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Author for correspondence:
Paige C. Geiger
e-mail: pgeiger@kumc.edu

†These authors contributed equally to this study.

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Exercise, heat shock proteins and insulin resistance

Ashley E. Archer†, Alex T. Von Schulze† and Paige C. Geiger

Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA

PCG, 0000-0002-3990-7246

Best known as chaperones, heat shock proteins (HSPs) also have roles in cell signalling and regulation of metabolism. Rodent studies demonstrate that heat treatment, transgenic overexpression and pharmacological induction of HSP72 prevent high-fat diet-induced glucose intolerance and skeletal muscle insulin resistance. Overexpression of skeletal muscle HSP72 in mice has been shown to increase endurance running capacity nearly twofold and increase mitochondrial content by 50%. A positive correlation between HSP72 mRNA expression and mitochondrial enzyme activity has been observed in human skeletal muscle, and HSP72 expression is markedly decreased in skeletal muscle of insulin resistant and type 2 diabetic patients. In addition, decreased levels of HSP72 correlate with insulin resistance and non-alcoholic fatty liver disease progression in livers from obese patients. These data suggest the targeted induction of HSPs could be a therapeutic approach for preventing metabolic disease by maintaining the body's natural stress response. Exercise elicits a number of metabolic adaptations and is a powerful tool in the prevention and treatment of insulin resistance. Exercise training is also a stimulus for increased HSP expression. Although the underlying mechanism(s) for exercise-induced HSP expression are currently unknown, the HSP response may be critical for the beneficial metabolic effects of exercise. Exercise-induced extracellular HSP release may also contribute to metabolic homeostasis by actively restoring HSP72 content in insulin resistant tissues containing low endogenous levels of HSPs.

This article is part of the theme issue 'Heat shock proteins as modulators and therapeutic targets of chronic disease: an integrated perspective'.

1. Introduction

Insulin resistance is a condition that impacts at least 86 million U.S. adults aged 20 or older [1]. Insulin resistance occurs when the islet cells in the pancreas secrete insulin but the hormone no longer effectively triggers glucose uptake in metabolic tissues. The inability of metabolic tissues to take up glucose results in hyperglycaemia and hyperinsulinaemia, both hallmark symptoms of insulin resistance. Most individuals with insulin resistance go undiagnosed and the condition can persist for 10–12 years. This decade plus of time can be especially damaging as insulin resistance is an independent risk factor for obesity, cardiovascular disease, hypertension and type 2 diabetes. This time frame represents a critical intervention window where progression towards metabolic dysfunction and type 2 diabetes can be prevented and reversed (figure 1).

Growing evidence suggests the heat shock response and/or heat shock proteins (HSPs) could play an important role in preventing insulin resistance and the development of type 2 diabetes. HSPs are a highly conserved family of proteins best identified for their role as molecular chaperones [2]. They play a critical role in maintaining cellular function via regulation of protein folding and degradation. Not surprisingly, changes in their expression profile and cellular localization are linked to numerous disease states. Several studies suggest that induction, transcription and translation of these cytoprotective HSPs decline with chronic disease like non-alcoholic fatty liver disease (NAFLD) [3], Huntington's disease [4] and type 2 diabetes [5]. Conversely, induction and/or transgenic

