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## Combating osteoporosis and obesity with exercise: leveraging cell mechanosensitivity

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### Abstract

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Osteoporosis, a condition of skeletal decline that undermines quality of life, is treated with pharmacological interventions that are associated with poor adherence and adverse effects. Complicating efforts to improve clinical outcomes, the incidence of obesity is increasing, predisposing the population to a range of musculoskeletal complications and metabolic disorders. Pharmacological management of obesity has yet to deliver notable reductions in weight and debilitating complications are rarely avoided. By contrast, exercise shows promise as a non-invasive and non-pharmacological method of regulating both osteoporosis and obesity. The principal components of exercise — mechanical signals — promote bone and muscle anabolism while limiting formation and expansion of fat mass. Mechanical regulation of bone and marrow fat might be achieved by regulating functions of differentiated cells in the skeletal tissue while biasing lineage selection of their common progenitors — mesenchymal stem cells. An inverse relationship between adipocyte versus osteoblast fate selection from stem cells is implicated in clinical conditions such as childhood obesity and increased marrow adiposity in type 2 diabetes mellitus, as well as contributing to skeletal frailty. Understanding how exercise-induced mechanical signals can be used to improve bone quality while decreasing fat mass and metabolic dysfunction should lead to new strategies to treat chronic diseases such as osteoporosis and obesity.

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The evolution of *Homo sapiens* from hunters to farmers, and then from agrarian cultures to the industrial era<sup>1,2</sup>, drove profound adaptations to our skeletal phenotype, including a decline in bone quantity and quality<sup>3</sup>. In parallel, as food has become more accessible and lifestyles less physically demanding, the composition of the human body has shifted towards increased body fat and reduced lean tissue mass, which is partly a consequence of a vestigial survival strategy that stockpiles calories when food is available<sup>4</sup>. The post-Industrial Revolution era and the sedentary lifestyle that it has promoted has fostered two major diseases — osteoporosis and obesity<sup>5-7</sup>. Basic, applied and translational sciences emphasize that these diseases can be managed by reintroducing physical activity into our lives<sup>8</sup>. Exercise, at a minimum, provides additive benefits to

pharmacological interventions to improve bone quality and reduce fat mass, and is frequently recommended to those capable of strenuous activity and sustaining high-magnitude loading. However, intense physical activity is often not achievable in ageing populations and for those with underlying musculoskeletal or metabolic conditions for whom exercise is simply not possible or could be dangerous. Alternative approaches that incorporate mechanical stimuli without the need to run a marathon or compete on a football pitch are being studied for clinical application in these less-mobile populations.

Osteoporosis, which is defined as decreased bone quantity and quality, has multiple aetiologies, ranging from age-related conditions (postmenopausal and ageing physiology) to genetic causes (for example, mutations that result in WNT1 deficiency), endocrine or disease-specific treatment modifiers (for example, glucocorticosteroids and aromatase inhibitors) and unloading (bed rest or paraplegia). Statistically, osteoporosis primarily affects postmenopausal women and elderly men, with 30% of women and 20% of men >50 years old predicted to experience an osteoporosis-related fracture in their lifetime<sup>9,10</sup>. Age-driven shifts in hormone status<sup>11-13</sup>, compounded by reduced physical activity, disrupt balanced bone remodelling, leading to elevated bone resorption and suppressed bone formation. The risk of fracture in the ageing population is further compounded by muscle wasting, or sarcopenia, which contributes to muscle weakness and decreased mobility, as well as an increase in instability that precipitates the risk of falls<sup>14</sup>. Although pharmaceutical approaches for sarcopenia have yet to be approved for use, interventions targeting osteoporosis have been well studied and demonstrate beneficial effects on fracture risk<sup>15</sup>. However, adverse effects<sup>16</sup> can accrue during long-term use of osteoporosis therapeutics<sup>17</sup>, and a lack of patient adherence to the drug therapy can occur<sup>18,19</sup>. In addition, poor patient adherence with standard osteoporotic recommendations<sup>20</sup> continues to be a barrier to effective treatment in our ageing populations.

Equally disconcerting, almost 40% of adults and nearly 20% of adolescents in the United States have obesity, with a continuing upwards trend: in the past 50 years, the prevalence of obesity has risen by 27% in adults and 47% among children<sup>21,22</sup>, which has been promoted by sedentary lifestyles and poor nutrition. Obesity markedly increases susceptibility to a range of associated diseases (for example, type 2 diabetes mellitus<sup>23</sup> and cardiovascular disease<sup>24</sup>), physical limitations (such as immobility<sup>25</sup> and atypical gait<sup>26</sup>) and chronic inflammation (for example, osteoarthritis<sup>27-29</sup>). Obesity not only increases the risk of many solid tumours<sup>30-32</sup> but also promotes cancer metastases<sup>33</sup>. Compounding these problems, accrual of adipose tissue within the bone marrow space can lead to an inflammatory state<sup>34</sup>, which increases bone resorption, disrupts differentiation of mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs)<sup>33</sup> and undermines regenerative and immune responses<sup>35,36</sup>. Decreasing adipose tissue mass or regulating the functions of the adipocytes in the bone marrow might present a target for controlling both bone quality and inflammation, which is of great interest for developing new therapeutic strategies<sup>37</sup>.

Weight-bearing exercise is a cornerstone in the treatment and prevention of postmenopausal and age-associated osteoporosis. The National Osteoporosis Foundation recommends skeletal loading with both high-impact and low-impact weight-bearing exercises for at least 30 min per day, 5-7 days a week<sup>38</sup>. Importantly, MSCs, the shared progenitor for bone and adipose cells, seem to be key to the inverse control of cell output, interpreting mechanical signals as stimulatory for bone

and inhibitory for adipose<sup>39</sup>. Furthermore, muscle strengthening exercises are now recommended as complimentary to weight-bearing exercises to improve posture, reduce fall risk and promote musculoskeletal anabolism<sup>8</sup>. The dynamic ground-reaction forces generated during exercise transduce a range of signals across the skeleton and musculature, subjecting cells, tissues and organs to mechanical strain (deformation) and acceleration. Key beneficial outcomes of exercise include increased lean muscle mass, increased bone mineralization and turnover rate and decreased systemic inflammation.

A central tenet of this Review is that key regulatory signals are generated during exercise and that these factors are first and foremost mechanical in nature. Thus, a goal of this Review is to discuss how these ubiquitous signals arising from activity are first perceived by the cell population and then how the cells respond to them, with particular emphasis on the musculoskeletal and adipose systems. In addition, how metabolic and genetic disorders, as well as ageing, can disrupt this process is addressed. Finally, we consider how surrogates for exercise might serve to treat these conditions.

## Mechanical influences on bone

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### Bone adaptation to physiological extremes.

Bone mass and architecture are placed at risk by disuse<sup>40</sup> but can respond to exercise with increased mass and strength, leading to the ‘use it or lose it’ tenet of bone adaptation that is often referred to as Wolff’s law<sup>41</sup>. Retrospective studies illustrate the response of bone to physical extremes. Astronauts enduring microgravity lose as much as 2% of their hip BMD each month<sup>42</sup>, whereas professional tennis players have up to 35% more bone hypertrophy in the serving arm than in the arm that simply tosses the ball into the air<sup>43</sup>. Furthermore, several site-specific benefits correlate with the specialized tasks of elite sportspeople trained over extended periods<sup>44</sup>, where enhancement of bone morphology is greater in athletes challenged with intense impact training (for example, football and gymnastics) than in those engaged in ‘smoother’ sports such as cycling or swimming<sup>45</sup>. What is also clear is that commitment to exercise early in life will maximize potential gains, with strong correlations between physical activity and bone strength being evident from childhood to early adulthood<sup>46</sup>.

