Minireview

MOLECULAR METABOLISM

Chaperoning to the metabolic party: The emerging therapeutic role of heat-shock proteins in obesity and type 2 diabetes

Darren C. Henstridge [1], **Martin Whitham** [1], **Mark A. Febbraio***

ABSTRACT

Background: From their initial, accidental discovery 50 years ago, the highly conserved Heat Shock Proteins (HSPs) continue to exhibit fundamental roles in the protection of cell integrity. Meanwhile, in the midst of an obesity epidemic, research demonstrates a key involvement of low grade inflammation, and mitochondrial dysfunction amongst other mechanisms, in the pathology of insulin resistance and type 2 diabetes mellitus (T2DM). In particular, tumor necrosis factor alpha (TNFα), endoplasmic reticulum (ER) and oxidative stress all appear to be associated with obesity and stimulate inflammatory kinases such as c jun amino terminal kinase (JNK), inhibitor of NF-κβ kinase (IKK) and protein kinase C (PKC) which in turn, inhibit insulin signaling. Mitochondrial dysfunction in skeletal muscle has also been proposed to be prominent in the pathogenesis of T2DM either by reducing the ability to oxidize fatty acids, leading to the accumulation of deleterious lipid species in peripheral tissues such as skeletal muscle and liver, or by altering the cellular redox state. Since HSPs act as molecular chaperones and demonstrate crucial protective functions in stressed cells, we and others have postulated that the manipulation of HSP expression in metabolically relevant tissues represents a therapeutic avenue for obesity-induced insulin resistance.

Scope of Review: This review summarizes the literature from both animal and human studies, that has examined how HSPs, particularly the inducible HSP, Heat Shock Protein 72 (Hsp72) alters glucose homeostasis and the possible approaches to modulating Hsp72 expression. A summation of the role of chemical chaperones in metabolic disorders is also included.

Major Conclusions: Targeted manipulation of Hsp72 or use of chemical chaperiones may have clinical utility in treating metabolic disorders such as insulin resistance and T2DM.

Keywords Hsp72; Skeletal muscle; Insulin resistance; Type 2 diabetes; Obesity; Inflammation; Oxidative capacity; Mitochondria; Chemical chaperones

1. INTRODUCTION

Heat shock proteins (HSPs) were serendipitously discovered over 50 years ago by the Italian scientist Ferruccio Ritossa. Indeed, the inadvertent application of heat shock to drosophila salivary glands (by the accidental adjustment of incubator settings) and the subsequent observation of new HSP RNA synthesis remains a striking demonstration of environmentally induced changes in gene expression [1,2]. A rather different and no doubt, more complex collective gene response is occurring consequential of today's snowballing and problematic environment of nutrient excess. Rates of obesity continue to rise and the understanding of how this leads to metabolic diseases such as type 2 diabetes (T2DM) is improving. However, effective treatments remain elusive. Given the ubiquity of the highly conserved HSPs, it is perhaps unsurprising that these chaperone proteins have been implicated in the treatment of insulin resistance and obesity associated T2DM [3,4]. Attracting most attention in this regard is the inducible isoform of HSP70, Hsp72 (Hspa1a). Experimental models indicate that Hsp72 is likely to confer protection against disturbed metabolic homeostasis via multiple modes of action including, but not limited to, reducing inflammation [3,5,6] and improving skeletal muscle oxidation [3,6–8]. Importantly, we [3,8] and others [4,9] have been conducting experiments using a small molecule activator of HSP72 (a hydroxylamine derivate termed BGP-15). This agent improves insulin sensitivity and inflammation in a genetic mouse model of insulin resistance [3], increases mitochondrial volume and improves metabolic homeostasis in a rat model of T2DM [8]. This is most promising from a clinical and translational perspective since BGP-15 has now proceeded to Phase 2b clinical trials and has previously been used in humans without any side-effects [4,10]. This review summarizes the accumulating evidence for a

Cellular & Molecular Metabolism Laboratory, Division of Metabolism and Obesity, Baker IDI Heart & Diabetes Institute, 75 Commercial Rd, Melbourne, Victoria 3004, Australia

[1] Co-first authors: These authors contributed equally to this work.

*Corresponding author. Tel.: +61 3 8532 1767; fax: +61 3 8532 1100.

E-mails: Darren.Henstridge@bakeridi.edu.au (D.C. Henstridge), Martin.Whitham@bakeridi.edu.au (M. Whitham), mark.febbraio@bakeridi.edu.au (M.A. Febbraio).

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role of Hsp72 in glucose metabolism and discusses the therapeutic potential of Hsp72 raising agents in the treatment of conditions associated with insulin resistance.

2. HSP EXPRESSION IN INSULIN RESISTANCE AND DIABETES

Arguably the first link between HSPs and diabetes was derived from the observation that in insulin resistant and T2DM patients, HSP expression was markedly altered. Muscle biopsies taken from patients with T2DM showed significantly lower mRNA levels of Hsp72 than those taken from non-diabetic controls [11]. Furthermore, data collected in our laboratory supported this finding and demonstrated a marked relationship between Hsp72 mRNA and insulin stimulated glucose uptake during a hyperinsulemic-euglycemic clamp in T2DM patients [12]. We and others [13] later demonstrated that skeletal muscle Hsp72 protein expression reflected the same trend as Hsp72 mRNA, supporting the hypothesis that Hsp72 expression is decreased in T2DM [3]. Early speculation considered that Hsp72 expression might be affecting insulin sensitivity through a direct interaction with GLUT4 [11]. However, other studies found no reduction in GLUT4 gene expression in diabetic patients versus aged matched controls [12]. In the same study, we directly measured intramuscular triglyceride (IMTG) content in the muscle biopsy samples derived from T2DM patients and aged matched healthy controls. IMTG content was ~150% higher in the patient group. Allied to the finding of lowered Hsp72 expression in T2DM, these data provided a rationale for the examination of the role of HSP expression in the etiology of obesity induced insulin resistance.

