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ORIGINAL PAPER

Endurance training in mice increases the unfolded protein response induced by a high-fat diet

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Abstract Certain conditions, such as several weeks of high-fat diet, disrupt endoplasmic reticulum (ER) homeostasis and activate an adaptive pathway referred as the unfolded protein response. When the unfolded protein response fails, the result is the development of inflammation and insulin resistance. These two pathological states are known to be improved by regular exercise training but the mechanisms remain largely undetermined. As it has recently been shown that the unfolded protein response is regulated by exercise, we hypothesised that concomitant treadmill exercise training (HFD+ex) prevents ER homeostasis disruption and its downstream consequences induced by a 6-week high-fat diet (HFD) in mice by activating the protective unfolded protein response. Several well-documented markers of the unfolded protein response were measured in the soleus and tibialis anterior muscles as well as in the liver and pancreas. In HFD mice, an increase in these markers was observed (from 2- to 15-fold, $P<0.05$) in all tissues studied. The combination of HFD+ex increased the expression of several markers further, up to 100 % compared to HFD alone ($P<0.05$). HFD increased inflammatory markers both in the plasma (IL-6 protein, 2.5±0.52-fold; MIP-1α protein, 1.3±0.13-fold; $P<0.05$) and in the tissues studied, and treadmill exercise attenuated the inflammatory state induced by HFD ($P<0.05$). However, treadmill exercise could not reverse HFD-induced whole body glucose intolerance, assessed by OGTT (AUC, 1.8±0.29-fold, $P<0.05$). In conclusion, our results show that a HFD activated the unfolded protein response in mouse tissues in vivo, and that endurance training promoted this response. We speculate that the potentiation of the unfolded protein response by endurance training may represent a positive adaptation protecting against further cellular stress.

Keywords Skeletal muscle · ER stress · BiP/GRP78 · XBP1 · Caspase 12

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Introduction

The endoplasmic reticulum (ER) is a key organelle, in which the folding and post-translational modifications of proteins occur. It is also crucial in the selection and transport of proteins to other compartments both within and outside of the cell. Certain stress conditions, such as high lipids, glucose deprivation and increased synthesis of secretory proteins, disrupt ER homeostasis and lead to the accumulation of unfolded or misfolded proteins in the ER lumen [36]. To cope with this stress, cells activate a signal transduction system that links the ER lumen to the cytoplasm and the nucleus; this process is referred to as the unfolded protein response [22, 27, 33]. The unfolded protein response is required for the restoration of normal ER function through three main unfolded protein response transducers: ATF6 (activating transcription factor 6); IRE1α (inositol-requiring enzyme 1 alpha); and PERK (protein kinase R-like ER protein kinase). Each of these factors associates with the protein chaperone BiP/GRP78 (binding protein/glucose regulated-protein 78), a member of the Hsp70 (heat shock protein 70) family, in its inactive state. Upon accumulation of unfolded/misfolded proteins in the ER lumen, ATF6, IRE1α, and PERK are released from BiP/GRP78 and become activated. The downstream effectors of these three pathways induce the expression of genes, such as XBP1 (X box binding protein 1), CHOP [C/EBP (CCAAT/enhancer binding protein) homologous protein] and ATF4 (activating transcription factor 4), which encode proteins that function to augment the ER protein-folding capacity.

When the unfolded protein response fails, the result is cell death, usually in the form of apoptosis triggered by the cleavage of pro-caspase 12 at the ER membrane [30, 35]. In addition to apoptosis, ER stress can increase inflammatory state [18] and decrease glucose tolerance by two distinct processes. First, ER stress in pancreatic islets results in dysfunctional beta-cells and in immature insulin release in the blood [21, 33]. Second, ER stress in peripheral insulin-sensitive tissues such as liver and adipose tissue leads to an increase in JNK (c-Jun N-terminal kinase)-mediated serine phosphorylation of IRS-1 (insulin receptor-substrate 1) and thereby to inhibition of insulin action via decreased signalling to Akt/PKB (protein kinase B) [31].

However, it is not clear whether similar effects occur in skeletal muscle. Using two different models of high-fat-fed mice, we recently showed that the unfolded protein response was increased in skeletal muscles [6] although we could not confirm those results in human skeletal muscle [7]. Since exercise is known to prevent glucose intolerance and to reduce inflammatory state, endurance training has been hypothesised to reduce ER stress previously induced by high-fat feeding in rat adipose and hepatic tissues [5]. The conclusions of the previous study were that swimming reduced pro-inflammatory molecules and ER stress markers and increased Akt/PKB phosphorylation in adipose and hepatic tissues of diet-induced obese rats. While the results of the previous study are important for understanding the mechanisms of the beneficial effects of exercise in obesity and diabetes, one could ask if the same occurs in skeletal muscle, proportionally the most important tissue by weight in the body. The aim of the present study was thus to determine whether contractile activity alters the unfolded protein response in skeletal muscle and whole body glucose tolerance in 6-week high-fat-fed mice. Liver and pancreas were studied at the same time to detect whether the potential regulation of the unfolded protein response by exercise is tissue-specific.