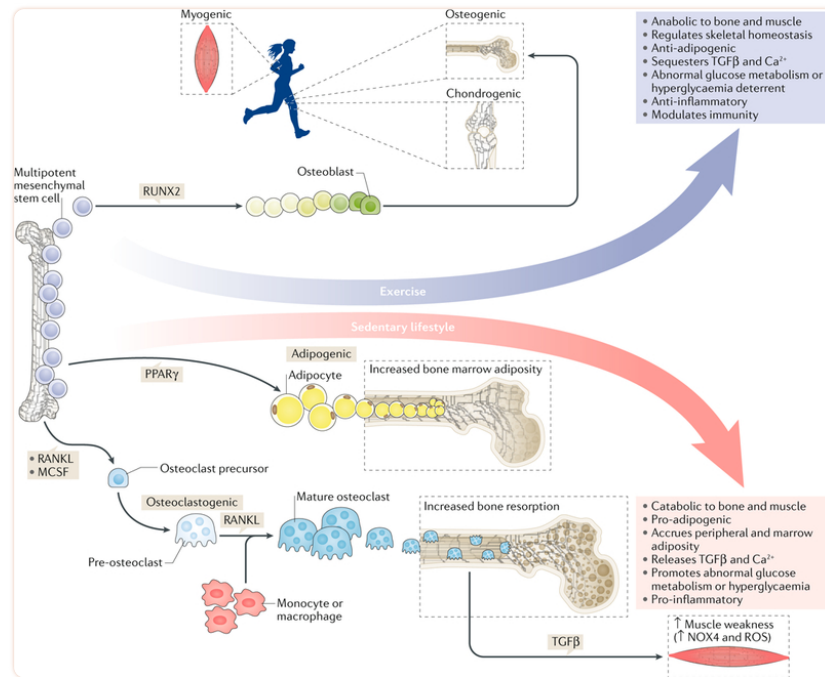
### Response to new exercise regimens.

The results of several prospective trials indicate that new loading challenges can induce system-level and site-specific accretions of bone mass. Intense exercise in young army recruits stimulated increases in BMD<sup>47</sup>, and a 10-month, high-impact strength-building regimen in children significantly (1.9% versus 3.8%,  $P = 0.002$ ) increased femoral neck BMD<sup>48</sup>. Despite the apparent anabolic nature of the mechanical signal, moderate exercise regimens generally result in modest (if any) increases in bone mass; for example, a 1-year high-resistance strength-training study in young women (mean age of  $23.8 \pm 5.0$  years) significantly increased muscle strength (14%,  $P = 0.001$ ) but failed to influence bone mass<sup>49</sup>. By contrast, high-intensity resistance plus impact train-

ing improved both BMD and physical function in postmenopausal women with osteoporosis<sup>8</sup>. The inherent complexity of exercise-generated mechanical challenges for skeletal tissues<sup>50</sup> indicates that some components of the load-bearing regimen might be more influential than others<sup>51,52</sup>.

### Mechanisms.

The ability of mechanical signals to increase musculoskeletal mass and quality is multifactorial, as it involves simultaneously repressing pathways involved in the formation of adipose tissue and the resorption of bone<sup>53,54</sup> (FIG. 1). Exercise exerts a range of forces across the appendicular and axial skeleton<sup>55</sup>; therefore, the musculoskeletal construct acts as a conduit to transduce both peaks of ground-reaction forces and the spectral content of muscle contraction, bombarding the bone tissue with both high-frequency and low-frequency mechanical signals. Transmitted through tissues to the cellular level, mechanical responses are mediated through cytoskeletal proteins and transmembrane-bound integrins that link the extracellular environment with the genetic machinery encased within the nucleus<sup>56-61</sup>. Propagation of mechanical signals along the WNT- $\beta$ -catenin pathway enhances osteogenic<sup>62,63</sup> (*RUNX2*, encoding Runt-related transcription factor 2) gene expression and chondrogenic (*SOX9*) growth while arresting adipogenesis<sup>64</sup>. By increasing both muscle and bone mass and strength, exercise succeeds in reducing the incidence of bone fracture (reduction in falls and improvement of fracture strength)<sup>65</sup>, achieved to a degree by upregulating the expression of osteogenic, chondrogenic or myogenic<sup>66</sup> growth factors while pathways conducive to PPAR $\gamma$ -driven adipogenesis are downregulated<sup>64,67-70</sup>.



[Fig. 1](#)

**Exercise and mechanical signals are anabolic to skeletal tissue and muscle and slow excessive bone resorption, counteracting the negative effects of a high-fat diet and sedentary lifestyle on bone and fat.**

Mesenchymal stem cell lineage selection as a function of mechanical signals drives osteogenic differentiation. Here, exercise is directly responsible for augmenting bone and muscle mass while suppressing the accumulation of fat. By contrast, sedentary lifestyles (the absence of mechanical stimuli, often accompanied by poor diet) increase adipogenic programmes, resulting in increased marrow adiposity that can obstruct the persistence of bone-remodelling cells. In the absence of mechanical load, osteoclast-mediated resorption is accelerated, in part, by the secretion of inflammatory adipokines released into the marrow, which results in the resorption of abnormal levels of bone that are not reciprocated by bone formation, as is the case in normal bone remodelling. Chronic destruction of bone matrix releases transforming growth factor- $\beta$  (TGF $\beta$ ), an inflammatory cytokine that leads to an impairment in the calcium gradient across muscle fibres. Therefore, mechanical signals are critical in regulating the dynamic between bone, muscle and fat. Depriving the body of mechanical stimuli in combination with a high-fat diet perpetuates extensive bone loss, muscle weakness and fatty-tissue accumulation around vital organs, which are tissue phenotypes that are conducive to the advancement of osteoporosis, impairments in glucose metabolism and chronic inflammation. NOX4, NADPH oxidase 4; RANKL, receptor activator of nuclear factor- $\kappa$ B ligand; ROS, reactive oxygen species; RUNX2, Runt-related transcription factor 2.

Whereas exercise delivers large quantities of mechanical information to the musculoskeletal system, absence of this regulatory information as a consequence of disuse (such as during chronic bed rest, exposure to microgravity, immobilization due to a cast or reduced physical activity)<sup>71,72</sup> results in conditions in which muscles, tendons and ligaments undergo catabolism and bone is rapidly resorbed<sup>73-76</sup>. Concurrently, studies show that extended bed rest drives increased marrow adipogenesis<sup>77</sup>, which exacerbates the consequences of inactivity. For example, disuse will in-

crease the expression of PPAR $\gamma$  in MSCs and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) in bone marrow, which promotes osteoclast-mediated bone resorption, yet both are rapidly suppressed via introduction of mechanical stimuli<sup>64,78</sup>.

### Benefits of exercise in patients.

As outlined, the musculoskeletal system of healthy individuals is highly sensitive to mechanical signals. The mechanosensitivity of bone and muscle also suggests that patients with osteoporosis might benefit from incorporating exercise into a treatment regimen; however, the degree of the regenerative effects of exercise might be limited by cell senescence, radiation damage and the effects of ageing (where stem and progenitor cells that have undergone apoptosis are already removed from the potential pool of replacement cells)<sup>79,80</sup>. Indeed, activities such as strength training are recognized as critical to achieving and maintaining a robust musculoskeletal system and to reducing the risk of fracture<sup>81</sup>. These activities have the added benefits of suppressing adiposity, the onset of obesity and the development of obesity-induced diabetes mellitus<sup>82</sup>.

Postmenopausal women<sup>83</sup> and men with low testosterone levels<sup>84</sup>, who have an increased risk of fracture<sup>85</sup> and elevated levels of visceral adiposity, are encouraged to incorporate exercise into their daily regimen to improve bone strength<sup>86</sup> and muscle mass<sup>87</sup>. In children and adolescents with chronic diseases, such as cancer<sup>88</sup> or type 1 diabetes mellitus<sup>89</sup>, concomitant low bone density and suboptimal muscle mass and/or function are prevalent and persist into adulthood<sup>90,91</sup>. The addition of exercise, when tolerated, is key to conditioning in children with chronic diseases. Exercise is also recommended for individuals with secondary osteoporosis as a result of cancer (such as breast cancer) or as an adverse effect of certain treatments (such as aromatase inhibitors); however, the effects of the disease or the treatment can prevent these individuals from participating in enough exercise to see benefits and might actually cause the fracture it is intended to prevent<sup>92</sup>. This effect is particularly evident in patients who are too frail to undertake exercise with sufficient impact to improve bone end points; the frail state thus aggravates bone loss. Furthermore, studies have shown that variations in osteocyte sensitivity and their lacunar morphology persist with ageing<sup>93,94</sup>. However, in vitro studies performed on cells collected from the bones of ageing women (between the ages of 53 and 80 years)<sup>95,96</sup> have shown that anabolic responses (for example, production of bone matrix and prostaglandin E<sub>2</sub>) can be upregulated if the signals are dynamic (that is, a time-varying, as opposed to static, or constant, signal) in nature<sup>97</sup>.

