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REVIEW ARTICLE

Pathophysiological mechanisms of reduced physical activity: Insights from the human step reduction model and animal analogues

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Abstract

Physical inactivity represents a heavy burden for modern societies and is spreading worldwide, it is a recognised pandemic and is the fourth cause of global mortality. Not surprisingly, there is an increasing interest in longitudinal studies on the impact of reduced physical activity on different physiological systems. This narrative review focuses on the pathophysiological mechanisms of step reduction (SR), an experimental paradigm that involves a sudden decrease in participants' habitual daily steps to a lower level, mimicking the effects of a sedentary lifestyle. Analogous animal models of reduced physical activity, namely, the "wheel-lock" and the "cage reduction" models, which can provide the foundation for human studies, are also discussed. The empirical evidence obtained thus far shows that even brief periods of reduced physical activity can lead to substantial alterations in skeletal muscle health and metabolic function. In particular, decrements in lean/muscle mass, muscle function, muscle protein synthesis, cardiorespiratory fitness, endothelial function and insulin sensitivity, together with an increased fat mass and inflammation, have been observed. Exercise interventions seem particularly effective for counteracting these pathophysiological alterations induced by periods of reduced physical activity. A direct comparison of SR with other human models of unloading, such as bed rest and lower limb suspension/immobilisation, is presented. In addition, we propose a conceptual framework aiming to unravel the mechanisms of muscle atrophy and insulin resistance in the specific context of reduced ambulatory activity. Finally, methodological considerations, knowledge gaps and future directions for both animal and human models are also discussed in the review.

KEYWORDS

cage reduction, disuse, inactivity, insulin sensitivity, muscle atrophy, wheel-lock

1 | INTRODUCTION

Physical inactivity is a major cause of chronic diseases[1] and has been recognised as the fourth cause of global death,[2] representing a heavy economic burden for modern society.[3,4] Physical inactivity is considered a pandemic,[5,6] requiring global action for public health.[7] About 30% of the population is estimated to be physically inactive, a trend growing considerably in high-income countries.[8] From an evolutionary perspective, physical inactivity represents a strategy for energy-saving and reducing the risk of predation, snake bites and musculoskeletal injury.[9] However, our hunter-gatherer ancestors, due to the dominant urge to collect food to eat, never experienced low levels of physical activity that could be harmful to their health. Thus, no corresponding mechanism for avoidance of physical inactivity evolved.[9]

Since the seminal work of Morris et al. in the 1950s,[10] showing a higher occurrence of coronary disease in bus drivers (a sedentary work) compared with bus conductors (a physically active work), most indirect evidence examining the detrimental effects of physical inactivity has been derived from epidemiological (mainly cross-sectional) studies. However, there is mounting interest in inactivity experimental (longitudinal) studies to investigate the mechanisms by which physical inactivity impacts different physiological systems. Most of the previous studies investigating these aspects have been conducted in laboratory settings employing extreme models of disuse/unloading (e.g. bed rest, dry-immersion, unilateral limb suspension, knee bracing).[11–17] Such unloading models have provided essential knowledge in the understanding of the remarkable skeletal muscle plasticity and how physical inactivity leads to muscle wasting and metabolic dysfunction in different populations.[11–17] Bed rest and dry immersion, by forcing volunteers to lay down for days or weeks, represent excellent models for comprehending the systemic effects of unloading,[17,18] while single limb disuse models (i.e. unilateral limb suspension, knee bracing) afford the opportunity for investigating unloading mostly at a local muscle level, although some effects at systemic level are still observed.[16]

More recently, an additional physical inactivity systemic model, termed step reduction (SR), has been proposed.[19,20] SR consists in inducing a sudden reduction in participants' habitual daily steps (generally assessed by a pedometer and/or an accelerometer) to a lower maximal steps limit, ranging from ~750 to ~4500 steps/day.[21] When compared with the traditional disuse models, SR is considered a less extreme form of physical inactivity, since participants are still exposed to loading stimuli.[21–23] However, SR represents an attractive model as it is closer to real-life conditions and more appropriate to mimic the deleterious effects of a sedentary lifestyle.[21,23–25] In fact, SR appears well suited for mimicking a decrease in everyday life activities which supports most daily energy consumption and has been shown to increase health risk.[26,27] In addition, it is important to consider that periods of reduced physical activity occur more frequently than events of prolonged bed rest or limb immobilisation,[21] as for instance occurred during the COVID-19 pandemic.[28] Low number of daily steps is also strongly associated with an increased risk of all-cause mortality.[29–31]

This narrative review aims to examine the impact of SR, focusing on skeletal muscle physiology and metabolic impairment. As analogous models of reduced physical activity used in rodents can provide the foundation for human investigations, they are also discussed first. In addition, methodological considerations, knowledge gaps and future directions for both animal and human models are described.

2 | REDUCED PHYSICAL ACTIVITY MODELS IN RODENTS

Physical inactivity is a serious threat to animals' health and in particular to the skeletal muscle. Several models have been proposed to study its effects in rodents, including hindlimb unloading (also known as "tail suspension") and cast immobilisation.[32,33] These models are considered severe forms of unloading, therefore comparable with human best rest, unilateral limb suspension and immobilisation. In contrast, there are relatively few studies on the effects of reduced daily ambulatory activity in animal models. These studies have typically used either the "wheel-lock" model or the cage reduction model.

2.1 | Wheel-lock model

2.1.1 | Impact of wheel-lock on insulin sensitivity

The wheel-lock model, sometimes also reported as cessation of voluntary wheel running, was the first attempt in the literature to mimic a sedentary lifestyle. Originally developed by Rhodes et al.,[34,35] the wheel-lock model was then employed extensively to study the effects of acute physical inactivity on metabolic dysfunction.[36,37] In this model, rodents are provided with running wheels and allowed to voluntarily run for several weeks (3–6 weeks), after which the wheels are locked, causing the cessation of animals' normal activity and thus promoting physical inactivity. Voluntary wheel running is an intermittent activity, performed under non-stressed conditions and does

not require the direct intervention of the researcher.[38] Therefore, wheel locking prevents animals' primary source of physical activity, determining the transition from the habitual level of locomotion to a lower daily amount, simulating SR studies in humans.

Wheel-lock studies produced several novel findings related to metabolic maladaptation to physical inactivity. In these studies, rats whose wheels were locked for only 5 h (WL5) constituted the control group, while rats whose wheels were locked for longer periods, including 29 h (WL29), 53 h (WL53), and in some studies also 173 h (WL173), represented the groups were inactivity was induced. Rats that never had access to voluntary wheel running constituted the sedentary group. In their first WL study,[36] insulin-stimulated 2-deoxyglucose uptake into the epitrochlearis muscle was lower in WL53 and the sedentary rats compared with WL5, indicating a rapid reduction in insulin sensitivity induced by the intervention. In addition, muscle insulin receptor ligation and signalling alterations, associated with reduced GLUT4 protein levels, were observed.[36] Noteworthy, another study showed higher plasma insulin and triglyceride concentrations in WL53 rats when compared with animals that had continuous running wheel access.[39] A recent investigation excluded the hypothesis that changes in muscle ceramides, a family of waxy lipid molecules considered involved in insulin sensitivity, are involved in this inactivity-induced insulin resistance.[40] Differently, a decreased gene expression of two key mechanical stretch sensors (Ankrd2 and Csrp3) that play a role in skeletal muscle metabolism and hypertrophy was reported.[41]

2.1.2 | Impact of wheel-lock on fat mass and inflammation

Adipose mass changes rapidly in response to wheel-lock.[37,42–45] Fifty-three hours of wheel-lock increased relative omental and epididymal fat masses as well as triacylglycerol synthesis rates,[37] independently from food intake/energy balance.[42] Rector et al.,[43] employing a rat model of obesity, after 16 weeks of voluntary wheel running with subsequent wheel-lock, observed increased omental and retroperitoneal fat pad masses, hepatic triglycerides and protein markers of fatty acid synthesis in the sedentary group with respect to WL5-, WL53- and WL173-hour animals. Another study carried out in obese rats observed higher lipid peroxidation levels in epitrochlearis muscles of the sedentary group than WL5, WL53 and WL173 rats.[46] An additional investigation from the same group reported that the transition from high to low physical activity levels caused a reduction in fatty acid oxidative capacity in skeletal muscle, liver, and adipocytes, accompanied by fat pad mass increase, attenuated growth of lean body mass and reduced PGC1-α mRNA in both skeletal muscle and liver.[44] Company et al. examined the role of age (49–56 vs. 70–77 days of age) on the growth of adipose tissue mass and adipocyte size following 7 days of wheel-lock.[45] Compared with rats that always had wheel access, 70- to 77-day-old animals had increased rates of gain in fat mass, greater adipocyte number, more small adipocytes and greater cyclin A1 mRNA in epididymal and perirenal adipose tissue.