Materials and methods

Animals and diets

Two-month-old female C57BL/6 J mice (Laboratory of Experimental Surgery) were housed in groups of six or seven per cage at 22 °C in a 12 h light/dark cycle and were given free access to diet and water. After 1 week acclimation, mice were randomly assigned to either a control group (Ctrl, $n=7$), a sedentary high-fat diet group (HFD, $n=7$) and a high-fat diet group that was simultaneously exercise trained (HFD+ex, $n=6$). The control group ate standard chow, while the two other groups received a diet containing 49.5 g fat (corn oil and lard)/100 g, 37 g protein (cow milk casein)/100 g and <1 g carbohydrate/100 g. This represents, in % total energy: 72 % fat, 28 % protein, and <1 % carbohydrate (UAR). This diet is known to induce a marked diabetic and metabolic stress state [2, 3]. Although a lack of carbohydrates per se has been shown to activate ER stress [13], we recently evidenced that a 45 % HFD containing 35 % carbohydrates induced the unfolded protein response in a way similar to the

present diet, indicating that a lack of carbohydrates is not the principal factor inducing the unfolded protein response in skeletal muscle [7]. All mice experiments were approved by the local committee and the housing conditions were as specified by the Belgian Law of November 14, 1993 on the protection of laboratory animals (agreement no. LA 1220548).

Exercise training protocol

The results of the Ctrl and the HFD groups have been partially published in a previous study [6], the purpose of which was to evidence the presence of ER stress in skeletal muscle of sedentary high-fat-fed mice. The data of the HFD+ex group were acquired at the same time as the Ctrl and HFD groups. In order to study the effect of contractile activity, mice assigned to the HFD +ex group exercised on a treadmill 5 days per week for 6 weeks. Initially, mice were progressively acclimated to the treadmill during a period of 2 weeks from 7 m/min for 30–40 min to 12 m/min for 60 min. For the remainder of the experiment, mice trained at a treadmill speed of 12 m/min for 60 min. During the whole experiment, the slope of the treadmill was equal to zero. According to pre-experiments on mice of exactly the same strain, same origin (Laboratory of Experimental Surgery), same gender and same age, this speed corresponds to approximately 70 % of the maximal velocity of the mice. The maximal velocity was determined thanks to an incremental exercise test on a flat treadmill mill (no slope). The starting velocity was 8 $m \cdot min^{-1}$ and was increased by 2 $m \cdot min^{-1}$ every 2 min until exhaustion. The maximal velocity was defined as the velocity of the last stage completed by the animals.

Tissue and blood samples

At the end of the 6 weeks and 48 h after the last exercise session, 6-h-fasted mice were terminally anaesthetized by intra-peritoneal injection of sodium pentobarbital solution (using 60 mg/kg of body weight, Nembutal®, Sanofi). Tibialis anterior and soleus muscles, liver, pancreas and visceral fat were removed as fast as possible and immediately frozen in liquid nitrogen. The visceral fat corresponds to the mesenteric fat present along the gastrointestinal tract. This fat depot was separated from the pancreas and the mesenteric ganglion was precisely dissected. Cava vein blood samples were collected in EDTA tubes; after centrifugation (10 min at 1,500×*g*), plasma was stored at −80 °C.

Protein extraction, SDS/PAGE and immunoblotting

Approximately 10–20 mg of frozen tissue were ground in a mortar and homogenized in ice-cold buffer [20 mM Tris, pH 7.0, 270 mM sucrose, 5 mM EGTA, 1 mM EDTA, 1 % Triton X-100, 1 mM sodium orthovanadate, 50 mM sodium β-glycerophosphate, 5 mM sodium pyrophosphate, 50 mM sodium fluoride, 1 mM DTT (1,4-dithiothreitol) and a protease inhibitor cocktail containing 1 mM EDTA (Roche Applied Science)] for 5 min on ice. The homogenates were then centrifuged for 10 min at 10,000×*g* and the supernatants were immediately stored at −80 °C. Protein concentration was determined using the DC protein assay kit (Bio-Rad Laboratories).

Cell lysates (60 μg for skeletal muscle proteins and 30 μg for pancreatic and hepatic proteins) were combined with Laemmli sample buffer and separated by SDS/PAGE. After electrophoretic separation at 40 mA for 1 h, the proteins were transferred to a PVDF membrane at 80 V for 2 h for western blot analysis. Membranes were then incubated in a 5 % Blotto solution. Subsequently, membranes were incubated with the following antibodies overnight at 4 °C: BiP, PDI (protein disulfide isomerase), MBTPS2 (membrane-bound transcription factor protease site 2), IRE1α, PERK, phospho-PERK Thr 980, phospho-p38 (p38 protein kinase) Thr 180/Tyr 182, phospho-JNK Thr 183/Tyr 185, phospho-IKK (I-kappa-B kinase) Ser 176/180, SDH (succinate dehydrogenase) and GAPDH. All antibodies were from cell signalling except PERK and GAPDH from Abcam and SDH from Santa Cruz.

Membranes were washed in TBST and incubated for 1 h at room temperature in a secondary antibody conjugated to horseradish peroxidase (1:10,000, Sigma). After an additional three washes, chemiluminescence detection was carried out using an Enhanced Chemiluminescent Western blotting kit (ECL Plus, Amersham Biosciences). The films were then scanned on an ImageScanner using the Labscan software and quantified with the Image Master 1D Image Analysis Software (Amersham Biosciences). Results are reported relative to GAPDH. A value of 1 was arbitrarily assigned to the control conditions which were

used as a reference for the high-fat and high-fat plus exercise values.