### Age influences the response to exercise.

Indeed, ageing might affect skeletal sensitivity to mechanical information. It is clear that younger people more rapidly accrue bone in response to exercise than do older people<sup>81</sup>, an observation supported by animal studies<sup>98,99</sup>. Ageing could also affect the ability of loading to deform bone<sup>99</sup> and results in a reduction in the number of available stem cells in older mice<sup>100</sup>. Other factors that are associated with ageing, such as a change in osteocyte morphology<sup>93</sup>, might also contribute to reduced load sensation. A great deal of exciting new information regarding the nature of tissue senescence suggests fundamentally new ways to think about ageing. It appears that within many tissues, including bone, joint and muscle, some cells become 'senescent' and secrete cytokines that lead to disruption of normal physiology<sup>101</sup>. In the case of bone, senescent osteoblasts secrete a pro-in-



inflammatory panel of factors that lead to resorption and decreased repair<sup>102</sup>. Indeed, senolytic compounds (such as navitoclax and quercetin) and targeted destruction of senescent cells have demonstrated promise in overcoming apoptotic programmes<sup>103</sup> and preventing bone<sup>102</sup> and muscle<sup>104</sup> loss<sup>105</sup>. It will be fascinating to determine if exercise can delay the appearance of these ageing-associated cells or modify their secretory profiles.

## Ageing, exercise and muscle phenotype

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### Muscle in ageing.

Fracture risk is coupled to muscle health; thus, in order to address the effect of mechanical input on bone, one should also consider how exercise influences muscle mass and function<sup>106</sup>. Age<sup>107,108</sup>, disease, cell senescence<sup>80,109</sup>, reduced physical activity<sup>110</sup> and diminished synthesis of sex steroids (androgens and oestrogens) contribute to reduced muscle cross-sectional area and mass<sup>111</sup>, alterations in which types of muscle fibre are present, lipid infiltration of muscle, decreased protein synthesis and decreased muscle-specific force, which collectively lead to sarcopenia<sup>112,113</sup>. The definition of sarcopenia, which might seem obvious to clinicians, is still controversial in clinical studies<sup>114</sup> but can be diagnosed using dual-energy X-ray absorptiometry (DXA) to measure skeletal mass and predict disease progression as well as all-cause mortality. Decline in muscle function is also hard to precisely diagnose. Measurable muscle decline begins at approximately 30 years of age, with one report estimating sarcopenia to affect almost 50% of the US population >60 years old<sup>115</sup> and other reports suggesting numbers below 20%<sup>116</sup>. Men and women are equally predisposed to the development of age-related sarcopenia, but muscle decline begins earlier in women than in men<sup>117</sup> and might contribute to fall risk, ultimately increasing fracture risk. A consensus definition, as well as more studies, will be necessary to confirm the degree to which sarcopenia affects the health of the ageing population.

### Muscle–bone crosstalk.

Crosstalk between bone and skeletal muscle is mediated by mechanical signal transduction and soluble factors<sup>118</sup>. Skeletal muscle strength is dependent on protein integrity, which is regulated by calcium, specifically ryanodine receptors (RyRs; intracellular calcium channels). These receptors regulate<sup>119</sup> potentials maintained across the sarcoplasmic reticulum of muscle fibres, thereby orchestrating contractile forces. Maladaptive post-translational modifications to the RyR1 channel, including oxidation or nitrosylation, lead to the dissociation of its stabilizing subunit calstabin 1. As a result of this lack of stabilization, pathological leaks of  $\text{Ca}^{2+}$  occur, which contribute to muscle weakness in ageing, chronic muscle fatigue, heart disease and muscular dystrophy<sup>120-122</sup>.

Accelerated bone destruction as a consequence of bone metastases<sup>123,124</sup> or other high-turnover bone diseases, such as Camurati–Engelmann disease<sup>125</sup>, releases transforming growth factor- $\beta$  (TGF $\beta$ ) stored within the mineralized bone matrix<sup>126</sup> into the circulation. In mice and humans, this bone-derived TGF $\beta$  upregulates NADPH oxidase 4 (NOX4)-mediated production of reactive oxygen species (ROS), which leads to instability of the RyR1–calstabin 1 complex, leakage of  $\text{Ca}^{2+}$  and muscle weakness<sup>127</sup>. Bisphosphonate inhibitors of osteoclastic bone resorption reduce circulating

levels of TGF $\beta$ <sup>128</sup> and prevent muscle weakness in mice with osteolytic breast cancer bone metastases, which suggests that pathological bone remodelling is involved in muscle weakness in cancer-induced osteolysis as well as other states of bone loss<sup>129</sup>. As physical activity decreases in those with osteoporosis<sup>124</sup>, other bone maladies or obesity<sup>130,131</sup>, MSC fate selection is biased towards adipocytes in lieu of bone<sup>68</sup>, further degrading musculoskeletal integrity and strength. This process represents a feed-forward cycle in which bone loss promotes muscle weakness, resulting in further reductions in bone and increases in adipose tissue.

### Effects of exercise on muscle.

The management of sarcopenia includes improved nutrition, protein and/or pharmacological supplementation and physical training. Use of either synthetic or endogenous growth hormone has had mixed efficacy as unexpected biochemical alterations have led to adverse cardiovascular and endocrine function<sup>132-134</sup>. These poor results have meant that the pharmaceutical industry has been unwilling to address common age-associated sarcopenia. Incorporating various forms of exercise<sup>135,136</sup> and physical activity into our daily lives improves muscle function, offsets age-related changes to muscle morphology<sup>136</sup> and improves insulin resistance<sup>137,138</sup>. Physical activity increases the oxidative capacity of muscle and encourages anabolic growth and function through the mammalian target of rapamycin complex 1 (mTORC1)<sup>139,140</sup>, PI3K-AKT and NF- $\kappa$ B pathways<sup>141</sup>. At the molecular level, exercise is a potent inhibitor of the FOXO<sup>142</sup> family of muscle-controlled transcription factors that are tightly linked to muscle atrophy<sup>143</sup> (such as FOXO3) and attenuation of bone formation through WNT suppression<sup>144</sup>. Inhibition of FOXO3 is mediated by resistance exercise through activation of the PI3K-AKT-mTOR pathway<sup>145,146</sup>. Therefore, by default, the introduction of mechanical stimuli through exercise facilitates pathways conducive to the maintenance and growth of bone.

Force production at the level of the skeletal myocyte depends on the proper handling of Ca<sup>2+</sup> between the sarcoplasmic reticulum and the cytosol. During excitation contraction coupling, sarcoplasmic-reticulum-sequestered Ca<sup>2+</sup> is released through activated RyR1 into the cytoplasm, which permits Ca<sup>2+</sup>-dependent actin-myosin cross bridging. Disruption of the RyR1 complex caused by oxidative stress has been implicated in muscle weakness<sup>122</sup> due to ageing, congestive heart failure, muscular dystrophy and cancer-associated osteolysis<sup>127</sup>. In the latter setting, the muscle weakness is mediated by release of bone-derived TGF $\beta$  and has implications for any state of increased bone resorption, including ageing, sex steroid deprivation, cancer and/or drug-treatment-induced osteoporosis. Thus, the prevention of bone loss through exercise intervention might have positive indirect downstream effects on muscle. Maladaptation of RyR1 has been shown in other disorders of muscle weakness and bone loss. For instance, in humans, muscle atrophy as a consequence of 60 days of bed rest resulted in dysfunctional Ca<sup>2+</sup> homeostasis with increased S-nitrosylation of the RyR1 and malfunction of the SERCA1 pump<sup>147</sup>. This maladaptive nitrosylation of RyR1 was rescued with a combination of resistive exercise and low-intensity vibration (an exercise surrogate) but not with resistive exercise alone<sup>147</sup>. In addition to reducing bed rest-induced RyR1 S-nitrosylation, low-magnitude mechanical signals increased protein expression of RyR1 (REF.<sup>148</sup>) and nuclear factor erythroid 2-related factor 2 (NRF2)<sup>147</sup>, a critical transcriptional regulator of antioxidant protein expression, which protects against oxidative damage. These low-inten-



sity signals also served to protect the actual number of satellite cells (precursors to differentiated skeletal muscle cells) available within the muscle when challenged with endocrinopathy<sup>149</sup> or obesity<sup>150</sup>.

### Osteocalcin.

Bone-derived osteocalcin has also been demonstrated to regulate skeletal muscle function, mass and exercise capacity in mice<sup>151</sup>. At the molecular level, osteocalcin signalling in skeletal muscle promotes the uptake and subsequent catabolism of glucose and free fatty acids<sup>152</sup>, effects that are similar to those of IL-6 (REF.<sup>153</sup>). This metabolic response and optimization of energy utilization in myofibrils might contribute to gains in muscle performance following the delivery of mechanical forces to bone. Interestingly, muscle-derived IL-6 increases the production of bioactive osteocalcin<sup>154</sup> and, similarly, these same preclinical studies demonstrated that osteocalcin stimulated the expression and secretion of IL-6 from skeletal muscle. These data support a feedforward mechanism of adaptation to exercise mediated by osteoblast expression of osteocalcin and skeletal muscle expression of IL-6 during mechanical loading of the musculoskeletal system.