3. OBESITY, INFLAMMATION AND INSULIN RESISTANCE

3.1. Low grade inflammation

Numerous lines of evidence suggest a link between obesity and inflammation. Prolonged or chronic inflammation is associated with a cluster of metabolic diseases, including T2DM and is referred to as "low grade" or meta-inflammation [14]. While the cascade of molecules involved in inflammation is complex, the pro-inflammatory cytokine, TNFα appears to demonstrate a prominent role in mediating downstream transduction cascades that affect insulin signaling. For example, TNFα is increased in the adipose tissue of obese mice [15]. Moreover, in loss-of-function experiments in obese mice, null mutations in the gene encoding TNFα and its receptors resulted in improved insulin sensitivity [16]. Significantly, a multitude of metabolic stressors appear capable of inducing inflammatory signaling pathways. In addition to extracellular TNFα., stressors originating from within the cell appear influential. For example, obesity places overload on the endoplasmic reticulum (ER) due to an accumulation of misfolded proteins, lipid oversupply and increased demand on the synthetic machinery [17]. Indeed, in both high fat diet (HFD) and genetic (*ob/ob*) models of murine obesity, indicators of ER stress such as PKR-like kinase (PERK) and eIF2α are significantly phosphorylated in liver extracts from obese versus lean animals [18]. Elevated glucose metabolism can also cause an increase in reactive oxygen species (ROS) in the mitochondria. Interestingly, gene expression analysis has suggested a role for ROS in both TNFα and glucocorticoid models of insulin resistance [19]. Given that both ER and oxidative stress are known to induce inflammatory signaling cascades [17,20], these stressors provide additional means by which obesity might disrupt insulin signaling.

3.2. Inflammatory kinases and the disruption of insulin signaling

Since the inflammatory serine/threonine kinases, c jun amino terminal kinase (JNK), inhibitor of NF-κβ kinase (IKK), and protein kinase C (PKC) disrupt insulin signaling [21–23] blocking their action provides a possible means for therapeutic intervention to treat insulin resistance. In particular, the MAPK kinase JNK has emerged as a possible key regulator of metabolic alterations in insulin sensitivity. Indeed, three lines of evidence highlight this. Firstly, JNK activity is elevated in both dietary and genetic models of obesity [24,25] and deletion of two of the three JNK isoforms, JNK1 [24,26] and JNK2 [26] protects mice from HFD-induced insulin resistance. Rats fed a high fat "western" diet for 30 days showed higher JNK activity in liver, muscle and hypothalamus, compared with controls [25]. In addition, JNK phosphorylation is elevated in liver, muscle and adipose tissues taken from leptin deficient (*ob/ob*) mice, a commonly used genetic model of murine obesity [24]. Secondly, JNK is activated by free fatty acids (FFA), TNFα, ER stress and ROS [17,20,27–29], all of which are known to contribute to insulin resistance. Finally, JNK serine phosphorylates IRS-1 (ser307) which disrupts IRS-1 and IR interaction, the proximal step in insulin signaling [30,31]. Taken together, these findings provide support for the notion that JNK1 inhibition might provide a promising therapeutic avenue for the treatment of T2DM.

4. HSPS AND INFLAMMATION

A key feature of HSPs is their ability to provide cytoprotection. Early experiments demonstrated that if cells were heat treated to 43 °C, (which increased HSP synthesis) the number of cells surviving a subsequent insult of heat shock increased [32]. Once it became understood that HSPs can provide cytoprotection against other stressors, interest in their therapeutic value increased. For example, preheating of human leukemic cells led to reduced cell death following a subsequent heat shock, which was associated with an inhibition of JNK1 and p38 activation [33]. That this effect might be mediated by HSPs was assessed using ectopic over-expression of Hsp72 in the human PEER cell line. Indeed, overexpression of Hsp72 suppressed the apoptotic and stress kinase activating effects of heat, osmotic shock, H_2O_2 and UV irradiation [33]. Experimental evidence suggests several potential mechanisms by which HSP72 can downregulate JNK. Experiments utilizing Hsp72 transfected mouse embryonic fibroblasts, suggested that Hsp72 suppresses the JNK1 signaling pathway through physical association and prevention of JNK1 phosphorylation by its upstream kinase SEK1 [34]. In addition, Daviau et al. demonstrated a role for duel leucine zipper-bearing kinase (DLK) in the mechanism by which HSP72 can down-regulate JNK. DLK is a member of the mixed linage kinase family which are known mitogen activated kinase kinases [35]. DLK is a known upstream activator of JNK. These authors [35] showed in COS-7 cells that HSP72 associates with the HSP co-chaperone CHIP, a known ubiquitin ligase, which can negatively regulate DLK expression and activity. These data suggest that the mechanism by which HSP72 blocks JNK activity is via CHIP mediated DLK ubiquitination. Finally, others [36] demonstrated a role for the upstream phosphatase MAP kinase phosphatase-1 (MKP-1) in HSP72 mediated down-regulation of JNK.

5. HSP72 AND THE PREVENTION OF INSULIN RESISTANCE

Meta-inflammation appears to disrupt insulin signaling and HSPs appear to have the potential to inhibit inflammatory kinases. Therefore, there is a strong rationale to investigate the activation and/or upregulation of HSPs as a means to treat insulin resistance. Interestingly, one preliminary report has suggested that heat therapy in general might have therapeutic potential. T2DM patients using a hot tub daily for three weeks exhibited improvements in glycaemia by unknown