RNA extraction and quantitative real-time PCR

About 10–20 mg of frozen tissue samples were homogenized in TriPure reagent (Roche) using a Polytron. Total RNA was extracted according to the instructions provided by the manufacturer (Roche Diagnostics). RNA was quantified by spectrophotometry (260 nm) and its concentration adjusted to 1 μg/μl using RNase-free water. Since soleus muscles were very small (about 5–6 mg), the RNA concentrations obtained after extraction were too low to perform the reverse transcription reaction. For tibialis anterior and liver, cDNA was prepared by reverse transcription of 1 μg total RNA using the reverse transcription system (Promega). Real-time PCR was performed with a STEP one PLUS instrument and software (Applied Biosystems) using SYBR®Green PCR Master Mix (Applied Biosystems) for detection. Real-time PCR primers were designed (Table 1) for mouse CHOP, ATF4, spliced (s) XBP1, unspliced (u) XBP1, IL-1 (interleukin-1), IL-6 (interleukin-6), NADPHox (nicotinamide adenine dinucleotide phosphate oxidase) and RPL-19 (ribosomal protein L19). Specific primers were designed to recognize the spliced, or active, form of XBP1 (XBP1s) versus the unspliced form (XBP1u). RPL-19 was used as the "house keeping" gene. All tissues were run in duplicate in a single 96-well reaction plate (MicroAmp Optical, Applied Biosystems) and data were analysed according to the $2^{-\Delta CT}$ method. The identity and purity of the amplified product was checked through analysis of the melting curve carried out at the end of amplification. A value of 1 was arbitrarily assigned to the control condition to which the high-fat and the high-fat plus exercise values were reported.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) (gavage with 1 mg glucose/g body weight; 20 % glucose solution) was performed on 6-h-fasted mice at the end of the 6-week treatment and 24 h after the last training session. Blood glucose was determined with a glucose meter (Roche Diagnostics) on 3.5 μl of blood collected from the tip of the tail vein, 30 min before and 0, 15, 30, 60, 90 and 120 min following glucose injection.

Cytokines quantification

Cytokines were determined in 12 μl of plasma using a kit (Bio-Plex Multiplex; Bio-Rad) and measured using Luminex technology (Bio-Plex; Bio-Rad) as previously described [4]. Insulin was measured in 5 μl of plasma using an ELISA kit (Mercodia, Upssala, Sweden).

Statistical analysis

The difference between the three groups was tested for significance using a one-way analysis of variance. When significant, Student–Newman–Keuls post hoc tests were applied. The significance threshold was set to $P<0.05$. Results are presented as means±SEM.

Table 1 Primer sequences

	Forward	Reverse
CHOP	CCT AGC TTG GCT GAC AGA GG	CTG CTC CTT CTC CTT CAT GC
ATF4	GAG CTT CCT GAA CAG CGA AGT G	TGG CCA CCT CCA GAT AGT CAT C
XBP1u	TGA GAA CCA GGA GTT AAG AAC ACG C	TTC TGG GTA GAC CTC TGG GAG TTC C
XBP1s	GAG TCC GCA GCA GGT G	GTG TCA GAG TCC ATG GGA
IL-1	TCG CTC AGG GTC ACA AGA AA	CAT CAG AGG CAA GGA GGA AAA C
IL-6	ACA AGT CGG AGG CTT AAT TAC ACA T	TTG CCA TTG CAC AAC TCT TTT C
NADPHox	TTG GGT CAG CAC TGG CTC TG	TGG CGG TGT GCA GTG CTA TC
RPL-19	GAA GGT CAA AGG GAA TGT GTT CA	CCT TGT CTG CCT TCA GCT TGT

Sequences of primers used for mRNA quantification by real-time RT-PCR CHOP

C/EBP (CCAAT/enhancer binding protein) homologous protein, *ATF4* activating transcription factor 4, *XBP1u* unspliced X Box binding protein 1, *XBP1s* spliced X Box binding protein 1, *IL-1* interleukin-1, *IL-6* interleukin-6, *NADPHox* nicotinamide adenine dinucleotide phosphate oxidase, *RPL-19* ribosomal protein L19

Results

Endurance training reduces gain in body weight induced by high-fat diet

Throughout the 6 weeks of experiment, mice fed with a normal diet increased body weight by 20 % (Fig. 1a). At the end of the 6 weeks, HFD mice weighed 10 % more than control mice ($P<0.05$ at week 5 and $P=0.088$ at week 6). Running reduced the body weight gain induced by the HFD by about a half ($P<0.05$). In the same way, HFD increased visceral fat content by 50 % ($P<0.05$, Fig. 1b–c) compared to control mice whereas running while eating a HFD tended to reduce this gain.

Endurance training increases succinate dehydrogenase expression

The efficacy of the endurance training protocol was assessed by measuring SDH expression. The latter almost doubled in soleus ($P<0.001$) and tibialis anterior ($P<0.01$) muscles, as compared to untrained mice, whereas it was not modified in the liver (Fig. 2).