### Types of exercise regimen.

Exercise modalities can halt or reverse muscle loss. For example, resistance and endurance training are both effective countermeasures to slow muscle loss<sup>155</sup> and promote gain in mass and neuronal activation<sup>155</sup>. In addition, resistance training induces muscle hypertrophy<sup>156</sup>, which increases muscle mass and strength through morphological changes to muscle fibres<sup>157</sup>. Although resistance training ensures dramatic effects, individuals with sarcopenia and osteoporosis cannot endure (or risk) the higher magnitudes of resistance training<sup>158</sup>. Alternatively, adherence to exercise regimens might be more achievable through endurance training in ageing adult populations, particularly in those with obesity, which would inhibit weight gain and maintain healthier muscle by stimulating satellite cell proliferation and increasing metabolic muscle output<sup>159</sup>. Even the low-intensity nature of yoga, which is known to enhance musculature and improve balance<sup>160</sup>, has been introduced as a means to treat the effects of muscle wasting in patients with cancer-associated cachexia, a strategy that is also encouraged in those with sarcopenia who have restricted mobility<sup>161-163</sup>.

### Muscle and bone outcomes.

The hypertrophy of muscle mass and bone mass are positively correlated to each other in response to mechanical stimuli<sup>164</sup> as exemplified by exercise-induced increases in satellite cells, increased fibre size and muscle hypertrophy. The dependency of bone outcomes on muscle is also apparent in embryonic paralysis<sup>165</sup> and genetic muscle dysfunction<sup>166</sup>, as well as in humans with muscular dystrophy who develop skeletal abnormalities. Translating this observation to humans, patients adversely affected by myopathy-inducing pharmacological treatments have benefited from incorporating exercise into the treatment strategy. Exercise surrogates, such as low-intensity vibration, have been hypothesized to play a similar role; low-intensity vibration increases the satellite cell pool<sup>149</sup>, limits fatty infiltration of skeletal muscle<sup>167</sup>, downregulates pro-inflammatory

gene expression<sup>168</sup> and upregulates the expression of anti-inflammatory molecules. Therefore, through mechanical intervention, whether strenuous or as general maintenance of adequate muscle health, bone outcomes are improved.

## Factors that promote marrow fat

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### Marrow adipocytes.

In excess, adipose tissue can contribute to a host of metabolic conditions — the most extreme of these being obesity. However, the degree of adipose accrual does not need to reach a BMI  $\geq 35$  kg/m<sup>2</sup> to be harmful: fat is found in bone marrow, muscle, joints and liver and has a myriad of functional consequences when present in excess. In the marrow microenvironment, which is a constrained space consisting of MSCs and HSCs, the impact of excess adipose tissue probably depends on age, aetiology and inflammatory status<sup>37</sup>. Ageing, postmenopausal status, undernutrition, some pharmacological therapies and an absence of physical activity can all drive marrow adiposity<sup>169</sup>. Marrow adipose tissue (MAT), which first develops in the prenatal skeleton, is estimated to occupy 70% of the marrow space by young adulthood<sup>170</sup>. Marrow adipocytes secrete a multitude of adipokines (such as adiponectin and IL-6), some of which induce inflammation<sup>171</sup> and osteoclastogenesis<sup>172</sup>, which can disrupt haematopoiesis<sup>173</sup> and aggravate bone loss<sup>174</sup>. For instance, IL-6 induces the expression of RANKL on osteoclasts and their precursors, which increases recruitment of haematopoietic macrophage precursors into the osteoclast lineage and increases bone resorption<sup>175</sup>. Adiponectin, another adipokine highly expressed by marrow adipocytes<sup>176</sup>, stimulates RANKL expression on mature osteoclasts and is associated with low BMD in elderly men and women<sup>177,178</sup>. With these findings in mind, slowing the expansion of adipose tissue throughout the marrow space might protect and preserve the MSC and HSC niche, permitting progenitors to retain their regenerative (MSC) and immune (HSC) functions and counteracting osteoporosis and inflammatory disease.

Exercise suppresses the formation of MAT, even when MAT is stimulated by an anti-diabetic thiazolidinedione drug or a high-fat dietary intervention<sup>39</sup>. As such, exercise might help preserve the morphology and phenotype of the marrow microniche where osteoprogenitors, as well as HSCs, reside. For example, in contrast to their non-exercised counterparts, 6 weeks of daily running increased bone quantity, improved bone quality and suppressed MAT accumulation in mice fed either regular or high-fat diets (HFDs)<sup>39</sup>. Furthermore, treatment with a PPAR $\gamma$  agonist (rosiglitazone) increases stem cell adipogenesis in rodents<sup>179,180</sup> and humans<sup>181</sup>. These outcomes are suppressed by dynamic (time-varying) mechanical signals *in vitro*<sup>67</sup>. *In vivo*, treadmill running in rosiglitazone-treated mice suppressed an adipogenic shift in the marrow phenotype<sup>180</sup>.

### Obesity.

Obesity predisposes the body to a wide-range of perturbations and morphological changes, such as adipocyte hypertrophy from excess lipid storage<sup>182</sup>, including within the marrow space. Multiple studies have demonstrated that MAT increases as total fat mass increases in mouse models of obesity<sup>39,183–187</sup>. For instance, 6 weeks of a mild HFD (45% kcal from fat) led to a 2.6-fold

increase in MAT in young female mice<sup>39</sup>. Six weeks of higher-fat-supplemented chow (60% kcal from fat) led to a fourfold increase in MAT in young male C57BL/6 mice<sup>183</sup>. After 12 weeks of a similar diet, which was fed from weaning, MAT increased more than fivefold<sup>185</sup>. In addition, 3 months of a diet consisting of 45% kcal from fat increased MAT and adipocyte hypertrophy<sup>187</sup> (FIG. 2).

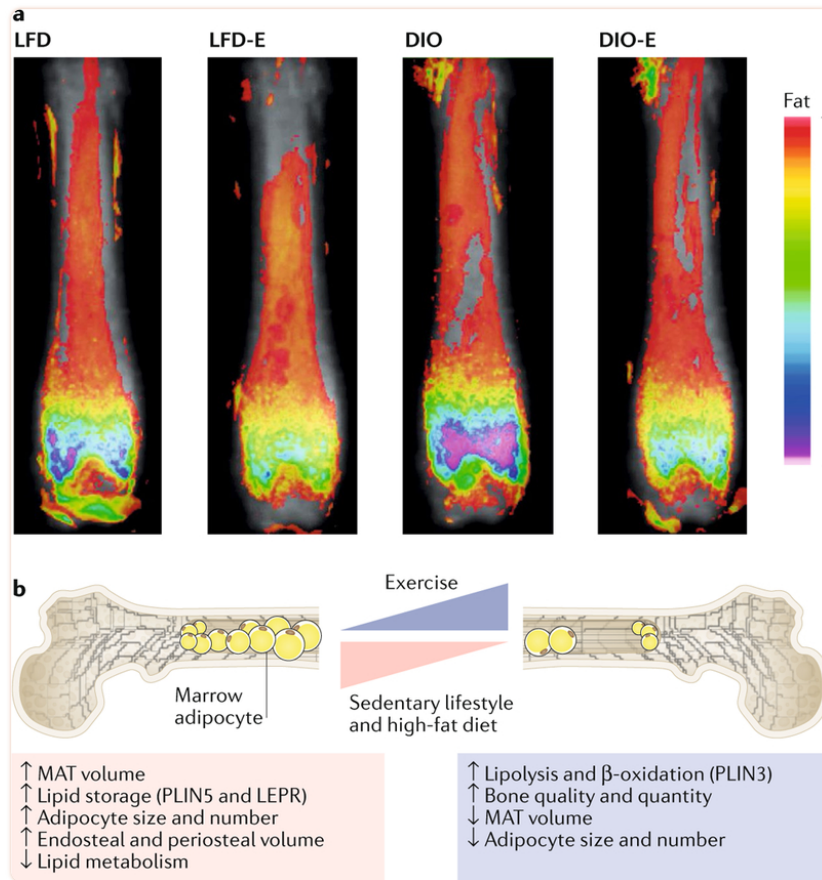


Fig. 2 |

### Exercise suppresses expansion of marrow adipocytes and strengthens bone in obese mice.

**a** | Obese (diet-induced obesity (DIO)) and lean (low-fat diet (LFD)) mice were allocated to running exercise (DIO-E and LFD-E, respectively) or sedentary groups for 6 weeks ( $n = 6$  per group). The images are a visualization of femoral marrow adipose tissue (MAT) in mice measured by MRI with advanced image analysis. Each image represents six images superimposed on each other. The heat map demonstrates the relative lipid quantity. **b** | Schematic representation of marrow adipocytes in the setting of obesity with or without exercise. DIO increases adipocyte size and number and expression of the lipid droplet marker PLIN5, resulting in expansion of cortical endosteal and periosteal bone surfaces. By contrast, exercise increases bone quantity and quality relying on  $\beta$ -oxidation of lipids in the marrow, as supported by a reduced number of adipocytes in the marrow and their cross-sectional area and increased expression of oxidation and lipolysis markers (for example, PLIN3). Part **a** reproduced with permission from REF. <sup>187</sup>, Wiley-VCH.