Alterations in mRNA and protein expression in the iliac artery tissue of genes associated with inflammation (TNFR1 and ET-1) and oxidative stress (LOX-1) in the WL173 group were also observed.[47] Another study, besides reporting increases in fat mass and body fat percentage, observed 646 differentially expressed transcripts in perirenal adipose tissue comparing rats with continued wheel access and wheel-lock rats.[48] These findings suggest that reduced mobility promotes alterations of multiple pathways related to extracellular matrix remodelling, macrophage infiltration, immunity and pro-inflammatory function, some of which may exacerbate the development of obesity.

2.1.3 | Impact of wheel-lock on brain and neural function

The wheel-lock model and other very similar models have been employed by other research groups to study the effects of physical inactivity on other factors of animal health, including the brain and neural function. For instance, differential short-term changes in brain activity (evaluated through Fos-positive cells) in numerous brain regions were detected in mice blocked from reaching their wheel compared with those always having access to the wheel.[35] Mice housed in a running wheel cage for 8 weeks and subsequently moved to a standard cage were more anxious and exhibited impaired hippocampal neurogenesis.[49] Similarly, another report suggested that forced cessation of voluntary wheel running increases anxiety-like behaviours in rodents and exercise-induced stress resistance endured following wheel-lock.[50] Furthermore, a study reported a rapid decrease in BDNF mRNA in the hippocampus of hypertensive rats after the cessation of long-term voluntary wheel running,[51] while conversely, a second study reported an increase in BDNF protein in the hippocampus.[52]

2.2 | Cage reduction models

The cage reduction model represents an alternative approach for reducing physical activity in rodents. This

model is based on the modification of rodents' home cage size and features, thereby reducing their voluntary ambulatory activity. Indeed, despite locomotor behaviour being already limited in captivity, rodents do walk around and lid climb in their cages.[53] Although the use of small cages is not new in rats,[54–59] this approach has been re-adapted also in a few recent investigations in mice in the context of muscle adaptations and insulin resistance.[60,61] Employing this model, reducing the living space by varying degrees, muscle atrophy,[54,57,59,62] reduced muscle protein synthesis (MPS)[59] and reduction in local[61,62] and whole-body[54,61] insulin sensibility have been shown to occur.

In a study recently conducted,[60] the authors placed a plexiglass spacer in the middle of a standard type 2 macrolon cage, thus reducing the available cage volume.[60] To prevent lid climbing, a sheet of plastic having small holes was positioned under the standard wire lid and fixed using cable ties.[60] In addition, drinking bottles were placed so that the nozzle did not stick out and a piece of plastic was placed in all other wire lids to prevent possible licking or gnawing on the plastic. In this study, mice were allocated in six conditions: one for each of three different cage sizes with lids that either allowed or prevented lid climbing. Employing this model, the authors found that preventing climbing reduced motor coordination, muscle strength and muscle stamina after 5 and 10 weeks of intervention.[60] In addition, a further reduction in cage size affected motor coordination but not grip strength or muscle stamina. Moreover, preventing climbing increased visceral fat mass but did not induce muscle atrophy over 19 weeks.

2.3 | Methodological considerations and future directions for animal studies

In summary, both the wheel-lock and cage reduction seem appropriate models for studying the mechanisms by which reduced physical activity impacts the function of different physiological systems in animals (Figure 1). Blocking wheel access or changing cage type (i.e. from a cage equipped with a running wheel to a standard cage) should be preferred to conventional wheel-lock because mice do climb in their wheels also when they are locked.[34] Cage reduction volume should be selected carefully based on the study aim, as an excessive reduction could almost abolish animals movements, making this model more akin to an animal physiological analogue for bed rest.[58] A possible approach for future studies to further exacerbate inactivity-induced effects may be to apply both models in the same study design, reducing cage size after the cessation of daily voluntary wheel running.[63] This has only been implemented in a limited number of studies thus far.[55,61] Individual housing could represent a more severe physical inactivity model compared with partner or social housing.[64,65] For a better quantification of ambulatory activity reduction, future studies should evaluate in-cage habitual physical activity levels. Individual voluntary wheel running activity can be monitored via running wheels' number of revolutions or different commercially available systems.[53,66] Moreover, in-cage spontaneous physical activity can be recorded through video-tracking or other wireless procedures.[67,68] Finally, using these models, very little attention has been paid to the impact of reduced physical activity on neuromuscular and cardiovascular systems; these aspects should be further investigated. Methodological considerations for animal models are summarised in Figure 2.

3 | STEP REDUCTION MODEL IN HUMANS

In 2008, the SR model was originally designed to study changes in insulin sensitivity and adiposity in response to reduced physical activity in humans.[19,20] Ever since this model has been employed to investigate the impact of reduced physical activity also on other physiological systems (Table 1 and Figure 1).

3.1 | Impact of SR on metabolic function, body composition and inflammation

The seminal study by Olsen et al. was the first one to show the pathophysiological consequences of SR.[19] Young healthy participants reduced their habitual daily steps from ~10 000 steps/day to ~1300 steps/day, simply through a change in lifestyle behaviour (e.g. taking the elevator instead of stairs and using cars instead of walking or cycling). After 3 weeks, participants underwent metabolic changes indicative of decreased insulin sensitivity (assessed with the oral glucose tolerance test) and attenuation of postprandial lipid metabolism (evaluated with oral fat tolerance test), accompanied by increased intra-abdominal fat and decreased total fat-free mass.[19] Alterations in insulin sensitivity and body composition following periods of SR (ranging from 3 days to 2 weeks) were confirmed also by later studies in young subjects,[20,69–76] middle-aged men[77] and older adults,[78–80] independently from whether the SR interventions were carried out in combination with overfeeding or not.