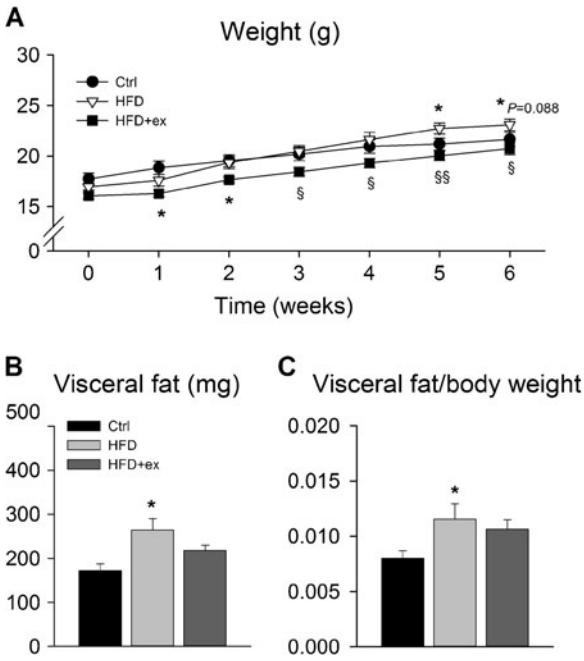

Fig. 1 *Body weight and visceral fat content*. Mice were weighed each week (**a**). Visceral fat content was weighed at the end of the 6 weeks (**b**) and reported to body weight (**c**). Results are expressed as the means±SEM. Ctrl, control; HFD, high-fat diet; HFD+ex, high-fat diet plus exercise. *$P<0.05$ vs Ctrl; §$P<0.05$, §§$P<0.01$ vs HFD

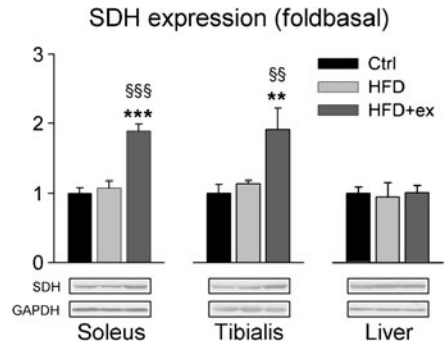

Fig. 2 *Succinate dehydrogenase as a marker of endurance training*. SDH expression in soleus, tibialis anterior and liver after 6 weeks HFD or HFD+ex. Results are expressed as the means± SEM. Ctrl, control; HFD, high-fat diet; HFD+ex, high-fat diet plus exercise. **$P<0.01$, ***$P<0.001$ vs Ctrl; §§$P<0.01$, §§§$P<0.001$ vs HFD

High-fat diet increases unfolded protein response in skeletal muscle

Several unfolded protein response markers were higher in skeletal muscles of HFD than in control mice (Fig. 3a and b and [6]). The changes were more pronounced in slow-type soleus muscle (Fig. 3a) than in fast-type tibialis anterior muscle (Fig. 3b). In the soleus, high-fat diet increased the protein expression of BiP ($P<0.05$) and MBTPS2 ($P<0.001$) eightfold as well as IRE1α ($P<0.05$) and PERK ($P<0.05$) five-fold. The role of MBTPS2 is to cleave ATF6 into a functional transcription factor. In soleus, PERK was only detectable in its unactivated non-phosphorylated form. In tibialis anterior, HFD doubled BiP ($P<0.05$) and IRE1α ($P<0.05$) levels and increased the expression of PERK 15-fold ($P<0.001$). Since PERK phosphorylation was unchanged by HFD, the ratio of phospho-PERK/PERK was decreased, suggesting that the relative activity of PERK was reduced in HFD (Fig. 3b). High-fat diet also induced the unfolded protein response in liver (Fig. 3c) and in pancreas (Fig. 3d). PDI, IRE1α and MBTPS2 protein levels were more than doubled and PERK phosphorylation was increased fourfold in the liver ($P<0.05$). In the pancreas, IRE1α expression was increased by three-fold ($P<0.05$), MBTPS2 by twofold ($P<0.05$) and total PERK by sixfold ($P<0.01$). It is of note that the antibody against PDI used in this study resulted in a