Six months of a diet consisting of 45% kcal from fat in C57BL/6 mice led to a 20–25% increase in total numbers of cells in the bone marrow without affecting the fraction of different cell types; thus, factors secreted by elevated amounts of MAT, such as leptin, increased haematopoietic and lymphopoietic populations, which indicates that an HFD heavily dysregulates immunity<sup>188</sup>. Similarly, 12 weeks of a diet of 60% kcal from fat led to bone marrow hyperplasia (28% increase in nucleated cells) in Wistar rats<sup>189</sup>. Conversely, 18 weeks of a diet of 45% kcal from fat led to decreased HSCs and progenitor cells in the marrow, owing partly to both reduced proliferation and increased differentiation of short-term HSCs and progenitor cells into multipotent progenitor cells<sup>190</sup>. By contrast, 16 weeks of an HFD led to increased long-term HSC populations in the marrow<sup>191</sup>.

Most studies on obesity suggest that the condition is associated with impaired lymphopoiesis and increased myelopoiesis. In rats, obesity led to an increased number of osteoblasts, segmented neutrophils and eosinophils, whereas no notable difference was observed in the number of marrow-bound lymphocytes<sup>189</sup>. In mice, the effects of obesity on immunity in the marrow have been associated with reduced numbers of B cells and T cells and an increased number of myeloid cells<sup>183,192</sup>. By contrast, another mouse study demonstrated a 10–18% increase in lymphocyte progenitors within the marrow during obesity, resulting in an enrichment of total lymphocyte counts in the circulation of 70–125%<sup>188</sup>. Furthermore, HSCs harvested from a marrow environment that was high in fat have an elevated capacity to produce macrophages<sup>191</sup>.

Obesity in humans has been linked with elevated systemic inflammation, much of which is directly associated with macrophage infiltration of extra-marrow adipose depots and increased numbers of immune cells in the circulation<sup>193–197</sup>. Interestingly, a positive correlation between BMI and blood leukocyte count is found in individuals who are insulin resistant<sup>195</sup>. Increased infiltration of immune cells into the visceral cavity and increased secretion of pro-inflammatory cytokines perpetuate in the obese phenotype<sup>34,198–200</sup>. In mice, just 2 weeks of an HFD facilitates rapid weight gain and diffuse visceral adiposity<sup>68,201</sup>. White adipose tissue-mediated secretion of pro-inflammatory cytokines (adipokines)<sup>34</sup> and ROS (which drive macrophage and cytotoxic T cell production) further promotes the state of chronic inflammation<sup>198</sup> through release of matrix metalloproteinases (MMPs), tumour necrosis factor (TNF) and IL-6, among others. In humans, these factors predispose the individual to developing insulin resistance<sup>202</sup> and glucose intolerance, conditions that can lead to the onset of type 2 diabetes mellitus<sup>203</sup> and ultimately contribute to deficits in cortical bone density, trabecular microarchitecture and bone size<sup>65</sup>. Inflammation and increased levels of pro-inflammatory cytokines, such as TNF, IL-1, IL-6 and IL-17, are associated with increased bone loss<sup>204–206</sup>. Pro-inflammatory cytokines promote bone loss by increasing the expression of macrophage colony-stimulating factor and RANKL by osteoblasts and fibroblasts in the marrow<sup>204</sup>. Inhibiting the function of IL-1 or TNF, which can be secreted by adipocytes, prevented bone loss in ovariectomized mice<sup>207</sup>. In addition, multiple animal studies have shown increased adipogenesis resulting from a HFD that led to an obese phenotype exhibited, in parallel, with suppression of osteoblastogenesis<sup>68,192,201</sup>. Translated to humans, these findings demonstrate that the damage associated with poor diet and obesity to bone and immunity are exacerbated by increased inflammation. Whether the rise in marrow adiposity as a consequence of HFDs contrib-

utes to elevated inflammation and dysregulated immunity or outcompetes osteoprogenitors for marrow space, the resulting phenotype is conducive to increased pathological outcomes across a range of systems.

## Transducing a mechanical signal

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### Mechanical forces.

The array of mechanical forces experienced by all cells in bone, including MSCs and HSCs in the marrow, is complex and multifactorial. Spatially, MSCs reside in bone marrow niches near the bone surface and are exposed to matrix deformations<sup>55,208-211</sup>, accelerations<sup>212-217</sup>, muscle activity<sup>218-220</sup>, fluid flow<sup>221-225</sup> and changes in intramedullary pressure<sup>226-228</sup>, each of which cannot be disentangled from the other<sup>229</sup>. During locomotion, the bone matrix encounters strains in the range of 2,000–3,500 microstrain ( $\mu\epsilon$ )<sup>208</sup>. Owing to bone's porous structure, local strain concentrations create pressure gradients and induce local fluid flow in and out of the bone matrix, similar to squishing a kitchen sponge. In vivo, even fairly low strains (400  $\mu\epsilon$ ) that might correspond to seemingly gentle activities such as walking can produce fluid flow within the lacunar–canalicular network that is as high as 5 Pa (REF.<sup>230</sup>). In this way, MSCs that reside on or in proximity to bone surfaces are also subjected to exercise-derived fluid flow. Within the marrow, small motions at the interface between marrow and bone, such as those induced by exercise, will generate a fluid shear that is independent of strain-derived fluid flow<sup>231</sup>. Dynamic shear forces<sup>232,233</sup>, such as pulsatile fluid flow, can promote osteogenesis in rat calvarial cells and represent a key physical factor in mechanotransduction.

During moderate running, tibial accelerations approach 2.0 g (where 1.0 g (or 9.8 m/s<sup>2</sup>) is Earth's gravitational pull), and the ground-reaction force of Olympic sprinters can exceed 3.0 g (that is, three times their body weight)<sup>234</sup>. In silico studies reveal that when using vibration to introduce subtle mechanical oscillations (with a range of 0.1–0.5g), marrow-filled trabecular compartments<sup>235,236</sup> generate fluid shear stresses up to 2.0 Pa (REFS<sup>231,237</sup>), which is a mechanical signal capable of influencing MSC function<sup>237</sup>. The viscosity of red marrow was found to be much higher (400 cP) than that of fatty marrow (40 cP)<sup>238</sup>, which implies that fluid shear at the bone marrow interface, and within the marrow itself, can change dramatically because of marrow's fluid dynamic properties<sup>239</sup>. Red and fatty bone marrow can replace one another<sup>240</sup>, and conditions such as ageing and osteoporosis result in an increase in adipose tissue volume in the marrow while depleting the bone<sup>241</sup>.

### Pathways.

Osteoblasts have more cytoskeletal constructs and interconnections that link to the nucleus than adipocytes; culturing human MSCs and osteoblasts on hard surfaces improves osteogenesis and is associated with the development of a complex cytoskeleton<sup>242,243</sup>. The application of physical forces in vitro, which dynamically increases cytoskeletal actin structure<sup>244</sup>, inhibits adipogenesis, which preserves the multipotentiality of MSCs and their ability to enter the osteoblast lineage<sup>63,245,246</sup>. In vitro application of mechanical strain to MSCs is associated with recruitment of



signalling complexes to focal adhesions, where AKT activation inhibits the effect of GSK3 $\beta$  targeting  $\beta$ -catenin for destruction, thereby increasing  $\beta$ -catenin signalling<sup>247</sup>. AKT activation also leads to increased levels of GTP-bound active RhoA and production of highly connected cytoplasmic actin connections<sup>245</sup> that are involved in molecule translocation as well as transmitting forces directly into the nucleus<sup>248</sup> (FIG. 3). Thus, the dynamic remodelling of cytoskeletal elements in response to the local bone marrow mechanical environment orchestrates the delivery of this mechanical information from the plasma membrane and/or other sites, such as the nucleus itself, in order to regulate gene expression programmes in cells<sup>60</sup>.

