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Review

Sedentary behaviour and cardiometabolic health: Integrating the potential underlying molecular health aspects

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ABSTRACT

During the last decades, sedentary behaviour has been recognised as an interdependent risk factor for cardiometabolic health and premature mortality. Prolonged sedentary behaviour is associated with increased risks for chronic non-communicable diseases (NCDs) such as obesity, chronic respiratory diseases, type 2 diabetes mellitus, cardiovascular diseases and cancer due to disturbances in cardiometabolic health. However, despite the increased evidence supporting these associations, the underlying molecular mechanisms to the development of these NCDs remain largely unknown. In this review, we therefore discuss the existing evidence with regard to the potential underlying molecular mechanisms of sedentary behaviour-induced perturbations in cardiometabolic health. Here, various potential mechanisms related to carbohydrate metabolism, lipid metabolism, oxidative stress, inflammation and micro- and macro vascular function will be outlined. In addition, we summarise the current evidence on various strategies to interrupt sedentary behaviour and their effects on cardiometabolic health outcomes, including insulin sensitivity, blood lipid profiles, and cardiovascular health. Finally, we highlight key research gaps in the field of sedentary behaviour in relation to the underlying molecular mechanisms.

1. Background

Chronic non-communicable diseases (NCDs) such as obesity, chronic respiratory diseases, type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD) and cancer are an important public health concern worldwide [1]. In fact, according to the World Health Organization (WHO), it is estimated that each year >41 million people die from NCDs, which is equivalent to 71 % of all global deaths [1]. As a result, NCDs now are the leading cause of mortality worldwide and constitute one of the most important challenges for the 21st century [1,2].

Although the exact aetiology and origin have not yet been identified, their presence appears to be multifactorial and arising from a combination of socioeconomic, cultural and environmental determinants, genetic predisposition and modifiable risk factors [3,4]. The latter are predominantly lifestyle factors such as tobacco use, harmful use of alcohol, unhealthy diet and physical inactivity [3,4]. Here, physical inactivity is one of the major contributing factors. This is defined as performing insufficient amounts of moderate-to-vigorous physical activity (MVPA), as recommended by the 2020 WHO guidelines on physical activity and sedentary behaviour [5]. They recommend practicing a weekly volume of 150–300 min of moderate intensity (e.g. brisk walking), 75–150 min of vigorous intensity (e.g. running) or an equivalent combination of MVPA. This can be performed in different ways as part of work, during leisure time, domestic chores, transportation or participation in structured exercise or sports activities. Of interest, a recent report from the WHO indicated that 28 % of the adult and even 80 % of the adolescent population is still physically inactive [6]. As such, finding effective strategies that may improve long-term adherence to adequate daily physical activity is one of the greatest challenges of the future decades.

Research has mainly focused on physical activity so far, and it is clear that inactive individuals in general have a worse cardiometabolic health

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compared to physically active individuals, irrespective of age, sex and educational level [7]. Nonetheless, it appears that high levels of MVPA do not always fully protect against cardiometabolic risk factors and NCD development [7,8]. As such, sedentary behaviour, next to the time spent in MVPA, appears to be another important key player to determine cardiometabolic health and NCD development. According to the Sedentary Behaviour Research Network, sedentary behaviour encompasses 'any waking behaviour characterized by a low energy expenditure (≤1.5 metabolic equivalents), while being in a sitting or reclining posture' [9]. It is possible to meet or exceed the physical activity guidelines along with spending a lot of time sedentary. Therefore, sedentary behaviour can coexist and mitigate physical activity related health benefits. During the past decade, emerging evidence clearly disclosed that spending too much time in sedentary behaviour, typically involving prolonged periods of sitting, is also an important and interdependent contributor to cardiometabolic diseases and all-cause mortality, even in the presence of regular MVPA [10,11]. Therefore, the WHO incorporated sedentary behaviour recommendations into its revised guidelines in 2020 [5]. Here, individuals are now advised to not only participate in a weekly volume moderate-to-vigorous intensity physical activity, but also to limit the amount of time spent being sedentary [5]. In addition, they stated that replacing sedentary time with physical activity of any intensity (including light intensity) provides health benefits [5]. This means that both physical activity and sedentary behaviour are clinically relevant targets and major opportunities for interventions to change lifestyle behaviours, improve general health and reduce the risk of developing NCDs. In this respect, the ongoing quest to find ways for a healthier balance between sedentary behaviour and physical activity is of utmost importance for public health.

Within the field of (preventive) medicine, a necessary tenet is to better understand the pathophysiological mechanisms of NCDs in such a way that effective treatments and preventive interventions can be developed.

While previous reviews have primarily examined the metabolic consequences of sedentary behaviour through observable clinical markers [12] and some general molecular mechanisms [13], our approach delves deeper into the cellular and molecular pathways that drive these changes. By highlighting key mechanisms such as mitochondrial dysfunction, inflammatory signalling, lipid metabolism alterations, and insulin signalling pathways, we offer a more mechanistic understanding of how prolonged sedentary behaviour and physical inactivity contributes to cardiometabolic disease development. This molecular-level insight enhances our ability to identify potential therapeutic targets and interventions, distinguishing our review from previous work in the field.

2. Sedentary behaviour related effects on health

In the past decade, an exponential growth in research on 'sedentary behaviour' and its detrimental effects on cardiometabolic health has been observed. The harmful effects of prolonged sitting were first highlighted in the 1950s during the London Busmen Study [14]. Here, Morris et al. investigated the incidence rates of coronary heart disease within drivers and conductors of the Central ("red") Buses and identified a twofold increase in the risk of a myocardial infarction in bus drivers, who mainly sit during work, compared to more physically active bus conductors [14]. In the following 70 years, a plethora of research has focused on establishing the associations between MVPA and health, thereby overlooking the potentially important distinction between sedentary behaviour and physical activity. More recent objective measures have demonstrated that the average adult in Westernized societies spend between 8 and 12 h of their day in sedentary pursuits (up to 70 % of adult wake time) [15]. Within this harmonised meta-analysis Ekelund et al. also revealed a dose-response association between sedentary time and mortality risk increased gradually from 7.5 to 9 h per day [15]. Of interest, Patterson et al. already found a threshold of 6–8 h per day of total sitting, resulting in increased risks for the development of cardiometabolic health related diseases such as T2DM and CVD [16]. Over the past decade, epidemiologic evidence has revealed that, next to an increased risk for all-cause mortality, sedentary time is also an interdependent risk factor for cardiometabolic health outcomes including waist circumference, cholesterol, glucose concentration [7,17] and endothelial function and blood pressure [18,19]. It is important to note that the current literature on this topic is very limited, and further experimental research is necessary. Therefore, the results presented in the following chapters should be interpreted with caution, as there are also negative and mixed findings concerning these underlying mechanisms.

2.1. Experimental models regarding sedentary behaviour research

Changes in cardiometabolic health caused by sedentary behaviour can be studied using various experimental models, such as bed rest [20–22], limb immobilisation (i.e. casting) [23,24] and prolonged sitting or interventions that modify or interrupt prolonged sitting [25]. These models create different levels of sedentary behaviour exposure, offering complementary insights into its effects on cardiometabolic health outcomes. Understanding the advantages and disadvantages of each approach is crucial for accurately interpreting and generalising findings within the field of sedentary behaviour research on cardiometabolic health and has already explain in detail by Pinto et al. [12]. Briefly, limb immobilisation is a valuable model for studying muscle disuse and localised inactivity, offering a highly controlled environment to investigate molecular pathways involved in cardiometabolic changes within skeletal muscle tissue. However, since it primarily affects one limb rather than inducing full-body inactivity, it has limitations in assessing the systemic effects of prolonged sedentary behaviour on cardiometabolic health. To gain a more comprehensive understanding, combining limb immobilisation with other models, such as prolonged sitting or bed rest, may be beneficial.

Bed rest effectively simulates extreme sedentary behaviour by minimising all weight-bearing activity in a highly controlled environment, making it a strong model for studying systemic effects on cardiometabolic health. However, due to its extreme nature, it does not fully represent real-world sedentary behaviour conditions and, therefore, findings should be interpreted with caution when generalising to everyday sedentary lifestyles. However, because of upper body movements and fidgeting movements in the bed, the energy expenditure measured during bed rest studies is similar to that of sedentary individuals [26,27]. In addition, it is important to note that participants in these models are both sedentary and highly physically inactive, making it difficult to isolate the specific effects of sedentary behaviour. Since the detrimental effects of sedentary behaviour and physical inactivity generally align, their combined impact leads to more severe cardiometabolic health consequences than sedentary behaviour alone [28].

Unlike bed rest or immobilisation models, a relatively new approach such as prolonged sitting closely replicates everyday sedentary behaviour, such as office work and screen time, making it a highly applicable model for studying both whole-body and local skeletal muscle effects on cardiometabolic health. This model can be used to examine both acute (hours to days) and long-term (weeks to months) effects. However, extended sitting studies require strict participant adherence, which can be challenging to maintain, particularly in free-living conditions outside a controlled laboratory setting. Additionally, prolonged sedentary behaviour models can be combined with interventions that vary in intensity, frequency, and duration to explore strategies for mitigating its negative effects [29].

2.2. Sedentary behaviour and metabolic health – gene expression and proteomic insights

Several epidemiological studies using accelerometer-measured sedentary time have reported negative associations between sedentary behaviour and metabolic health [30,31]. Surprisingly, these associations were also found in individuals who reached the recommended levels of MVPA, which suggests that sedentary behaviour is a stand-alone factor in the relationship between physical activity and cardiometabolic health [30,31]. This means that sedentary behaviour should be considered as a separate behaviour that resides in a different mechanistic plane, rather than mirroring the effects of physical activity within a physical activity continuum. This concept was consolidated by Alibegovic et al., who were one of the first to show that four weeks of intensive physical activity did not completely normalize mRNA expression levels of genetic pathways involved in glucose and lipid transport and metabolism after 9 days of bed rest [32]. This was recently confirmed by a study from Pillon et al. who identified physical activity and sedentary behaviour responsive genes involved in cardiometabolic health [33]. Here, they found 897 responsive genes for acute aerobic physical activity, 2404 for acute resistance-based physical activity and 1576 for sedentary behaviour [33]. Taken together, these findings suggest that prolonged sitting induced impairments in physiological mechanisms, other than or partly overlapping those responsive to physical activity, comprising carbohydrate metabolism, lipid metabolism, oxidative stress, mitochondrial dysfunction and systemic inflammation (Fig. 1).

2.2.1. Carbohydrate metabolism

The interaction between sedentary behaviour and carbohydrate metabolism has been extensively investigated in both experimental and epidemiological studies. As skeletal muscle is responsible for 70–80 % of the postprandial glucose disposal in the body [34], a reduced metabolic activity due to prolonged sitting may ultimately lead to metabolic disruptions. In this regard, it has been hypothesized that the sedentary behaviour-induced disruption in carbohydrate metabolism is due to an impaired skeletal muscle insulin signalling and oxidative capacity of glucose.

2.2.1.1. Insulin Signalling. It has been shown that prolonged periods of sedentary behaviour are associated with substantial increases in insulin resistance [35–37] and reduced (insulin-mediated) skeletal muscle glucose uptake [37]. In humans, the earliest detectable reduction in insulin sensitivity (±17–35 %) was already found after one to three days of complete bed rest [22,38–40]. Therefore, an impaired insulin signalling appears an early metabolic response to prolonged sedentary behaviour and is maintained in the long-term after 56 day of bed rest [21].

