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)45γ, 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)1α. 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}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-κB: 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 phenomen 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 κB kinase β, 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 activites (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-γ coactivator (PGC)-1α (8). A transient induction of HSP72 expression and synthesis of HSP72, HSP73 and glucose-regulated proteins75 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**
liver	**3**	**22**	**352**	**2**	**10**	**184**
soleus muscle	**0**	**1**	**72**	**1**	**2**	**90**

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
MAPK signaling pathway	16
Cytokine-cytokine receptor interaction	8
PPAR signaling pathway	7
Insulin signaling pathway	7
Focal adhesion	6
Arachidonic acid metabolism	5
Toll-like receptor signaling pathway	5
Wnt signaling pathway	5
Adipocytokine signaling pathway	5
VEGF signaling pathway	5
Jak-STAT signaling pathway	4
Calcium signaling pathway	4
GnRH signaling pathway	4
Glycolysis/Gluconeogenesis	4
p53 signaling pathway	4
Axon guidance	4
Fatty acid metabolism	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-1α 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)45γ, 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
Gadd45g	*Growth arrest and DNA-damage-inducible 45 gamma*	**I**
Dusp8	*Dual specificity phosphatase 8*	**I**
Rasa2	*RAS p21 protein activator 2*	**I**
Dusp6	*Dual specificity phosphatase 6*	**I**
Fos	*FBJ osteosarcoma oncogene*	**I**
Mapk8	*Mitogen activated protein kinase 8*	**I**
Jun	*Jun oncogene*	**I**
Il1b	*Interleukin 1 beta*	**I**
Hspa2	*Heat shock protein 2*	**I**
Dusp4	*Dual specificity phosphatase 4*	**I**
Mapkapk3	*Mitogen-activated protein kinase-activated protein kinase 3*	**I**
Mapkapk2	*Mitogen-activated protein kinase-activated protein kinase 2*	**I**
Ppm1b	*Protein phosphatase 1B, magnesium dependent, beta isoform*	**D**
Gadd45a	*Growth arrest and DNA-damage-inducible 45 alpha*	**D**
Rac2	*RAS-related C3 botulinum substrate 2*	**D**
Mapk11	*Mitogen activated protein kinase 11*	**D**

phosphorylation of an ERK isoform and to a lesser extend 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. GADD45γ, 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 pro-apoptotic 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)-1β, 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
Cntfr	*Ciliary neurotrophic factor receptor*	**I**
Il11ra1	*Interleukin 11 receptor, alpha chain 1*	**I**
Il1b	*Interleukin 1 beta*	**I**
Cxcl13	*Chemokine (C-X-C motif) ligand 13*	**I**
Lifr	*Leukemia inhibitory factor receptor*	**I**
Inhbe	*Inhibin beta E*	**D**
Cxcl1	*Chemokine (C-X-C motif) ligand 1*	**D**
Ccl1	*Chemokine (C-C) motif ligand 1*	**D**

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 GADD45γ 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

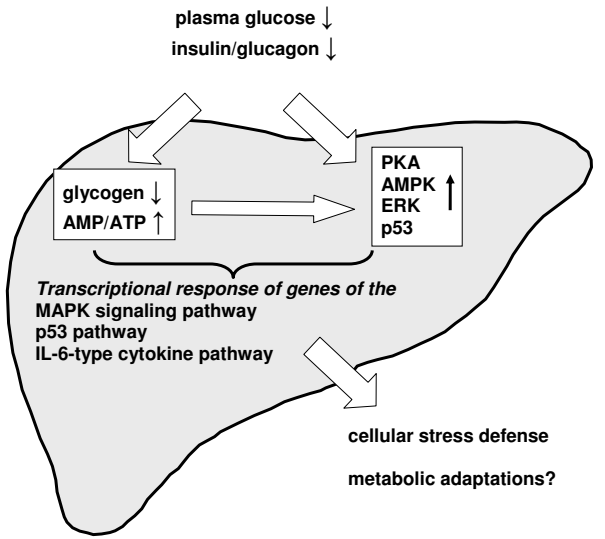

Fig. 1. Relationship of energetic stress in the liver induced by acute exercise and the transcriptional regulation of stress response-related signaling pathways

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 (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

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-1α, peroxisome proliferator-activated receptor-γ coactivator-1α;
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