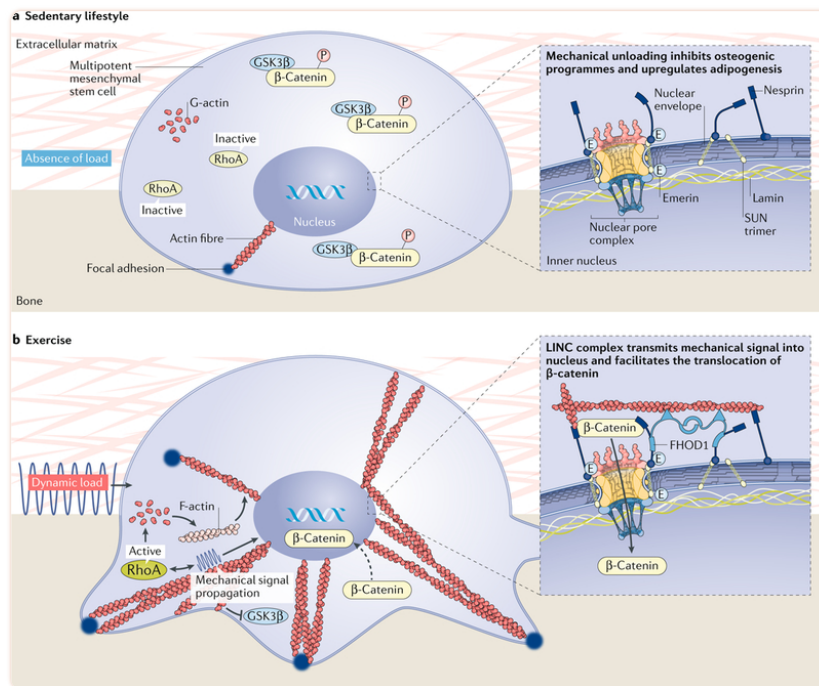


Fig. 3 |

**Mechanotransductive responses of mesenchymal stem cells to dynamic mechanical stimuli are achieved through the internal stiffening of the cell via cytoplasmic-bound actin proteins.**

**a** | The absence of mechanical forces prevents the polymerization of actin fibres, preventing the dephosphorylation of  $\beta$ -catenin, which remains bound to GSK3 $\beta$ . As such,  $\beta$ -catenin does not translocate to the nucleus, resulting in the expression of PPAR $\gamma$ -driven adipogenic pathways. **b** | By contrast, mechanical stimuli recruit actin fibres to the interface of the cell membrane and the substrate surface. These focal adhesions become stronger and denser in response to dynamic mechanical stimuli, permitting the movement of  $\beta$ -catenin into the nucleus and an ensuing osteogenic response. FHOD1, FH1/FH2 domain-containing protein 1; LINC, linker of nucleoskeleton and cytoskeleton.

Perhaps the most widely recognized mechanoreceptive pathways are those that sense mechanical information at the plasma membrane and transmit it to the nucleus (termed outside-in signalling). Focal adhesions, which are maintained by cytoskeletal tension<sup>249</sup> and extracellular force, act as signalling relays for extracellular (and intracellular) cues<sup>250</sup>. In response to mechanical challenges,



structural proteins, such as vinculin, Paxillin and talin, are recruited into focal adhesions<sup>251-253</sup>, whereas others (such as zyxin) leave focal adhesions and localize themselves onto actin fibres to recruit actin nucleation and the branching factor actin-related protein 2 (ARP2)-ARP3 complex<sup>254-256</sup>. Structural changes in focal adhesions are accompanied by the recruitment of signalling molecules, including focal adhesion kinase (FAK) and SRC kinases as well as AKT, a known activator of Rho GTPases<sup>63,245</sup>, such as RhoA, RAS and CDC42 (REF.<sup>257</sup>). RhoA activity increases the cell tension through its effector protein ROCK, which activates myosin light-chain kinases, leading to activation of the dimerized motor protein myosin II<sup>258,259</sup>. These mechanically driven changes in RhoA-ROCK activity have been implicated in the osteogenic commitment of MSCs as they increase the activity of two early-stage osteogenic markers, osterix and RUNX2 (REF.<sup>260</sup>). Reinforcing the role of RhoA in MSC osteogenesis, our group recently showed that regulation of RhoA activity through leukaemia-associated Rho guanine nucleotide exchange factor (LARG; also known as ARHGEF12) and Rho GTPase-activating protein 18 (ARHGAP18) regulates osteogenic commitment in MSCs<sup>244</sup>.

### Signalling molecules.

In parallel to cytoskeletal restructuring, mechanical signals also activate a number of signalling molecules, including MAP kinases (such as ERK and JNK) and the WNT effector  $\beta$ -catenin. Perhaps the most studied signalling protein in bone,  $\beta$ -catenin counteracts an adipogenic stimulus when activated, which inhibits adipogenesis of bone-marrow-derived MSCs as demonstrated by reduced levels of lipids and decreased expression of PPAR $\gamma$  and adiponectin<sup>60,245,260</sup>. Following a mechanical challenge, FAK operates in conjunction with the SRC kinase FYN to activate mTORC2, which then initiates the signal cascade of increased levels of AKT leading to decreased levels of GSK3 $\beta$ , thereby increasing levels of  $\beta$ -catenin<sup>247,261</sup>. In this way, the increase in the number of focal adhesions after application of an acute mechanical challenge amplifies the downstream response to force, as demonstrated by a greater induction of  $\beta$ -catenin with a subsequent application of force<sup>70</sup>. Thus, a transient adaptation of the cell increases its sensitivity to follow-on mechanical signals, yet the absence of mechanical signals could systematically dismantle these 'antennae' and leave the system unresponsive to input<sup>63</sup>. Translating this finding to the clinic, strategic delivery of physical interventions during rehabilitation might have the potential to ratchet up the mechanical sensitivity and response of cell populations; however, leaving a system unstimulated for long periods of time (for example, as in long-term bed rest) might undermine the adaptive machinery's capacity to protect the patient.

### The nucleus.

Emerging evidence suggests that the nuclear envelope houses a number of mechanoregulatory proteins and has an active role in both cytoskeletal dynamics and nuclear access to molecular transducers of mechanical information. Mechanically, the cytoskeleton couples to the nucleus through the linker of nucleoskeleton and cytoskeleton (LINC) complex protein<sup>262</sup>. F-actin binds to a nesprin protein (nesprin 1 or nesprin 2), which are spectrin repeat proteins that pierce the nuclear envelope, connecting via its KASH (Klarsicht, ANC1, SYNE homology) domain to intramembrane leaflet SUN proteins (SUN1 and SUN2)<sup>262</sup>. As LINC elements, SUN1 and SUN2 partly regulate nuclear mechanical integrity<sup>263</sup>. Mechanically, the nuclear envelope transmembrane protein

emerin is known to accelerate actin polymerization<sup>264</sup>, shifting between inner and outer nuclear membranes. By contrast, external mechanical challenges to epidermal stem cells cause emerin enrichment at the opposing nuclear envelope, and emerin accumulation is accompanied by the recruitment of non-muscle myosin IIA to promote local actin polymerization that reduces nuclear actin levels and promotes perinuclear actin accumulation<sup>265</sup>. This finding is consistent with our recent findings that nuclear actin levels and its polymerizing state are powerful determinants of MSC differentiation into osteogenic and adipogenic lineages<sup>246,266</sup>.

Another protein that is active in the outer nuclear envelope, FH1/FH2 domain-containing protein 1 (FHOD1), binds to the spectrin repeat domain of nesprin 2G to increase the coupling strength between LINC and F-actin<sup>267</sup>. Supporting the regulatory role of the LINC complex in cytoskeletal dynamics, depletion of nesprin 1 (REF.<sup>268</sup>) or SUN1 (REF.<sup>269</sup>) alters focal adhesion kinetics by increasing focal adhesion strength, whereas deletion of SUN2 results in the opposite effect, decreasing focal adhesions<sup>270</sup>. Co-depletion of LINC elements SUN1 and SUN2, as well as disconnecting LINC through overexpressing the nesprin–SUN binding domain KASH, accelerates MSC osteoblastogenesis and impedes cell mechanosensitivity to subtle mechanical signals, such as low-intensity vibration<sup>261</sup>. Although this finding suggests that LINC has a role in regulating mechanosensitivity, both LINC-depleted MSCs and cells without nuclei remain responsive to high-magnitude substrate strain and activate FAK at Tyr397 in response to strain. This observation suggests that LINC has a nuanced function in cell mechanosensitivity. Moreover, the LINC complex serves an important role in the nuclear access of important mechanotransducers, such as  $\beta$ -catenin and YAP1 (REFS<sup>271,272</sup>).

