

## *The stress response of the liver to physical exercise*

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### ABSTRACT

*Recent research on the effectiveness of training interventions indicates major alterations of hepatic lipid metabolism and suggests a substantial and beneficial adaptation of the liver to regular physical activity in humans. However, while various data demonstrate the response of the working skeletal muscle to acute exercise and training, considerably less is known about the molecular events in the liver during and after increased physical activity. Here we discuss recent studies performed in rodents, that elucidate the acute hepatic response to one single bout of exercise with particular emphasis on stress response-related pathways. The acute transcriptional response to one exercise bout comprises three-times more hepatic transcripts than those expressed in soleus muscle, with a significantly more pronounced up- or downregulation of hepatic genes. Evaluation of the affected pathways shows that the liver responds to acute exercise with a rapid activation of the mitogen-activated protein kinase (MAPK) signalling pathway, of the p53 protein, and of interleukin (IL)-6-type cytokine signalling pathways, resulting in a marked transcriptional upregulation of stress response genes (e.g. transcription factors of the Fos/Jun-family, growth arrest and DNA damage (GADD)45 $\gamma$ , and p53-target genes) and genes typically induced by energy depletion, e.g. insulin-like growth factor binding protein (IGFBP)-1, peroxisome proliferator-activated receptor coactivator (PGC)1 $\alpha$ . One explanation for the marked differential expression of hepatic genes immediately after exercise is the induction of energetic stress. After non-exhaustive exercise energy depletion predominantly occurs in the liver, not as much in the working muscle, and during exercise, the liver is exposed to altered concentrations of insulin and glucagon in the portal vein. Furthermore, lower plasma glucose levels post-exercise are related to increased expression levels of stress response genes. It appears that the unique function of the liver to supply glucose for the working muscle renders this organ*

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*especially susceptible for exercise-induced cellular stress that leads to the marked induction of defense adaptations. These results give rise to the question whether these molecular events are linked not only to stress defense but to the metabolic adaptations of the liver to exercise.*

**Key words:** MAPK, p53, energy depletion, oxidative stress, glucose output

## INTRODUCTION

Regular physical activity is known to have multiple health benefits including the maintenance of insulin sensitivity and of cardiorespiratory fitness, and it is a major factor to prevent the onset of coronary heart disease and type 2 diabetes mellitus (23, 36, 84, 87, 104). Physical inactivity has the opposite effect: the reduction of ambulatory activity for two weeks in young healthy men causes a decline of peripheral insulin sensitivity (63). The molecular mechanisms which are responsible for the beneficial effects of exercise on the peripheral tissues are incompletely understood. The importance to elucidate these mechanism is augmented by the fact that there is a large variability in the individual outcome of training interventions on mitochondrial function, on changes in aerobic physical fitness and on insulin sensitivity (108) and that not all people exhibit an apparent improvement of their individual insulin sensitivity by performing regular exercise (14).

The working skeletal muscle plays an outstanding role during exercise as the most directly affected organ. Researchers have mainly focused on the molecular response of the contracting muscle. The invasive nature of these investigations is a clear limitation for studies of other tissues like the liver and therefore little data are available. However, exercise is a major challenge also for other organs, particularly for the liver due to its central role in the maintenance of glucose and lipid homeostasis and its function as energy supplier for the working muscle (34, 59, 121, 123). Recent research on the effectiveness of training interventions shows major alterations of hepatic lipid content and suggests a significant adaptation of hepatic metabolism to regular physical activity (53, 105, 106, 111, 113). These findings gave rise to the hypothesis that the liver is strongly affected by exercise and initiated studies on molecular events induced by physical activity in the liver. Due to the mentioned limitations to investigate the hepatic response in humans, rodent models of exercise are used to elucidate the exercise-dependent regulation of signal transduction pathways, gene expression and protein levels in the liver. Data from these studies will be discussed with a specific emphasis on the stress response of the liver to acute exercise.