Investigating possible mechanisms behind these alterations, a recent study found a decline in maximal citrate synthase activity (a marker of mitochondrial content) and an increase in the protein content of p-glycogen synthase (P-GS^{S641}; a marker of reduced glycogen synthase activation) following 7 days of SR (<1500 steps/day) in the skeletal muscle.[81] Differently, these authors

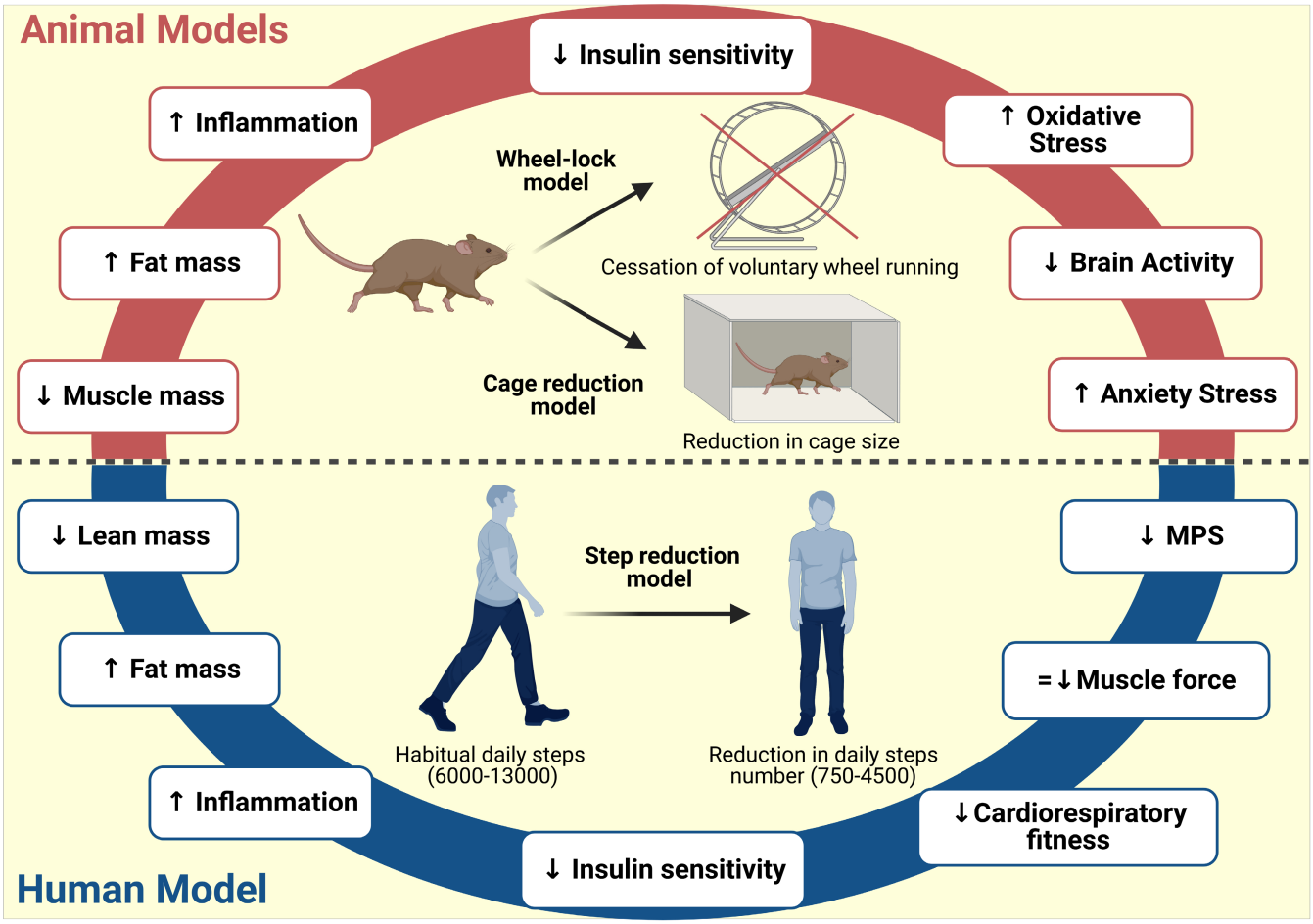

FIGURE 1 Deleterious effects of step reduction and analogous animal models (wheel-lock and cage reduction). Created with BioRender.com. MPS, muscle protein synthesis.

reported no changes in total protein or phosphorylation content of markers of insulin-mediated signalling, mitochondrial function (e.g. oxidative phosphorylation complex I-V), oxidative metabolism (e.g. PGC1-α, AMPKa), or mitochondrial dynamics (e.g. FIS1, DRP1, and MFN2), possibly due to the short duration of the intervention. In addition, another study reported only modest changes in ceramides content following reduced activity and recovery.[80] In studies conducted in older adults, a rise in inflammatory cytokines (levels of TNF-α, IL-6 and CRP),[78,79] associated with changes in muscle inflammation cell signalling (JNK (Thr183/Tyr185), IκBα, AKT (Ser473), TLR4 and SPT2)[80] and increase in muscle macrophages,[82] were detected in response to SR, contrary to what was observed in young[20,74,75] and middle-aged[77] adults. It has been hypothesised[21] that this difference in the inflammatory response could lead to an impaired regenerative capacity that may explain why younger individuals recover from SR,[71,74] while older individuals not always.[79]

Investigating potential countermeasures, one study examined the effects of 45 min of daily treadmill aerobic training on young healthy subjects undergoing a week of SR combined with overfeeding.[76] The exercise intervention was effective in counteracting most of the alterations in metabolic function and adipose tissue metabolism that were observed in the control group.[76] Despite this interesting finding, it should be considered that background inactivity (i.e. a low number of daily steps) can blunt metabolic benefits in response to both acute[83–85] and chronic[86] exercise (for a recent review on this topic we refer the reader to Coyle et al.[87]). An additional countermeasure that could be potentially interesting for limiting the metabolic impact of SR without actually increasing the number of the daily steps would be to increase the non-exercise activity thermogenesis (NEAT),[27] employing standing workstations and gymnastic balls.[88]

3.2 | Impact of SR on muscle protein turnover

Skeletal muscle is highly malleable tissue that is very sensitive to changes in mechanical loading. Loss of lean/