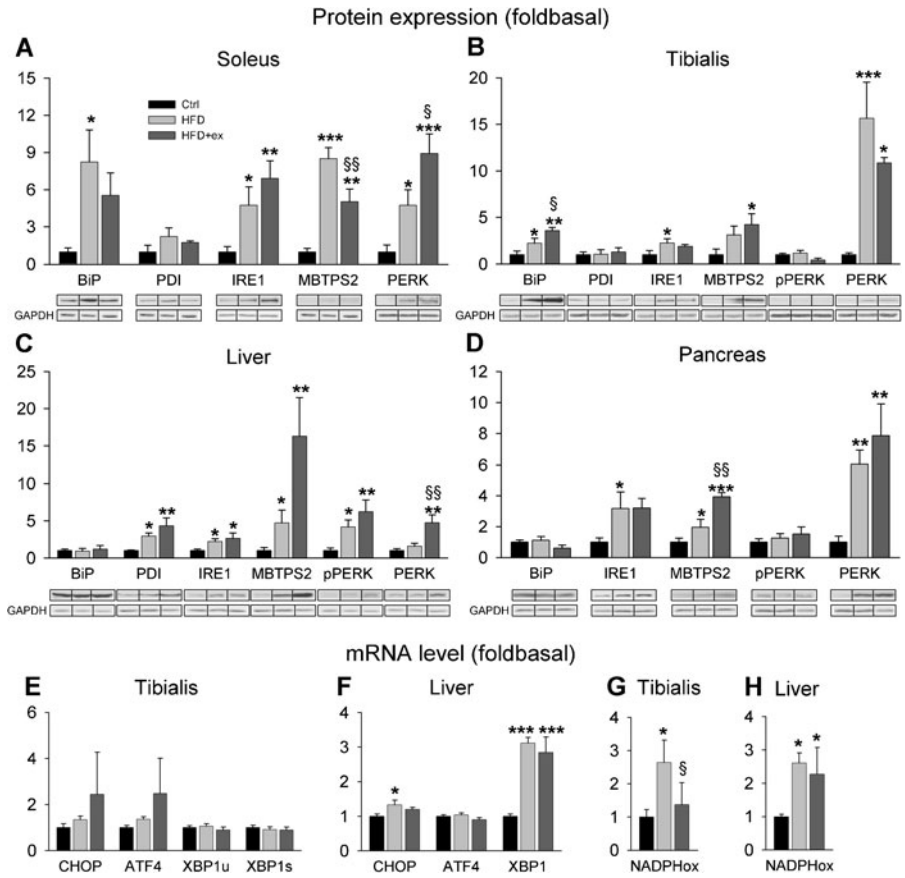

Fig. 3 *Endoplasmic reticulum stress markers*. Protein expression of BiP, PDI, IRE1α, MBTPS2, PERK and phosphorylation state of PERK in soleus (**a**), tibialis anterior (**b**), liver (**c**) and pancreas (**d**) and mRNA level of CHOP, ATF4, XBP1s, XBP1u (**e**, **f**) and NADPHox (**g**, **h**) in tibialis anterior (**e**, **g**) and liver (**f**, **h**) after 6 weeks HFD or HFD+ex. Results are expressed as the means±SEM. A value of 1 was arbitrarily assigned to the control conditions to which the HFD and HFD+ex values were reported and expressed as foldbasal. *BiP* binding protein, *PDI* protein disulfide isomerase, *IRE1* inositol-requiring enzyme 1, *MBTPS2* membrane-bound transcription factor protease site 2, *PERK* protein kinase R-like ER protein kinase, *CHOP* C/EBP (CCAAT/enhancer binding protein) homologous protein, *ATF4* activating transcription factor 4, *XBP1u* unspliced X Box binding protein 1, XBP1s, spliced X Box binding protein 1 *NADPHox* nicotinamide adenine dinucleotide phosphate oxidase. *Ctrl* control, *HFD* high-fat diet, *HFD+ex* high-fat diet plus exercise. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs Ctrl; $^{§}P<0.05$, $^{§§}P<0.01$, $^{§§§}P<0.001$ vs HFD

diffuse signal that was not quantifiable due to the two isoforms expressed only in the pancreas [8].

Since ER stress induces responses at both the translational and the transcriptional levels, we also analysed the mRNA levels of some well-documented ER stress markers: CHOP, ATF4, XBP1s and XBP1u (Fig. 3e and f) [11, 16]. Whereas the mRNA level of these markers was not affected by HFD in the tibialis anterior (Fig. 3e), CHOP was increased by 30 % ($P<0.05$) and XBP1u by 200 % ($P<0.001$) in the liver (Fig. 3f). NADPH oxidase is a major player in the production of reactive oxygen species which are known to trigger ER stress and to play a role in the pathogenesis of diabetes [14]. High-fat diet doubled NADPHox mRNA level in both the tibialis anterior muscle (Fig. 3g) and the liver (Fig. 3h) ($P<0.05$).

Compared to the effects of HFD alone, endurance training did not induce many profound changes in ER stress markers. Endurance training increased the expression of a few markers more than HFD alone, as observed for PERK in the soleus ($P<0.05$, Fig. 3a) and in the liver ($P<0.01$, Fig. 3c), for BiP in the tibialis anterior ($P<0.05$, Fig. 3b) and for MBTPS2 in the pancreas ($P<0.01$, Fig. 3d). Interestingly, endurance training reversed the increase in NADPHox mRNA level induced by a HFD in the tibialis anterior ($P<0.05$, Fig. 3g).

Endurance exercising while fed with a high-fat diet increases caspase 12 expression and cleavage

High-fat diet alone had no effect on caspase 12 expression or cleavage in any tissue studied. The cleavage of caspase 12 however was increased in HFD+ex condition in the soleus ($P<0.05$, Fig. 4a), tibialis anterior ($P<0.001$, Fig. 4b), liver ($P<0.01$, Fig. 4c) and pancreas ($P<0.05$, Fig. 4d). It is of note that cleaved caspase 12 was not detectable in control conditions in the tibialis anterior (Fig. 4b) and in the liver (Fig. 4c) whereas total caspase 12 was observed. Endurance exercising while fed with a HFD also increased the total form of caspase 12 compared to control or HFD alone in the soleus ($P<0.05$, Fig. 4a) and liver ($P<0.01$, Fig. 4c).

Regulation of inflammation markers is tissue specific

JNK and IKK/NFκB have been implicated in insulin resistance and inflammation induced by ER stress through the IRE1α pathway [12, 14]. In our hands, these two pathways seem to be regulated independently since phospho-IKK was increased by HFD in the soleus ($P<0.05$, Fig. 5a) whereas in the liver ($P<0.05$, Fig. 5c) and in the pancreas ($P<0.001$, Fig. 5d), HFD increased phospho-JNK without affecting phospho-IKK. Phospho-p38 was only enhanced in the tibialis anterior ($P<0.05$, Fig. 5b). All these results indicate that the pathways