As skeletal muscle is the primary tissue for maintaining glucose homeostasis through glucose uptake, a decreased insulin signalling in this tissue is a major contributor to whole-body insulin sensitivity. Prolonged sitting rapidly decreases muscle insulin sensitivity in association with an

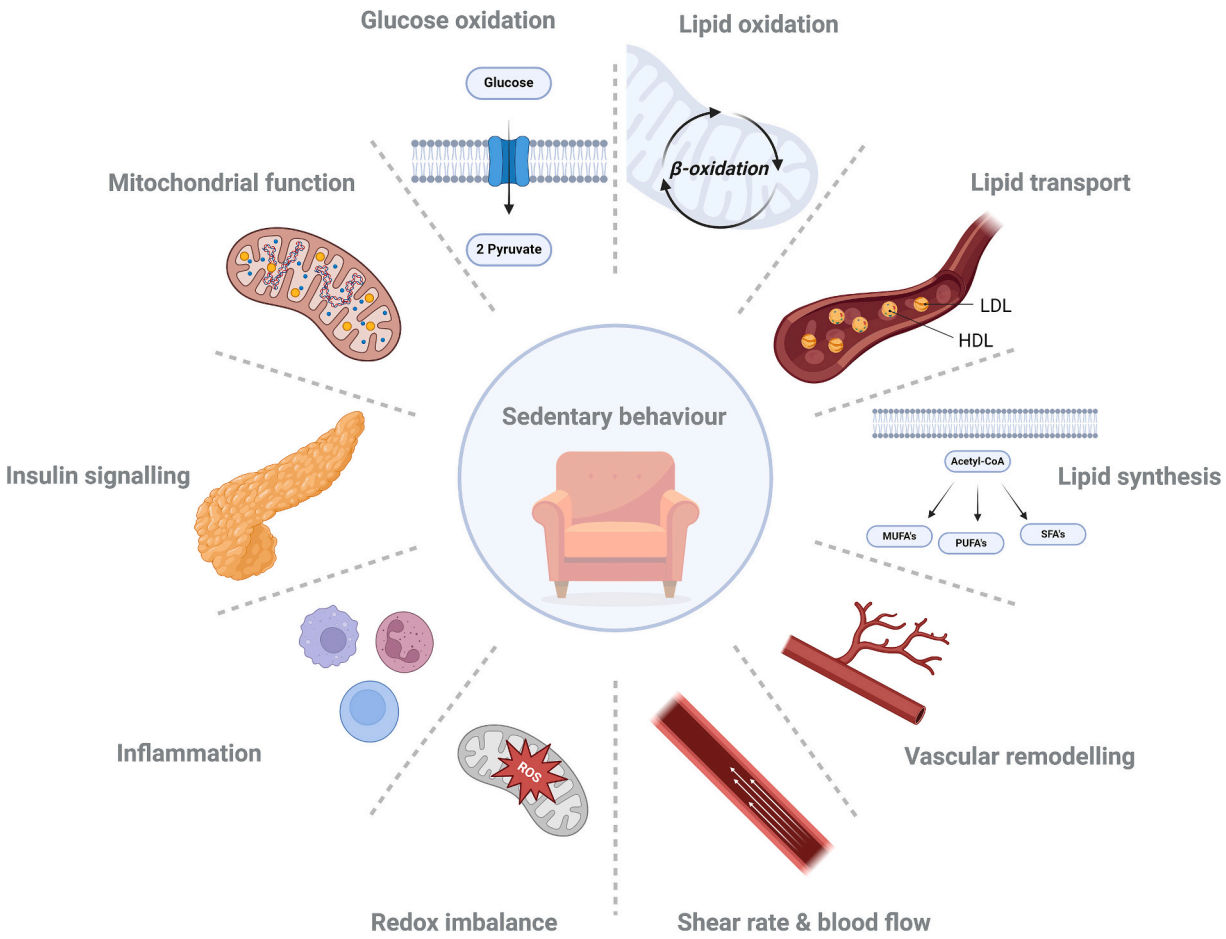

Fig. 1. Cardiometabolic hallmarks of sedentary behaviour. The scheme illustrates the ten hallmarks described within the current article. Prolonged periods of sedentary behaviour lead to numerous adverse effects, including impairments in glucose and lipid oxidation, lipid transport, lipid synthesis, vascular remodeling, a lower shear rate and blood flow, redox imbalance, inflammation, reduced insulin signalling and mitochondrial dysfunction. Abbreviations: HDL = High-density lipoprotein, LDL = low-density lipoprotein, MUFA = Monounsaturated fatty acids, PUFA = Polyunsaturated fatty acid, SFA = Saturated fatty acid.
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altered activity and expression of important signalling proteins involved in glucose transport and metabolism [20,25,41]. In this respect, several studies found that bed rest-induced insulin resistance was associated with reduced insulin-stimulated glycogen synthase activity and Akt signalling, as well as decreased activity and protein levels of the glucose transporter type 4 (GLUT4) after 6–9 days (short-term) [20,42] and 55 days (long-term) [21] of bed rest at the skeletal muscle tissue level, likely reducing the potential of the entire glucose oxidative system. In addition, after a 10-day bed rest period, an increase in ras related glycolysis inhibitor and calcium channel regulator (*RRAD*) expression, a protein that negatively affects muscle glycolysis, has been found [32]. Taken together, current evidence points to an impaired glucose uptake during sedentary behaviours, resulting from both decreased glucose transport/phosphorylation as well as decreased non-oxidative glucose metabolism in skeletal muscle tissues.

It could be suggested that the absence of muscle use during prolonged sitting leads to the lack of stimulation of transcriptional and translational processes normally seen during physical activity [33]. In particular, Alibegovic et al. identified an altered gene expression level of the most prominent down- and up-regulated transcriptional alterations that contribute to an impaired insulin response and glucose transport within skeletal muscle, suggesting that sedentary behaviour induces modifications in gene expression levels similar to those observed in people with T2DM [32].

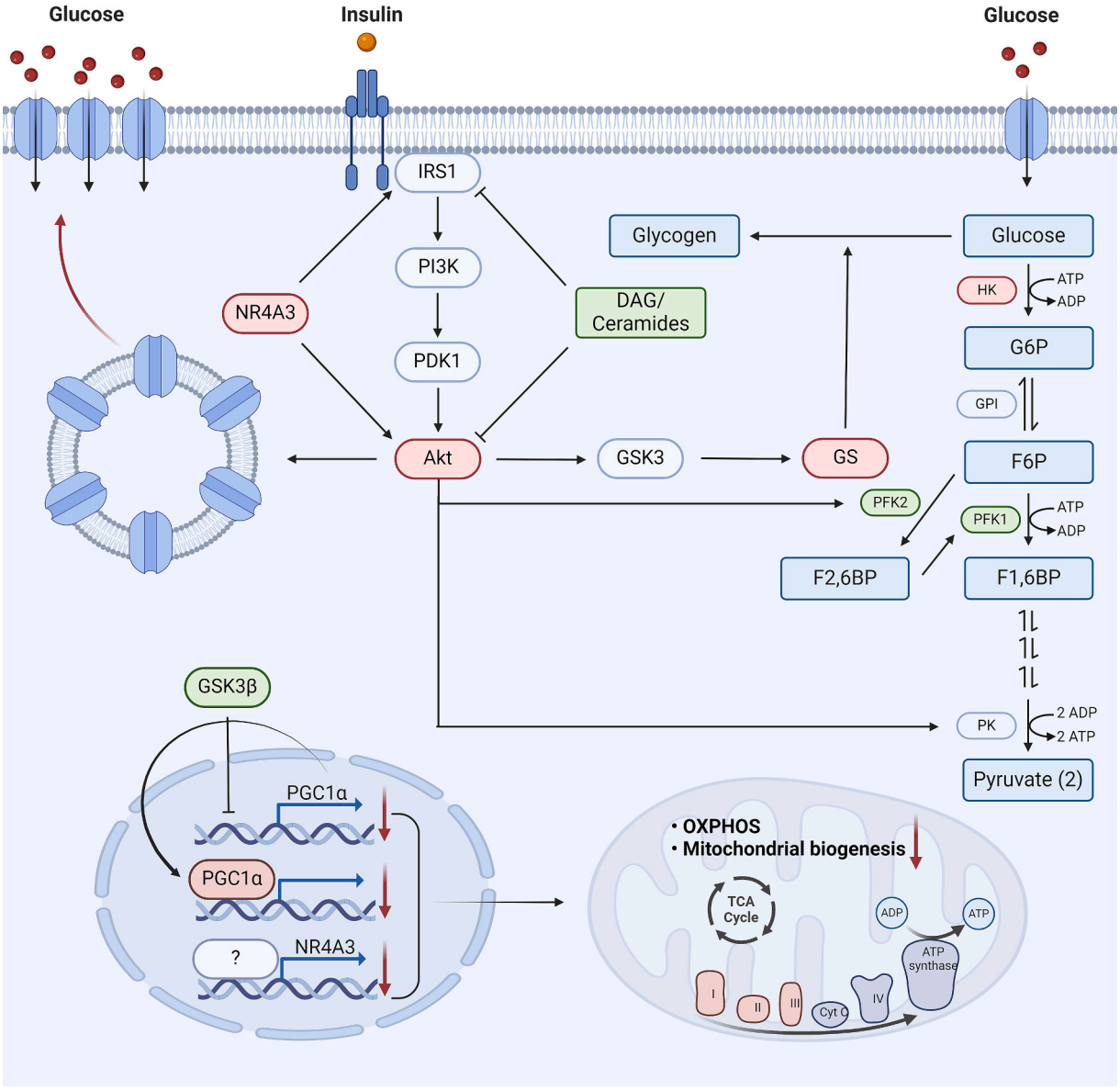

Fig. 2. Mechanisms underlying sedentary behaviour on carbohydrate metabolism and mitochondrial function in skeletal muscle tissue. Prolonged periods of sedentary behaviour result in impaired glucose uptake and oxidation, primarily due to disrupted insulin signalling. Additionally, the downregulation of key transcription factors involved in mitochondrial function and biogenesis contributes to a reduced oxidative metabolism and mitochondrial biogenesis. Metabolites and/or enzymes in green or red indicate decreased or increased transcription or activity, respectively. Abbreviations: IRS-1 = Insulin receptor substrate 1, PI3K = Phosphatidylinositol 3-kinase, PDK1 = Pyruvate dehydrogenase kinase 1, GSK3 = Glycogen synthase kinase-3, GS = Glycogen synthase, PFK1 = Phosphofructokinase 1, PFK2 = Phosphofructokinase 2, G6P = Glucose 6-phosphate, F6P = Fructose 6-phosphate, ATP = Adenosine triphosphate, ADP = Adenosine diphosphate, F1,6BP = Fructose 1,6-bisphosphate, F2,6BP = Fructose 2,6-bisphosphate, PK = Pyruvate kinase, PGC1α = Peroxisome proliferator-activated receptor-γ coactivator-1α, NR4A3 = Nuclear receptor subfamily 4 group A member 3, OXPHOS = Oxidative phosphorylation, CytC = Cytochrome c. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
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With regard to these metabolic changes during or after prolonged sitting, it is tempting to speculate on the involvement of the nuclear receptor subfamily 4 group A member 3 (NR4A3 or Nor-1), since this master regulatory transcription factor is involved in the expression of multiple proteins with regard to energy metabolism (fatty acid oxidation, glycogen synthesis, glycolysis, the tricarboxylic acid cycle and oxidative phosphorylation) [33,43]. An increased muscle-specific expression of *NR4A3* augments insulin signalling transduction due to an increased phosphorylation and activation of insulin receptor substrate-1 (IRS-1), Akt (protein-kinase B), and translocation of GLUT4 to the plasma membrane (Fig. 2) [44]. However, it has previously been shown by Pillon et al., that this pleiotropic transcription factor was downregulated in skeletal muscle following 5–10 days of bed rest or limb immobilisation, whereas no effects were found in the long-term (after 84 days of bedrest) [33].

Along with the mechanisms discussed so far, Peddie et al. suggest that energy surplus plays a key role in the acute metabolic responses to prolonged sedentary behaviour [45]. An overabundance of particularly fatty acids and glucose inhibits key components related to the insulin signalling pathways within skeletal muscle tissue [46,47]. Other complex mechanisms potentially involved in a reduced insulin action following sedentary behaviour are increased circulating counter-regulatory hormones as glucagon, epinephrine and cortisol [48]. In addition, short-term (7 days after bed rest) hemodynamic changes, including a decreased insulin-mediated muscle blood flow and capillary recruitment are also possibly involved [37]. Furthermore, a reduced expression of the peroxisome proliferator-activated receptor-γ coactivator-1α gene (*PPARGC1A* or *PGC1α*), an important transcriptional regulator orchestrating the expression of metabolic and vascular genes including vascular endothelial growth factor A (*VEGFA*) has been observed following 10 days of sedentary behaviour [32], thereby impacting insulin-related vasoactivity and glucose clearance [32,49,50].

As a result of all these metabolic adjustments in terms of gene transcription, translation and protein activities, the insulin concentration may increase to compensate for the reduction in glucose uptake via the signalling transduction pathway, which in turn causes hyperinsulinaemia and a further decline in skeletal muscle insulin sensitivity.