REFERENCES

1. Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS and Yan Z. Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway. J Biol Chem 280: 19587-19593, 2005.
2. Aldred HE, Perry IC and Hardman AE. The effect of a single bout of brisk walking on postprandial lipemia in normolipidemic young adults. Metabolism 43: 836-841, 1994.
3. Annuzzi G, Jansson E, Kaijser L, Holmquist L and Carlson LA. Increased removal rate of exogenous triglycerides after prolonged exercise in man: time course and effect of exercise duration. Metabolism 36: 438-443, 1987.
4. Anthony TG, Anthony JC, Lewitt MS, Donovan SM and Layman DK. Time course changes in IGFBP-1 after treadmill exercise and postexercise food intake in rats. Am J Physiol Endocrinol Metab 280: E650-E656, 2001.
5. Aoi W, Ichiishi E, Sakamoto N, Tsujimoto A, Tokuda H and Yoshikawa T. Effect of exercise on hepatic gene expression in rats: a microarray analysis. Life Sci 75: 3117-3128, 2004.
6. Aronson D, Violan MA, Dufresne SD, Zangen D, Fielding RA and Goodyear LJ. Exercise stimulates the mitogen-activated protein kinase pathway in human skeletal muscle. J Clin Invest 99: 1251-1257, 1997.
7. Baldwin KM, Reitman JS, Terjung RL, Winder WW and Holloszy JO. Substrate depletion in different types of muscle and in liver during prolonged running. Am J Physiol 225: 1045-1050, 1973.
8. Banzet S, Koulmann N, Simler N, Sanchez H, Chapot R, Serrurier B, Peinnequin A and Bigard X. Control of gluconeogenic genes during intense/prolonged exercise: hormone-independent effect of muscle-derived IL-6 on hepatic tissue and PEPCK mRNA. J Appl Physiol 107: 1830-1839, 2009.
9. Bejma J, Ramires P and Ji LL. Free radical generation and oxidative stress with ageing and exercise: differential effects in the myocardium and liver. Acta Physiol Scand 169: 343-351, 2000.
10. Berglund ED, Lee-Young RS, Lustig DG, Lynes SE, Donahue EP, Camacho RC, Meredith ME, Magnuson MA, Charron MJ and Wasserman DH. Hepatic energy state is regulated by glucagon receptor signaling in mice. J Clin Invest 119: 2412-2422, 2009.
11. Boppart MD, Aronson D, Gibson L, Roubenoff R, Abad LW, Bean J, Goodyear LJ and Fielding RA. Eccentric exercise markedly increases c-Jun NH(2)-terminal kinase activity in human skeletal muscle. J Appl Physiol 87: 1668-1673, 1999.
12. Boppart MD, Asp S, Wojtaszewski JF, Fielding RA, Mohr T and Goodyear LJ. Marathon running transiently increases c-Jun NH2-terminal kinase and p38 activities in human skeletal muscle. J Physiol 526 Pt 3: 663-669, 2000.
13. Borsheim E, Knardahl S and Hostmark AT. Short-term effects of exercise on plasma very low density lipoproteins (VLDL) and fatty acids. Med Sci Sports Exerc 31: 522-530, 1999.