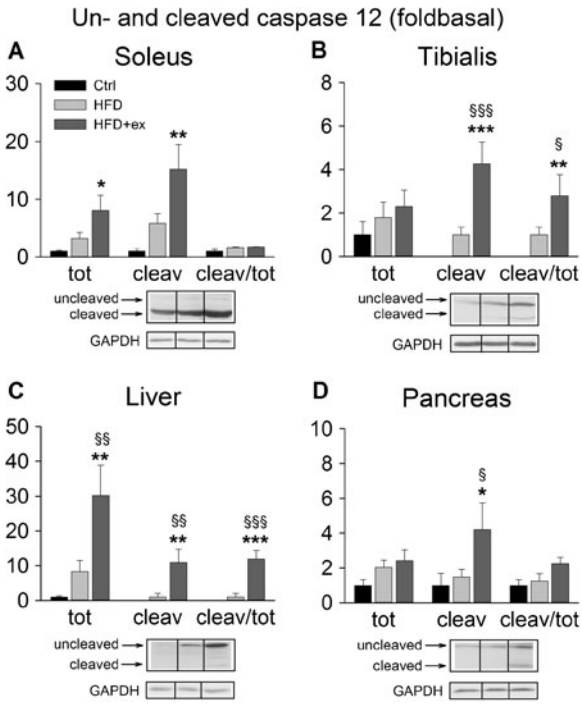

Fig. 4 *Apoptosis marker*. Caspase 12 expression and cleavage in soleus (**a**), tibialis anterior (**b**), liver (**c**) and pancreas (**d**) after 6 weeks HFD or HFD+ex. Results are expressed as the means± SEM. A value of 1 was arbitrarily assigned to the control conditions to which the HFD and HFD+ex values were reported and expressed as foldbasal. *Ctrl* control, *HFD* high-fat diet, *HFD+ex* high-fat diet plus exercise. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs Ctrl; §$P<0.05$, §§$P<0.01$, §§§$P<0.001$ vs HFD

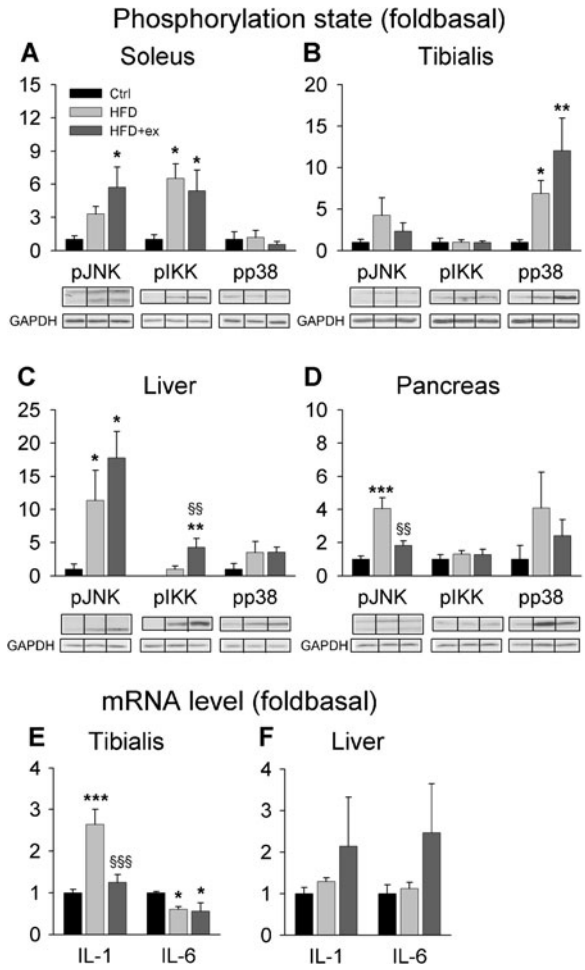

Fig. 5 *Inflammation markers*. Phosphorylation state of JNK, IKK and p38 in soleus (**a**), tibialis anterior (**b**), liver (**c**) and pancreas (**d**) and mRNA level of IL-1 and IL-6 in tibialis anterior (**e**) and liver (**f**) after 6 weeks HFD or HFD+ex. Results are expressed as the means±SEM. A value of 1 was arbitrarily assigned to the control conditions to which the HFD and HFD +ex values were reported and expressed as foldbasal. *JNK* Jun N-terminal kinase, *IKK* I-kappa-B kinase, *p38* p38 protein kinase, *IL-1* interleukin-1, *IL-6* interleukin-6. *Ctrl* control, *HFD* high-fat diet, *HFD+ex* high-fat diet plus exercise. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs Ctrl; §§$P<0.01$, §§§$P<0.001$ vs HFD

known to be switched on by inflammation were regulated in a tissue-specific manner by HFD feeding and that exercise did not significantly reverse this effect except in the pancreas, in which phospho-JNK mice reached a similar level in control and HFD+ex mice.

To evaluate the further implication of this inflammation-induced signalling by HFD, we measured the mRNA level of IL-1 and IL-6 in the tibialis anterior (Fig. 5e) and liver (Fig. 5f) as well as the plasma level of several pro- and anti-inflammatory cytokines (Table 2). IL-1 is a master mediator and initiator of inflammation in several tissues [24] and its mRNA expression has recently been shown to be also regulated in mouse skeletal muscle [20]. IL-1 mRNA level was increased ($P<0.001$, Fig. 5e) and IL-6 decreased ($P<0.05$, Fig. 5e) by HFD in the tibialis anterior and were unchanged in the liver (Fig. 5f). Exercise reversed the HFD-induced increase in IL-1 ($P<0.001$, Fig. 5e) but not the decrease in IL-6 mRNA in the tibialis anterior. The effects of exercise were more pronounced at the plasma level where it reduced the high-fat diet increase in IL-6 and MIP-1α concentrations ($P<0.05$, Table 2). Endurance exercising while high-fat fed also decreased the levels of circulating IL-15 compared to sedentary normally fed mice and MCP-1 compared to sedentary normally fed and high-fat-fed mice ($P<0.05$, Table 2).