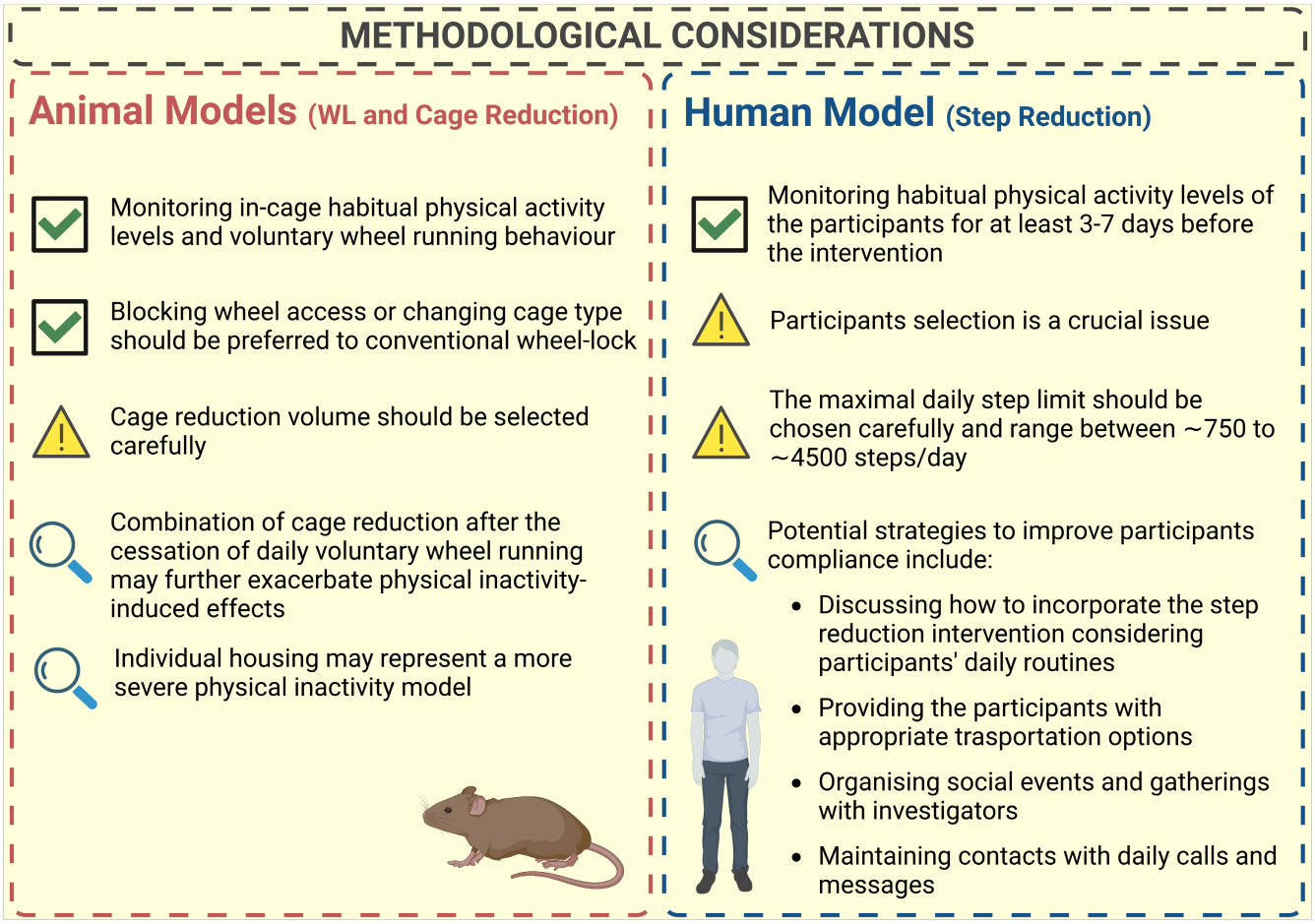

FIGURE 2 Summary of the methodological considerations for animals and human models of reduced physical activity. Created with BioRender.com. WL, wheel-lock.

muscle mass is evident and consistent both in young[20,71] and older adults[78–80,89,90] already with the mild unloading stimulus induced from SR. Since declines in MPS are considered one of the predominant mechanisms underpinning the loss of muscle mass in human models of disuse/unloading,[91–93] leading experts in protein metabolism have extensively investigated the changes in integrated rates of MPS in response to SR in older populations.[78,79,89,90] After 2 weeks of SR (750–1500 steps/day), several studies found reductions in MPS,[78,79,90] supporting the concept of muscle disuse-induced "anabolic resistance".[92,94] Notably, one of these investigations observed a failed recovery in MPS rates after 2 weeks of resumption to habitual activity in overweight pre-diabetic older adults.[79] In the same cohort, the non-targeted metabolite profile assessed from multisegment injection-capillary electrophoresis-mass spectrometry on fasting plasma samples highlighted changes in circulatory metabolites associated with a decline in muscle energy metabolism and protein degradation.[95] This altered metabolite profile was not fully restored after resuming normal ambulatory activity.[95] Another recent SR study with ~80% reduction in daily step number for 2 weeks, leveraging an innovative combined RNA sequencing and ribosomal profiling approach, showed decreased baseline and leucine-stimulated translation of mRNAs encoding for ribosomal proteins and alterations of circadian regulators which may precede adaptations to muscle size and metabolic function.[96] The rapid dysregulation of MPS in response to reduced ambulatory activity has also been confirmed by a recent study on young healthy adults following one-week SR (<1500 steps/day).[72] In this investigation, changes in MPS were accompanied by altered insulin sensitivity and expression of mRNA genes involved in muscle mass regulation and oxidative metabolism. Indeed, myostatin and MAFbx were upregulated after the intervention, whereas mTOR, p53 and PDK4 were downregulated.[72]

A series of studies investigated the effects of exercise and nutritional countermeasures on these aspects during periods of SR. In two different studies from the same SR campaign conducted on older adults,[89,97] unilateral low-load resistance exercise training (three sessions/week) has been employed to counteract SR-induced muscle alterations. This intervention increased leg lean mass, and muscle

function and maintained feeding-induced MPS rates in the exercised vs. unexercised leg.[89] In addition, the training protocol preserved type I and II fibre cross-sectional area, Pax7+ positive cells content and capillarisation.[97] The study by Devries et al.[89] was the first one to examine whether different nutritional strategies (20 g whey protein isolate plus 15 g glycine or micellar-whey with 5 g citrulline or 15 g glycin) could attenuate the SR-induced anabolic resistance. However, the authors concluded that none of the proposed supplements attenuated the reduction in MPS following SR.[89] More recently, older participants were kept in energy balance for one week, then underwent one week of energy restriction, followed by a 2-week combination of energy restriction and SR (<750 steps/day), before a recovery period.[90] A supplementation of whey protein or collagen peptides was provided during the intervention. Despite these nutritional strategies did not protect participants against muscle mass loss, whey protein supplementation increased leg lean mass and MPS rates during the recovery phase.[90]

3.3 | Impact of SR on muscle function and physical performance

While changes in metabolic function, body composition, muscle mass and MPS rates have been consistently observed in response to SR, evidence regarding muscle and physical function alterations is more controversial. Indeed, in older adults, decreases in knee extensors maximum isometric strength was found in some,[80,98] but not all[78,79,89] SR studies, despite all having the same duration (2 weeks). Inconsistencies among studies might be due to differences in familiarisation procedures,[21] knee angle set during maximum voluntary contraction and rest between trials. Following 2 weeks of combined energy restriction and SR, another investigation[98] observed an unexpected increase in maximum isometric tension in type IIA muscle fibres accompanied by augmented maximum power production in type I and IIA vastus lateralis fibres, despite a reduction in knee extensor maximum isometric strength at whole muscle level. To date, no studies have examined changes in muscle strength following SR periods in younger populations. In addition, older adults generally presented unchanged outcomes in physical performance tests (e.g. short physical performance battery, time up and go, 6-meter walking test and thirty seconds chair-to-stand) after 2 weeks of reduced ambulatory activity.[78,80,98] Differently, as presented above, young adults showed considerable decreases in cardiorespiratory fitness.[20,71,73–75] This finding may be at least partially due to endothelial dysfunction, reported in different SR studies,[73,99,100] similarly to what was observed with short-term bed rest,[17] and changes in mitochondrial content.[81]

3.4 | Unexplored topic I: Does SR impact neuromuscular function?

Integrity of the neuromuscular system represents a pillar for muscle force production and motor control. However, to date, no studies have investigated these aspects in SR experiments. Recent evidence from severe models of unloading (bed rest, unilateral limb suspension) suggests that disuse is associated with initial signs of myofibre denervation,[12,15,16] impairment of excitation-contraction coupling,[15] neuromuscular junction instability[15,16] and downregulation of skeletal muscle ion channels genes.[16] From an electrophysiological perspective, changes in neural drive,[101] corticospinal excitability[102] and motor unit potential properties[16,103] are considerably affected by disuse. Overall these findings highlight remarkable plasticity of the neuromuscular system in conditions of disuse. Future SR studies are warranted in order to determine whether these adaptations of the neuromuscular integrity and function could also be detected with a less severe form of inactivity, such as SR.

3.5 | Unexplored topic II: Does SR impact brain activity, neurogenesis and cognitive function?

A sedentary lifestyle has been associated with decreased brain activity, cognitive function and brain structural remodelling, being also considered a risk factor for several neurological disorders, including dementia.[102,104–106] This may be due, in part, to a blunted release of myokines involved in muscle–brain crosstalk (e.g. BDNF and IGF-1)[107] and impaired cerebrovascular perfusion[105] with physical inactivity. The brain's adaptations in function and structure, as well as the physiological factors that contribute to these changes, are largely unexplored in SR studies. Thus, specific attention should be placed on these aspects, especially after long-term SR. This seems particularly relevant in light of the reductions in brain function and neurogenesis observed in mice undergoing wheel-lock[35,49,51] (Section 2.1.3).

3.6 | Comparison of SR with other human physical inactivity models

In order to fully appreciate the potential applications, pros and cons of the SR model, we propose here a direct comparison with other traditional complete disuse models with regards to both pathophysiological impact and technical/practical aspects.