2.2.1.2. Glucose oxidation. Long periods of sedentary behaviour resulted in increased phosphofructokinase (PFK) mRNA expression, a rate limiting enzyme within the glycolysis [51]. This was in line with a study from Mahmassani et al., where a significant increase in 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*PFKFB3*) mRNA expression was identified. This gene encodes for the enzyme phosphofructokinase (PFK) 2, whose product stimulates the glycolysis through the synthesis of fructose-2,6-bisphosphate and a subsequent enhancement of PFK-1 activity [24]. In contrast, a reduced hexokinase II (HK2) content and activity (after both 7 and 84 days of bed rest), together with a reduction in *HK-2* gene expression levels after 84 days of bed rest was found by others [20,42]. However, despite the controversial molecular effects of sedentary behaviour on glucose metabolism and whether or not there is a shift from oxidative towards glycolytic metabolism, it is postulated that there is a general impairment of energy metabolism [52]. In addition, a recent study of Shur et al. suggested that widespread transcriptional events occur in an early stage (days) of sedentary behaviour, accounting for the shift in fuel metabolism seen after long periods (weeks) of prolonged sitting [53]. Thus, for future research it is important to discriminate between effects of sedentary behaviour on the short and long-term.

2.2.1.3. Mitochondrial dysfunction. Within skeletal muscle tissue, a reduction in mitochondrial content or dysfunction plays an important role in the development of insulin resistance [54]. In this respect, Alibegovic et al. showed that 54 % of 162 transcripts involved in the oxidative phosphorylation (OXPHOS) pathways was decreased after 9 days of continuous bed rest [32]. They hypothesized that the induction of insulin resistance was a causal factor for the downregulation of the genes involved in the OXPHOS pathway. Here, important genes involved are *PGC1α*, a master regulator of mitochondrial biogenesis and oxidative metabolism [55] and OXPHOS genes [52,56]. Although there is compelling evidence that PGC1α is down regulated after longer periods of sedentary behaviour [32,42,52,56], few studies are contradictory [57,58]. The sedentarism-induced downregulation of *PGC1α* might be mediated by glycogen synthase kinase-3 beta (GSK-3β), a negative regulator of PGC1α expression [59], which has been described to increase upon 10 days of leg immobilisation (Fig. 2) [23]. Moreover, sedentary individuals showed a significantly lower content and activity of proteins reflecting mitochondrial biogenesis and function in skeletal muscle tissue, including citrate synthase, Complex II, Complex III and COX subunits-I and II [60]. These results were confirmed by Alibegovic et al., who found a decline in NADH dehydrogenase 1 beta subcomplex subunit 6 (*NDUFB6*) expression, a subunit of the NADH dehydrogenase complex (Complex I), after 9 days of bed rest [32]. In addition, Eggelbusch et al. observed that long-term bed rest (55 days) reduced muscle mitochondrial density and protein concentrations of the mitochondrial complexes II, III, and IV and ATP synthase, whereas short-term (6 days) bed rest did not [21]. Pillon et al. recently profiled the skeletal muscle transcriptome after sedentary behaviour and showed a reduction in the expression of genes related to OXPHOS proteins and lipid regulating enzymes following both short-term (5–10 days) and long-term (60–84 days) bed rest or limb immobilisation [33]. Here, a decrease in oxygen consumption was related to a decrease in mitochondrial OXPHOS complexes. Interestingly, this was accompanied by a decreased expression of *NR4A3* (*Nor-1*). Therefore, it could be speculated that NR4A3 acts as a central regulator of the deleterious effects of sedentary behaviour on cardiometabolic health. Other studies confirmed these results and showed that a decreased *NR4A3* expression attenuated oxidative and fatty acid metabolism [61]. Interestingly, it seems that the repression of NR4A3 is induced by a lower activity of a cAMP-dependent pathway involving protein kinase A and/or C, p38 MAPK and the transcription factor cAMP response element binding protein (CREB) [62,63]. Given that PGC1α is a nuclear receptor activator, this master regulator is probably also involved (as coactivator) in the regulation of NR4A3 [64]. Other mechanisms participating in the repression of NR4A3 are epigenetic processes, including DNA methylation and histone acetylation, and the repression of NR4A3 by CREB [65]. However, to what extent epigenetic modifications are involved in the molecular mechanism of sedentary behaviour should be further investigated. From current sedentary behaviour literature, it could be suggested that a reduced oxidative capacity leads to a lower muscle energy turnover, resulting in an increased content of intramuscular lipid intermediates including triacylglycerol (TAG), diacylglycerol (DAG) and/or ceramides. Skeletal muscle tissue concentration of TAG and ceramides increased with long-term (55 days) but not short-term bed rest [21]. These lipotoxic intermediates in turn putatively interfere with serine kinases, thereby reducing insulin signalling transduction and thus insulin-stimulated GLUT4 translocation to the plasma membrane [66,67].

2.2.2. Lipid metabolism

A sedentary lifestyle is also a moderator of lipid metabolism and especially contributes to hyperlipidaemia [68–70]. Despite the abundance of studies using a prolonged sitting approach to investigate lipid metabolism, both fasting and postprandial plasma lipid responses have been less consistent compared to glucose and/or insulin responses [39,71]. It has been shown that prolonged sitting leads to significantly higher levels of plasma triglycerides (±18–63 %), and atherogenic lipid particles including low-density lipoprotein (LDL; ±4 %) and very low-density lipoprotein (VLDL) cholesterol (±55 %), and lower levels of high-density lipoproteins (±6–23 %) [72–74], although these findings

are controversial [39,40]. These disruptions in lipid metabolism are possibly due to changes in lipid oxidation and/or synthesis and transport.

2.2.2.1. Lipid oxidation and synthesis. Sedentary behaviour is related to a decreased mitochondrial oxidative capacity, a lower muscle energy turnover and an increased content of lipotoxic intermediates [32]. Another reason for this increase in lipotoxic intermediates might be found in the reduction of the lipogenic capacity of the skeletal muscle tissue. Of interest, Schenk et al. revealed a decreased expression of mitochondrial glycerol-3-phosphate acyltransferase (m*GPAT*), diacylglycerol O-acyltransferase 1 (*DGAT1*) and stearoyl-CoA 9-desaturase-1 (SCD1) after an acute one-day sedentary intervention period [75]. As mGPAT and DGAT1 are the first and second step of the triacylglycerol biosynthesis, respectively, reduced expression levels of these lipogenic enzymes lead to a lower disposal of fatty acids towards the intramuscular triacylglycerol (TAG) synthesis and consequently results in the accumulation of lipotoxic intermediates in skeletal muscle tissue (Fig. 3) [75,76]. Moreover, these lipotoxic consequences of ectopic lipid accumulation following prolonged sitting may be extrapolated to other important metabolic tissues such as liver and cardiac tissue, dependent on the degree of disbalance between lipid supply and oxidation [13,77]. In this respect, it has for example been shown that during 40–90 days of bed rest both aspartate aminotransferase and alanine aminotransferase were increased by almost 50 % [13], being predictive for non-alcoholic fatty liver disease and the development of liver insulin resistance [78].

Opposite mechanisms reflected by an increased triacylglycerol synthesis have also been proposed [23,52,79]. First, a blunted fat oxidation might be due to a decreased expression of carnityl palmitoyl transferase I (*CPT-1*), an important regulator of the entrance to the beta-oxidation of fatty acids at the mitochondrial matrix. Studies revealed a significantly decreased gene expression of *CPT-1* after both 10 days [32] and 60 days [79] of bed rest, possibly leading to a reduced beta-oxidation and resulting in intramuscular lipid accumulation. This was also confirmed by a cross-sectional study where a significantly reduced CPT-1 enzyme activity in skeletal muscle tissue of sedentary subjects was shown, compared to trained subjects [60]. In addition, it is hypothesized that there is a disbalance between CPT-1 and mGPAT activity, both localised

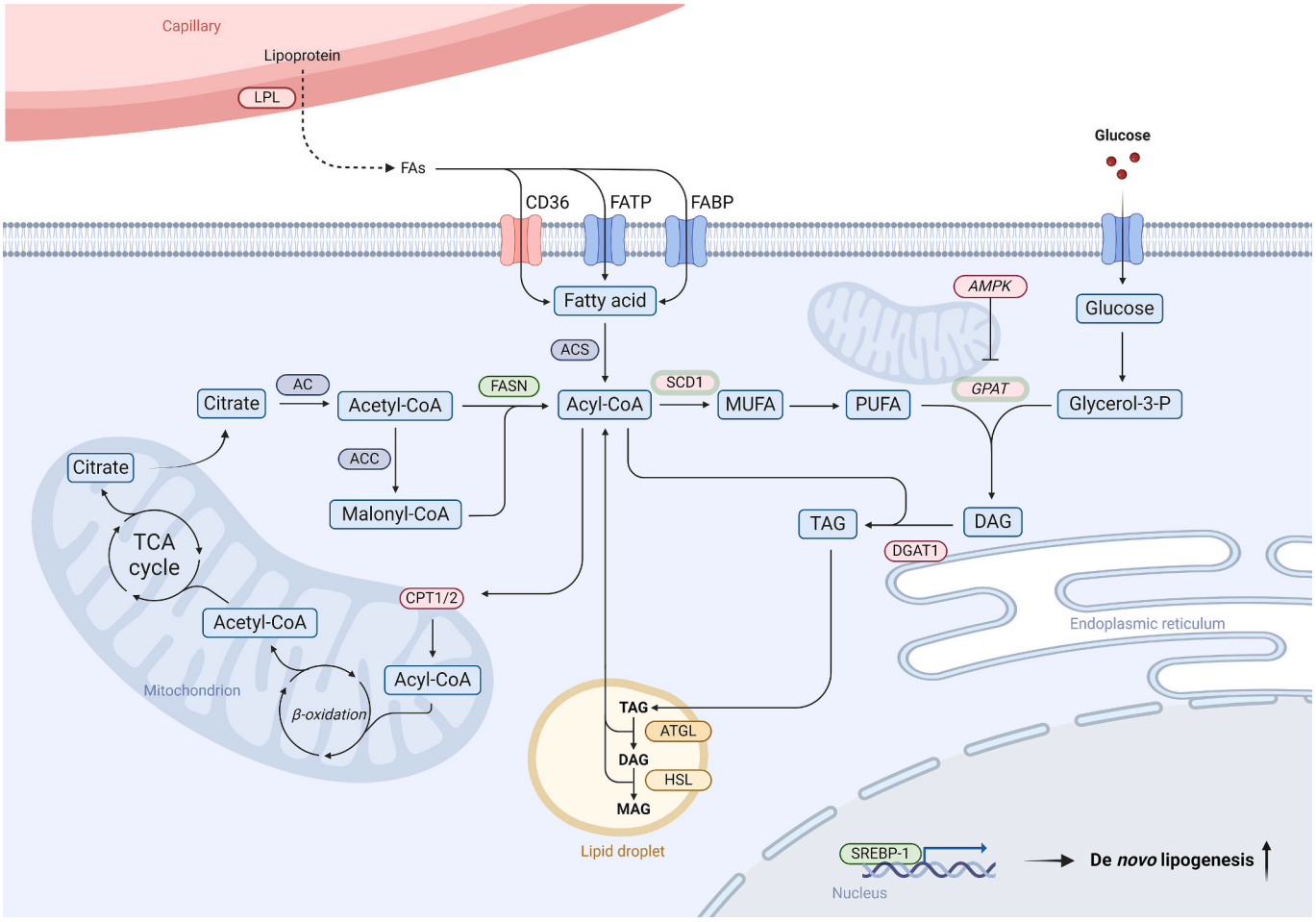

Fig. 3. Mechanisms underlying sedentary behaviour on lipid metabolism in skeletal muscle tissue. Prolonged sedentary behaviour significantly impacts lipid metabolism. In skeletal muscle, the activity of lipoprotein lipase and fatty acid transporters decreases, impairing the release and uptake of free fatty acids by muscle cells. This reduction is accompanied by altered lipogenic capacity and diminished beta-oxidation, leading to the accumulation of lipotoxic intermediates such as diacylglycerol and ceramides, which can contribute to metabolic dysfunction over time. Metabolites, transporters and/or enzymes in green or red indicate decreased or increased transcription or activity, respectively. Abbreviations: LPL = Lipoprotein lipase, CD36 = Cluster of differentiation 36, FATP = Fatty acid transport protein, FABP = Fatty acid-binding protein, ACS = Acetyl-CoA synthetase, ACLY = ATP citrate lyase, FASN = Fatty acid synthase, SCD1 = Stearoyl-CoA desaturase-1, MUFA = Monounsaturated fatty acid, PUFA = Polyunsaturated fatty acid, GPAT = Glycerol-3-phosphate acyltransferase, AMPK = AMP-activated protein kinase, MAG = Monoacylglycerol, DAG = Diacylglycerol, TAG = Triacylglycerol, DGAT1 = Diacylglycerol acyltransferase-1, ACC = Acetyl-CoA carboxylase, CPT = Carnitine palmitoyltransferase, TCA = Tricarboxylic acid cycle, ATGL = Adipose triglyceride lipase, HSL = Hormone-sensitive lipase, SREBP = Sterol regulatory element-binding protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
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within the outer mitochondrial membrane. Due to this location they compete for acyl-CoAs, which means that there is a reciprocal regulation between lipid synthesis (mGPAT) and oxidation (CPT-1), orchestrated by AMP-dependent protein kinase (AMPK). It is postulated that AMPK expression and activity is reduced during sedentary behaviours, which attenuates the inhibition of mGPAT and a subsequent increase in triacylglycerol synthesis [80]. However, AMPK (in)activation during sedentary behaviours [52] is controversial and, therefore, more research is necessary regarding the expression and activity of AMPK to elucidate the exact regulatory mechanisms. In particular, AMPK phosphorylation and the expression of the different subunits (α, β and γ) should be investigated in more detail.