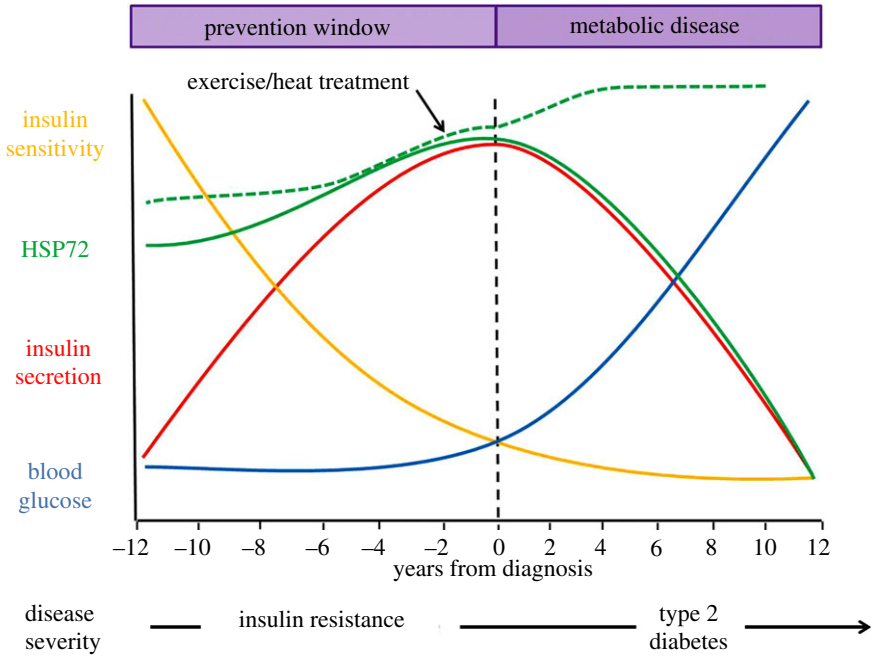

Figure 1. Targeting heat shock proteins in the prevention of insulin resistance. Schematic depicting the timeline of metabolic disease from insulin resistance to type 2 diabetes. Insulin resistance can persist for 10–12 years prior to clinical diagnosis of type 2 diabetes, a time period that represents an increased risk for cardiovascular disease, obesity and type 2 diabetes. During insulin resistance, insulin secretion (red line) from pancreatic beta cells increases in an effort to maintain blood glucose (blue line). Insulin sensitivity declines (yellow line) resulting in a gradual increase in blood glucose and the development of type 2 diabetes. Insulin resistance represents a window of time when progression towards more severe metabolic disease can be prevented by lifestyle interventions like diet and exercise. HSPs are robustly induced by exercise, and HSP72 mRNA and protein expression are significantly reduced in the skeletal muscle of type 2 diabetic patients. However, very little is known about HSP expression patterns and regulation during insulin resistance. We hypothesize that HSP72 expression, in particular, may demonstrate an inverted parabolic relationship wherein initial increases in HSP72 combat metabolic dysfunction, but expression levels eventually peak and decline with disease severity and time spent under metabolic strain (solid green line). Exercise and heat treatment represent potential targeted therapies that could maintain and even increase HSP expression to prevent metabolic disease (dashed green line).

overexpression of HSPs results in ample metabolic benefit in animal models of obesity/metabolic disease [5–11]. Less clear, however, are the factors that regulate HSP expression in the pathological development of metabolic disease. In particular, very little is known regarding skeletal muscle HSP expression levels throughout the progression of obesity, insulin resistance and type 2 diabetes.

As skeletal muscle is the primary tissue responsible for insulin-stimulated glucose uptake [12], many researchers have investigated changes in skeletal muscle HSP expression during obesity, insulin resistance and type 2 diabetes (summarized in table 1). Skeletal muscle HSP72 (the corollary of HSP70 in animals) expression is inversely related to body fat percentage and blood glucose in healthy subjects [9,15]. Additionally, both HSP72 mRNA and protein expression are significantly reduced in the skeletal muscle of type 2 diabetic patients and subjects with insulin resistance [5,13,14,17,18]. Therefore, many have asserted that HSP72 expression levels are tightly correlated to adiposity and decrease through the progression from obesity to metabolic disease (i.e. insulin resistance and type 2 diabetes). It is also possible that glucose levels may partially regulate HSP expression levels [21–23].