Table 2 The cytokine concentrations are expressed in pg/ml

	Ctrl	HFD	HFD+ex
IL-1α	34±11.3	20±3.5	17±2.7
IL-1β	26±6.2	18±2.5	32±11.3
IL-6	22±3.3	56±11.3*	35±6.1**
IL-10	43±14.0	112±24.3	232±122.5
IL-15	306±27.2	257±17.8	210±16.5*
IL-18	440±107.7	593±189.8	228±52.0
MCP-1	107±13.1	120±12.4	72±7.5*·**
MIP-1α	139±10.6	178±18.7*	129±12.6**
TNF-α	26±6.4	14±1.2	16±3.6

Values are expressed as the means±SEM

IL-1α interleukin-1 alpha, *IL-1β* interleukin-1 beta, *IL-6* interleukin-6, *IL-10* interleukin-10, *IL-15* interleukin-15, *IL-18* interleukin-18, *MCP-1* monocyte chemotactic protein-1, *MIP-1α* macrophage inflammatory protein-1 alpha, *TNFα* tumor necrosis factor alpha, *Ctrl* control, *HFD* high-fat diet, *HFD+ex* high-fat diet plus exercise

*$P<0.05$ vs Ctrl; **$P<0.05$ vs HFD

High-fat diet-induced glucose intolerance is not reversed by endurance training

Mice fed a HFD were glucose intolerant as indicated by the OGTT performed at the end of the 6 weeks (Fig. 6a). For the same amount of glucose given orally, plasma glucose concentrations remained elevated up to 90 min after gavage ($P<0.05$) whereas plasma insulin concentrations were not different between groups (Fig. 6a). The area under the curve was increased by 1.8-fold by the HFD ($P<0.05$, Fig. 6b) and was not reduced by exercise. The OGTT indicates that low intensity running while eating a HFD did not reverse the glucose intolerant state induced by HFD alone.

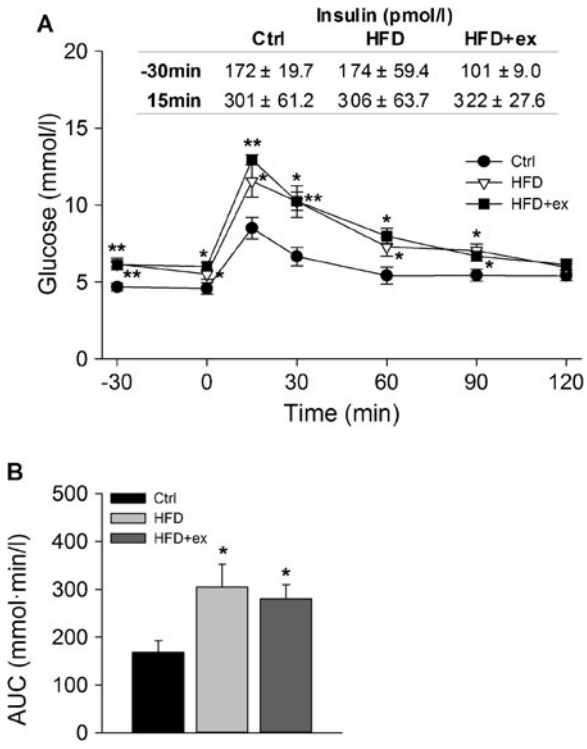

Fig. 6 *OGTT test*. An oral glucose tolerance test (OGTT) was performed on 6-h-fasted mice at the end of the 6-weeks treatment and 24 h after the last training session. (**a**) Plasma glucose and insulin concentrations after the oral glucose load. (**b**) Area under curve (*AUC*) of the glucose excursion after the oral glucose load. Results are expressed as the means±SEM. *Ctrl* control, *HFD* high-fat diet, *HFD+ex* high-fat diet plus exercise. *$P<0.05$, **$P<0.01$ vs Ctrl

Discussion

The main findings of the present study are that: (1) endurance training attenuated the inflammatory state induced by a HFD in mice without reversing whole body glucose intolerance; (2) endurance training increased the unfolded protein response induced by a HFD in mice.

The present results are different from those obtained in a recently published study showing that swimming reduced ER stress in adipose and hepatic tissues of diet-induced obese rats [5]. One major difference of the later study with the current study is that rats were high-fat-fed for 2 months before starting exercise training. One could postulate that rats were accustomed to the HFD and that a new homeostasis level was reached before the stress of physical activity was added, so that the beneficial effects of exercise could be exerted. This was not the case in the present study as both HFD and treadmill exercise began at the same time, each stress potentially exacerbating the other. It is also possible that the lipid percentage or nature of the diet and/or the intensity or nature of exercise could explain the opposite results. These hypotheses are further discussed below.