14. Boule NG, Weisnagel SJ, Lakka TA, Tremblay A, Bergman RN, Rankinen T, Leon AS, Skinner JS, Wilmore JH, Rao DC and Bouchard C. Effects of exercise training on glucose homeostasis: the HERITAGE Family Study. Diabetes Care 28: 108-114, 2005.
15. Boveris A and Navarro A. Systemic and mitochondrial adaptive responses to moderate exercise in rodents. Free Radic Biol Med 44: 224-229, 2008.
16. Camacho RC, Donahue EP, James FD, Berglund ED and Wasserman DH. Energy state of the liver during short-term and exhaustive exercise in C57BL/6J mice. Am J Physiol Endocrinol Metab 290: E405-E408, 2006.
17. Chen YW, Nader GA, Baar KR, Fedele MJ, Hoffman EP and Esser KA. Response of rat muscle to acute resistance exercise defined by transcriptional and translational profiling. J Physiol 545: 27-41, 2002.
18. Christensen NJ and Galbo H. Sympathetic nervous activity during exercise. Annu Rev Physiol 45: 139-153, 1983.
19. Coker RH, Koyama Y, Denny JC, Camacho RC, Lacy DB and Wasserman DH. Prevention of overt hypoglycemia during exercise: stimulation of endogenous glucose production independent of hepatic catecholamine action and changes in pancreatic hormone concentration. Diabetes 51: 1310-1318, 2002.
20. Coker RH, Krishna MG, Lacy DB, Bracy DP and Wasserman DH. Role of hepatic alpha- and beta-adrenergic receptor stimulation on hepatic glucose production during heavy exercise. Am J Physiol 273: E831-E838, 1997.
21. Coker RH, Simonsen L, Bulow J, Wasserman DH and Kjaer M. Stimulation of splanchnic glucose production during exercise in humans contains a glucagon-independent component. Am J Physiol Endocrinol Metab 280: E918-E927, 2001.
22. Coker RH, Williams RH, Yeo SE, Kortebein PM, Bodenner DL, Kern PA and Evans WJ. The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. J Clin Endocrinol Metab 94: 4258-4266, 2009.
23. Colberg SR. Physical activity, insulin action, and diabetes prevention and control. Curr Diabetes Rev 3: 176-184, 2007.
24. Colombo M, Gregersen S, Kruhoeffer M, Agger A, Xiao J, Jeppesen PB, Orntoft T, Ploug T, Galbo H and Hermansen K. Prevention of hyperglycemia in Zucker diabetic fatty rats by exercise training: effects on gene expression in insulin-sensitive tissues determined by high-density oligonucleotide microarray analysis. Metabolism 54: 1571-1581, 2005.
25. Coombes JS, Powers SK, Rowell B, Hamilton KL, Dodd SL, Shanely RA, Sen CK and Packer L. Effects of vitamin E and alpha-lipoic acid on skeletal muscle contractile properties. J Appl Physiol 90: 1424-1430, 2001.
26. Cullinane E, Siconolfi S, Saritelli A and Thompson PD. Acute decrease in serum triglycerides with exercise: is there a threshold for an exercise effect? Metabolism 31: 844-847, 1982.
27. Davies KJ, Quintanilha AT, Brooks GA and Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 107: 1198-1205, 1982.
28. Febbraio MA and Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 16: 1335-1347, 2002.
29. Febbraio MA, Steensberg A, Walsh R, Koukoulas I, van Hall G, Saltin B and Pedersen BK. Reduced glycogen availability is associated with an elevation in HSP72 in contracting human skeletal muscle. J Physiol 538: 911-917, 2002.

30. Fehrenbach E and Niess AM. Role of heat shock proteins in the exercise response. Exerc Immunol Rev 5: 57-77, 1999.
31. Fiebig RG, Hollander JM and Ji LL. Exercise down-regulates hepatic fatty acid synthase in streptozotocin-treated rats. J Nutr 131: 2252-2259, 2001.
32. Fiebig RG, Hollander JM, Ney D, Boileau R, Jeffery E and Ji LL. Training down-regulates fatty acid synthase and body fat in obese Zucker rats. Med Sci Sports Exerc 34: 1106-1114, 2002.
33. Fritsche L, Hoene M, Lehmann R, Ellingsgaard H, Hennige AM, Pohl AK, Haring HU, Schleicher ED and Weigert C. IL-6 deficiency in mice neither impairs induction of metabolic genes in the liver nor affects blood glucose levels during fasting and moderately intense exercise. Diabetologia Epub ahead of print: 2010.
34. Fritsche L, Weigert C, Haring HU and Lehmann R. How insulin receptor substrate proteins regulate the metabolic capacity of the liver - implications for health and disease. Curr Med Chem 15: 1316-1329, 2008.
35. Gauthier MS, Couturier K, Charbonneau A and Lavoie JM. Effects of introducing physical training in the course of a 16-week high-fat diet regimen on hepatic steatosis, adipose tissue fat accumulation, and plasma lipid profile. Int J Obes Relat Metab Disord 28: 1064-1071, 2004.
36. Gill JM. Physical activity, cardiorespiratory fitness and insulin resistance: a short update. Curr Opin Lipidol 18: 47-52, 2007.
37. Gill JM, Mees GP, Frayn KN and Hardman AE. Moderate exercise, postprandial lipaemia and triacylglycerol clearance. Eur J Clin Invest 31: 201-207, 2001.
38. Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J and Vina J. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87: 142-149, 2008.
39. Gonzalez B and Manso R. Induction, modification and accumulation of HSP70s in the rat liver after acute exercise: early and late responses. J Physiol 556: 369-385, 2004.
40. Goodyear LJ, Chang PY, Sherwood DJ, Dufresne SD and Moller DE. Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle. Am J Physiol 271: E403-E408, 1996.
41. Griffiths MA, Fiebig R, Gore MT, Baker DH, Esser K, Oscai L and Ji LL. Exercise down-regulates hepatic lipogenic enzymes in food-deprived and refed rats. J Nutr 126: 1959-1971, 1996.
42. Halverstadt A, Phares DA, Wilund KR, Goldberg AP and Hagberg JM. Endurance exercise training raises high-density lipoprotein cholesterol and lowers small low-density lipoprotein and very low-density lipoprotein independent of body fat phenotypes in older men and women. Metabolism 56: 444-450, 2007.
43. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G and Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374: 1-20, 2003.
44. Herd SL, Hardman AE, Boobis LH and Cairns CJ. The effect of 13 weeks of running training followed by 9 d of detraining on postprandial lipaemia. Br J Nutr 80: 57-66, 1998.
45. Hoene M, Franken H, Fritsche L, Lehmann R, Pohl AK, Häring H-U, Zell A, Schleicher ED and Weigert C. Activation of the mitogen-activated protein kinase (MAPK)