Depleting nesprin 1 inhibits strain-induced nuclear entry of transcriptional co-activator YAP1. Findings published in 2017 indicate that the access of transcriptional co-activator YAP1 to the nucleus is regulated through stretching of nuclear pores during cytoskeletal tension, which facilitates the transfer of transcriptional co-activator YAP1 to the nucleus<sup>273</sup>. Our group has also demonstrated that LINC has an important role in  $\beta$ -catenin access to the nucleus.  $\beta$ -Catenin does not have a classic nuclear localization signal but, instead, enters through the nuclear leaflets via direct contact with the nuclear pore complex (NPC)<sup>274,275</sup>.  $\beta$ -Catenin transiently localizes to the LINC element nesprin, which might provide a 'launching pad' for subsequent nuclear entry<sup>276</sup>. Untethering of nesprin 2 from the nuclear envelope via co-depletion of both SUN1 and SUN2 proteins displaces  $\beta$ -catenin and decreases its levels in the nucleus<sup>271</sup>. Thus,  $\beta$ -catenin generated through exercise appears to be a critical event in transmitting these signals into the nuclear-mediated transcription of osteogenic genes.

Lamin A/C is a well-known intranuclear, mechanoadaptive intermediate filament system housed inside the nucleus. Nuclear levels of lamin A/C positively correlate with resident tissue stiffness in a linear manner<sup>242</sup>, and lamin A/C plays a major role in regulating nuclear stiffness<sup>277,278</sup>. Multipotent MSCs, which enter musculoskeletal cell lineages that have mechanically demanding functions, have a more robust lamin A/C network and increased LINC connectivity than multipotent embryonic stem cells<sup>242</sup>. As embryonic stem cells differentiate into somatic cell lineages, levels of LINC and lamin A/C increase<sup>279</sup>. In bone, levels of lamin A/C increase when MSCs enter the osteogenic lineage<sup>280</sup>, a change that contributes to the increased cellular stiffness of osteoblasts<sup>242,281</sup>. Furthermore, lamin A/C overexpression promotes osteogenic differentiation<sup>282</sup>. By contrast, levels of lamin A/C decrease when MSCs undergo adipogenesis<sup>283</sup>,

and both partial and complete deletion of lamin A/C promote an adipogenic programme in MSCs<sup>284-286</sup>. In this way, stiffness of resident tissues, such as the hard tissues of the skeleton, exerts control on the cell itself, both at the material level (higher effective modulus or stiffer bone) and through regulating nuclear stiffness (stiffer cell) to bias MSC differentiation towards osteogenesis and against adipogenesis.

## Bone cells.

Resident bone cells exhibit distinct responses to mechanical loading, including increased  $\beta$ -catenin signalling (for example, osteoblasts<sup>287</sup> and osteocytes have dendritic processes that are embedded throughout the bone matrix that function as a mechanosensor array)<sup>288</sup>. Oestrogen has a distinct role in regulating bone homeostasis as osteoblast apoptosis is prevented<sup>289</sup> by the phosphorylation of oestrogen receptor- $\alpha$  (ER $\alpha$ ) and upregulation of MAPK expression<sup>290</sup>, the latter perhaps similar in nature to activation of MAPK by mechanical strain<sup>78</sup>. In states of oestrogen depletion in mice, achieved via ovariectomy, osteoclast activity is heavily upregulated, yet incorporation of mechanical strain via low-intensity vibration still enhances bone formation during fracture callous healing through increased expression of ER $\alpha$ <sup>291</sup>. In vitro, mechanical strain of pre-osteoblasts increases the matrix mineralization of osteocalcin and osteopontin, which, when extrapolated to exercise challenges at the level of the organism, should improve bone strength<sup>292</sup>. When stimulated by fluid shear stress,  $\beta$ -catenin signalling increases in osteocytes and osteoblasts<sup>293</sup>. Finally, bringing us back to how these bone cells might recognize and best respond to mechanical signals through their dynamic cytoskeletal apparatus, we have shown that inclusion of a 3 h refractory period between successive bouts of mechanical challenges, in this case by low-intensity vibration, improves the ability of these mechanical signals to suppress adipogenesis<sup>70</sup>. To a degree, this effect is achieved, as discussed in a previous section, because the second bout of mechanical force is given after an increase in focal adhesion number and connectivity through a RhoA-based signalling cascade, taking advantage of an adapted cell better suited to perceive the mechanical challenges<sup>63</sup>. Separating the refractory period even further to 5 h between mechanical bouts, which were delivered in vivo, also increased the MSC population<sup>52</sup>.

Collectively, these findings indicate that MSCs residing throughout the bone marrow utilize both cytoskeleton remodelling and biochemical transducers to facilitate information flow between two critical mechanosensory centres (focal adhesions and the nuclear envelope) in response to mechanical challenges. In addition, in vivo exposure to multiple mechanical events that are separated by sufficient time for the system to adapt results in the promotion of osteogenic and anti-adipogenic outcomes<sup>52,294</sup>. In deciphering how exercise regulates MSC fate, consideration should be given as to how the marrow mechanical environment and MSCs residing within bone evolve with age and disease state, as well as treatment of diseases, and adapt to loading demands during exercise. For instance, in the setting of cancer, certain treatments, such as radiation<sup>295</sup> and hormone deprivation<sup>296,297</sup>, significantly increase bone marrow adiposity. The implications of marrow fat for musculoskeletal health are unclear in humans but will be important areas of study. Understanding the role of mechanical signals in improving musculoskeletal function will be important, as elderly individuals and individuals who are infirm, injured or obese are often unlikely to adhere to an exercise prescription no matter how beneficial it might be.

## Low-intensity vibration

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### Mechanical effects of exercise.

Exercise is often presumed to suppress adiposity through metabolic pathways, such as increasing caloric expenditure<sup>298</sup>, and it is assumed that the longer and more strenuous the activity the more effective it becomes. Indeed, our group showed that exercise (both treadmill and wheel running) in mice (aged 4–8 weeks)<sup>39,299</sup>, even under conditions conducive to adipogenesis, such as a HFD or treatment with thiazolidinedione, suppressed adiposity. The salutary effect of exercise on the skeleton, however, is generally believed to be energy-independent and is instead regulated by osteoblast or osteocyte signalling, or concerted effort from both cells<sup>300</sup>, generated by load-induced bone strain<sup>55</sup>, enhanced fluid flow<sup>301</sup>, intramedullary pressure<sup>302</sup> and/or streaming potentials<sup>303</sup>. Furthermore, once a threshold of loading is surpassed, no additional influence is realized<sup>304</sup>. These mechanical parameters correlate more strongly to the dynamics (time-varying) of the load environment (that is, impact<sup>305</sup>, strain rate<sup>209</sup>, strain gradients<sup>306</sup> and cycle number<sup>208</sup>) than to load magnitude, a conclusion strengthened when considering that static challenges (that is, upright stance and balance) fail to serve as anabolic stimuli<sup>307</sup>. Departing from a ‘more is better’ strategy, several groups have reported that extremely small mechanical signals, induced at high frequency using low-intensity vibration, are anabolic to bone<sup>212,216,308–315</sup> and suppress the formation of adipose tissue<sup>316,317</sup>.