Interestingly, multiple studies using animals fed a high-fat diet (HFD) highlight that this relationship is much more complex (table 1). For instance, investigations in primates and rodents show that short-term high-fat feeding (16 and 6–12 weeks, respectively) results in hallmark symptomology of insulin resistance but does not significantly reduce skeletal muscle HSP72 expression [6,7,10,16,19,20]. In fact, HSP72 expression may increase after short-term high-fat feeding, suggesting a possible compensatory response to combat metabolic dysfunction [16,19]. However, long-term high-fat feeding (6 years) appears to cause significant reductions in skeletal muscle HSP72 expression similar to the phenomenon described in type 2 diabetics [16]. Therefore, it is possible that skeletal muscle HSP72 expression can be characterized as an inverted parabolic relationship wherein initial increases in skeletal muscle HSP72 combat metabolic dysfunction, but these levels will eventually peak and decline depending on the severity and time spent under metabolic strain (solid green line, figure 1).

Discrepancies in the data regarding skeletal muscle HSP72 reductions during obesity, insulin resistance and type 2 diabetes may also be due to the model being used and the muscle type analysed. For example, investigations reporting significant reductions in HSP72 expression during obesity, insulin resistance and type 2 diabetes primarily analysed the vastus lateralis muscle from human subjects [13,14,17,18]. Alternatively, primate and rodent investigations observing no significant reductions in HSP72 expression in response to short-term high-fat feeding analysed the biceps femoris, soleus, and extensor digitorum longus muscles [6,7,10,16,19,20]. Thus, it is also possible that organismal differences and/or muscle fibre type differences, variations in muscle oxidative capacity, and muscle size could contribute to inter-study data variations (table 1). It is critical that future investigators address these inconsistencies when designing studies to address the role of HSP72 expression in metabolic disease.

Table 1. Changes in skeletal muscle HSP72 levels during metabolic disease. HFD, high-fat diet; n.a., not available.

investigators	model	muscle	status	duration HFD	HSP72 levels
Kurucz *et al.* [13]	human	vastus lateralis	type 2 diabetic	n.a.	HSP72 mRNA reduced
Bruce *et al.* [14]	human	vastus lateralis	type 2 diabetic	n.a.	HSP72 mRNA reduced
Chung *et al.* [5]	human and rodent	not reported	obese and insulin resistant	unknown for humans, 16 weeks for rodents	HSP72 protein reduced in humans, not reported in rodents
Gupte *et al.* [6]	rodent	soleus	impaired glucose tolerance	6 weeks	HSP72 protein unaffected by diet
Gupte *et al.* [7]	rodent	soleus and extensor digitorum longus	impaired glucose tolerance	12 weeks	HSP72 protein unaffected by diet
Henstridge *et al.* [15]	human	vastus lateralis	healthy	n.a.	HSP72 protein content inversely related to body fat percentage
Kavanagh *et al.* [16]	primate	biceps femoris	healthy and insulin resistant	16 weeks and 6 years	HSP72 protein increased at 16 weeks and reduced at 6 years
Rodrigues-Krause *et al.* [17]	human	vastus lateralis	type 2 diabetic	n.a.	HSP72 protein reduced
Henstridge *et al.* [8]	rodent	quadriceps	obese/insulin resistant	10 weeks	no change in HSP72 protein reported
Matos *et al.* [18]	human	vastus lateralis	healthy, obese – insulin sensitive and obese – insulin resistant	n.a.	basal and exercise-induced HSP72 protein reduced in both obese – sensitive and obese – insulin resistant subjects
Silverstein *et al.* [11]	rodent	gastrocnemius	aged mice; heat or geranylgeranylacetone treated	14 weeks	changes in basal HSP72 protein not reported; HSP72 induction positively impacted metabolic measures
Marineli *et al.* [19]	rodent	soleus	impaired glucose tolerance	12 weeks	HSP72 protein increased
Bock *et al.* [20]	rodent	soleus	insulin resistant	28 weeks	HSP72 protein unaffected by diet
Kavanagh *et al.* [9]	primate	vastus lateralis	aged or young, both healthy and pre-diabetic/diabetic	n.a.	HSP72 protein inversely related to fasting blood glucose
Rogers *et al.* [10]	rodent	extensor digitorum longus (EDL) and soleus	high-capacity or low-capacity runners (HCR and LCR respectively)	3 days	basal HSP72 protein unaffected by diet; HSP72 induction via heat blunted in LCRs in EDL

A greater understanding of the regulation of skeletal muscle HSPs during insulin resistance will allow future development of targeted therapies to maintain and even increase HSP expression to prevent metabolic disease (figure 1, dashed green line).