TABLE 1 Summary of human step reduction studies.

Reference	Participants	Reduction in daily steps	Intervention duration	Physical and muscle function
Bowen-Davies et al.[71]	Young adults $n=45$ (18 M and 27 F)	12 780 to 2495 steps/day	2 weeks +2 weeks resuming activity	↓ treadmill VO_2 Max
Bowen-Davies et al.[73]	Young adults $n=28$ (18F and 10 M)	12 624 to <1500 steps/day	2 weeks +2 weeks resuming activity	↓ treadmill VO_2 Max
Boyle et al.[99]	Recreationally active young adults $n=11$ M	12 550 to <5000 steps/day	5 days	Not measured
Breen et al.[78]	Older adults $n=10$ (5 M and 5 F)	5962–1413 steps/day	2 weeks	= isometric MVC = Physical function (SPPB)
Burton and Coyle[85]	Recreationally active young adults $n=10$ (7 M and 3 F)	10 648 to a different number of daily steps based on the experimental conditions NORMAL: 2675 LIMITED: 4759 LOW: 8481	2 days for each condition 1-h bout of running on the evening of the second day	Not measured
Devries et al.[89]	Older adults $n=30$ M SR+ unilateral retraining 3 groups based on supplements	~6200–7700 to-1160–1280 steps/day	2 weeks	= isometric MVC = 1RM (↑ in the leg with RT)
Dixon et al.[77]	Middle-aged adults Group 1 (lean) = 9 M Group 2 (overweight) = 9 M	~13 000 to-4000 steps/day	1 week	Only baseline measures reported
Edwards et al.[81]	Recreationally active young adults $N=11$ M	13 054 to 1192 steps/day	1 week	Not measured
Knudsen et al.[74]	Young adults $n=9$ M	10 028 to 1521 steps/day + overfeeding	2 weeks +2 weeks resuming activity (alterations already after 3 days)	↓ Cycle VO_2 Max
Krogh-Madsen et al.[20]	Young adults $n=10$ M	10 501 to 1344 steps/day	2 weeks	↓ Cycle VO_2 Max
Krogh-Madsen et al.[75]	Young adults Group 1: step reduction + overfeeding $n=10$ M Group 2: control + overfeeding $n=10$ M	10 948 to 1796 steps/day	2 weeks	↓ Cycle VO_2 Max
Mahmassani et al.[96]	Older adults $N=8$ (2 M and 6 F)	10 909 to 2258	2 weeks	= leg lean mass ↓(trend) type I fibre CSA
McGlory et al.[79]	Overweight, pre-diabetic older adults $n=22$ (12 M and 10 F)	7362 to 991 steps/day	2 weeks +2 weeks resuming activity	=isometric MVC
Mikus et al.[69]	Young adults $n=12$ (8 M and 4 F)	12 956 to 4319 steps/day	3 days	Not measured
Moore et al.[97]	Older adults $n=14$ M	7011 to <1500 steps/day SR+ unilateral retraining	2 weeks	Not measured

Muscle morphology and body composition	Effects of SR on: Muscle protein turnover/signalling pathway	Metabolism/Vascular function
DEXA: ↑fat mass, central and liver ↓leg lean mass = arms and trunk lean mass	Not measured	OGTT: ↑ insulin ↑ glucose ↓ Matsuda index = hepatic insulin resistance
DEXA: ↑fat mass, central and liver ↓leg lean mass = arms and trunk lean mass	Not measured	OGTT: ↓ Matsuda index Endothelial function: ↓ brachial artery FMD
Not measured	Not measured	Endothelial function: = brachial artery FMD ↓ popliteal artery FMD ↑ $CD31^{+}/CD42b^{-}$ endothelial microparticles
DEXA: = total body and fat mass ↑ trunk fat mass ↓ leg lean mass	VL biopsy: ↓ MPS = basal MPS ↑inflammatory markers (TNF-α and C-reactive protein) = intramuscular signaling proteins (mTor; 4E-BP1; Akt; p70S6K)	OGTT: ↑ insulin and glucose ↓ Matsuda index
Not measured	Not measured	HFTT: ↑postprandial plasma triglyceride (in LIMITED and LOW vs. NORMAL) ↓whole body fat oxidation (in LIMITED and LOW vs. NORMAL)
DEXA: = lean mass, fat mass, appendicular lean mass ↓leg lean mass (↑ in the leg with RT)	MPS assessed but not compared with baseline measures ↓MPS in the leg with no retraining	Assessed but not compared with baseline measures
Only baseline measures reported	=TNFα, IL6, CPR, ALT	OGTT: ↑ insulin and glucose = fasting triglyceride
Not measured	Not measured	↓ maximal citrate synthase activity ↑ protein content of p-glycogen synthase
DEXA: ↑ total body fat ↑ total fat mass = total lean mass MRI: ↑visceral fat	Not measured	OGTT: ↑ insulin = glucose ↓ Matsuda index (at 3 and 7 days) ↑ leptin and adiponectin H-E clamp: ↓ Peripheral insulin sensitivity = hepatic glucose prod
DEXA: ↓ leg lean mass = arm and trunk lean mass = fat mass	= inflammation (TNF-α, IL-6, IL-15, leptin, and adiponectin) VL biopsy: ↓ insulin-stimulated Akt phosphorylation = IR beta and AS160	H-E clamp: ↓ Peripheral insulin sensitivity = hepatic glucose prod. = fasting bloods
DEXA: ↑ fat mass = lean mass MRI: ↑ visceral adipose tissue ↑ abdominal subcutaneous adipose tissue	Not measured	OGTT: ↑insulin ↑glucose CGM: ↑ mean 24-h glucose ↑ maximum glucose ↑ FFA = TNF-α and IL6
Not measured	= Plasma leucine at baseline and in response to leucine ingestion Alterations in leucine-stimulated mRNA translation (protein synthesis)	OGTT: = Glucose tolerance = Insulin Sensitivity Index
DEXA: = total body fat and lean mass VL Biopsy: = fibres CSA and distribution	VL Biopsy: ↓ MPS Altered expression of mitochondrial-related genes	OGTT: ↑insulin ↑glucose ↓Matsuda index ↑TNF-α, CRP and IL6
Not measured	Not measured	OGTT: ↑insulin = glucose ↓ Matsuda index CMG: ↑ post prandial glucose
Muscle fibre CSA was assessed but not compared with baseline measures ↑CSA in the leg with RT	↑satellite content and capillarisation in the leg with RT = compared with baseline measures	Not measured

TABLE 1 (Continued)

Reference	Participants	Reduction in daily steps	Intervention duration	Physical and muscle function
Oikawa et al.[90]	Older adults $n=32$ (16 M and 16 F) SR + energy restriction + supplements two groups based on supplements	Group 1: 6237 to <750 steps/day Group 2: 8392 to <750 steps/day	2 weeks +2 weeks resuming activity	Not measured
Oikawa et al.[98]	Older adults $n=30$ (15 M and 15 F) SR + energy restriction	7315 to 920 steps/day	2 weeks +2 weeks resuming activity	↓ isometric MVC = time to peak torque ↓ gait power = 30s CST, TUG, and 6MWT VL Biopsy (n = 9): ↑ maximum isometric tension ↑ maximum power production
Olsen et al.[19]	Young adults Study 1: $n=8$ M Study 2: $n=10$ M	Study 1: 6203–1394 steps/day Study 2: 10 501 to 1344 steps/day	Study 1: 2 weeks Study 2: 3 weeks	Not measured
Reynolds et al.[70]	Young adults $n=14$ M	~12 000 to 4000 steps/day	5 days +1 day resuming activity	Not measured
Reidy et al.[80]	Older adults $n=12$ (7 M and 5 F)	9004 to 2994 steps/day	2 weeks +2 weeks resuming activity	↓isometric knee extension MVC = knee extension power = 6MWT
Reidy et al.[82]	Older adults $n=12$ (7 M and 5 F)	9004 to 2994 steps/day	2 weeks +2 weeks resuming activity	Not measured
Saoi et al.[95]	Overweight, pre-diabetic older adults $n=17$ (10 M and 7 F)	7550 to 980 steps/day	2 weeks +2 weeks resuming activity	Not measured
Shad et al.[72]	Young adults $n=11$ M	13 054 ± 1192 steps/day	1 week	Not measured
Teixeira et al.[100]	Recreationally active young adults $n=13$ M Condition: one foot exposed to hot water immersions	13 103 steps/day	5 days	Not measured
Walhin et al.[76]	Young adults Group 1: SR + overfeeding; $n=14$ M Group 2: SR + overfeeding + exercise; $n=12$ M	Group 1: 125 632 to 3520 steps/day Group 2: 10 544 to 3690 steps/day	1 week	Only baseline measures reported