### Frequency and magnitude of signals.

High-frequency, low-magnitude mechanical signals persist in the functional load regime<sup>220</sup>; low-magnitude mechanical signals are generated by the dynamics of muscle contraction<sup>318</sup>. The persistence of such signals is evident when considering that the daily history of bone strain consists of a few large mechanical events (for example, four loading events per day that are  $>2,000 \mu\epsilon$ )<sup>319</sup> as well as hundreds of thousands of daily events that are well below  $10 \mu\epsilon$  (REF.<sup>320</sup>). That low-magnitude mechanical signals, induced using low-intensity vibrations in the absence of weight bearing, can promote bone formation<sup>74,321</sup> led to the unexpected finding that low-intensity vibrations also inhibit adipogenesis and systemic adiposity in adult mice<sup>68,201,317</sup> while decreasing levels of triglycerides, free fatty acids and liver steatosis<sup>322</sup>. These findings suggest that adipose development might also have an energy-independent element; the reciprocal relationship of fat to bone<sup>323</sup> points to a novel target to control the bone versus fat phenotype — their shared MSC progenitor<sup>68,201,324,325</sup>. Furthermore, low-intensity vibrations alter the haematopoietic response of obese mice fed an HFD by restoring depleted B cell populations in gonadal fat pads, a mechanism suggested to have arisen through fate selection of HSCs towards B cell lymphopoiesis at the expense of osteoclastogenesis<sup>192</sup>. Therefore, by targeting both bone marrow MSCs and HSC immune progenitors of the marrow, low-intensity vibrations could mitigate the pernicious consequences of obesity on the immune system while suppressing adiposity. As age increases, however, the challenge of using low-intensity vibrations in adults to treat obesity (or osteoporosis) is that the sensitivity to mechanical stimulation might have already declined<sup>326</sup>.

### Cycles of signals and rest.

Growing evidence suggests that the incorporation of multiple cycles of mechanical signals within a given day, separated by periods of rest, can increase their effects in reducing adipogenesis and increasing osteogenesis. Low-intensity vibrations have the greatest impact in young participants (aged 5–20 years)<sup>61,327</sup>, an influence that reduces over time in ageing adults (65–85 years). Young mice (aged 5 weeks) receiving HFD chow are receptive to singular bouts of low-intensity vibrations over 12 weeks, which results in increased glucose and insulin metabolism<sup>328</sup>. However, aged HFD-fed mice (17 weeks old) benefit more in terms of offsetting adiposity and impaired glucose metabolism (hyperinsulinaemia) as a result of two 15 min bouts of low-intensity vibrations than from a singular 30min bout<sup>52</sup>.

Rest periods, ranging anywhere from 14 s to 8 h, introduced between mechanical inputs increase the anabolic response of bone<sup>329</sup>. Incorporating refractory periods into the low-intensity vibration loading scheme increases the expression of insulin receptor substrate 1 (IRS1), which is a negative regulator of the PI3K pathway<sup>330</sup>, in the perigonadal fat pads in HFD-fed mice, eliciting an even stronger effect than that seen in aged mice on control diets exposed to a single bout of low-intensity vibration treatment. In the clinical setting, exercise regimens to treat patients with obesity might elicit the anti-diabetic effects of mechanical loading, but the effects will probably be dependent on the age of the patient — the younger the patient is, the more responsive they will be. Altogether, targeting the immunosuppressive and anti-inflammatory capacity of bone marrow stem cells by inducing proliferation and lineage selection using exercise or exercise surrogates might collectively help address adipose tissue dysfunction.

### Effects of low-intensity vibrations.

In humans, low-intensity vibrations promote increased bone mass and quality, both of which contribute to bone strength and resistance to fracture, in children with disabling conditions, including cerebral palsy<sup>331,332</sup>, Duchenne muscular dystrophy<sup>333</sup> and adolescent girls with idiopathic scoliosis<sup>332</sup>. Low-intensity vibrations are anabolic to bone and muscle in young women (15–20 years old) with osteoporosis<sup>327</sup> and augment bone accretion in survivors of childhood cancer<sup>334</sup> and patients with Crohn's disease<sup>335</sup>. Acute studies (within 5 days) show that normal bone turnover can be restored in young women (aged  $16.3 \pm 1.9$  years) combating anorexia nervosa<sup>336</sup> and that markers for bone resorption are suppressed in healthy young women within 3 months<sup>313</sup>. In each of these studies, however, it is important to note that the salutary influence correlates with adherence; mechanical signals are effective only if you use them.

The design of the low-intensity vibration platform uses closed-loop acceleration feedback to ensure a high-fidelity signal<sup>337</sup>, a design that can safely<sup>229,338</sup> deliver these barely perceptible mechanical signals to participants, including frail elderly individuals<sup>339,340</sup> and those with spinal cord injuries<sup>341</sup>. Low-intensity vibrations are considered a nonsignificant risk by the FDA<sup>338</sup>, with an intensity considered safe for up to 4 h of exposure per day<sup>342</sup>. Other instruments providing mechanical stimulation operate in higher-magnitude and lower-frequency domains, making them less practical and even risky for use in patients whose skeletons are frail (such as those with postmenopausal osteoporosis or osteogenesis imperfecta)<sup>343</sup>. Although low-intensity vibrations cannot be considered a substitute for exercise, these studies indicate that they represent salutary mechanical signals to improve clinical end points in participants with limited exercise capacity<sup>344</sup> and



might be a means of priming responsiveness to exercise. Increasing sensitivity to, and thus efficacy of, exercise might ultimately make it more accessible to older adults (age  $\geq 60$  years) and infirm patients unable to exercise adequately to stimulate these integrated regulatory systems.

## Conclusion

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Living systems are affected by mechanical signals at the organ and tissue level (bone, muscle and fat) as well as at the level of the cell (MSCs, osteoblasts, myocytes and adipocytes). Gravity has been an inescapable physical signal across all life systems since the beginning of time, whereas other physical factors, such as light, temperature, geography, substrate, external threats or food availability, vary with time, both acutely and over aeons. Biological systems have been challenged to resist gravity, and by evolving to become mechanosensitive, their ability to survive improved<sup>345,346</sup>. Conversely, sedentary lifestyles, combined with ageing, have led to a degraded musculoskeletal system and increased adiposity. Mounting evidence indicates that these systemic stressors disrupt both MSC and HSC populations, in addition to biasing the fate selection of their progeny<sup>35</sup>, contributing to a compromised regenerative (MSC) and immune (HSC) system. Empirical evidence suggests that mechanical signals can be used to prevent and/or treat osteoporosis and obesity, guiding mesenchymal and satellite stem cell lineage selection towards an improved musculoskeletal system and suppressed adipose burden, salutary end points that mirror those of exercise and are enabled by an intact cytoskeletal and nuclear connectivity.

### Key points

- Ageing and inactivity each contribute towards a local and systemic environment conducive to poor bone quality, increased systemic adiposity, marrow adipogenesis and inflammation.
- Mesenchymal stem cells and their lineage-differentiated progeny (for example, osteoblasts) are mechanosensitive, such that increased mechanical signals (such as exercise) stimulate muscle and bone anabolism.
- Mechanical signals suppress obesity end points, including fat gain, adipocyte lipid acquisition, chronic inflammation and indices associated with type 2 diabetes mellitus.
- Transduction of mechanical signals across the plasma membrane of stem cells into the nucleus activates signalling cascades and cytoskeletal adaptations to initiate osteogenic, chondrogenic and myogenic differentiation and inhibit adipocyte differentiation.
- Mechanical signals, such as those induced through low-intensity vibration, need not be large in magnitude, or long in duration, to influence bone or fat phenotypes.

### Loading



In terms of mechanical loading, a singular or compound series of static or dynamic (time-varying) forces applied to a system via gravity or direct application from an external body, causing tension, shear or compression.

### Unloading

A cell or body is considered mechanically unloaded if no static or dynamic strain is present, such as what might occur with bed rest or spaceflight (that is, microgravity).

### Ground-reaction forces

As applicable to biomechanics, ground-reaction forces consist of the normal forces exerted by the ground on the body making contact with it, particularly resulting from a heel strike during walking or running.

### Spectral content

Muscle contractive forces, specifically on bone, resonate within a discrete frequency range.

### Load sensation

Mechanical loads are 'sensed' by cells through transduction of external or internal forces across cytoskeletal proteins into the nucleus.

### Tissue senility

The ageing process is associated with the quiescence of regenerative cell populations residing in tissues throughout the body.

### Muscle-specific force

Quantification of the contractile forces generated by muscles can be normalized to muscle size *ex vivo*.

### Fluid shear

Fluidic forces applied tangentially across cell membranes or tissues.

### Dynamic shear forces

Physiological fluids exert a gradient of pulsatile flow across vessel walls, mineralized bone and cells housed in the bone marrow microenvironment.

### Tissue stiffness

In terms of bone, the stiffness of the tissue is correlated to its ability to resist deformation.

### Nuclear stiffness

Nuclear stiffness refers to its rigidity and is directly related to polymeric structural proteins (that is, microtubules, intermediate filaments and microfilaments) found across the cytoskeleton, of which actin proteins provide substantial reinforcement.

## Footnotes

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Competing interests

C.T.R. is a founder of Marodyne Medical, Inc. and BTT Health and has several patents issued and pending related to the ability of mechanical signals to control musculoskeletal and metabolic disorders. The other authors declare no competing interests.

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