2. Heat shock protein mechanisms of action in insulin resistance

The complex, integrative and multi-organ nature of the HSP response makes the identification of specific mechanisms of action difficult. For instance, the most widely known HSP, HSP72, has varying roles and mechanisms of action in heart muscle, skeletal muscle, adipose tissue and the liver. Recent studies suggest decreasing inflammation, improving mitochondrial function/oxidative capacity, and maintaining proteostasis could be viable mechanisms of action for HSPs in metabolic tissues.

(a) Anti-inflammatory properties of HSP72

The ability of HSPs to decrease inflammation has centred on the proinflammatory protein c-Jun terminal kinase (JNK). Importantly, JNK activation is increased with the progression of insulin resistance and diabetes [24–29], while HSP72 expression is correspondingly decreased [5,13,14,17]. This inverse relationship between JNK activation and HSP expression also occurs during the progression from NAFLD to non-alcoholic steatohepatitis [3]. This relationship is of no coincidence. JNK activation indirectly inhibits HSP expression by maintaining heat shock factor 1 (HSF1), the primary HSP transcription factor, in its inactive monomeric state [30,31]. Beyond inactivation of HSF1 and HSP expression, there are other downstream targets of JNK that potentiate insulin resistance.

JNK is thought to drive insulin resistance through inhibitory phosphorylation of insulin receptor substrate 1 (IRS-1), a key protein in the insulin signalling cascade [25]. In addition, JNK can downregulate peroxisome proliferator-activated receptor α/fibroblast growth factor 21 (PPARα/FGF21) signalling in hepatocytes, leading to reduced fatty acid oxidation and the development of insulin resistance [32]. JNK activation also inhibits mitochondrial respiration, increases reactive oxygen species (ROS) production and causes apoptosis [33–37]. Previous studies suggest that HSP72 induction directly inhibits JNK activation, thereby improving insulin sensitivity and glucose tolerance at both skeletal muscle-specific and systemic levels [5,6,8,38,39]. For example, work by our laboratory has demonstrated that *in vivo* heat treatments decrease JNK activation in skeletal muscle of aged and HFD-fed rats [7,40]. Pharmacological activation of HSP72 also causes reduced JNK activation in skeletal muscle and liver [6,38]. Finally, overexpression of HSP72 in skeletal muscle decreased JNK activation in mice fed a HFD and was associated with beneficial metabolic outcomes [5]. In each instance, lowering of JNK activation resulted in improvements in insulin sensitivity and glucose tolerance, highlighting the importance of this HSP-mediated mechanism for insulin action.

HSP72 is proposed to regulate JNK activation through multiple mechanisms, including direct inhibition via protein–protein interaction with JNK [41], and/or inhibition of upstream JNK signalling pathways [42,43]. Evidence also exists suggesting that activation of HSP72 in the liver may decrease inflammation independently of JNK inhibition. Specifically, pharmacological activation of HSP72 decreases steatosis without decreasing JNK activation in HFD-fed rodents [44]. Although no change in JNK activation was observed, increased HSP72 expression resulted in inhibition of tumour necrosis factor α (TNFα) in the liver of rodents fed a HFD.

HSP72 may also play additional anti-inflammatory roles extracellularly or via localization in macrophages. For instance, HSP72 decreases during NAFLD progression in human Kupffer cells, liver-specific macrophages [3]. Interestingly, heat-induced upregulation of HSP72 in Kupffer cells coincides with suppression of TNFα [45,46]. Additionally, in myeloid cells, JNK activity is considered essential for activation of macrophages and a release of pro-inflammatory cytokines [47,48]. The ability of extracellular HSP72 to inhibit pro-inflammatory cytokine release from macrophages, lymphocytes and other immune cells [49–54] could be critical in decreasing local inflammation and attenuating the development of insulin resistance.