signalling pathway in the liver of mice is related to plasma glucose levels after acute exercise. Diabetologia 53: 1131-1141, 2010. Table 1 and 2 adapted with kind permission from SPRINGER SCIENCE + BUSINESS MEDIA © 2010

46. Hoene M, Lehmann R, Hennige AM, Pohl AK, Haring HU, Schleicher ED and Weigert C. Acute regulation of metabolic genes and insulin receptor substrates in the liver of mice by one single bout of treadmill exercise. J Physiol 587: 241-252, 2009.
47. Holloszy JO, Skinner JS, TORO G and CURETON TK. EFFECTS OF A SIX MONTH PROGRAM OF ENDURANCE EXERCISE ON THE SERUM LIPIDS OF MIDDLE-AGED MAN. Am J Cardiol 14: 753-760, 1964.
48. Hopkins NJ, Jakeman PM, Hughes SC and Holly JM. Changes in circulating insulin-like growth factor-binding protein-1 (IGFBP-1) during prolonged exercise: effect of carbohydrate feeding. J Clin Endocrinol Metab 79: 1887-1890, 1994.
49. Huang H, Iida KT, Sone H and Ajisaka R. The regulation of adiponectin receptors expression by acute exercise in mice. Exp Clin Endocrinol Diabetes 115: 417-422, 2007.
50. Ivy JL. Role of carbohydrate in physical activity. Clin Sports Med 18: 469-84, v, 1999.
51. Ji LL. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 222: 283-292, 1999.
52. Jiang Y, Cypess AM, Muse ED, Wu CR, Unson CG, Merrifield RB and Sakmar TP. Glucagon receptor activates extracellular signal-regulated protein kinase 1/2 via cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 98: 10102-10107, 2001.
53. Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW and George J. Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. Hepatology 50: 1105-1112, 2009.
54. Johnson NA, Walton DW, Sachinwalla T, Thompson CH, Smith K, Ruell PA, Stannard SR and George J. Noninvasive assessment of hepatic lipid composition: Advancing understanding and management of fatty liver disorders. Hepatology 47: 1513-1523, 2008.
55. Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, Birnbaum MJ and Thompson CB. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 18: 283-293, 2005.
56. Karlsson J and Saltin B. Diet, muscle glycogen, and endurance performance. J Appl Physiol 31: 203-206, 1971.
57. Kelly M, Keller C, Avilucea PR, Keller P, Luo Z, Xiang X, Giralt M, Hidalgo J, Saha AK, Pedersen BK and Ruderman NB. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. Biochem Biophys Res Commun 320: 449-454, 2004.
58. Khassaf M, Child RB, McArdle A, Brodie DA, Esanu C and Jackson MJ. Time course of responses of human skeletal muscle to oxidative stress induced by nondamaging exercise. J Appl Physiol 90: 1031-1035, 2001.
59. Kjaer M. Hepatic glucose production during exercise. Adv Exp Med Biol 441: 117-127, 1998.
60. Kjaer M, Engfred K, Fernandes A, Secher NH and Galbo H. Regulation of hepatic glucose production during exercise in humans: role of sympathoadrenergic activity. Am J Physiol 265: E275-E283, 1993.
61. Koyama K, Kaya M, Ishigaki T, Tsujita J, Hori S, Seino T and Kasugai A. Role of xanthine oxidase in delayed lipid peroxidation in rat liver induced by acute exhausting exercise. Eur J Appl Physiol Occup Physiol 80: 28-33, 1999.