Abbreviations: 1RM, one-repetition maximum; 6MWT, six minutes walking test; CGM, continuous glucose monitoring; CSA, cross-sectional area; CST, chair to stand; CT, computed tomography; DEXA, Dual-energy X-ray absorptiometry; F, females; FFA, free fatty acids; FMD, Flow Mediated Dilation; H-E clamp, hyperinsulinemic clamp; HFTT, high-fat tolerance test; IL-6, Interleukin 6; LDL, Low-density lipoprotein; M, males; MPS, muscle protein synthesis; MRI, magnetic resonance imaging; MVC, maximum voluntary contraction; OFTT, oral fat tolerance test; OGTT, oral glucose tolerance test; RT, resistance training; SPPB, short physical performance battery; TNF-α, tumor necrosis factor alfa; TUG, timed up and go; VL, vastus lateralis; VO_2 Max, maximal oxygen consumption.

3.6.1 | Pathophysiological effects

As presented above, SR is accompanied by decreases in lean and muscle mass. When compared with changes in leg lean mass reported after two weeks of bed rest in a recent meta-analysis (−8.5%),[108] the reduction observed in SR studies is about one-fourth: on average −2.1%, ranging from −1.2%[71] to −3.7%.[78] In the only SR study that evaluated muscle mass instead of lean mass, plantar flexors cross-sectional area was reduced by 2.4% in two weeks.[80] This is considerably lower than the 5.6%–8.4% plantar flexors cross-sectional area loss that could be expected with unilateral lower limb suspension or immobilisation after two weeks based on estimated median daily change (unilateral lower limb suspension: $-0.4\%\cdot day^{-1}$; unilateral lower limb immobilisation: $-0.6\%\cdot day^{-1}$)[101] and the 12% observed with longer 20-day bed rest studies.[109] Accordingly, MPS declines with SR are relevant

Muscle morphology and body composition	Effects of SR on: Muscle protein turnover/signalling pathway	Metabolism/Vascular function
DEXA: ↓lean body mass ↓ leg lean mass VL Biopsy: = fibres CSA and distribution	VL Biopsy: ↓ MPS ↑TNF-α, CRP and IL6	↑ fasted blood glucose
Not measured	Not measured	Not measured
DEXA: ↓ lean mass = fat mass MRI: ↑Intraabdominal fat	Not measured	OGTT and OFTT: ↑ insuline ↑ C-peptide ↑ triglyceride
Not measured	Not measured	OGTT: ↑ insulin = glucose ↓ Matsuda index CGM: ↑ peak postprandial glucose ↑ blood glucose variability
DEXA: ↓leg lean mass = trunk lean mass and fat mass CT: ↓ Mid-plantar flexor CSA VL biopsy: = fibres CSA and distribution	↑muscle inflammation cell signalling (JNK, IκBα, TLR4, Ser^{473}, SPT2) ↑Serum and intramuscular ceramides	H-E clamp: = resting and clamp insulin ↓insulin sensitivity
Not measured	↑ muscle macrophages ↑ satellite cells (slow fibre) = capillariation	↓H-E clamp (insulin sensitivity)
Not measured	Changes in circulatory metabolites involved in muscle energy metabolism, protein degradation and inflammation	OGTT: ↓ post OGTT glucose ($p = 0.07$)
Not measured	VL biopsy: ↓MPS Alterations in mRNA genes: ↑myostatin ↓mTor = p70S6K = MuRF1 ↑MAFbx = p53, PDK4 and PCG-1α	OGTT: ↑ insulin = glucose ↓ Matsuda index
Not measured	Not measured	Endothelial function: ↓ popliteal artery FMD (nonheated leg only)
DEXA: ↑total body mass ↑lean mass = fat mass	Adipose tissue biopsy: Altered expression of genes and proteins in adipose tissue remodelling	OGTT: Group1: ↑ insulin ↑ C-peptide ↓Matsuda Group 2: ↑ insulin Blood sample: ↑ adiponectin, total cholesterol, LDL cholesterol and whole blood white blood cell

(young adults: −27%[72]; older adults: −12%−26%[78,79,90]) but appear of lower magnitude compared with the ones induced by traditional disuse models (~40–60%).[22,110] Changes in knee extensors isometric muscle force with 2-week SR are trivial, ranging from +2.8% (non-significant increase)[79] to −7.1%.[80] These conflicting results contrast with the well-established marked loss of muscle strength with disuse, which is estimated, following a 2-week intervention, to be 13% for bed rest,[108] 14% for unilateral lower limb suspension,[101] and 23% for unilateral lower limb immobilisation.[111] Cardiorespiratory fitness declines with 2-week SR are 3.4%–6.4% in young adults[20,74,75] and 6.5% in older adults[71] and are contained compared with what was observed in a bed rest study of the same duration (young adults: −7.6%; older adults: −15.3%).[112] Differently, declines in insulin sensitivity assessed with the Matsuda index seem similar between SR (−17.6% to 22%)[73,78] and bed rest (−19.8%)[112] in healthy older adults over 2 weeks of intervention. Similarly, a ~30% reduction in the same parameter was observed in shorter SR (3–7 days) in young adults,[69,70,72] which is comparable with the impact of bed rest of similar durations (−24% to 31%).[113,114]

The comparison between SR and other disuse models confirmed that SR is a mild physical inactivity model. However, SR-induced alterations are consistent and