### EVIDENCE FOR A MARKED HEPATIC RESPONSE TO EXERCISE – HUMAN STUDIES

Application of noninvasive proton magnetic resonance spectroscopy ( $^1\text{H-MRS}$ ) enables the accurate quantification of liver fat content (54, 73, 110) and allows the independent measurement of the effectiveness of lifestyle interventions on body weight, visceral adipose mass and ectopic fat. Data from 181 subjects obtained in the Tuebingen Lifestyle Intervention Program revealed that after nine months of

reduced calorie intake and increased physical activity, the most prominent reduction was found in liver fat (mean - 32 %), while the mean decrease in visceral fat was 13 %, and the mean reduction of total body fat and subcutaneous fat was modest (105, 113). After these nine months of intervention, fasting glucose was decreased and peripheral insulin sensitivity was increased indicating the effectiveness of the program. A similar reduction in hepatic lipids was found after four weeks of aerobic training in 19 sedentary obese men and women in the absence of weight loss (53). Reduced liver fat without any changes in body weight was also reported in two others studies that combined exercise training with caloric restriction (64, 111). Although not all studies examining the impact of exercise intervention on liver fat could show a clear relationship of increased physical activity and reduced hepatic lipids (106) it appears that hepatic lipids are the fat depot with the highest responsiveness to exercise intervention. Moreover, endurance exercise training is known to improve plasma lipoprotein and lipid profiles (42, 62, 75). The reduction of the postprandial concentrations of plasma TG has been found both after one single bout of acute exercise (2, 26, 37, 74) and after regular exercise training (35, 44, 47, 69, 133). The contribution of distinct mechanisms leading to this hypotriglyceridemic effect of exercise is not completely understood, but it is predominantly a decrease in the very low density lipoprotein (VLDL)-TG fraction (13, 37, 75). This decrease has been attributed to an increased plasma clearance rate of VLDL-TG (3, 101, 117) and a reduced VLDL-TG secretion (116). The reduction of dyslipidaemia is, together with the anti-inflammatory effect of exercise (93) important for lowering the risk of cardiovascular disease (114). It has also been reported that increased physical activity could improve the insulin sensitivity of the liver in humans leading to reduced hepatic glucose output in the presence of insulin (22, 119), which might be related to the reduction in liver fat and to the anti-inflammatory effect of exercise. Given the central role of the liver in glucose and lipid metabolism and the putative pathological consequences of a dysregulated liver function (for review (107)), it appears necessary to consider the liver as an important exercise target and to elucidate the molecular mechanisms activated by training intervention and acute exercise that are responsible for the prevention or amelioration of hepatic dysregulation and thus help to avoid impaired insulin action, hepatic steatosis, and cardiovascular disease.

### **IS THE ACUTE STRESS RESPONSE TO EXERCISE ESSENTIAL FOR THE BENEFICIAL HEALTH EFFECTS OF TRAINING INTERVENTIONS?**

An often described but little understood feature of exercise is the acute and transient induction of oxidative, energetic and (in the working muscle) mechanical stress (reviewed in (51, 82, 96)), while performing regular exercise is a successful intervention to reduce low-grade systemic inflammation and to protect against stress system dysregulation (93, 95).

The acute stress response in humans has mainly been studied in skeletal muscle and peripheral mononuclear blood cells (PBMC). It includes the production of reactive oxygen species (ROS) such as superoxide and nitric oxide and other reactive nitrogen species (RNS) in the contracting muscle and in PBMCs (83, 132) that act as signaling molecules to modulate signal transduction pathways and redox-sensitive gene transcription. Exercise-regulated and ROS-modulated genes include antioxidant enzymes, DNA repair proteins and mitochondrial

electron transport proteins. Importantly, this occurs even if the work load is adequate, e.g. during concentric exercise in trained people, and is not dependent on muscular damage (15, 58). The stress response of the working muscle and of PBMCs also includes the induction of heat shock proteins (HSP) (30, 78, 130), which is mediated by oxidative stress and enhanced by glycogen depletion in the muscle (29). Moreover, exercise activates – partially mediated by the ROS/RNS production – mitogen-activated protein kinases (MAPK) and the transcription factor NF- $\kappa$ B: Low intensity exercise leads to activation of the extracellular signal regulated kinase (ERK)1/2 MAPKs in rodent and human skeletal muscle (6, 127) and to a lesser extent to activation of p38 MAPK (40, 127). One-legged cycling exercise induces MAPK phosphorylation in the exercised leg only, which suggests the involvement of a local rather than a systemic factor (6, 127). Eccentric exercise protocols also activate the c-Jun N-terminal kinase (JNK) pathway in the working muscle (11, 12).