62. Kraus WE, Houmard JA, Duscha BD, Knetzger KJ, Wharton MB, McCartney JS, Bales CW, Henes S, Samsa GP, Otvos JD, Kulkarni KR and Slentz CA. Effects of the amount and intensity of exercise on plasma lipoproteins. N Engl J Med 347: 1483-1492, 2002.
63. Krogh-Madsen R, Thyfault JP, Broholm C, Mortensen OH, Olsen RH, Mounier R, Plomgaard P, Hall GV, Booth FW and Pedersen BK. A two-week reduction of ambulatory activity attenuates peripheral insulin sensitivity. J Appl Physiol -[Epub ahead of print], 2009.
64. Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, Smith SR, Alfonso A and Ravussin E. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes Care 29: 1337-1344, 2006.
65. Lavoie C, Ducros F, Bourque J, Langelier H and Chiasson JL. Glucose metabolism during exercise in man: the role of insulin in the regulation of glucose utilization. Can J Physiol Pharmacol 75: 36-43, 1997.
66. Lavoie JM, Fillion Y, Couturier K and Corriveau P. Evidence that the decrease in liver glycogen is associated with the exercise-induced increase in IGFBP-1. J Appl Physiol 93: 798-804, 2002.
67. Lee KY, Kim SJ, Cha YS, So JR, Park JS, Kang KS and Chon TW. Effect of exercise on hepatic gene expression in an obese mouse model using cDNA microarrays. Obesity (Silver Spring) 14: 1294-1302, 2006.
68. Leick L, Wojtaszewski JF, Johansen ST, Kiilerich K, Comes G, Hellsten Y, Hidalgo J and Pilegaard H. PGC-1alpha is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. Am J Physiol Endocrinol Metab 294: E463-E474, 2008.
69. Leon AS and Sanchez OA. Response of blood lipids to exercise training alone or combined with dietary intervention. Med Sci Sports Exerc 33: S502-S515, 2001.
70. Leu JI and George DL. Hepatic IGFBP1 is a prosurvival factor that binds to BAK, protects the liver from apoptosis, and antagonizes the proapoptotic actions of p53 at mitochondria. Genes Dev 21: 3095-3109, 2007.
71. Lewis SF and Haller RG. The pathophysiology of McArdle's disease: clues to regulation in exercise and fatigue. J Appl Physiol 61: 391-401, 1986.
72. Liu J, Yeo HC, Overvik-Douki E, Hagen T, Doniger SJ, Chyu DW, Brooks GA and Ames BN. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. J Appl Physiol 89: 21-28, 2000.
73. Machann J, Thamer C, Schnoedt B, Stefan N, Haring HU, Claussen CD, Fritsche A and Schick F. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized 1H-MR spectroscopy. Magn Reson Med 55: 913-917, 2006.
74. Magkos F, Patterson BW, Mohammed BS and Mittendorfer B. A single 1-h bout of evening exercise increases basal FFA flux without affecting VLDL-triglyceride and VLDL-apolipoprotein B-100 kinetics in untrained lean men. Am J Physiol Endocrinol Metab 292: E1568-E1574, 2007.
75. Magkos F, Tsekouras YE, Prentzas KI, Basioukas KN, Matsama SG, Yanni AE, Kavouras SA and Sidossis LS. Acute exercise-induced changes in basal VLDL-triglyceride kinetics leading to hypotriglyceridemia manifest more readily after resistance than endurance exercise. J Appl Physiol 105: 1228-1236, 2008.

76. Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F and Hwang PM. p53 regulates mitochondrial respiration. Science 312: 1650-1653, 2006.
77. Mattson MP. Hormesis and disease resistance: activation of cellular stress response pathways. Hum Exp Toxicol 27: 155-162, 2008.
78. Morton JP, MacLaren DP, Cable NT, Bongers T, Griffiths RD, Campbell IT, Evans L, Kayani A, McArdle A and Drust B. Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute non-damaging treadmill exercise. J Appl Physiol 101: 176-182, 2006.
79. Nader GA and Esser KA. Intracellular signaling specificity in skeletal muscle in response to different modes of exercise. J Appl Physiol 90: 1936-1942, 2001.
80. Navarro A, Gomez C, Lopez-Cepero JM and Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol Regul Integr Comp Physiol 286: R505-R511, 2004.
81. Nguyen UN, Mougin F, Simon-Rigaud ML, Rouillon JD, Marguet P and Regnard J. Influence of exercise duration on serum insulin-like growth factor and its binding proteins in athletes. Eur J Appl Physiol Occup Physiol 78: 533-537, 1998.
82. Niess AM and Simon P. Response and adaptation of skeletal muscle to exercise--the role of reactive oxygen species. Front Biosci 12: 4826-4838, 2007.
83. Northoff H, Symons S, Zieker D, Schaible EV, Schafer K, Thoma S, Loffler M, Abbasi A, Simon P, Niess AM and Fehrenbach E. Gender- and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. Exerc Immunol Rev 14: 86-103, 2008.
84. O'Gorman DJ and Krook A. Exercise and the treatment of diabetes and obesity. Endocrinol Metab Clin North Am 37: 887-903, 2008.
85. Ogonovszky H, Sasvari M, Dosek A, Berkes I, Kaneko T, Tahara S, Nakamoto H, Goto S and Radak Z. The effects of moderate, strenuous, and overtraining on oxidative stress markers and DNA repair in rat liver. Can J Appl Physiol 30: 186-195, 2005.
86. Okoshi R, Ozaki T, Yamamoto H, Ando K, Koida N, Ono S, Koda T, Kamijo T, Nakagawara A and Kizaki H. Activation of AMP-activated protein kinase induces p53-dependent apoptotic cell death in response to energetic stress. J Biol Chem 283: 3979-3987, 2008.
87. Ostergard T, Jessen N, Schmitz O and Mandarino LJ. The effect of exercise, training, and inactivity on insulin sensitivity in diabetics and their relatives: what is new? Appl Physiol Nutr Metab 32: 541-548, 2007.
88. Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB and Saha AK. Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem 277: 32571-32577, 2002.
89. Park S, Hong SM and Sung SR. Exendin-4 and exercise promotes beta-cell function and mass through IRS2 induction in islets of diabetic rats. Life Sci 82: 503-511, 2008.
90. Patterson KI, Brummer T, O'Brien PM and Daly RJ. Dual-specificity phosphatases: critical regulators with diverse cellular targets. Biochem J 418: 475-489, 2009.
91. Pederson BA, Cope CR, Irimia JM, Schroeder JM, Thurberg BL, DePaoli-Roach AA and Roach PJ. Mice with elevated muscle glycogen stores do not have improved exercise performance. Biochem Biophys Res Commun 331: 491-496, 2005.