A second mechanism regarding lipid synthesis during sedentary behaviour has been shown by Brocca et al. They revealed that the expression of sterol regulatory element-binding protein 1 (*SREBP-1*), a transcription factor that plays an important role in the regulation of lipid metabolism within skeletal muscle tissue, was increased after 24 days of bed rest [52], potentially related to the concomitant hyper insulinemic state. SREBP-1 stimulates lipogenesis and triacylglycerol deposition by regulating several lipogenic enzymes including GPAT and SCD1 [81]. As such, an increased expression of SREBP-1 is indicative of lipid synthesis and intramuscular lipid accumulation, which subsequently may contribute to mitochondrial dysfunction and incomplete fatty acid oxidation [52]. A reduction in lipid oxidation favoured the incorporation of fatty acids, in particular palmitate, into intramuscular triacylglycerols [23,79]. These results were consistent with a study from Rimbert et al. who revealed a reduced muscle palmitate oxidative capacity of 46 % in sedentary older adults [60]. These proposed molecular mechanisms align with findings from other clinical trials, which show that mitochondrial oxidative capacity and lipid oxidation during sedentary behaviour are 22–40 % lower after 7, 10 and 90 days of bed rest, accompanied by increased palmitate incorporation into triacylglycerols, suggesting that a reduced oxidative capacity drives muscle cells to a higher intramuscular lipid synthesis and accumulation [23,35,82]. These findings are in line with experimental studies showing a reduced fatty acid oxidation capacity, indicated by decreased acylcarnitine concentrations with medium and long chain lengths, and resulting in an increased intramuscular lipid content after short-term and long-term bed rest and/or immobilisation [21,23].

Interestingly, increased lipid synthesis and intramuscular triacylglycerol accumulation are considered to be of less importance to induce insulin resistance compared to the lipotoxic intermediates (DAG and ceramides) [67]. Therefore, it seems to be that the increased triacylglycerol synthesis acts as a protective mechanism to prevent the development of insulin resistance during mitochondrial dysfunction. This means that mitochondrial dysfunction, reflected by a reduced mitochondrial oxidative capacity and skeletal muscle lipid oxidative capacity, contributes to a compromised insulin signalling rather than the increased intramuscular lipid accumulation [23].

All these mechanisms were consistent with other studies providing a unique glimpse of the transcriptome after bed rest or leg immobilisation [24,83,84]. These reveal a decreased lipid oxidation, reflected by lower *PPARGC1A* mRNA expression and important enzymes involved in the beta-oxidation, including Acyl-CoA dehydrogenase for long chain fatty acids (*ACADL*), acyl-coenzyme A dehydrogenase for medium-chain fatty acids (*ACADM*), acyl-coenzyme A dehydrogenase for very long chain fatty acids (*ACADVL*) and hydroxyacyl-coenzyme A dehydrogenase (*HADH*) after 5, 20 and 60 days of immobilisation or bed rest [24,84]. On the other hand, it has been shown that the lipid biosynthesis increases, in particular due to an increased expression of the fatty acid synthase gene (*FASN*). This gene codes for a key lipogenic enzyme that mainly converts acetyl-CoA to the fatty acid palmitate [85]. In addition, other studies also found alterations in fatty acid transport due to a decreased expression of the mitochondrial fatty acid transporter *SLC25A20* (also known as *CACT*), *CD36* and the intracellular *FABP3* after a 24- and 60-day bed rest period [52,84]. Interestingly, these changes coincided by a larger increase in insulin resistance and are in line with theories that sedentary behaviour-induced accumulation of intramuscular lipids may lead to insulin resistance [24,86]. Here, activation of protein kinase C (PKC) is speculated to be mechanistically linked with a subsequent inhibition of IRS1/2 and decreased translocation of GLUT4 to the cell membrane.

2.2.2.2. Lipid transport. The changes in lipoprotein particles and an increase in plasma triglyceride concentration may merge from a reduced systemic clearance of triglycerides. Here, it has been suggested that a lower hydrolysis of triglyceride-rich lipoproteins (VLDL and chylomicrons) by lipoprotein lipase (LPL), which is anchored to the capillary walls within adipose tissue, skeletal muscle and cardiac tissue [87], may be a key contributor [73]. Indeed, it has been shown that 5, 21 and 60 days of sedentary behaviour led to a decreased LPL concentration and activity, which in turn resulted in increased VLDL triglyceride levels and decreased HDL_2 cholesterol and HDL_3 cholesterol levels [24,73,79]. These reduced concentrations in HDL cholesterol subfractions raise the possibility of changes in the reverse cholesterol transport, which normally removes cholesterol from peripheral tissues. In contrast, some studies reported no differences in total cholesterol, HDL, LDL and triglyceride concentrations after prolonged sedentary behaviour [39,88].

Interestingly, it has been shown that the regulatory mechanisms affecting skeletal muscle LPL activity, and therefore lipid metabolism, during prolonged sitting were distinct from those during MVPA. In rodents, the absence of normal physical activity levels was found to decrease LPL activity (relative to ambulatory controls) in oxidative skeletal muscle fibers, whereas voluntary running had no effect on relative LPL activity [89]. These findings suggest that sedentary behaviour has a much greater effect on LPL regulation than supplementary exercise training [90]. Therefore, it could be speculated that MVPA and sedentary behaviour have independent effects on (cardio) metabolic pathways relevant to metabolic health. However, it is important to mention that these potential underlying mechanisms have to be revaluated in human experimental studies.

Although the exact mechanisms have not been elucidated yet, it has been proposed that during prolonged sedentary behaviours, an increased apoC-III/apoC-II ratio may impair LPL activity [73]. It has already been shown that apoC-III is a potent inhibitor of LPL, thereby explaining the inverse association between plasma apoC-III levels and lipolysis of VLDL [87]. In addition, other mechanisms such as insulin resistance, a decreased capillary perfusion and the influence of other molecular pathways during prolonged sitting are possibly associated with a decline in LPL activity [91,92].

In terms of lipid transport, Bergouignan et al. found a diminished expression of the fatty acid translocase CD36 in skeletal muscle tissue after 5 and 59 days of bed rest, which also contributes to a reduced muscle clearance of non-esterified fatty acids (NEFA) [24,79]. Next to CD36, Mahmassani et al. also showed an increased expression levels of *APOC-I*, which is involved in the inhibition of cholesteryl ester transfer protein (CETP), a plasma protein that facilitates the transport of cholesteryl esters and triglycerides between lipoproteins [24]. In addition, people who developed insulin resistance were characterized with muscle gene responses described to have a low capacity for triglyceride export within skeletal muscles, reflected by a decreased *APOB* expression, thereby reducing skeletal muscle triglyceride accumulation [93]. Interestingly, a study of Chen et al. found a dramatic upregulation of the *APOD* gene, a component of HDL, in skeletal muscle tissue after 5 days of immobilised legs [94]. Although the exact function of APOD is not fully known, many potential functions have been proposed, including the transport of small hydrophobic ligands and the formation of a protein complex with lecithin-cholesterol acyltransferase (LCAT). In addition, they also found an increased expression of the leptin receptor (*LEPR*) gene, which has been shown to interact with APOD [95]. The leptin receptor has been identified as a key regulator involved in energy

homeostasis and body weight regulation [95].

As described earlier, it is hypothesized that increased plasma lipids resulting from impaired lipid transport mechanisms enhances ectopic lipid accumulation (i.e. visceral adipose tissue, intramuscular and hepatic lipid storage) on the long-term [96]. Here, lipid accumulation in the liver stimulates de novo lipogenesis and increases synthesis of VLDL particles, as proposed by a recent study in free-living individuals who reduced their physical activity levels [97]. This increased secretion of VLDL further facilitates hyperlipidaemia and ectopic lipid storage, which in turn exacerbates inter-organ crosstalk and may lead to the development of insulin resistance.

2.2.3. Oxidative stress

Several experimental studies in humans indicated that oxidative stress occurred during prolonged sedentary behaviour, due to an imbalance of (anti)oxidant enzymes [52,98]. Although the source of reactive oxygen species (ROS) within skeletal muscle tissue remains incompletely understood, several oxidant production pathways, including xanthine oxidase, NADPH-oxidase, and the mitochondria might contribute to superoxide production. In addition, the production of nitric oxide by nitric oxide synthase possibly further promotes ROS formation. On the other hand, the aberrant increase in ROS levels could also result from a down-regulation and decreased activity of endogenous antioxidant defense systems such as superoxide dismutase (SOD, [SOD1 and mnSOD]), peroxiredoxin 3, carbonic anhydrase III and heat shock proteins (α- and β-crystallin, HspB6, HspB1 and Hsp70) after both 8 and 35 days of bed rest [52]. This suggests a persistent impairment of the myocellular response against ROS and is aligns with a cross-sectional study in which they showed a reduced mn-SOD activity in sedentary adults [56]. Libera et al. have shown an increased protein carbonylation, a marker of oxidative stress, accompanied by an antioxidant stress response characterized by the expression of the inducible heme oxygenase-1 (HO-1), a small heat shock protein (Hsp) highly induced by ROS and heme molecules, and changes in protein levels of the mitochondrial Hsp glucose-regulated protein-75 (Grp75) after 8 but not after 35 days of bed rest [98]. In contrast, the expression of the nuclear factor E2-related factor 2 (NRF2), a sensor of redox (im)balance and a key transcription factor involved in the expression levels of these antioxidant defense systems, was increased after 24 days of bed rest [52].

These findings suggest that sedentary behaviour leads to an impairment of the antioxidant defense systems along with a pro-oxidant environment, which may result in a mitochondrial redox crisis. The ROS-mediated toxicity during sedentary behaviour will in turn lead to an increase in free radicals oxidized macromolecules that would compromise mitochondrial and cellular functional capacity. Of interest, it is hypothesized that these processes are mediated by the activity of gene regulators involved in inflammation, including nuclear factor-kappa B (NF-κβ), which may also be involved in the development of insulin resistance [32,99].

2.2.4. Inflammation

Chronic low-grade inflammation is indicated by the production of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and the hepatocyte-derived C-reactive protein (CRP) [100]. A chronic low-grade inflammatory state has been found to be associated with the pathogenesis of insulin resistance and atherosclerosis [101,102]. Concerning sedentary behaviour research, experimental studies showed that 9–14 days of bed rest in young healthy volunteers activated a pro-inflammatory cascade as shown by increased circulating levels of CRP (±92 %) and IL-6 (±240 %), and decreased anti-inflammatory interleukin-10 (IL-10) and white blood cell mRNAs (±10–66 %) [32,103,104]. These findings were confirmed in large, representative population-based samples where sedentary time was negatively associated with various pro-inflammatory cytokines including CRP, IL-6 and TNF-α in both healthy individuals and individuals with T2DM [104–108]. In addition, a large cross-sectional population-based study showed that sedentary time (h/day) and prolonged sedentary bouts were positively associated with a composite score, consisted of high-sensitivity-CRP, IL-6, IL-8, TNF-α, serum amyloid A (SAA) and soluble intercellular adhesion molecule-1 (sICAM-1), of low-grade inflammation [109].