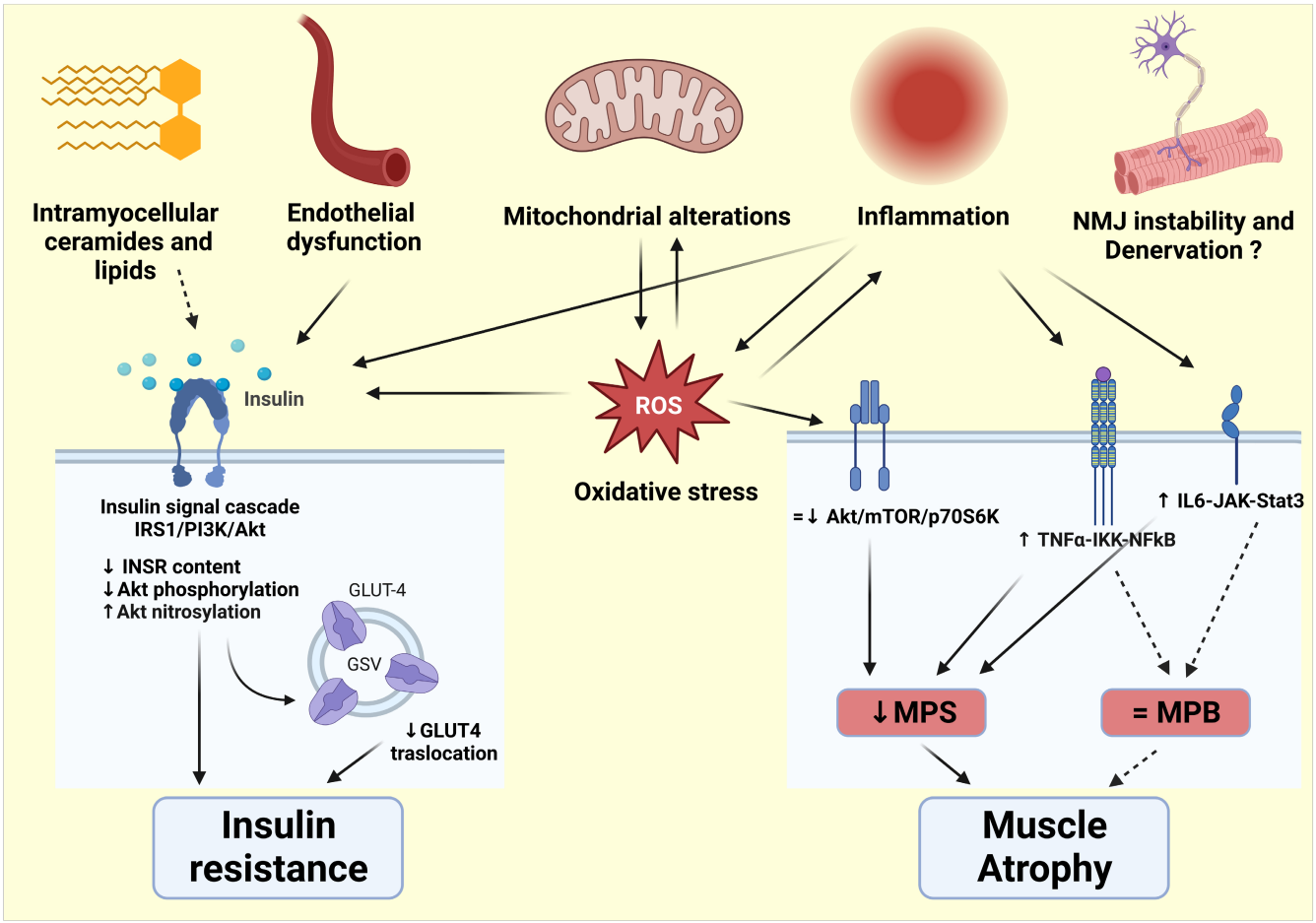

FIGURE 3 Conceptual framework of the mechanisms leading to muscle atrophy and insulin resistance with reduced ambulatory activity. Dotted lines express mechanisms that theoretically have an impact, but seem to not occur in the specific context of step reduction. Denervation could play a role both in the development of muscle atrophy and insulin resistance, but it is still unknown whether initial signs of denervation are induced by step reduction. Created with BioRender.com. GSV, GLUT4 storage vesicles; INSR, insulin receptor; MPB: muscle protein breakdown; MPS: muscle protein synthesis; ROS: reactive oxygen species.

should not be neglected, particularly concerning insulin sensitivity in which surprisingly SR might have an impact similar to bed rest.

3.6.2 | Technical and practical aspects

The first relevant difference between SR and more extreme models of physical inactivity resides in the context of application. These latter are indeed often employed in the context of microgravity, as analogues of spaceflight.[109,115] Despite differences between the effects of spaceflight and ground-based models exist, these are useful to unravel the mechanisms of muscle loss during mechanical unloading.[116] While the SR model represents probably a too mild inactivity stimulus for being applied in this scenario, it is instead very appealing for researchers interested in studying the effects of sedentarism.

One clear advantage of SR is a relatively inexpensive model compared for instance to bed rest. Moreover, SR has a limited impact on volunteers' private/social life with no particular health risks. Differently, during bed rest, symptoms such as musculoskeletal complaints (low back pain in particular), signs of anxiety and depression, vertigo, nausea, reduced appetite and gastroesophageal reflux can be occasionally experienced. It should also be considered that participants in BR studies are potentially exposed to an increased risk of renal calculi, urinary tract infections and deep vein thrombosis, this latter also reported in unilateral lower limb disuse models.[115] One of the most evident issues of the SR interventions is that compliance cannot be completely monitored, a limitation that is shared also with the unilateral lower limb suspension and immobilisation models, differently from full-time supervised bed rest campaigns. Procedures to ensure and assess compliance are hence needed (Section 3.9).

3.7 | Conceptual framework of the mechanisms involved in SR-induced muscle atrophy and insulin resistance

Despite some mechanisms remain poorly understood, we developed a conceptual framework in order to unravel the drivers leading to inactivity-induced muscle atrophy and insulin resistance, based on the evidence specifically obtained from SR studies and animals analogues (Figure 3). It is well known that the maintenance of muscle mass is regulated by the balance between rates of MPS and muscle protein breakdown.[110,117] Differently from what originally believed,[23,116] recent evidence suggests that muscle protein breakdown has no or little influence in the context of "uncomplicated" disuse (i.e. inactivity in absence of diseases or other catabolic processes).[110,117] Thus, it seems very unlikely to play a role in models with lower atrophic stimulus, as in the context of SR. Declines in MPS are therefore considered the primary driver of skeletal muscle disuse atrophy[110,117] and are indeed observed in several SR studies[78,79,89,90] (Section 3.2). However, molecular mechanisms regulating this process are still partially unknown in this scenario. Some alterations of the Akt/mTOR/p70S6K signalling cascade were found with SR in young adults,[72] but another study reported opposite results in older adults,[78] as also observed in more extreme models of disuse.[117] Molecular pathways such as TNFα-IKK-NFkB and IL6-JAK-Stat3 may be involved in SR-induced muscle atrophy as inflammation is commonly observed in SR studies,[78–80,82] at least in older adults (Section 3.1). Changes in mitochondrial content[81] and gene expression (e.g. COX7A2, ATP5E and MRPS36)[79] occur quickly with reduced ambulatory activity and could contribute to the atrophic program via increased oxidative stress, inducing calpain and caspase-3 activation and increasing expression of the ubiquitin-proteasome system.[118] MPS decreases could be also partially attributed to altered ribosome biogenesis and increased ribosome degradation that have been observed to regulate translational capacity in skeletal muscle during periods of disuse.[119] This hypothesis seems supported by recent evidence showing SR-induced deficits in ribosome production.[96] While it is still unknown whether initial signs of neuromuscular junction instability and denervation occur with SR (Section 3.4), they could ultimately have an influence on reducing MPS,[117] affecting the expression of a group of atrophy-related genes, such as Runx1, Trim63, Fbxo32 and Elk4[120] and causing expansion of the fibro-adipogenic precursor cells which induce an inflammatory response via the IL6-JAK-Stat3 pathway.[121]

Inflammation, mitochondrial alterations, oxidative stress and denervation could also trigger impairments in insulin sensitivity with SR.[122] In addition, endothelial dysfunction, reported in different cage reduction[56] and SR[73,99,100] studies, may also contribute in part to peripheral insulin resistance due to reduced blood flow.[73] Differently, ceramides accumulation seems to not have a great influence on early changes in insulin sensitivity caused by reduced physical activity.[40,80] Intramyocellular lipids, another well-established player in insulin resistance,[122] seem also unaffected by wheel-lock[46] and SR[73] interventions. It is well established that the two main molecular mechanisms that regulate glucose transport in skeletal muscle are the insulin signal transduction cascade and GLUT4 translocation.[122] Pooling together evidence from animal and human studies, it seems overall supported that skeletal muscle GLUT4 content and/or translocation may be affected by reduced ambulatory activity,[36,40,61,76] although not all studies are in agreement.[81,123] Findings regarding insulin signalling are more complex to interpret. Two weeks of SR decreased insulin-simulated skeletal muscle Akt phosphorylation,[20] while 7 days of SR were sufficient to increase the protein content of P-GS^{S641}, a marker of reduced glycogen synthase.[81] Wheel-lock studies seem also to support this finding showing reductions in the protein level of insulin receptor β-subunit[36] and increase in 4-hydroxynonenal, known to induce Akt nitrosylation.[46] However, other evidence points towards the absence of a marked involvement of the insulin signalling, highlighting unchanged protein content and/or phosphorylation state of insulin receptor,[81] IRS1,[40,81] PI3K[81] and Akt.[40,81,123]

3.8 | Do the SR studies support the findings of "theoretical" epidemiological studies?