(b) HSP72 regulation of mitochondrial integrity and function

Mitochondrial dysfunction is a primary contributor to the development of metabolic disease and is therefore a possible target for therapy [55–57]. Our laboratory and others have shown that heat treatment improves skeletal muscle mitochondrial function by improving fatty acid oxidation [7], increasing mitochondrial enzyme activity [7,58,59], and increasing mitochondrial biogenesis [60]. Transgenic overexpression of HSP72 in skeletal muscle also increases mitochondrial enzyme activity, mitochondrial content and endurance running capacity [5,8]. Thus, it is possible that the beneficial mitochondrial adaptations stemming from heat treatment are a result of HSP72 induction.

HSP72 induction may mediate mitochondrial improvements by regulating mitophagy, the targeted degradation of mitochondria through autophagy. For instance, mice lacking skeletal muscle HSP72 demonstrate a reduced ability to degrade mitochondria through mitophagy [61]. Additionally, these mice exhibit enlarged, dysmorphic mitochondria with reduced muscle respiratory capacity and increased lipid accumulation. Thus, activation of HSP72 may improve mitochondrial quality by enhancing the degradation of dysfunctional mitochondria.

(c) HSP72 regulation of the unfolded protein response and proteostasis

Cellular stress causes unfolded proteins to accumulate in the endoplasmic reticulum (ER), which activates the unfolded protein response (UPR) [62–64]. This response is important for cellular adaptation to ER stress and prevention of ER-stress-induced apoptosis [65,66]. ER stress and chronic activation of the UPR causes inflammation and contributes to the development of insulin resistance [67–76].

While HSP family proteins have been shown to be a part of the UPR [77–79], new evidence has also identified the cytoplasmic HSP72 as a part of the UPR. Specifically, HSP72 interacts with and upregulates inositol-requiring enzyme 1α (IRE1α) signalling. Activation of IRE1α by HSP72 enhances cell survival through prevention of ER-stress-induced apoptosis [80]. This mechanism may be important in HSP72-mediated

metabolic improvements, since activation of IRE1α also has been shown to suppress lipogenesis [81].

HSP72 may also impact metabolic health through the protein's additional responsibilities as a cellular chaperone. During stress, HSP72 is essential to refold misfolded proteins and to maintain proteostasis. HSP72 may maintain proteostasis by regulating proteosomal degradation and autophagy [82,83]. Degradation pathways via proteasomes and autophagy are well established, but it was recently demonstrated that mitochondria also function as sites for protein degradation [84]. Specifically, the chaperone HSP104 detangles protein aggregates allowing mitochondrial transporters to import proteins in the outer and inner mitochondrial membrane. Proteases in the mitochondrial matrix are then able to degrade the newly imported unfolded proteins. Importantly, defects in HSP70 activity resulted in increased transport of misfolded proteins into the mitochondria, causing increased mitochondrial damage and ROS production. This phenomenon was confirmed both in yeast and in human retinal pigment epithelium cells [84]. It is tempting to speculate that defects in HSP72 activity could contribute to mitochondrial dysfunction by triggering this alternative mitochondrial-dependent degradation pathway. This alternative pathway may contribute to the swollen, rounded appearance of the mitochondria during metabolic disease, as well as decreased ability for the mitochondria to function as a respiratory organelle. Future research investigating this mechanism in metabolic organs will be necessary.