The stress-activated pathways are clearly important to induce a stress defense including the upregulation of enzymes with antioxidative capacity and DNA repair proteins. Beyond that, the data suggest that an adequate stress response to physical activity is important to initiate essential adaptations to exercise, not only to prevent tissue damage but also to improve exercise performance and to achieve health benefits. The muscular stress response has been implicated in the upregulation of enzymes and co-activators important for lipid and glucose metabolism in rodents (1, 98, 118). Antioxidant treatment to reduce the exercise-dependent oxidative stress depresses muscle force production (25, 99), prevents training-induced adaptations in endurance performance and mitochondrial biogenesis (38) and abrogates improvements of insulin sensitivity (100) and cardiovascular parameters (129). This phenomenon of “stress response hormesis” with hormesis referring to the beneficial effects of a stimulus that at a higher intensity is harmful (77) might also be important in the response of the liver to exercise. In the following parts it will be shown that acute exercise induces a pronounced and rapid activation of signaling pathways in the liver leading to a cellular stress response.

### **THE HEPATIC RESPONSE TO EXERCISE TRAINING IN RODENTS**

Animal models, particularly rodents, are widely used to investigate the molecular mechanisms regulated by acute exercise or training interventions. Similar to human studies, most reports have focussed on the skeletal muscle. Some groups have investigated the effects of long-term exercise training over several weeks on the hepatic gene expression in rodents (5, 24, 32, 67, 128), mostly performed with obese or hyperglycaemic animals. These reports provide evidence not only for the expected regulation of hepatic metabolic enzymes involved in glucose and lipid metabolism, but also for an altered expression of signalling molecules such as kinases and transcription factors and proteins involved in anti-oxidant defense in the liver. Four weeks of regular running exercise of young, nonobese rats induced the expression of p38 MAPK, inhibitory  $\kappa$ B kinase  $\beta$ , and signal transducer and activator of transcription (STAT)-3 (5). After ten weeks of running the hepatic expression of superoxide dismutase and catalase in rat liver was found to be upregulated which, however, was not paralleled by changes in enzyme activities (128). Twelve weeks of swimming exercise normalized the expression of antioxi-

dant genes or heat shock proteins in obese mice, which was dysregulated due to high fat diet (67). Other studies investigated the effect of long-term exercise training on oxidative stress and mitochondrial function in the liver. Eight weeks of treadmill exercise of rats increased the ratio of reduced to oxidized glutathione in the exercised group (97). Moderate treadmill exercise from 28 to 52 weeks of age in mice decreased the aging-related increase in oxidative stress markers in hepatic mitochondria, prevented the decrease of antioxidant enzymes in the liver and the reduction of the enzymatic activity of respiratory complex IV (80). Thus training of rodents demonstrates a great impact of regular exercise performance on the hepatic metabolism shown as altered hepatic gene expression, prevention of hyperglycemia and hepatic steatosis (24, 67). But there is also clear evidence for the regulation of anti-oxidative defense mechanism in the liver, similar to the data obtained in skeletal muscle. Moreover, the data suggest an acute regulation of signal transduction pathways in the liver by exercise.

### THE HEPATIC TRANSCRIPTIONAL RESPONSE TO ACUTE EXERCISE IN RODENTS

Only few studies are available to date that investigated the acute response of the liver to one single bout of exercise. It has been reported that acute exercise reduces the expression of lipogenic enzymes in the liver of rodents (31, 41) and upregulates the expression of the gluconeogenic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (8), of adiponectin receptor 1, forkhead box O1 (49), and peroxisome proliferator-activated receptor- $\gamma$  coactivator (PGC)-1 $\alpha$  (8). A transient induction of HSP72 expression and synthesis of HSP72, HSP73 and glucose-regulated proteins 75 and 78 has been found in rat livers (39).

To obtain a more general view on the transcriptional events in the liver after acute exercise we have performed treadmill exercise studies with mice and applied whole genome expression analysis (45). The mice were 12 weeks of age and untrained, but habituated to treadmill running. Immediately after one single exercise bout of 60 min of non-exhaustive running, 536 transcripts in the liver were differentially regulated more than twofold. 352 of them were upregulated and 184 of them were downregulated. 37 hepatic transcripts were regulated more than tenfold. In comparison, in the soleus muscle of the same mice, 162 transcripts were differentially expressed more than twofold with more transcripts downregulated than upregulated (90 vs. 72), and only 4 of them were altered more than tenfold (Table 1a).

