinase; STAT, signal transducers and activators of transcription; TGF- β , transforming growth factor- β ; WNT/FZD, wingless-int1-frizzled.

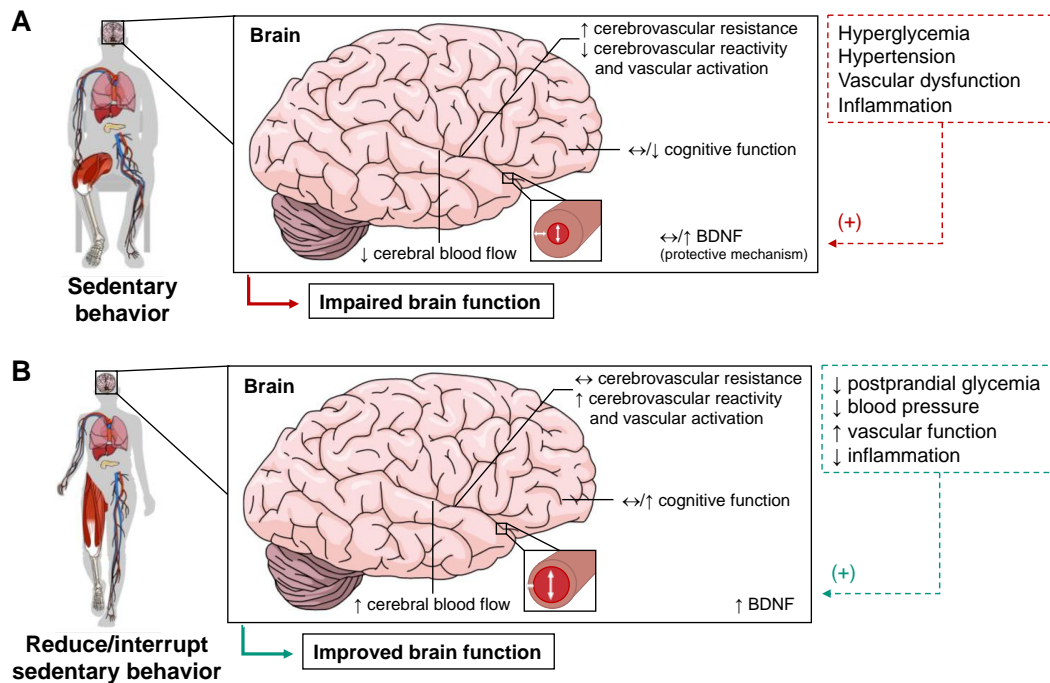


FIGURE 7. Cerebrovascular responses following exposures to prolonged sedentary behavior and to reducing/interrupting sedentary behavior. A) Exposures to prolonged sedentary behavior may result in decreased cerebral blood flow velocity, cerebrovascular reactivity, vascular activation, and increased cerebrovascular resistance. Additionally, poor glucose control, vascular dysfunction, low-grade inflammation, and altered levels of neurotrophic biomarkers and their precursors/metabolites may further impair brain and central nervous system function. B) Reducing/interrupting sedentary behaviors may be a potential strategy to attenuate sedentary behavior-induced maladaptations in the central nervous system function (28). Abbreviation: BDNF, brain-derived neurotrophic factor.