(d) Heat shock transcription factor regulation of oxidative capacity

One of the most important heat shock response functions in metabolic tissue may actually lie upstream of HSP72. HSP72 overexpression leads to an increase in mitochondrial content, oxidative capacity and insulin sensitivity [5,55–57,85]. Similarly, the absence of HSP72 expression results in mitochondrial dysfunction and insulin resistance [61]. In addition to increasing HSP72 content, and thereby the ability to enhance mitochondrial quality control, exercise also increases peroxisome proliferator-activated receptor γ coactivator 1-α (PGC1α) expression [86–88]. PGC1α is the primary transcriptional coactivator for mitochondrial biosynthesis [89,90]. Interestingly, recent investigations reveal that the upstream regulatory elements of the *PPARGC1A* gene contain a heat shock element (HSE) binding sequence. This HSE sequence provides a docking site for the primary HSP transcription factor, heat shock factor 1 (HSF1). Indeed, chromatin immunoprecipitation analyses show that HSF1 and PGC1α co-occupy the HSE sequence on the promoter of the *PPARGC1A* gene [91]. Through a myriad of HSF1 activation and knockdown experiments, the Mueller lab has provided compelling evidence that HSF1 is a primary regulator of mitochondrial biogenesis, enzymatic function and whole-body metabolism [91,92]. These data exemplify the elegant coordination of HSF1 downstream targets (i.e. HSPs and PGC1α) in regulating mitochondrial biogenesis, quality control, and enzymatic function under conditions of metabolic demand and/or chronic disease. Importantly, future research is needed to delineate the specific contributions of varying downstream HSF1 targets, as well as potential direct effects of HSF1 itself, with regard to metabolic outcomes. This information, combined with a greater understanding of HSP mechanisms of action in metabolic tissue, may provide novel therapeutic targets to ameliorate metabolic dysfunction.

3. Exercise-induced heat shock protein response

Exercise is a primary treatment modality for patients exhibiting symptoms of metabolic dysfunction. Specifically, regular exercise training is known to decrease metabolic and cardiovascular disease risk factors in patients suffering from obesity and metabolic dysfunction [93,94]. Exercise is also a potent inducer of HSP expression [78], with HSP72 showing the most robust and consistent upregulation with exercise. HSP72 induction via heat treatment, pharmacologic intervention, and transgenic overexpression results in metabolic effects similar to exercise in models of obesity and insulin resistance [5–7,40,78]. Thus, exercise-induced HSP72 expression may contribute to the beneficial metabolic effects observed with exercise training. There is already a significant amount of information available about exercise and HSPs; however, little is known regarding the role of exercise-induced HSP72 expression in treating metabolic disease.

(a) Complexity of the exercise heat shock protein response

The direct cause of exercise-induced HSP upregulation, primarily of HSP72, remains unknown. It is hypothesized that a variety of biochemical, metabolic and/or physical stressors may stimulate HSP72 expression post-exercise. For instance, common challenges to tissues during exercise such as mechanical stress, acidosis, hypoxia, ischaemia, ROS formation, and calcium signalling changes are shown to independently cause HSP induction [95–103]. Additionally, increased metabolic stress via depletion of bioenergetic substrates (i.e. glycogen) is shown to potentiate exercise-induced HSP72 expression [104]. A similar potentiation effect is observed when exercise bouts are completed in a hot environment, but this effect is blunted in a cold environment [105]. Thus, it appears that elevations in HSP72 expression post-exercise are a result of not one, but many physiological stressors associated with exercise.

Adding complexity is the understanding that exercise-induced HSP expression is training modality, intensity and duration dependent. In skeletal muscle, elevations in HSP72 expression occur with both aerobic and resistance training [106,107]. Importantly, HSP72 expression is dependent on exercise intensity. For instance, HSP72 expression displays a positive relationship with exercise intensity during both aerobic and resistance training [104,106,108,109]. This relationship also exists when comparing exercise intensity and metabolic outcomes [110], supporting the potential contribution of HSP72 induction to the metabolic benefits associated with exercise.