As described earlier, the accumulation of lipotoxic intermediates (i.e. diacylglycerol and ceramides) within skeletal muscle tissue may activate proinflammatory pathways such as the IKK/NF-κβ and c-Jun N-terminal kinase (JNK) signalling. In this respect, Schenk et al. indeed showed a significant increase in activation of the IKK/NF-κβ pathway after an acute sedentary behaviour intervention, compared to exercise [75]. Increased activation of proinflammatory pathways leads to an aberrant cascade of cellular events which have been shown to impair insulin signalling and induce skeletal muscle insulin resistance [67,110,111].

Given that pro-inflammatory markers negatively affect insulin sensitivity and their association with an increased risk of cardiovascular diseases, inflammation may be a key pathway through which prolonged sitting has an impact on NCD development [112,113]. Therefore, long-term interventions are necessary to examine the exact independent relations between sedentary behaviour and inflammatory markers.

2.3. Cardiovascular health and sedentary behaviour

Global leading organizations such as the American Heart Association, the American College of Cardiology, and the European Society of Cardiology emphasize that sedentary behaviour and physical inactivity are major modifiable risk factors for the development of cardiovascular diseases (CVD). Strikingly, it has been shown that two decades of a sedentary lifestyle were associated with a doubled risk of premature all-cause and cardiovascular death [114]. Considering the interdependence between sedentary time and MVPA, prospective cohort studies have shown that populations who even meet the recommended physical activity guidelines still display adverse associations between sedentary behaviour and cardiovascular disease risk factors and mortality [115,116]. In contrast, although Stamatakis et al. found that higher sitting times were associated with higher CVD mortality risk, a relation being restricted to people not meeting the physical activity recommendations (i.e. spending ≥150 min/week in MVPA) [117]. In addition, Pandey et al. suggested that an increased CVD risk was only independently associated with total sedentary time at very high levels (>10 h/d) [118]. Despite some potential mechanisms have been proposed, the exact dose-response relationship between sedentary time and cardiovascular health and the underlying mechanisms should be further elucidated.

So far, prolonged sedentary behaviour has been shown to induce detrimental effects on the cardiovascular system [119], with recent studies indicating substantial impairments in both the macro- and microcirculation of the lower extremities [19,120–122]. It has been suggested that these vascular perturbations may be due to different physiological mechanisms such as alterations in local hemodynamic-induced changes in circulating molecules, arterial bending and reduced arterial shear stress. An important early marker of CVD is endothelial dysfunction and accumulating evidence has demonstrated that short (1–6 h) acute bouts of sedentary behaviour, already impairs endothelial function (measured by a reduced flow-mediated dilation) in the lower extremity vasculature including the popliteal (±60 %), femoral (±92 %), and posterior tibial arteries (±23 %) of healthy adults [123–129]. It could therefore be hypothesized that prolonged exposure to this sitting-induced endothelial dysfunction may be a key contributor driving the association between sedentary behaviour and CVD development. It has been proposed that this endothelial dysfunction is a consequence of a disturbed laminar shear stress response [120,130], which mediates the synthesis of both autocrine and paracrine substances released from the endothelium [131]. Here, it has been suggested that an unfavourable shift from anti-atherogenic, vasodilatory to vasoconstrictive pro-thrombotic substances may be a key mechanism underlying

the induction of endothelial dysfunction with excessive sitting [120,130,132]. More specifically, a reduced synthesis of nitric oxide (NO; ±32 %) and an increased synthesis of endotelin-1 (ET-1; ±27 %) and angiotensin II (ANGII; ±23 %) have been observed after 10–20 days of bed rest and after 8 weeks of detraining [133–135]. This imbalance may upregulate endothelial derived ROS, which in turn disrupts endogenous antioxidant mechanisms, and reduces total plasma nitrate and nitrite (markers of NO bioavailability) [129,130,136]. In addition, previous work has shown that a reduced laminar shear stress down-regulates the expression of endothelial nitric oxide synthase (eNOS) and, as a consequence, a lower NO production (Fig. 4) [137–139].

Although the majority of studies regarding prolonged sitting have been centred around alterations in macrovascular endothelial function, the microvascular circulation also appears to be affected after prolonged sitting [129,140]. It has been proposed that the same contributing mechanisms (i.e. reduced laminar shear stress and an imbalance of vasoactive substances) may be responsible for microvascular dysfunction. Thus, it is plausible that the combination of these mechanisms mediates a proatherogenic endothelial cell phenotype in both the micro- and macro vasculature after prolonged sitting periods. However, more research is necessary to clarify these (and other) mechanisms in more detail.

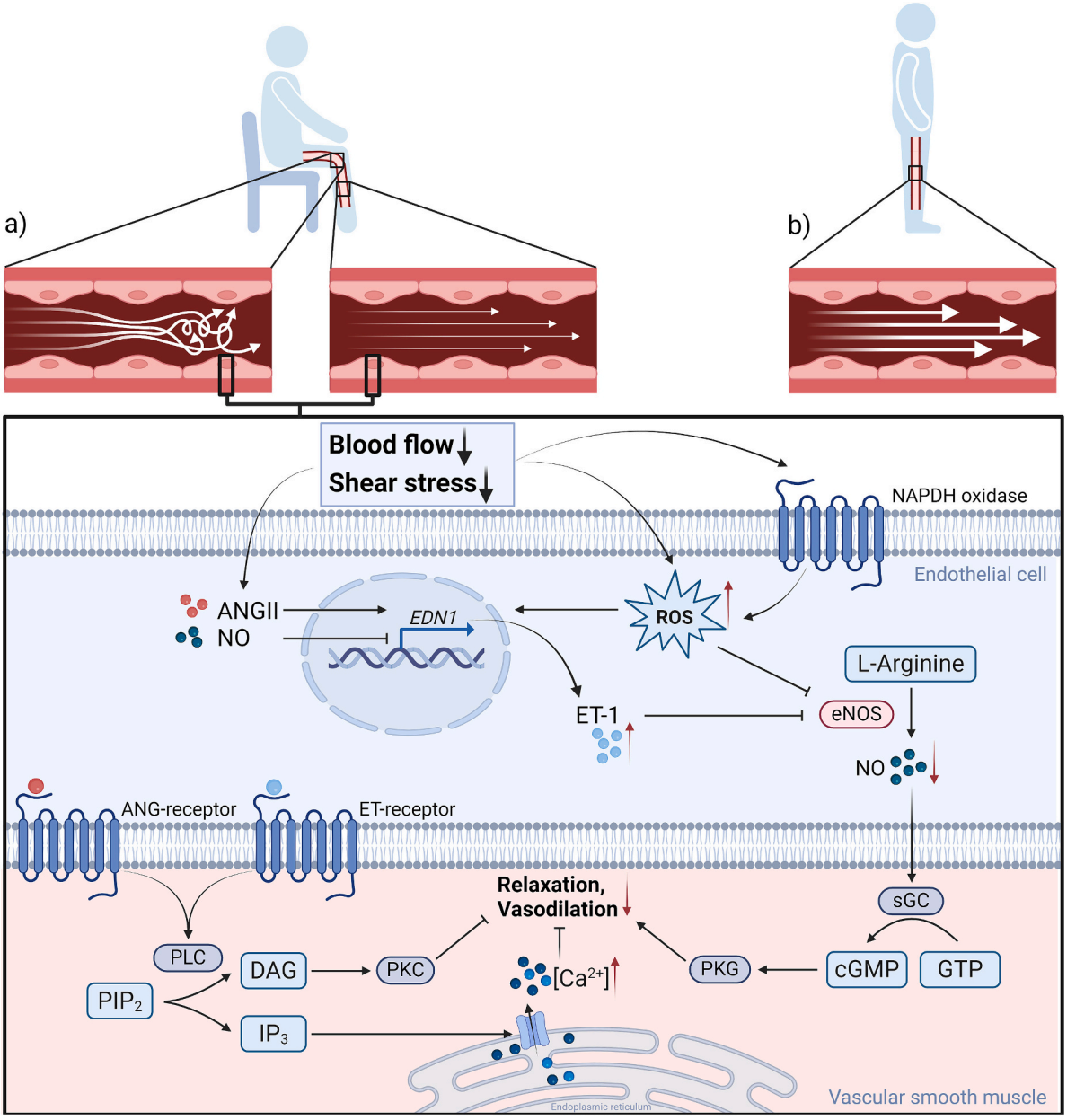

Fig. 4. Vascular endothelial function following exposures to prolonged sedentary behaviour. Prolonged sedentary behaviour reduces blood flow and shear stress (b), as compared to a standing position or physical activity (b). This, in turn, increases the production and activity of reactive oxygen species and angiotensin-converting enzyme II, disrupting the balance between endothelin-1 (increased) and nitric oxide (decreased). As a result, vascular smooth muscle relaxation and flow-mediated dilation in the lower limbs is reduced. Metabolites, transporters and/or enzymes in green or red indicate decreased or increased transcription or activity, respectively. Abbreviations: ET-1 = Endothelin-1, NO = Nitric oxide, NADPH = Nicotinamide adenine dinucleotide phosphate, ANGII = Angiotensin II, ROS = Reactive oxygen species, eNOS = Endothelial nitric oxide synthase, PLC = Phospholipase C, PIP_2 = Phosphatidylinositol 4,5-bisphosphate, DAG = Diacylglycerol, IP_3 = Inositol 1,4,5-triphosphate, PKC = Protein kinase C, PKG = Protein kinase G, cGMP = Cyclic guanosine monophosphate, GTP = Guanosine Triphosphate, sGC = Soluble guanylyl cyclase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Figure created with Biorender.

The observed endothelial dysfunction after prolonged sitting and related molecular alterations may contribute to structural remodeling of the vasculature [141]. For example, 25–60 days of bed rest leads to a significant reduction of the conduit arterial lumen size (±16–24 %) [142,143]. It has also been shown that sedentary behaviour may be related to an increased arterial wall thickness, as evidenced by the increase in superficial femoral artery wall thickness after 60 days of bed rest (±14 %) [143]. Interestingly, and with respect to the interdependency between sedentary behaviour and physical activity, it has been suggested that these behaviours differ with regard to the time course of vascular remodeling and changes in endothelial function. Here, Thijssen et al. showed that sedentary behaviour leads to a negative impact on artery structure, whereas at least 4 weeks of physical activity were necessary to induce positive effects [141].

Interestingly, recent studies investigating the effects of prolonged sitting on vascular function found that arterial angulations or 'bending' at the hip and knee joints may be an important contributing factor to sitting induced impairments in macro- and microvascular function [130,144]. In support of this hypothesis, McDaniel et al. demonstrated that passive knee flexion reduced femoral artery blood flow, whereas knee extension caused opposite effects, increasing blood flow by 90 % (125 ml/min) across an 80° range of motion [145]. These findings suggest that sitting-induced arterial bending mimics a similar environment as shown by arterial bifurcations (Fig. 4). It is generally known that arterial bifurcations may induce atherosclerotic plaques due to an unfavourable local haemodynamic environment. This subsequently leads to an imbalance of the vasoactive and vasoconstrictor substances and ROS production. Indeed, it has already been revealed by Walsh et al. that 3 h of prolonged sitting resulted in haemodynamic disturbances in the popliteal artery (FMD −60 %) compared to a straight limb (FMD +25 %) [144]. Therefore, and based on the current available studies, it can be postulated that arterial bending due to prolonged sitting behaviour may induce arterial haemodynamic disruptions, subsequently leading to a transient pro-inflammatory and atherogenic state. This has already been identified by cross-sectional studies in which they showed that spending a lot of time in a sitting position was associated with subclinical atherosclerosis and arterial disease in the lower extremities [146,147].