Analysis of the pathways mainly affected by the exercise-regulated gene expression suggested a predominant regulation of genes important for glucose and lipid metabolism, namely the metabolic pathways glycolysis, gluconeogenesis, fatty

Table 1a. Sum of differentially expressed transcripts in liver and soleus muscle after acute exercise (table adapted from (45)).

	increase			decrease		
	> 40-fold	>10-fold	>2-fold	> 40-fold	>10-fold	>2-fold
<b>liver</b>	<b>3</b>	<b>22</b>	<b>352</b>	<b>2</b>	<b>10</b>	<b>184</b>
<b>soleus muscle</b>	<b>0</b>	<b>1</b>	<b>72</b>	<b>1</b>	<b>2</b>	<b>90</b>

acid oxidation and synthesis (Table 1b) (46). Even more affected, at least as estimated from the number of differentially expressed genes, were signal transduction pathways, particularly the MAPK signalling pathway (Table 1b). Comparison of the

Table 1b. Predominantly regulated pathways in the liver after acute exercise in descending order of the number of participating differentially expressed genes (table adapted from (45)).

Pathway description	Number of differentially expressed genes
<b>MAPK signaling pathway</b>	16
<b>Cytokine-cytokine receptor interaction</b>	8
<b>PPAR signaling pathway</b>	7
<b>Insulin signaling pathway</b>	7
<b>Focal adhesion</b>	6
<b>Arachidonic acid metabolism</b>	5
<b>Toll-like receptor signaling pathway</b>	5
<b>Wnt signaling pathway</b>	5
<b>Adipocytokine signaling pathway</b>	5
<b>VEGF signaling pathway</b>	5
<b>Jak-STAT signaling pathway</b>	4
<b>Calcium signaling pathway</b>	4
<b>GnRH signaling pathway</b>	4
<b>Glycolysis/Gluconeogenesis</b>	4
<b>p53 signaling pathway</b>	4
<b>Axon guidance</b>	4
<b>Fatty acid metabolism</b>	4

exercise-regulated expression of genes in liver and skeletal muscle after validation by real-time PCR reveals four different groups of genes. Some of the genes, that were strongly induced immediately after a single treadmill run in the liver, have no relevant expression levels in skeletal muscle, e.g. insulin-like growth factor binding protein (IGFBP)-1 and glucose-6-phosphatase (46). The expression of several genes related to metabolic or signalling function was similarly induced in liver and skeletal muscle, e.g. c-Fos, insulin receptor substrate (IRS)-2, angiopoietin-like-4, and pyruvate dehydrogenase kinase-4. Some were expressed in both, skeletal muscle and liver, but only the hepatic expression was significantly upregulated, e.g. of serum/glucocorticoid-regulated kinase (Sgk)1. Moreover, further studies including 3 h of recovery phase after the treadmill run revealed that the kinetics of exercise-induced gene expression could be different in liver and skeletal muscle. The expression of the known exercise-responsive gene PGC-1 $\alpha$  was induced in the liver immediately after exercise, while a strong upregulation of skeletal muscle expression was only found in the recovery phase (M. Hoene, C. Weigert, unpublished data). These results indicate that the transcription of hepatic genes is highly responsive to a moderately intense exercise bout, and this acute response appears to be more pronounced than the response in the working skeletal muscle, at least than in the investigated soleus muscle. Moreover, the quantification of the expression of hepatic genes immediately after exercise and after 3 h of recovery reveals that the induction of the

majority of genes in the liver is rapid but transient and declines to values of sedentary mice in the first hours of the recovery phase.

### ACTIVATION OF THE MAPK SIGNAL TRANSDUCTION PATHWAY IN THE LIVER BY ACUTE EXERCISE

The pathway analysis of the transcriptional response of the liver indicates a pronounced activation of the MAPK signaling pathway. The upregulation of the transcription factors c-Fos, c-Jun, FosB, and JunB, of growth arrest and DNA-damage-inducible (GADD)45 $\gamma$ , and of DUSP1 and 6 was verified by real-time PCR, and the induction of further target genes of MAPK was indicated by the whole genome expression analysis, e.g. MAPK activated protein kinases 2 and 3 (Table 2) (45). Among the MAPKs tested we found a strong

Table 2. Differentially expressed hepatic genes of the MAPK signaling pathway (table adapted from (45)).