HSP72 expression also varies based on the duration of the training regimen (i.e. acute versus chronic training). Acute exercise bouts cause dramatic elevations in HSP72 within 24 h [106], while chronic training regimens typically result in minimal elevations in HSP72 post-exercise [102]. Similarly, untrained subjects exhibit lower basal HSP72 expression and a higher degree of change in HSP expression post-exercise compared with fit subjects [102,111]. The minimal degree of change in HSP72 expression observed during long-duration training protocols and in fit subjects is likely a result of adaptation to

exercise. This phenomenon, referred to as the repeated bout effect [112,113], is exemplified by the lack of potentiated HSP induction in recurring exercise bouts (specifically HSP72 and HSP27) [114]. However, cessation of exercise in trained subjects will cause basal HSP expression to return to levels comparable to those observed pre-exercise [115].

(b) Aerobic capacity and exercise training impact heat shock protein expression and induction

Recently, our laboratory has published data suggesting that intrinsic aerobic capacity, or the ability of the body to take up and use oxygen, is coupled to HSP induction and metabolic flexibility [10]. Low aerobic capacity increases susceptibility to developing metabolic dysfunction. Importantly, it is estimated that 50–70% of one's aerobic capacity is attributable to inheritable traits [116]. This genetic/phenotypic phenomenon is exemplified by rodent models selectively bred for high-capacity or low-capacity running (HCR and LCR respectively) [117]. Specifically, these models have drastic differences in susceptibility to metabolic complications [118–121]. For instance, the HSP72 response is blunted in LCR rodents after heat treatment and they require the heat intervention to maintain metabolic flexibility/protection when acutely challenged with a HFD [10]. Conversely, HCR rodents maintain the ability to upregulate HSP72 expression in skeletal muscle via heat treatment and display metabolic flexibility/protection independent of intervention when metabolically challenged. These data suggest that intrinsic aerobic capacity is coupled to the HSP72 response in skeletal muscle and that these two factors are primary contributors to whole-body metabolic health. As mentioned, unfit subjects with metabolic dysfunction, and most likely low aerobic capacity, have markedly low levels of HSP72 expression compared with healthy controls [5,13,14]. Thus, chronic exercise may restore basal HSP72 expression levels to that of healthy subjects. The restoration of basal HSP72 expression via exercise may directly impact organ-specific insulin sensitivity.

(c) Tissue-specific heat shock protein expression and induction

As mentioned, exercise increases skeletal muscle HSP72 expression. However, the levels of both basal HSP72 expression and exercise-induced HSP72 expression are dependent on muscle fibre type. For instance, muscles predominantly composed of type I fibres have higher basal HSP72 expression compared with muscles composed of type II fibres [122,123]. Furthermore, the magnitude of HSP72 upregulation is much greater in type II muscle fibres post-exercise compared with type I fibres [106,124]. This may explain the intensity dependent increases in HSP72 expression post-exercise, as higher intensity activities cause the recruitment of fast-twitch muscle fibres, resulting in a greater overall change in HSP72 expression. As type II muscle fibres are inherently glycolytic and have a high dynamic range of HSP72 expression, this invites the possibility that the positive metabolic effects seen with HSP72 overexpression may be primarily mediated by changes in type II fast-twitch muscles.

Exercise is also known to increase HSP72 expression in the liver, kidney, lungs, heart and brain [125–127]. During states of metabolic dysfunction, HSP72 expression in the liver is of primary concern owing to the organ's role in maintaining whole-body metabolic homeostasis. Pharmacologic HSP72 induction in the liver is shown to improve insulin sensitivity and glucose tolerance in models fed an HFD [38]. This protective effect may stem from the enhancement of HSP72-mediated mitochondrial quality control and the restoration of the insulin signalling pathway in hepatocytes—both of which occur with exercise and HSP72 upregulation in skeletal muscle. Thus, exercise-induced HSP72 expression in the liver may act to restore liver insulin sensitivity by mechanisms similar to those observed in skeletal muscle. However, future studies are needed to confirm this notion.