Population-based epidemiological research is essential for investigating the associations between physical activity/inactivity and health-related outcomes. A rapidly emerging area in this field, called time-use research,[124] has been recently applied in the context of physical activity/inactivity. These studies employ novel statistical approaches such as compositional data analysis and isotemporal modelling to study the impact of reallocation of a movement behaviour (e.g. sedentary behaviours, sitting, stepping, low and moderate intensity physical activity) to another one.[125,126] SR model essentially follows the same principle (i.e. reallocating stepping with sedentary behaviours) but tests this relationship experimentally. Thus, time-use research provides estimates that could theoretically align with the findings from SR experimental studies (and vice versa).

A recent interesting study used a compositional data analysis approach to model the association between physical behaviour and markers of metabolic health (including fasting glucose, two-hour glucose, Matsuda index and HOMA index) in individuals at high risk of developing diabetes, as well as evaluating the impact of time reallocation.[127] Interestingly, a SR study conducted in a similar population of prediabetic older adults (HBA1c: 5.90 ± 0.30 vs. 5.7 ± 0.5%) with comparable age (69 ± 4 vs. 66 ± 7.4 years old) investigated the same outcomes measures.[79] Considering that in this study a reduction on average of ~6400 steps/day was induced and assuming a stepping cadence in this population of 70 steps/min,[128] we can estimate that the SR intervention reduced daily stepping by approximately 1 h and 30 min in favour of sedentary behaviours. In the same study,[79] the alterations reported in circulating metabolic biomarkers were as follow: glucose fasting: 4.5% difference, two-hour glucose: 7.5%, Matsuda index: 35% and HOMA index: 23%. Interestingly, based on the study leveraging the compositional data analysis approach,[127] reallocating stepping with sitting for 1 h and 30 min would result in comparable changes of 3%, 19%, 44% and 20% for glucose fasting, 2-h glucose, Matsuda index and HOMA index, respectively.

Direct comparison of other SR and epidemiological studies is complex because most of the time-use investigations in the literature report only the reallocation from sedentary behaviours to stepping but not the opposite.[129,130] This is a relevant issue as the reallocation from one behaviour to another does not necessarily result in the same association as the inverse reallocation.[127,131] However, some further insights can be obtained from time-use studies using accelerometers to measure physical activity levels, assuming that most of the low-intensity physical activity levels derive from stepping. In these epidemiological studies, replacing low-intensity physical activity with sedentary time leads to increases in fat mass,[126] in agreement with SR experimental observations.[71] Conversely, this reallocation seems to not impact lean mass[132] and cardiorespiratory fitness,[133] differently from what reported in SR literature. However, it has to be considered that during SR interventions, participants are generally asked to refrain also from structured physical activities. Thus, SR studies are likely to induce a reduction also in moderate-to-vigorous physical activity levels, which reallocation to sedentary behaviours is instead associated with alterations of the aforementioned parameters in epidemiological studies.[132,133]

To the best of the authors' knowledge, this is the first article to link time-use epidemiological research with experimental SR studies. Overall, similarities in the findings of these two closely related topics are observed. Our article may pave the way for future studies leveraging this multidisciplinary epidemiological and physiological approach to further investigate associations between physical inactivity and health markers.

3.9 | Methodological considerations and future directions for SR studies

In summary, the SR model has been successfully employed to study the effects of reduced physical activity on various physiological systems. Habitual physical activity levels of the participants should be monitored for at least 3–7 days before the intervention via pedometers and accelerometers; in addition, a detailed description of their habitual exercise routine should be recorded. The maximal daily step limit (ranging from ~750 to ~4500 steps/day) in these interventions should be chosen carefully based on the population and research outcomes of interest. Interestingly, daily steps performed by hospitalised patients are on average ~740,[134] thus in line with the lower end of daily step count (~750 steps/day) used in steps SR studies.[90,98] An alternative approach may be to ask participants to reduce their daily step counts by a predetermined percentage of their baseline habitual steps activity level, ranging from 65% to 90%.[9,81,95] Selection of the participants and monitoring their compliance are other crucial issues that should not be underestimated. Volunteers should be recruited only after a careful evaluation regarding their ability to incorporate a SR intervention considering their daily routines and responsibilities. Strategies for reducing daily steps should be planned and discussed with the investigators before the beginning of the SR study. In order to facilitate adherence to the selected daily steps limit, investigators should consider providing participants with public transportation tickets, transport assistance or other appropriate transportation options, such as electric scooters. In addition, in order to promote compliance, it is advisable to foster a sense of camaraderie and collaboration among participants and investigators through the organisation of social events and gatherings, as previously proposed for other physical inactivity models.[115] Daily calls and messages to maintain contact with the participants are also encouraged. One potential strategy to evaluate retrospectively participants' compliance could be to instruct the participants to wear continuously the accelerometers and then, at the end of the intervention, apply algorithms[135] to differentiate non-wear time and inactivity time from the data obtained from the accelerometers. Methodological considerations for the SR model are summarised in Figure 2.

Future studies should focus on the effects of SR on other aspects that to date are largely unexplored, such as the neuromuscular (Section 3.4) and brain/cognitive

(Section 3.5) functions. Molecular mechanisms regulating declines in muscle mass and insulin sensitivity with SR are only partially understood (Section 3.7). Most of the SR studies lasted 1–2 weeks; studies of longer duration would be more relevant for deeper insights into the effects of sedentarism, given its chronic nature.[23] In addition, the time course of alterations is still poorly investigated. Some populations are significantly underrepresented as yet using this model. Females represent only ~24% of the participants included in SR studies (Table 1), despite a strong rationale for potential sex differences in the context of disuses-related muscle metabolism impairment and atrophy exists.[116,136] Moreover, only one SR study[77] was conducted in middle-aged adults, a population of particular interest as commonly subjected to periods of sedentarism due to work and family responsibilities peculiar to this period of life. The effects of different types of countermeasures (e.g. comparison of different types of training, NEAT and diet interventions) should be further investigated. Lastly, all the exercise countermeasures studies were performed exclusively during periods of SR, but none have investigated the effects of training programs following SR (i.e. during recovery from such inactivity periods). This is an important gap in this research field.

4 | CONCLUSIONS

Human body quickly develops adaptive responses to altered environmental conditions, such as physical inactivity. The SR model represents a unique opportunity to mimic the effects of a sedentary lifestyle and to study the pathophysiological mechanisms by which reduced physical activity impacts different physiological systems. Analogous models in rodents, which can provide the foundation for human studies, have been developed (Figure 1). Despite SR represents a less severe form of disuse compared with traditional severe unloading models, brief periods of reduced ambulatory activity have been shown to induce marked alterations in skeletal muscle health and metabolic function. These include reductions in lean mass, muscle function and protein synthesis, impairments in cardiorespiratory fitness, endothelial function and insulin sensitivity, accompanied by increased fat mass and inflammation (Table 1 and Figure 1). Exercise, NEAT-increasing and nutritional interventions ought to be developed for counteracting the deleterious alterations induced by periods of reduced physical activity.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

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