gene	description	change
<b>Gadd45g</b>	<i>Growth arrest and DNA-damage-inducible 45 gamma</i>	<b>I</b>
<b>Dusp8</b>	<i>Dual specificity phosphatase 8</i>	<b>I</b>
<b>Rasa2</b>	<i>RAS p21 protein activator 2</i>	<b>I</b>
<b>Dusp6</b>	<i>Dual specificity phosphatase 6</i>	<b>I</b>
<b>Fos</b>	<i>FBJ osteosarcoma oncogene</i>	<b>I</b>
<b>Mapk8</b>	<i>Mitogen activated protein kinase 8</i>	<b>I</b>
<b>Jun</b>	<i>Jun oncogene</i>	<b>I</b>
<b>Il1b</b>	<i>Interleukin 1 beta</i>	<b>I</b>
<b>Hspa2</b>	<i>Heat shock protein 2</i>	<b>I</b>
<b>Dusp4</b>	<i>Dual specificity phosphatase 4</i>	<b>I</b>
<b>Mapkapk3</b>	<i>Mitogen-activated protein kinase-activated protein kinase 3</i>	<b>I</b>
<b>Mapkapk2</b>	<i>Mitogen-activated protein kinase-activated protein kinase 2</i>	<b>I</b>
<b>Ppm1b</b>	<i>Protein phosphatase 1B, magnesium dependent, beta isoform</i>	<b>D</b>
<b>Gadd45a</b>	<i>Growth arrest and DNA-damage-inducible 45 alpha</i>	<b>D</b>
<b>Rac2</b>	<i>RAS-related C3 botulinum substrate 2</i>	<b>D</b>
<b>Mapk11</b>	<i>Mitogen activated protein kinase 11</i>	<b>D</b>

phosphorylation of an ERK isoform and to a lesser extent of JNK (45). Phosphorylation of both was transient and not observable after 3 h of recovery phase. The detected ERK isoform has an apparent molecular weight of 46-48 kDa and must differ in the C-terminal part from ERK1/2, since it is not recognized by the ERK protein antibody used, which binds to the C-terminal part of ERK1/2. The phosphorylation of this isoform was not detectable in any of the muscle types studied, while ERK1/2 were not phosphorylated in the hepatic tissue after exercise. This liver-specific regulation could be explained by the activation of DUSP isoforms found predominantly in the liver, which lead to the dephosphorylation of ERK1/2 (90). Of note, a 46-48 kDa ERK isoform named ERK1b appears not to be a good target for DUSP and can be activated despite induction of the phosphatases (131). A further difference between the exercise-induced activation of MAPKs in liver and muscle is that the phosphorylation of MAPKs in the exercised muscle could also be observed in the early recovery phase, when the phosphorylation of hepatic MAPK is no longer detectable (45, 79).

### ACTIVATION OF THE p53 PATHWAY IN THE LIVER BY ACUTE EXERCISE

The pathway analysis of the hepatic transcriptional response points also to an activation of the p53 pathway (Table 1b). The intracellular levels of the p53 tumor suppressor protein are low in unstressed conditions and tightly regulated by ubiquitin-proteosomal-dependent degradation. The activation of this pathway could be confirmed by the increased abundance of the p53 protein and upregulation of the target genes p53 inducible nuclear protein (trp53inp) and p21 (45). An exercise-induced activation of p53 also occurs in rodent muscle, particularly after eccentric exercise (17, 102). Further putative target genes of p53 were upregulated in the liver after exercise, e.g. GADD45 $\gamma$ , Sgk1 and IGFBP-1 (45), although the specific role of p53 herein needs to be evaluated. In this context it might be interesting to consider the rapid and pronounced exercise-induced increase in hepatic IGFBP-1 levels. Since the upregulation of IGFBP-1 expression results in increased circulating IGFBP-1 protein concentrations in humans (48, 81) and rodents (4, 66) it has been suggested that it may play a role in glucoregulation during exercise to neutralize the insulin-like effects of IGF-1. A further function of the increased expression of IGFBP-1 in the liver might be to upregulate the intracellular levels of this protein as an antiapoptotic defense mechanism. It has been shown that intracellular IGFBP-1 can bind to the protein BAK, thereby antagonizing the proapoptotic function of this protein and protecting the liver from apoptosis (70). This is discussed as one explanation for the surpassing resistance of the liver to p53-induced apoptosis. Thus upregulation of IGFBP-1 during exercise might be a feedback mechanism to prevent p53-induced apoptosis. Following this consideration, the upregulation of hepatic IRS-2 during acute exercise (46) could also be important not only for the regulation of hepatic glucose and lipid metabolism post-exercise, but also for the anti-apoptotic defense of the liver. This function of IRS-2 has been already suggested for the exercise-induced IRS-2 expression in pancreatic beta-cells (89).