3. Research gaps and future perspectives

While the epidemiological links between prolonged SB and increased risk of cardiometabolic diseases are well established, the molecular mechanisms driving these associations remain largely unexplored. In particular, there is a lack of evidence regarding how prolonged SB alters key molecular pathways involved in glucose and lipid metabolism (e.g., AMPK and insulin receptor signalling and β-oxidation regulation), mitochondrial function, and systemic inflammation. Additionally, the influence of SB on oxidative stress, endothelial nitric oxide signalling, and the expression of pro-inflammatory cytokines is poorly characterized.

In addition to identifying key molecular pathways affected by prolonged sitting, this review highlights inconsistencies in the existing literature. One possible explanation for these discrepancies is the prevailing assumption in the field of physical activity and sedentary behaviour biology that transient changes in mRNA levels during or after activity reliably reflect changes in protein content [148]. However, the relative contributions of transcriptional, translational, and post-translational mechanisms to protein abundance are often overlooked [149]. To comprehensively investigate the molecular effects of prolonged sitting on these pathways, it is essential to integrate gene expression analysis with proteomic, metabolomic profiling, and enzyme activity. While transcriptomic data provide valuable insights into how sedentary behaviour alters gene regulation (i.e. mRNA levels), these changes do not always translate directly to functional protein abundance or activity. In this respect, proteomics offers a deeper understanding of the actual proteins synthesised and post-translational modification in response to prolonged sedentary behaviour, while metabolomics captures dynamic shifts in cellular metabolism and systemic biochemical pathways. By combining these omics approaches, future research can better map the multi-layered molecular adaptations to prolonged sedentary behaviour, and uncover potential targets for interventions. This systems-level view is crucial for understanding how prolonged sitting contributes to cardiometabolic disease risk.

Moreover, the current body of literature is heavily skewed towards examining acute mechanistic cardiometabolic responses, with few studies investigating chronic molecular adaptations. As a result, in this review, data from different time points (mainly after 10 and/or 50 days of bed rest) were used interchangeably to explain potential underlying molecular mechanisms. However, due to the limited evidence on the timeframe of these molecular changes, further experimental research is needed to determine the specific timeframes in which molecular adaptations to prolonged SB exactly occur.

Since much of the existing evidence is based on models that combine extreme sedentary behaviour with physical inactivity, there is a clear need for future research to adopt models that can isolate the specific effects of sedentary behaviour within real-world contexts. To address this, we propose the use of step reduction protocols (physical activity replaced by sedentary behaviour) and sitting interventions in which sedentary time can be systematically manipulated. These approaches offer a more ecologically valid framework for understanding the interdependent molecular consequences of sedentary behaviour in terms of the frequency, duration, and pattern of sedentary behaviour. Moreover, the dose-response relationship between the duration or pattern of SB and these mechanistic pathways is poorly defined and, therefore, large observational studies in which tissue or liquid biopsies will be taken are necessary. Even more important is to also investigate the underlying molecular mechanisms when sedentary behaviour is interrupted in terms of differences in frequency, intensity, and duration. This would further inform physical activity guidelines.

Furthermore, research has predominantly focused on healthy populations, leaving a lack of mechanistic data in clinical subgroups such as individuals with metabolic syndrome, T2DM, or CVD, as well as potential sex-specific or age-related differences in physiological adaptations, is also underexplored. The absence of comprehensive molecular data constrains the ability to design targeted interventions and translate findings into precise clinical or public health recommendations.

Finally, it would be valuable to investigate the underlying mechanistic effects of prolonged sitting on other organ systems such as kidney function, neurobiological processes, brain function, and adipose tissue metabolism, that may help explain the observed associations between SB and organ-specific health outcomes. Gaining a more comprehensive understanding of these mechanisms is essential to clarify how prolonged sitting contributes to the development of cardiometabolic diseases. Although some studies have already explored these relationships [150,151], further experimental research is needed to elucidate the specific molecular and physiological pathways involved. Therefore, measuring the structure, function, blood flow, and metabolism of these tissues/organs should be included in future well-designed experimental studies using advanced imaging techniques (functional MRI, MR spectroscopy, ultrasound or positron emission tomography) or blood-derived biomarkers.

4. Concluding remarks

Sedentary behaviour is an often-overlooked interdependent behavioural risk factor for the development of many NCDs and all-cause mortality. Adults spend on average between 8 and 12 h a day in sedentary behaviours [15] and, therefore, it is important to further explore the effects of sedentary time on human health in order to unravel the mechanisms behind the associated harmful health effects. This review provides an overview of sedentary behaviour induced changes in

cardiometabolic health and the current available literature with regard to interventions to counteract the detrimental effects of prolonged sitting. Spending much time in sedentary behaviours leads to changes in glucose and lipid metabolism, mitochondrial function, systemic inflammation, redox balance and cardiovascular adaptations. This information could open up new avenues to optimise therapeutic strategies and targets to minimise the detrimental cardiometabolic health effects during prolonged sitting.

CRediT authorship contribution statement

Wouter M.A. Franssen: Supervision, Conceptualization, Writing – review & editing, Writing – original draft. **Ine Nieste:** Conceptualization, Writing – review & editing, Writing – original draft. **Kenneth Verboven:** Conceptualization, Writing – review & editing, Writing – original draft. **Bert O. Eijnde:** Conceptualization, Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] WHO. Global action plan for the prevention and control of noncommunicable diseases 2013–2020. Geneva: World Health Organization; 2013.
[2] Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the global burden of disease study 2013. Lancet 2015;386:743–800.
[3] Collaborators RF. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the global burden of disease study 2016. Lancet (London, England) 2017;390:1345–422.
[4] Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the global burden of disease study 2010. Lancet 2012;380:2224–60.
[5] Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. Br J Sports Med 2020;54:1451–62.
[6] Guthold R, Stevens GA, Riley LM, Bull FC. Worldwide trends in insufficient physical activity from 2001 to 2016: a pooled analysis of 358 population-based surveys with 1·9 million participants. Lancet Glob Health 2018;6:e1077–86.
[7] Knaeps S, Bourgois JG, Charlier R, Mertens E, Lefevre J, Wijndaele K. Ten-year change in sedentary behaviour, moderate-to-vigorous physical activity, cardiorespiratory fitness and cardiometabolic risk: independent associations and mediation analysis. Br J Sports Med 2018;52:1063–8.
[8] Farrahi V, Rostami M, Dumuid D, Chastin SFM, Niemelä M, Korpelainen R, et al. Joint profiles of sedentary time and physical activity in adults and their associations with cardiometabolic health. Med Sci Sports Exerc 2022;54:2118–28.
[9] Tremblay MS, Aubert S, Barnes JD, Saunders TJ, Carson V, Latimer-Cheung AE, et al. Sedentary behavior research network (SBRN) - terminology consensus project process and outcome. Int J Behav Nutr Phys Act 2017;14:75.
[10] van der Berg JD, Stehouwer CD, Bosma H, van der Velde JH, Willems PJ, Savelberg HH, et al. Associations of total amount and patterns of sedentary behaviour with type 2 diabetes and the metabolic syndrome: the Maastricht study. Diabetologia 2016;59:709–18.
[11] Wilmot EG, Edwardson CL, Achana FA, Davies MJ, Gorely T, Gray LJ, et al. Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. Diabetologia 2012;55:2895–905.
[12] Pinto AJ, Bergouignan A, Dempsey PC, Roschel H, Owen N, Gualano B, et al. Physiology of sedentary behavior. Physiol Rev 2023;103:2561–622.
[13] Bergouignan A, Rudwill F, Simon C, Blanc S. Physical inactivity as the culprit of metabolic inflexibility: evidence from bed-rest studies. J Appl Physiol (1985) 2011;111:1201–10.
[14] Morris JN, Heady JA, Raffle PA, Roberts CG, Parks JW. Coronary heart-disease and physical activity of work. Lancet 1953;262:1053–7.
[15] Ekelund U, Tarp J, Steene-Johannessen J, Hansen BH, Jefferis B, Fagerland MW, et al. Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: systematic review and harmonised meta-analysis. BMJ 2019;366:l4570.
[16] Patterson R, McNamara E, Tainio M, de Sá TH, Smith AD, Sharp SJ, et al. Sedentary behaviour and risk of all-cause, cardiovascular and cancer mortality, and incident type 2 diabetes: a systematic review and dose response meta-analysis. Eur J Epidemiol 2018;33:811–29.
[17] Chastin SF, Palarea-Albaladejo J, Dontje ML, Skelton DA. Combined effects of time spent in physical activity, sedentary behaviors and sleep on obesity and cardio-metabolic health markers: a novel compositional data analysis approach. PloS One 2015;10:e0139984.
[18] Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN. Sedentary behavior, exercise, and cardiovascular health. Circ Res 2019;124:799–815.
[19] Paterson C, Fryer S, Zieff G, Stone K, Credeur DP, Barone Gibbs B, et al. The effects of acute exposure to prolonged sitting, with and without interruption, on vascular function among adults: a meta-analysis. Sports Med 2020;50:1929–42.
[20] Biensø RS, Ringholm S, Kiilerich K, Aachmann-Andersen NJ, Krogh-Madsen R, Guerra B, et al. GLUT4 and glycogen synthase are key players in bed rest-induced insulin resistance. Diabetes 2012;61:1090–9.
[21] Eggelbusch M, Charlton BT, Bosutti A, Ganse B, Giakoumaki I, Grootemaat AE, et al. The impact of bed rest on human skeletal muscle metabolism. Cell Rep Med 2024;5:101372.
[22] Stephens BR, Granados K, Zderic TW, Hamilton MT, Braun B. Effects of 1 day of inactivity on insulin action in healthy men and women: interaction with energy intake. Metabolism 2011;60:941–9.
[23] Bilet L, Phielix E, van de Weijer T, Gemmink A, Bosma M, Moonen-Kornips E, et al. One-leg inactivity induces a reduction in mitochondrial oxidative capacity, intramyocellular lipid accumulation and reduced insulin signalling upon lipid infusion: a human study with unilateral limb suspension. Diabetologia 2020;63:1211–22.
[24] Mahmassani ZS, Reidy PT, McKenzie AI, Stubben C, Howard MT, Drummond MJ. Disuse-induced insulin resistance susceptibility coincides with a dysregulated skeletal muscle metabolic transcriptome. J Appl Physiol (1985) 2019;126:1419–29.
[25] Latouche C, Jowett JB, Carey AL, Bertovic DA, Owen N, Dunstan DW, et al. Effects of breaking up prolonged sitting on skeletal muscle gene expression. J Appl Physiol (1985) 2013;114:453–60.
[26] Blanc S, Normand S, Ritz P, Pachiaudi C, Vico L, Gharib C, et al. Energy and water metabolism, body composition, and hormonal changes induced by 42 days of enforced inactivity and simulated weightlessness. J Clin Endocrinol Metab 1998;83:4289–97.
[27] Bergouignan A, Momken I, Schoeller DA, Normand S, Zahariev A, Lescure B, et al. Regulation of energy balance during long-term physical inactivity induced by bed rest with and without exercise training. J Clin Endocrinol Metab 2010;95:1045–53.
[28] Ekelund U, Steene-Johannessen J, Brown WJ, Fagerland MW, Owen N, Powell KE, et al. Does physical activity attenuate, or even eliminate, the detrimental association of sitting time with mortality? A harmonised meta-analysis of data from more than 1 million men and women. Lancet (London, England) 2016;388:1302–10.
[29] Bellini A, Nicolò A, Bazzucchi I, Sacchetti M. Effects of different exercise strategies to improve postprandial glycemia in healthy individuals. Med Sci Sports Exerc 2021;53:1334–44.
[30] Dunstan DW, Howard B, Healy GN, Owen N. Too much sitting—a health hazard. Diabetes Res Clin Pract 2012;97:368–76.
[31] Edwardson CL, Gorely T, Davies MJ, Gray LJ, Khunti K, Wilmot EG, et al. Association of sedentary behaviour with metabolic syndrome: a meta-analysis. PloS One 2012;7:e34916.
[32] Alibegovic AC, Sonne MP, Højbjerre L, Bork-Jensen J, Jacobsen S, Nilsson E, et al. Insulin resistance induced by physical inactivity is associated with multiple transcriptional changes in skeletal muscle in young men. Am J Physiol Endocrinol Metab 2010;299:E752–63.
[33] Pillon NJ, Gabriel BM, Dollet L, Smith JAB, Sardón Puig L, Botella J, et al. Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. Nat Commun 2020;11:470.
[34] Nair KS. Aging muscle. Am J Clin Nutr 2005;81:953–63.
[35] Blanc S, Normand S, Pachiaudi C, Fortrat JO, Laville M, Gharib C. Fuel homeostasis during physical inactivity induced by bed rest. J Clin Endocrinol Metab 2000;85:2223–33.
[36] Hamburg NM, McMackin CJ, Huang AL, Shenouda SM, Widlansky ME, Schulz E, et al. Physical inactivity rapidly induces insulin resistance and microvascular dysfunction in healthy volunteers. Arterioscler Thromb Vasc Biol 2007;27:2650–6.
[37] Mikines KJ, Richter EA, Dela F, Galbo H. Seven days of bed rest decrease insulin action on glucose uptake in leg and whole body. J Appl Physiol 1991;70:1245–54.
[38] Lipman RL, Raskin P, Love T, Triebwasser J, Lecocq FR, Schnure JJ. Glucose intolerance during decreased physical activity in man. Diabetes 1972;21:101–7.
[39] Lyden K, Keadle SK, Staudenmayer J, Braun B, Freedson PS. Discrete features of sedentary behavior impact cardiometabolic risk factors. Med Sci Sports Exerc 2015;47:1079–86.
[40] Nieste I, Franssen WMA, Duvivier B, Spaas J, Savelberg H, Eijnde BO. Replacing sitting with light-intensity physical activity throughout the day versus 1 bout of vigorous-intensity exercise: similar cardiometabolic health effects in multiple sclerosis. A randomised cross-over study. Disabil Rehabil 2023;45:3293–302.
[41] Alibegovic AC, Højbjerre L, Sonne MP, van Hall G, Stallknecht B, Dela F, et al. Impact of 9 days of bed rest on hepatic and peripheral insulin action, insulin secretion, and whole-body lipolysis in healthy young male offspring of patients with type 2 diabetes. Diabetes 2009;58:2749–56.