Our data show that several markers of the unfolded protein response were up-regulated following 6 weeks on a high-fat diet and that the effect was greatest in the postural muscles. In the tonically active soleus muscle, BiP, IRE1α, MBTPS2 and PERK protein levels were increased more than fivefold. In the more phasically active tibialis anterior muscle, these changes were less pronounced. The difference could be due to greater susceptibility of oxidative fibers to a high-fat diet, an additive effect of contractile activity and high-fat diet on ER stress, or increased uptake of fatty acids in the soleus due to its higher metabolic requirements.

When the unfolded protein response is not sufficient to cope with ER stress, inflammation and insulin resistance may develop. The inflammation and insulin resistance may directly result from an activation of IRE1α during the unfolded protein response, since IRE1α, in turn, triggers the JNK and IKK/NFkappaB pathways [12, 31]. Indeed, high-fat feeding activated IRE1α as well as JNK and IKK, a kinase upstream of NFkappaB. However, in no tissue was a simultaneous activation of JNK and IKK observed. High-fat diet also increased plasma levels of cytokines such as IL-6 and MIP-1α and this was associated with whole body glucose intolerance as demonstrated by the OGTT. Taken together, these data confirm that mild chronic inflammatory state and glucose intolerance were induced by our high-fat diet.

After having shown that a high-fat diet induced the unfolded protein response, a mild inflammatory state and glucose intolerance, we tested whether contractile activity in the form of endurance exercise could reduce these responses. The intensity of the exercise (12 m/min) corresponded to ~70 % of the average maximal running speed of untrained mice, as measured in preliminary experiments, or to ~75 % of VO_2max [28]. We chose this intensity as it has been shown to enhance lipid oxidation in mice skeletal muscle [9, 25]. Hence, we expected this form of exercise to reduce the deleterious effects of a high-fat diet by oxidizing more of the consumed fatty acids. The increase in muscle SDH expression observed after 6 weeks in the exercised mice indicates that the training was efficient in increasing aerobic metabolism. At the end of the training period, we observed an increase in markers of the unfolded protein response, some of them being even more elevated than in the HFD group without exercise. The increase in the unfolded protein response indicates a greater degree of ER stress with concomitant high-fat diet and exercise. Since exercise at 75 % of VO_2max increases the mobilization of fatty acids, exercising while consuming a high-fat diet could potentially result in a greater increase in fatty acid uptake [26] and therefore a lipid-dependent ER stress. Although not mentioned, it is likely that the intensity of swimming was less than running and that mobilization of fatty acids was less in the study of da Luz [5], potentially partially explaining the fact that exercise was able to reduce ER stress in obese rats.

Since exercise is known to improve glucose tolerance, we hypothesized that if ER stress is implicated in the development of glucose intolerance, exercise training would simultaneously reduce ER stress and glucose intolerance. Six weeks of treadmill running did reduce the plasma levels of several pro-inflammatory cytokines (IL-6, MCP-1 and MIP-1α). However, the exercise protocol used in the current study was not effective in reducing either ER stress or whole body glucose intolerance induced by a high-fat diet. It is possible that, in the present study, the intensity and/or the duration of the exercise sessions were not sufficient to counteract glucose intolerance caused by a high-fat diet. It has been reported that treadmill

exercise had only a mild effect on high-fat diet-induced glucose intolerance when training sessions were performed for 1 h during 6–8 weeks [10, 32] or for 3 h during 3 weeks [29]. However, when animals have had free access to a wheel, and exercise for approximately five- to tenfold longer periods of time, insulin resistance induced by high-fat feeding had been shown to be improved [1, 19]. Another possibility is that the fat content of the diet was too high for allowing beneficial effect of exercise on whole body glucose tolerance to be observed. Yet, this percentage of fat is seen in diets used in human during carbohydrates free regimen. In the current study, 70 % of the energy in the high-fat diet was derived from fat, whereas studies showing a positive effect of exercise on glucose tolerance, either due to wheel running, treadmill running, or swimming, used diets containing about 40–45 % fat [1, 10] or used diets containing the same percentage of fat (60–70 %) for a shorter period of time (3 weeks) [15, 19]. It is also possible that the fatty acid composition of the diet, i.e. highly saturated, could have contributed to the lack of effect of the exercise training.

We recognize that a limitation of the present study is the lack of an exercised group not receiving a high-fat diet. This would have allow to discriminating the effect of exercise per se as high-fat feeding and a lack of carbohydrates are known to induce metabolic adaptations and to influence exercise performance [23]. However, the purpose was to determine molecular mechanisms by which exercise exerts beneficial effects in high-fat-fed and obese animals and our results allow to answer this question. Our results support the idea that exacerbation of the high-fat diet-induced unfolded protein response by endurance exercise might improve ER homeostasis and consequently might protect against inflammation and extend previous similar results in lean mice [34] and human [17]. A single moderate-intensity exercise bout activated the unfolded protein response in skeletal muscle of lean mice, whereas the activation was less or even repressed for some markers after several training sessions. The authors concluded that moderate exercise and the accompanying physiological ER stress in skeletal muscle may lead to adaptation and protect skeletal muscle against further stress [34]. The same potential mechanism of defence has been observed after a 200-km run in human skeletal muscle [17].

In conclusion, we have shown that endurance training attenuated the inflammatory state induced by a HFD in mice without reversing whole body glucose intolerance and that endurance training increased the unfolded protein response induced by a HFD in mice. We speculate that the potentiation of unfolded protein response by endurance training may represent a positive adaptation protecting against further cellular stress. Different exercise protocols, in terms of frequency, intensity and duration should be tested to identify the effect of exercise per se on ER stress markers in skeletal muscle.

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