### REGULATION OF JAK-STAT-3 SIGNALING IN THE LIVER BY ACUTE EXERCISE

Cytokine/Cytokine receptor signaling was also one of the most prominently regulated pathways in the liver after acute exercise as shown by the pathway analysis (Table 1b). Interleukin (IL)-1 $\beta$ , the receptors for the ciliary neurotrophic factor (CNTF), for IL-11 and for leukaemia inhibitory factor (LIF) were found among the upregulated genes (Table 3), but up to now we did not verify these results by

Table 3. Differentially expressed hepatic genes of the cytokine/cytokine receptor pathway

gene	description	change
<b>Cntfr</b>	<i>Ciliary neurotrophic factor receptor</i>	<b>I</b>
<b>Il1ra1</b>	<i>Interleukin 11 receptor, alpha chain 1</i>	<b>I</b>
<b>Il1b</b>	<i>Interleukin 1 beta</i>	<b>I</b>
<b>Cxcl13</b>	<i>Chemokine (C-X-C motif) ligand 13</i>	<b>I</b>
<b>Lifr</b>	<i>Leukemia inhibitory factor receptor</i>	<b>I</b>
<b>Inhbe</b>	<i>Inhibin beta E</i>	<b>D</b>
<b>Cxcl1</b>	<i>Chemokine (C-X-C motif) ligand 1</i>	<b>D</b>
<b>Ccl1</b>	<i>Chemokine (C-C) motif ligand 1</i>	<b>D</b>



quantitative real-time PCR. Of note, CNTF, IL-11 and LIF belong to the IL-6-type family of cytokines that lead to the activation of the JAK/STAT pathway and upregulation of suppressor of cytokine signalling (SOCS)-3 (43). Another major signal transduction pathway for these cytokines is the MAPK cascade. The best studied IL-6-type cytokine in the context of physical activity is IL-6 itself, since its plasma levels are increased after exercise due to its enhanced production and secretion from the working muscle (28). A recent study describes the acute and transient activation of the IL-6-dependent JAK/STAT-3 pathway in the liver after exhaustive exercise (8). In our moderately intense exercise model we found also evidence for the activation of STAT-3 and induction of its target gene SOCS-3 in the liver. However, since we observed a similar upregulation of SOCS-3 in IL-6-deficient mice (33) it is unclear whether IL-6 is responsible for the regulation of this pathway in wildtype mice. Here, further research is needed to elucidate the relevance of IL-6-type cytokine signalling (with IL-6 or other cytokines belonging to this family as stimulus) for the activation of the hepatic stress response to acute exercise.

### **IS OXIDATIVE STRESS THE TRIGGER FOR THE HEPATIC STRESS RESPONSE?**

Increased oxidative stress is discussed as an important stimulus for the induction of cellular stress in the working muscle and it could also be involved in the regulation of the different signalling pathways found to be activated after acute exercise (82). Studies aiming to evaluate the effect of acute exercise on oxidative stress in the liver determined parameters of increased generation of ROS such as lipid peroxidation, thiobarbituric acid reactive substances (TBARS), and accumulation of nuclear 8-hydroxydeoxyguanosine. These markers were found to be increased after acute exercise or in the early recovery phase in some studies (27, 61, 72), but others did not detect evidence for oxidative stress in the liver (9, 85). The upregulation of heat shock proteins in the liver after acute exercise has also been suggested to be a consequence of oxidative stress (39, 103). The induction of oxidative stress in the liver by endurance exercise might be influenced by nutrients (109). In a recent study we fed a group of mice an antioxidant-rich diet over 4 weeks before the single bout of 60 min treadmill exercise (45). The activation of c-Fos and GADD45 $\gamma$  mRNA expression and the phosphorylation of the hepatic ERK isoform was similarly induced in both exercised groups independent of the diet. Since we did not observe a clear upregulation of anti-oxidant enzymes metallotheonine-1, heme oxygenase-1, and superoxide dismutase-1 and-2 in either exercised group, neither in mice fed the control diet nor in mice fed the anti-oxidant-rich diet, our study could not answer the question whether acute exercise induces oxidative stress in the liver. This might strongly depend on the intensity and duration of the exercise bout. However, the study could clearly show that oxidative stress is not the stimulus for the acute activation of the hepatic MAPK signaling pathway by moderately intense exercise.