Interestingly, exercise also results in the release of extracellular HSPs (eHSPs) from the hepatosplanchnic viscera and brain into the circulation [104,128], and other potential sites of origin include epithelial cells [129] and immune cells [130,131]. eHSP72 function in general is associated with activation of the immune system [132], and in contrast to the anti-inflammatory actions of intracellular/cytosolic HSP72 (iHSP72), can induce activation of proinflammatory pathways. Based on this antagonistic action of HSP72 on the inflammatory response, the Chaperone Balance Hypothesis contends that the balance between eHSP72 and iHSP72 (eHSP72/iHSP72) could determine the extent of tissue inflammation, and thereby also influence the pathogenesis of insulin resistance and type 2 diabetes [133]. According to this hypothesis, an intervention that lowers the eHSP72/iHSP72 ratio could in effect improve insulin sensitivity. Long-term exercise training in effect results in decreased eHSP72 and increased iHSP72 expression (as in skeletal muscle), supporting this hypothesis. Importantly, the eHSP72/iHSP72 ratio could be a valuable biomarker for assessment of the inflammatory response in insulin resistance and diabetes.

However, the specific tissue contributions, mechanism(s) of action, and physiological consequences of eHSPs during exercise remain unknown. It is hypothesized that exercise-induced eHSPs may provide metabolic crosstalk between organs, contribute to exercise adaptation, and/or act as a stress-sensor or stress-messenger [78,104,128]. Exercise-induced eHSP release may also contribute to metabolic homeostasis by actively restoring HSP72 content in insulin resistant tissues containing low endogenous levels of HSPs. For example, existing evidence suggests that HSPs can be produced in tissues like muscle and adipose and released in the circulation via exosomes, small membrane vesicles that are secreted by numerous cell types [134]. In this manner, intracellular HSP72 transmission mediated by exosomes represents a novel mechanism for maintenance of HSP72 expression among different tissues. Future studies are needed to characterize the physiological outcomes of eHSPs both in healthy subjects and those with metabolic dysfunction.

4. Summary

Current lifestyle interventions for obesity and metabolic disease include dietary modification and exercise training. While effective at reducing body mass and enhancing insulin sensitivity, compliance is often low in patient populations and therefore alternative approaches are needed. Acute or short-term passive heating ($\leq$3 weeks) has been investigated with promising improvements in metabolic parameters in humans [135]. In 1999, Philip Hooper performed the first study to suggest heat therapy (HT) could be beneficial for metabolic disease. In diabetic patients, fasting plasma glucose

and haemoglobin A1c (HbA1c) levels were significantly decreased after only three weeks of HT by water immersion (30 min, 6 days/week) in which core body temperature was increased by an average of 0.8°C each session [135]. Despite this exciting phenomenon, only a handful of studies have examined the effects of HT in obese and/or type 2 diabetic patients [136–139].

Importantly, the first comprehensive investigation of long-term heat treatment in young, sedentary humans was recently performed [140]. Brunt *et al*. [140] found that eight weeks of repeated hot water immersion resulted in increased endothelial function (measured via flow-mediated dilation), reduced arterial stiffness, reduced mean arterial and diastolic blood pressure, and reduced carotid intima media thickness. Incredibly, these cardiovascular adaptations were on par with what is typically observed with exercise training in previously sedentary subjects. Despite ample evidence in animal studies demonstrating the beneficial effects of heat treatment on whole body metabolism and the anti-inflammatory and neuroprotective functions of HSP72 *in vivo*, heat treatment studies in insulin resistant or diabetic patients are lacking. Mild heat therapy treatment in patients with heart failure is remarkably effective [141], and the most promising application of mild, chronic heat treatment in humans could be in combination with exercise training. Novel, integrative research studies to examine both cellular mechanisms and systemic metabolic adaptations of heat therapy in humans could lead to new interventions for insulin resistance, obesity and cardiometabolic disease.

Data accessibility. This article has no additional data.

Authors' contributions. A.E.A., A.T.V.S. and P.C.G. wrote and edited all sections of the manuscript. P.C.G. had final editorial approval of the manuscript.

Competing interests. We declare we have no competing interests.

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