92. Pederson BA, Cope CR, Schroeder JM, Smith MW, Irimia JM, Thurberg BL, DePaoli-Roach AA and Roach PJ. Exercise capacity of mice genetically lacking muscle glycogen synthase: in mice, muscle glycogen is not essential for exercise. J Biol Chem 280: 17260-17265, 2005.
93. Petersen AM and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98: 1154-1162, 2005.
94. Petersen KF, Price TB and Bergeron R. Regulation of net hepatic glycogenolysis and gluconeogenesis during exercise: impact of type 1 diabetes. J Clin Endocrinol Metab 89: 4656-4664, 2004.
95. Ploeger HE, Takken T, de Greef MH and Timmons BW. The effects of acute and chronic exercise on inflammatory markers in children and adults with a chronic inflammatory disease: a systematic review. Exerc Immunol Rev 15: 6-41, 2009.
96. Powers SK and Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 88: 1243-1276, 2008.
97. Radak Z, Chung HY, Naito H, Takahashi R, Jung KJ, Kim HJ and Goto S. Age-associated increase in oxidative stress and nuclear factor kappaB activation are attenuated in rat liver by regular exercise. FASEB J 18: 749-750, 2004.
98. Raney MA, Yee AJ, Todd MK and Turcotte LP. AMPK activation is not critical in the regulation of muscle FA uptake and oxidation during low-intensity muscle contraction. Am J Physiol Endocrinol Metab 288: E592-E598, 2005.
99. Reid MB. Nitric oxide, reactive oxygen species, and skeletal muscle contraction. Med Sci Sports Exerc 33: 371-376, 2001.
100. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR and Bluher M. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A 106: 8665-8670, 2009.
101. Sady SP, Cullinane EM, Saritelli A, Bernier D and Thompson PD. Elevated high-density lipoprotein cholesterol in endurance athletes is related to enhanced plasma triglyceride clearance. Metabolism 37: 568-572, 1988.
102. Saleem A, Adhihetty PJ and Hood DA. Role of p53 in mitochondrial biogenesis and apoptosis in skeletal muscle. Physiol Genomics 37: 58-66, 2009.
103. Salo DC, Donovan CM and Davies KJ. HSP70 and other possible heat shock or oxidative stress proteins are induced in skeletal muscle, heart, and liver during exercise. Free Radic Biol Med 11: 239-246, 1991.
104. Sato Y, Nagasaki M, Nakai N and Fushimi T. Physical exercise improves glucose metabolism in lifestyle-related diseases. Exp Biol Med (Maywood) 228: 1208-1212, 2003.
105. Schafer S, Kantartzis K, Machann J, Venter C, Niess A, Schick F, Machicao F, Haring HU, Fritsche A and Stefan N. Lifestyle intervention in individuals with normal versus impaired glucose tolerance. Eur J Clin Invest 37: 535-543, 2007.
106. Shojaee-Moradie F, Baynes KC, Pentecost C, Bell JD, Thomas EL, Jackson NC, Stolinski M, Whyte M, Lovell D, Bowes SB, Gibney J, Jones RH and Umpleby AM. Exercise training reduces fatty acid availability and improves the insulin sensitivity of glucose metabolism. Diabetologia 50: 404-413, 2007.
107. Stefan N, Kantartzis K and Haring HU. Causes and metabolic consequences of Fatty liver. Endocr Rev 29: 939-960, 2008.
108. Stefan N, Thamer C, Staiger H, Machicao F, Machann J, Schick F, Venter C, Niess A, Laakso M, Fritsche A and Haring HU. Genetic variations in PPARD and PPARGC1A determine mitochondrial function and change in aerobic physical fit-

ness and insulin sensitivity during lifestyle intervention. J Clin Endocrinol Metab 92: 1827-1833, 2007.
109. Sun L, Shen W, Liu Z, Guan S, Liu J and Ding S. Endurance exercise causes mitochondrial and oxidative stress in rat liver: effects of a combination of mitochondrial targeting nutrients. Life Sci 86: 39-44, 2010.
110. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH and Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 288: E462-E468, 2005.
111. Tamura Y, Tanaka Y, Sato F, Choi JB, Watada H, Niwa M, Kinoshita J, Ooka A, Kumashiro N, Igarashi Y, Kyogoku S, Maehara T, Kawasumi M, Hirose T and Kawamori R. Effects of diet and exercise on muscle and liver intracellular lipid contents and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab 90: 3191-3196, 2005.
112. Terjung RL, Baldwin KM, Winder WW and Holloszy JO. Glycogen repletion in different types of muscle and in liver after exhausting exercise. Am J Physiol 226: 1387-1391, 1974.
113. Thamer C, Machann J, Stefan N, Haap M, Schafer S, Brenner S, Kantartzis K, Claussen C, Schick F, Haring H and Fritsche A. High visceral fat mass and high liver fat are associated with resistance to lifestyle intervention. Obesity (Silver Spring) 15: 531-538, 2007.
114. Thompson PD, Buchner D, Pina IL, Balady GJ, Williams MA, Marcus BH, Berra K, Blair SN, Costa F, Franklin B, Fletcher GF, Gordon NF, Pate RR, Rodriguez BL, Yancey AK and Wenger NK. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease: a statement from the Council on Clinical Cardiology (Subcommittee on Exercise, Rehabilitation, and Prevention) and the Council on Nutrition, Physical Activity, and Metabolism (Subcommittee on Physical Activity). Circulation 107: 3109-3116, 2003.
115. Trimmer JK, Schwarz JM, Casazza GA, Horning MA, Rodriguez N and Brooks GA. Measurement of gluconeogenesis in exercising men by mass isotopomer distribution analysis. J Appl Physiol 93: 233-241, 2002.
116. Tsekouras YE, Magkos F, Kellas Y, Basioukas KN, Kavouras SA and Sidossis LS. High-intensity interval aerobic training reduces hepatic very low-density lipoprotein-triglyceride secretion rate in men. Am J Physiol Endocrinol Metab 295: E851-E858, 2008.
117. Tsekouras YE, Magkos F, Prentzas KI, Basioukas KN, Matsama SG, Yanni AE, Kavouras SA and Sidossis LS. A single bout of whole-body resistance exercise augments basal VLDL-triacylglycerol removal from plasma in healthy untrained men. Clin Sci (Lond) 116: 147-156, 2009.
118. Turcotte LP, Raney MA and Todd MK. ERK1/2 inhibition prevents contraction-induced increase in plasma membrane FAT/CD36 content and FA uptake in rodent muscle. Acta Physiol Scand 184: 131-139, 2005.
119. van der Heijden GJ, Toffolo G, Manesso E, Sauer PJ and Sunehag AL. Aerobic exercise increases peripheral and hepatic insulin sensitivity in sedentary adolescents. J Clin Endocrinol Metab 94: 4292-4299, 2009.
120. van Dijk G, Balkan B, Lindfeldt J, Bouws G, Scheurink AJ, Ahren B and Steffens AB. Contribution of liver nerves, glucagon, and adrenaline to the glycaemic response to exercise in rats. Acta Physiol Scand 150: 305-313, 1994.