### **THE ROLE OF ENERGETIC STRESS IN THE LIVER FOR THE ACTIVATION OF MAPK AND p53 SIGNALING**

The liver has an outstanding function during prolonged exercise since it supplies glucose derived from glycogenolysis and gluconeogenesis for the working mus-

cle. Accordingly, glycogen concentrations in the liver significantly drop already after 30 min of moderate treadmill running in mice (16), they are reduced to 50 % of preexercise levels after intense, exhaustive exercise for 60 min (91, 92) and hepatic glycogen is almost depleted after running till exhaustion for an average running time of 84 min (16). Concomitantly, hepatic ATP concentrations decrease, while AMP is increased, resulting in a moderate decrease in the hepatic energy charge after short-term exercise and a marked decrease after exhaustive exercise (16). Of note, the energy state of the gastrocnemius muscle was unchanged in these mice, which is supported by a recent study that also did not detect alterations of AMP, ADP and ATP nucleotide concentrations in the gastrocnemius muscle after a 60 min treadmill run of moderate intensity (68). The existence of energetic stress in the liver during exercise is also evidenced by the enhanced phosphorylation and activation of the AMP-activated kinase (AMPK) in this organ (16, 46, 57, 88).

Could the energetic stress be responsible for the hepatic transcriptional response of the MAPK pathway and the p53 pathway to acute exercise? Exercise of moderate intensity leads to slightly reduced plasma glucose levels and decreased insulin levels, which result in a significant reduction of the insulin/glucagon ratio (46, 65, 126). Peripheral glucagon concentrations are often found not to be increased after exercise, but plasma glucagon concentrations do not necessarily reflect the effective glucagon levels in the portal vein (124), and the hepatic glucagon receptors might be exposed to higher concentrations during exercise (125). Stimulation of these receptors increases cAMP levels, leading to activation of protein kinase A, which subsequently activates ERK (52). Moreover, a recent study could show for the first time that hepatic glucagon action regulates the hepatic energy state and amplifies AMPK signalling (10). It thus appears likely that the activation of glucagon receptors is implicated in the pronounced transcriptional regulation of hepatic genes found after acute exercise, although a causal relationship needs to be proven.

The energetic stress is also known to elevate plasma concentrations of catecholamines, which could lead, via the activation of hepatic adrenergic receptors, to MAPK activation (18). While the question whether or not catecholamines are involved in the exercise-induced gene expression in the liver has not been addressed yet, strategies designed to specifically investigate the function of hepatic adrenergic stimulation in the activation of hepatic glucose output during exercise, e.g. hepatic adrenoreceptor blockade in dogs (20), or inhibition of hepatic innervation in rats (120) or humans (60) do not support an important role for catecholamines herein. Of course this does not exclude the possibility that hepatic innervation is involved in the activation of hepatic MAPK signalling during exercise.

Moreover, evidence exists that the fall in plasma glucose concentrations has stimulatory effects on the liver independent of pancreatic hormones and of catecholamine action leading to enhanced glucose output (19, 21). The results obtained in these studies suggest that either a yet to be determined signal related to the fall in plasma glucose or the decrease in glucose concentrations per se regulates the hepatic response to exercise. Of note, in our recent study we could show that lower plasma glucose levels are related to increased expression not only of well-known responders to hepatic energy depletion, e.g. IGFBP-1 (4, 66), but also

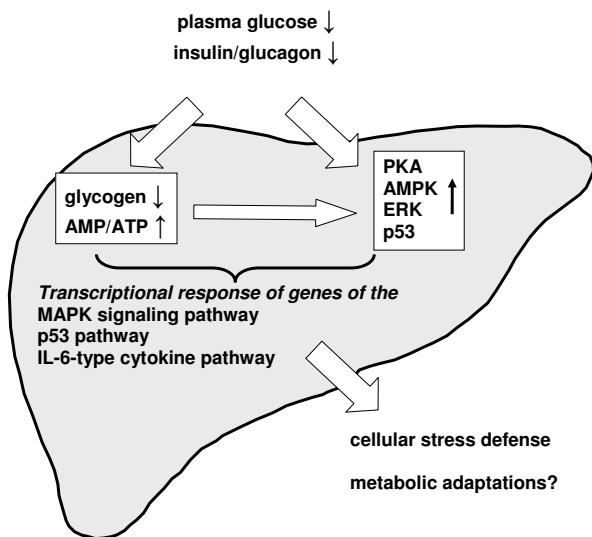


Fig. 1. Relationship of energetic stress in the liver induced by acute exercise and the transcriptional regulation of stress response-related signaling pathways

(86). Thus clear indication exists that energetic stress, the existence of which is detectable in the liver during moderately intense exercise (16, 68), is a major stimulus for the activation of cellular stress response-related pathways in this organ (Fig. 1).