[42] Irimia JM, Guerrero M, Rodriguez-Miguelez P, Cadefau JA, Tesch PA, Cussó R, et al. Metabolic adaptations in skeletal muscle after 84 days of bed rest with and without concurrent flywheel resistance exercise. J Appl Physiol (1985) 2017;122:96–103.
[43] Pearen MA, Goode JM, Fitzsimmons RL, Eriksson NA, Thomas GP, Cowin GJ, et al. Transgenic muscle-specific Nor-1 expression regulates multiple pathways that effect adiposity, metabolism, and endurance. Mol Endocrinol 2013;27:1897–917.
[44] Fu Y, Luo L, Luo N, Zhu X, Garvey WT. NR4A orphan nuclear receptors modulate insulin action and the glucose transport system: potential role in insulin resistance. J Biol Chem 2007;282:31525–33.
[45] Peddie MC, Bone JL, Rehrer NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. Am J Clin Nutr 2013;98:358–66.
[46] Patti ME. Nutrient modulation of cellular insulin action. Ann N Y Acad Sci 1999;892:187–203.
[47] Krebs M, Roden M. Nutrient-induced insulin resistance in human skeletal muscle. Curr Med Chem 2004;11:901–8.
[48] Vernikos-Danellis J, Leach CS, Winget CM, Goodwin AL, Rambaut PC. Changes in glucose, insulin, and growth hormone levels associated with bedrest. Aviat Space Environ Med 1976;47:583–7.
[49] Handschin C, Spiegelman BM. The role of exercise and PGC1alpha in inflammation and chronic disease. Nature 2008;454:463–9.
[50] Sullivan MJ, Binkley PF, Unverferth DV, Ren JH, Boudoulas H, Bashore TM, et al. Prevention of bedrest-induced physical deconditioning by daily dobutamine infusions. Implications for drug-induced physical conditioning. J Clin Invest 1985;76:1632–42.
[51] Fernandez-Gonzalo R, Irimia JM, Cusso R, Gustafsson T, Linné A, Tesch PA. Flywheel resistance exercise to maintain muscle oxidative potential during unloading. Aviat Space Environ Med 2014;85:694–9.
[52] Brocca L, Cannavino J, Coletto L, Biolo G, Sandri M, Bottinelli R, et al. The time course of the adaptations of human muscle proteome to bed rest and the underlying mechanisms. J Physiol 2012;590:5211–30.
[53] Shur NF, Simpson EJ, Crossland H, Chivaka PK, Constantin D, Cordon SM, et al. Human adaptation to immobilization: novel insights of impacts on glucose disposal and fuel utilization. J Cachexia Sarcopenia Muscle 2022;13:2999–3013.
[54] Kim JA, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. Circ Res 2008;102:401–14.
[55] Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ 2006;30:145–51.
[56] Safdar A, Hamadeh MJ, Kaczor JJ, Raha S, Debeer J, Tarnopolsky MA. Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. PloS One 2010;5:e10778.
[57] Ringholm S, Biensø RS, Kiilerich K, Guadalupe-Grau A, Aachmann-Andersen NJ, Saltin B, et al. Bed rest reduces metabolic protein content and abolishes exercise-induced mRNA responses in human skeletal muscle. Am J Physiol Endocrinol Metab 2011;301:E649–58.
[58] Moriggi M, Vasso M, Fania C, Capitanio D, Bonifacio G, Salanova M, et al. Long term bed rest with and without vibration exercise countermeasures: effects on human muscle protein dysregulation. Proteomics 2010;10:3756–74.
[59] Theeuwes WF, Gosker HR, Schols A, Langen RCJ, Remels AHV. Regulation of PGC-1α expression by a GSK-3β-TFEB signaling axis in skeletal muscle. Biochim Biophys Acta Mol Cell Res 2020;1867:118610.
[60] Rimbert V, Boirie Y, Bedu M, Hocquette JF, Ritz P, Morio B. Muscle fat oxidative capacity is not impaired by age but by physical inactivity: association with insulin sensitivity. FASEB J 2004;18:737–9.
[61] Pearen MA, Muscat GEO. The nuclear receptor Nor-1 is a pleiotropic regulator of exercise-induced adaptations. Exerc Sport Sci Rev 2018;46:97–104.
[62] Darragh J, Soloaga A, Beardmore VA, Wingate AD, Wiggin GR, Peggie M, et al. MSKs are required for the transcription of the nuclear orphan receptors Nur77, Nurr1 and Nor1 downstream of MAPK signalling. Biochem J 2005;390:749–59.
[63] Pearen MA, Myers SA, Raichur S, Ryall JG, Lynch GS, Muscat GE. The orphan nuclear receptor, NOR-1, a target of beta-adrenergic signaling, regulates gene expression that controls oxidative metabolism in skeletal muscle. Endocrinology 2008;149:2853–65.
[64] Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, et al. Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J Biol Chem 2005;280:19587–93.
[65] Pattamaprapanont P, Garde C, Fabre O, Barrès R. Muscle contraction induces acute hydroxymethylation of the exercise-responsive gene nr4a3. Front Endocrinol (Lausanne) 2016;7:165.
[66] James DE, Stöckli J, Birnbaum MJ. The aetiology and molecular landscape of insulin resistance. Nat Rev Mol Cell Biol 2021;22:751–71.
[67] Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes 2002;51:2005–11.
[68] Petibois C, Cassaigne A, Gin H, Déléris G. Lipid profile disorders induced by long-term cessation of physical activity in previously highly endurance-trained subjects. J Clin Endocrinol Metab 2004;89:3377–84.
[69] Slentz CA, Houmard JA, Johnson JL, Bateman LA, Tanner CJ, McCartney JS, et al. Inactivity, exercise training and detraining, and plasma lipoproteins. STRRIDE: a randomized, controlled study of exercise intensity and amount. J Appl Physiol (1985) 2007;103:432–42.
[70] Zderic TW, Hamilton MT. Physical inactivity amplifies the sensitivity of skeletal muscle to the lipid-induced downregulation of lipoprotein lipase activity. J Appl Physiol (1985) 2006;100:249–57.
[71] Miyashita M, Park JH, Takahashi M, Suzuki K, Stensel D, Nakamura Y. Postprandial lipaemia: effects of sitting, standing and walking in healthy normolipidaemic humans. Int J Sports Med 2013;34:21–7.
[72] Crichton GE, Alkerwi A. Physical activity, sedentary behavior time and lipid levels in the observation of cardiovascular risk factors in Luxembourg study. Lipids Health Dis 2015;14:87.
[73] Yanagibori R, Kondo K, Suzuki Y, Kawakubo K, Iwamoto T, Itakura H, et al. Effect of 20 days' bed rest on the reverse cholesterol transport system in healthy young subjects. J Intern Med 1998;243:307–12.
[74] Olsen RH, Krogh-Madsen R, Thomsen C, Booth FW, Pedersen BK. Metabolic responses to reduced daily steps in healthy nonexercising men. Jama 2008;299:1261–3.
[75] Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest 2007;117:1690–8.
[76] Bergman BC, Perreault L, Hunerdosse DM, Koehler MC, Samek AM, Eckel RH. Increased intramuscular lipid synthesis and low saturation relate to insulin sensitivity in endurance-trained athletes. J Appl Physiol (1985) 2010;108:1134–41.
[77] Rector RS, Thyfault JP. Does physical inactivity cause nonalcoholic fatty liver disease? J Appl Physiol (1985) 2011;111:1828–35.
[78] Sattar N, Scherbakova O, Ford I, O'Reilly DS, Stanley A, Forrest E, et al. Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. Diabetes 2004;53:2855–60.
[79] Bergouignan A, Trudel G, Simon C, Chopard A, Schoeller DA, Momken I, et al. Physical inactivity differentially alters dietary oleate and palmitate trafficking. Diabetes 2009;58:367–76.
[80] Bergouignan A, Momken I, Schoeller DA, Simon C, Blanc S. Metabolic fate of saturated and monounsaturated dietary fats: the Mediterranean diet revisited from epidemiological evidence to cellular mechanisms. Prog Lipid Res 2009;48:128–47.
[81] Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 2004;86:839–48.
[82] Bergouignan A, Schoeller DA, Normand S, Gauquelin-Koch G, Laville M, Shriver T, et al. Effect of physical inactivity on the oxidation of saturated and monounsaturated dietary fatty acids: results of a randomized trial. PLoS Clin Trials 2006;1:e27.
[83] Lammers G, Poelkens F, van Duijnhoven NT, Pardoel EM, Hoenderop JG, Thijssen DH, et al. Expression of genes involved in fatty acid transport and insulin signaling is altered by physical inactivity and exercise training in human skeletal muscle. Am J Physiol Endocrinol Metab 2012;303:E1245–51.
[84] Chopard A, Lecunff M, Danger R, Lamirault G, Bihouee A, Teusan R, et al. Large-scale mRNA analysis of female skeletal muscles during 60 days of bed rest with and without exercise or dietary protein supplementation as countermeasures. Physiol Genomics 2009;38:291–302.
[85] Jones SF, Infante JR. Molecular pathways: fatty acid synthase. Clin Cancer Res 2015;21:5434–8.
[86] Peluso G, Petillo O, Margarucci S, Mingrone G, Greco AV, Indiveri C, et al. Decreased mitochondrial carnitine translocase in skeletal muscles impairs utilization of fatty acids in insulin-resistant patients. Front Biosci 2002;7:a109–16.
[87] Borén J, Packard CJ, Taskinen M-R. The roles of ApoC-III on the metabolism of triglyceride-rich lipoproteins in humans. Front Endocrinol 2020;11.
[88] Duvivier BM, Schaper NC, Bremers MA, van Crombrugge G, Menheere PP, Kars M, et al. Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable. PloS One 2013;8:e55542.
[89] Bey L, Hamilton MT. Suppression of skeletal muscle lipoprotein lipase activity during physical inactivity: a molecular reason to maintain daily low-intensity activity. J Physiol 2003;551:673–82.
[90] Hamilton MT, Hamilton DG, Zderic TW. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. Diabetes 2007;56:2655–67.
[91] Panarotto D, Rémillard P, Bouffard L, Maheux P. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. Eur J Clin Invest 2002;32:84–92.
[92] Hamilton MT, Hamilton DG, Zderic TW. Exercise physiology versus inactivity physiology: an essential concept for understanding lipoprotein lipase regulation. Exerc Sport Sci Rev 2004;32:161–6.
[93] Bartels ED, Ploug T, Størling J, Mandrup-Poulsen T, Nielsen LB. Skeletal muscle apolipoprotein B expression reduces muscular triglyceride accumulation. Scand J Clin Lab Invest 2014;74:351–7.
[94] Chen YW, Gregory CM, Scarborough MT, Shi R, Walter GA, Vandenborne K. Transcriptional pathways associated with skeletal muscle disuse atrophy in humans. Physiol Genomics 2007;31:510–20.
[95] Liu Z, Chang GQ, Leibowitz SF. Apolipoprotein D interacts with the long-form leptin receptor: a hypothalamic function in the control of energy homeostasis. FASEB J 2001;15:1329–31.
[96] Belavý DL, Möhlig M, Pfeiffer AF, Felsenberg D, Armbrecht G. Preferential deposition of visceral adipose tissue occurs due to physical inactivity. Int J Obes (Lond) 2014;38:1478–80.
[97] Damiot A, Demangel R, Noone J, Chery I, Zahariev A, Normand S, et al. A nutrient cocktail prevents lipid metabolism alterations induced by 20 days of daily steps