121. Wahren J and Ekberg K. Splanchnic regulation of glucose production. Annu Rev Nutr 27: 329-345, 2007.
122. Wahren J, Felig P, Ahlborg G and Jorfeldt L. Glucose metabolism during leg exercise in man. J Clin Invest 50: 2715-2725, 1971.
123. Wasserman DH and Cherrington AD. Hepatic fuel metabolism during muscular work: role and regulation. Am J Physiol 260: E811-E824, 1991.
124. Wasserman DH, Lacy DB and Bracy DP. Relationship between arterial and portal vein immunoreactive glucagon during exercise. J Appl Physiol 75: 724-729, 1993.
125. Wasserman DH, Spalding JA, Lacy DB, Colburn CA, Goldstein RE and Cherrington AD. Glucagon is a primary controller of hepatic glycogenolysis and gluconeogenesis during muscular work. Am J Physiol 257: E108-E117, 1989.
126. Wasserman DH, Williams PE, Lacy DB, Goldstein RE and Cherrington AD. Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. Am J Physiol 256: E500-E509, 1989.
127. Widegren U, Jiang XJ, Krook A, Chibalin AV, Bjornholm M, Tally M, Roth RA, Henriksson J, Wallberg-Henriksson H and Zierath JR. Divergent effects of exercise on metabolic and mitogenic signaling pathways in human skeletal muscle. FASEB J 12: 1379-1389, 1998.
128. Wilson DO and Johnson P. Exercise modulates antioxidant enzyme gene expression in rat myocardium and liver. J Appl Physiol 88: 1791-1796, 2000.
129. Wray DW, Uberoi A, Lawrenson L, Bailey DM and Richardson RS. Oral antioxidants and cardiovascular health in the exercise-trained and untrained elderly: a radically different outcome. Clin Sci (Lond) 116: 433-441, 2009.
130. Yamada P, Amorim F, Moseley P and Schneider S. Heat shock protein 72 response to exercise in humans. Sports Med 38: 715-733, 2008.
131. Yung Y, Yao Z, Hanoch T and Seger R. ERK1b, a 46-kDa ERK isoform that is differentially regulated by MEK. J Biol Chem 275: 15799-15808, 2000.
132. Zieker D, Fehrenbach E, Dietzsch J, Fliegner J, Waidmann M, Nieselt K, Gebicke-Haerter P, Spanagel R, Simon P, Niess AM and Northoff H. cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. Physiol Genomics 23: 287-294, 2005.
133. Ziogas GG, Thomas TR and Harris WS. Exercise training, postprandial hypertriglyceridemia, and LDL subfraction distribution. Med Sci Sports Exerc 29: 986-991, 1997.