### IS THE ENERGETIC STRESS FOR THE LIVER DURING EXERCISE COMPARABLE IN HUMANS AND RODENTS?

The evidence for a high responsiveness of the liver to acute exercise and the role of hepatic energy depletion in the transcriptional adaptation is mainly based on rodent studies. In humans, the liver has a comparably unique function and is the primary source of glucose for the working muscle (for review (121)). There is also clear evidence in humans for hepatic glycogenolysis during physical activity (94, 122) and for a fall in plasma glucose concentrations during strenuous and long-lasting exercise (115). However, it has been suggested from data on glycogen depletion in liver and muscle that hepatic glycogen stores and availability might be more important for exercise capacity in rodents than in humans (7, 112). Mice store more glycogen in the liver than in the skeletal muscle, while the opposite is true in humans due to the difference in the relative contribution of muscle and liver mass to total body weight in mice and humans (50). Genetically modified mice lacking muscle glycogen or with excess of muscle glycogen have normal exercise capacity (91, 92), while depletion or ineffective utilization of muscle glycogen in humans leads to impaired exercise tolerance (56, 71). It may well be that the rodent liver is more affected by energetic stress during moderately intense exercise than the liver of humans, but it is difficult to compare the intensity of exercise applied to humans and rodents. Moreover, hepatic glycogen depletion

of genes encoding MAPK signalling proteins in the liver (45). In the same study, this correlation was not found for stress response genes in the soleus muscle.

Recent investigations also relate the activation of the p53 pathway to energy depletion and limited carbohydrate availability (76). It has been shown that AMPK-dependent phosphorylation of p53 leads to the stabilization of p53 protein (55) and that activation of AMPK induces the expression of p53

and hypoglycaemia is also found in humans after prolonged and intense exercise, and the human liver is highly responsive to exercise interventions. Therefore it could be expected that the relationship of energetic stress and the subsequent induction of transcriptional and posttranslational adaptive mechanisms in the liver is not only true for rodents, but also for humans.

## CONCLUDING REMARKS

Investigation of the molecular events that occur in the liver of rodents during and after exercise has just begun. The data available thus far describe the liver as an intensely affected organ during non-exhaustive exercise. This is evidenced by the activation of several signal transduction pathways and a marked transcriptional response. Many of the regulated pathways and genes have also been described as part of the exercise response of the working muscle, although the intensity and the kinetics of this response may vary between the tissues. Energetic stress during physical exercise appears to be an important determinant for the induction of hepatic genes, but not for those in the soleus muscle. A major challenge for future research will be to elucidate the relevance of the hepatic stress response for the beneficial metabolic adaptations of the liver to regular physical activity.

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## ABBREVIATIONS/GLOSSAR

**AMPK**, AMP-activated protein kinase;  
**DUSP**, dual-specificity phosphatase; MAPK phosphatase  
**ERK**, extracellular signal regulated kinase; MAPK  
**GADD**, growth arrest and DNA damage;  
**GnRH**, gonadotropin releasing hormone;  
**HSP**, heat shock protein;  
**IGFBP-1**, insulin-like growth factor binding protein;  
**IL-6**, interleukin-6;  
**IRS**, insulin receptor substrate;  
**JAK**, Janus tyrosine kinase; phosphorylates STAT-3 upon cytokine stimulation  
**JNK**, c-Jun N-terminal kinase; MAPK  
**MAPK**, mitogen-activated protein kinase; serine/threonine kinases including ERK and JNK  
**PGC-1 $\alpha$** , peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$ ;  
**PBMC**, peripheral blood mononuclear cells;  
**ROS**, reactive oxygen species;  
**SOCS**, suppressor of cytokine signalling;

**STAT**, signal transducer and activator of transcription;  
**trp53inp1**, tumor suppressor protein 53 inducible nuclear protein 1

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