reduction and fructose overfeeding: result from a randomized study. J Appl Physiol (1985) 2019;126:88–101.
[98] Dalla Libera L, Ravara B, Gobbo V, Tarricone E, Vitadello M, Biolo G, et al. A transient antioxidant stress response accompanies the onset of disuse atrophy in human skeletal muscle. J Appl Physiol (1985) 2009;107:549–57.
[99] Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF-κB signaling. Cell Res 2011;21:103–15.
[100] Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, et al. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 2018;233:6425–40.
[101] Emanuela F, Grazia M, Marco DR, Maria Paola L, Giorgio F, Marco B. Inflammation as a link between obesity and metabolic syndrome. J Nutr Metab 2012;2012:476380.
[102] Lee YS, Olefsky J. Chronic tissue inflammation and metabolic disease. Genes Dev 2021;35:307–28.
[103] Bosutti A, Malaponte G, Zanetti M, Castellino P, Heer M, Guarnieri G, et al. Calorie restriction modulates inactivity-induced changes in the inflammatory markers C-reactive protein and pentraxin-3. J Clin Endocrinol Metab 2008;93:3226–9.
[104] Rodas L, Riera-Sampol A, Aguilo A, Martínez S, Tauler P. Effects of habitual caffeine intake, physical activity levels, and sedentary behavior on the inflammatory status in a healthy population. Nutrients 2020;12.
[105] Allison MA, Jensky NE, Marshall SJ, Bertoni AG, Cushman M. Sedentary behavior and adiposity-associated inflammation: the multi-ethnic study of atherosclerosis. Am J Prev Med 2012;42:8–13.
[106] Falconer CL, Cooper AR, Walhin JP, Thompson D, Page AS, Peters TJ, et al. Sedentary time and markers of inflammation in people with newly diagnosed type 2 diabetes. Nutr Metab Cardiovasc Dis 2014;24:956–62.
[107] Henson J, Yates T, Edwardson CL, Khunti K, Talbot D, Gray LJ, et al. Sedentary time and markers of chronic low-grade inflammation in a high risk population. PloS One 2013;8:e78350.
[108] Reidy PT, Lindsay CC, McKenzie AI, Fry CS, Supiano MA, Marcus RL, et al. Aging-related effects of bed rest followed by eccentric exercise rehabilitation on skeletal muscle macrophages and insulin sensitivity. Exp Gerontol 2018;107:37–49.
[109] Van der Cappellen EJ, Koster A, Savelberg H, Eussen S, Dagnelie PC, Schaper NC, et al. Sedentary behaviour and physical activity are associated with biomarkers of endothelial dysfunction and low-grade inflammation-relevance for (pre)diabetes: the Maastricht study. Diabetologia 2022;65:777–89.
[110] Watt MJ, Hevener A, Lancaster GI, Febbraio MA. Ciliary neurotrophic factor prevents acute lipid-induced insulin resistance by attenuating ceramide accumulation and phosphorylation of c-Jun N-terminal kinase in peripheral tissues. Endocrinology 2006;147:2077–85.
[111] Hirosumi J, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, et al. A central role for JNK in obesity and insulin resistance. Nature 2002;420:333–6.
[112] Xourafa G, Korbmacher M, Roden M. Inter-organ crosstalk during development and progression of type 2 diabetes mellitus. Nat Rev Endocrinol 2023;20:27–49.
[113] Alfaddagh A, Martin SS, Leucker TM, Michos ED, Blaha MJ, Lowenstein CJ, et al. Inflammation and cardiovascular disease: from mechanisms to therapeutics. Am J Prev Cardiol 2020;4:100130.
[114] Moholdt T, Skarpsno ES, Moe B, Nilsen TIL. It is never too late to start: adherence to physical activity recommendations for 11-22 years and risk of all-cause and cardiovascular disease mortality. The HUNT study. Br J Sports Med 2020;55:743–50.
[115] Healy GN, Dunstan DW, Salmon J, Shaw JE, Zimmet PZ, Owen N. Television time and continuous metabolic risk in physically active adults. Med Sci Sports Exerc 2008;40:639–45.
[116] Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. Ann Intern Med 2015;162:123–32.
[117] Stamatakis E, Gale J, Bauman A, Ekelund U, Hamer M, Ding D. Sitting time, physical activity, and risk of mortality in adults. J Am Coll Cardiol 2019;73:2062–72.
[118] Pandey A, Salahuddin U, Garg S, Ayers C, Kulinski J, Anand V, et al. Continuous dose-response association between sedentary time and risk for cardiovascular disease: a meta-analysis. JAMA Cardiol 2016;1:575–83.
[119] Liang ZD, Zhang M, Wang CZ, Yuan Y, Liang JH. Association between sedentary behavior, physical activity, and cardiovascular disease-related outcomes in adults-a meta-analysis and systematic review. Front Public Health 2022;10:1018460.
[120] Pekas EJ, Allen MF, Park SY. Prolonged sitting and peripheral vascular function: potential mechanisms and methodological considerations. J Appl Physiol (1985) 2023;134:810–22.
[121] Bowden Davies KA, Norman JA, Thompson A, Mitchell KL, Harrold JA, Halford JCG, et al. Short-term physical inactivity induces endothelial dysfunction. Front Physiol 2021;12:659834.
[122] Higgins S, Pomeroy A, Bates LC, Paterson C, Barone Gibbs B, Pontzer H, et al. Sedentary behavior and cardiovascular disease risk: an evolutionary perspective. Front Physiol 2022;13:962791.
[123] Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, Padilla J. Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. Am J Physiol Heart Circ Physiol 2016;311:H177–82.
[124] Morishima T, Restaino RM, Walsh LK, Kanaley JA, Padilla J. Prior exercise and standing as strategies to circumvent sitting-induced leg endothelial dysfunction. Clin Sci (Lond) 2017;131:1045–53.
[125] Restaino RM, Holwerda SW, Credeur DP, Fadel PJ, Padilla J. Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function. Exp Physiol 2015;100:829–38.
[126] Thosar SS, Bielko SL, Mather KJ, Johnston JD, Wallace JP. Effect of prolonged sitting and breaks in sitting time on endothelial function. Med Sci Sports Exerc 2015;47:843–9.
[127] Thosar SS, Bielko SL, Wiggins CC, Wallace JP. Differences in brachial and femoral artery responses to prolonged sitting. Cardiovasc Ultrasound 2014;12:50.
[128] Credeur DP, Miller SM, Jones R, Stoner L, Dolbow DR, Fryer SM, et al. Impact of prolonged sitting on peripheral and central vascular health. Am J Cardiol 2019;123:260–6.
[129] Park SY, Wooden TK, Pekas EJ, Anderson CP, Yadav SK, Slivka DR, et al. Effects of passive and active leg movements to interrupt sitting in mild hypercapnia on cardiovascular function in healthy adults. J Appl Physiol 2022;132:874–87.
[130] Padilla J, Fadel PJ. Prolonged sitting leg vasculopathy: contributing factors and clinical implications. Am J Physiol Heart Circ Physiol 2017;313:H722–h8.
[131] Di Francescomarino S, Sciartilli A, Di Valerio V, Di Baldassarre A, Gallina S. The effect of physical exercise on endothelial function. Sports Med 2009;39:797–812.
[132] Morawietz H, Talanow R, Szibor M, Rueckschloss U, Schubert A, Bartling B, et al. Regulation of the endothelin system by shear stress in human endothelial cells. J Physiol 2000;525(Pt 3):761–70.
[133] Bestle MH, Norsk P, Bie P. Fluid volume and osmoregulation in humans after a week of head-down bed rest. Am J Physiol Regul Integr Comp Physiol 2001;281:R310–7.
[134] Haruna Y, Bonde-Petersen F, Takenaka K, Suzuki Y, Kawakubo K, Gunji A. Effects of the renin-angiotensin-aldosterone system on the cardiovascular system during 20-days bed rest. J Gravit Physiol 1997;4:S62–8.
[135] Maeda S, Miyauchi T, Kakiyama T, Sugawara J, Iemitsu M, Irukayama-Tomobe Y, et al. Effects of exercise training of 8 weeks and detraining on plasma levels of endothelium-derived factors, endothelin-1 and nitric oxide, in healthy young humans. Life Sci 2001;69:1005–16.
[136] Chao Y, Ye P, Zhu L, Kong X, Qu X, Zhang J, et al. Low shear stress induces endothelial reactive oxygen species via the AT1R/eNOS/NO pathway. J Cell Physiol 2018;233:1384–95.
[137] Laufs U, Wassmann S, Czech T, Münzel T, Eisenhauer M, Böhm M, et al. Physical inactivity increases oxidative stress, endothelial dysfunction, and atherosclerosis. Arterioscler Thromb Vasc Biol 2005;25:809–14.
[138] Carter S, Hartman Y, Holder S, Thijssen DH, Hopkins ND. Sedentary behavior and cardiovascular disease risk: mediating mechanisms. Exerc Sport Sci Rev 2017;45:80–6.
[139] Dunstan DW, Dogra S, Carter SE, Owen N. Sit less and move more for cardiovascular health: emerging insights and opportunities. Nat Rev Cardiol 2021;18:637–48.
[140] Vranish JR, Young BE, Stephens BY, Kaur J, Padilla J, Fadel PJ. Brief periods of inactivity reduce leg microvascular, but not macrovascular, function in healthy young men. Exp Physiol 2018;103:1425–34.
[141] Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT, Green DJ. Impact of inactivity and exercise on the vasculature in humans. Eur J Appl Physiol 2010;108:845–75.
[142] Bleeker MW, De Groot PC, Rongen GA, Rittweger J, Felsenberg D, Smits P, et al. Vascular adaptation to deconditioning and the effect of an exercise countermeasure: results of the Berlin Bed Rest study. J Appl Physiol (1985) 2005;99:1293–300.
[143] van Duijnhoven NT, Green DJ, Felsenberg D, Belavy DL, Hopman MT, Thijssen DH. Impact of bed rest on conduit artery remodeling: effect of exercise countermeasures. Hypertension 2010;56:240–6.
[144] Walsh LK, Restaino RM, Martinez-Lemus LA, Padilla J. Prolonged leg bending impairs endothelial function in the popliteal artery. Physiol Rep 2017;5.
[145] McDaniel J, Ives SJ, Richardson RS. Human muscle length-dependent changes in blood flow. J Appl Physiol (1985) 2012;112:560–5.
[146] Lim SS, Huang CC, Hsu PF, Lin CC, Wang YJ, Ding YZ, et al. Prolonged sitting time links to subclinical atherosclerosis. J Chin Med Assoc 2022;85:51–8.
[147] Asfour M, Baskovski E, Esenboğa K, Kumbasar D. Association between lower extremity arterial disease and various sitting positions. Anatol J Cardiol 2022;26:180–8.
[148] Bishop DJ, Hawley JA. Reassessing the relationship between mRNA levels and protein abundance in exercised skeletal muscles. Nat Rev Mol Cell Biol 2022;23:773–4.
[149] Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 2012;13:227–32.
[150] Volaklis K, Mamadjanov T, Meisinger C. Sedentary behavior and kidney function in adults: a narrative review. Wien Klin Wochenschr 2021;133:144–52.
[151] Zou L, Herold F, Cheval B, Wheeler MJ, Pindus DM, Erickson KI, et al. Sedentary behavior and lifespan brain health. Trends Cogn Sci 2024;28:369–82.