cytosolic area with fluorescence staining also could be observed and their unique pattern of mitochondrial dynamics represented was discussed.

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8P2

Hepatic mitochondrial bioenergetic and dynamic behaviour adaptations in response to high-fat feeding

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Introduction: High-fat feeding induces hepatic lipid accumulation associated with concomitant mitochondrial fat oxidation enhancement and electron-chain impairment that result in excessive formation of reactive oxygen species. Little is known about hepatic mitochondrial dynamic behaviour adaptation to high-fat feeding. Mitochondria are dynamic organelles that frequently undergo fission and fusion processes, imbalances in which have recently emerged as important etiological factors in obesity and insulin-resistance. Bearing in mind that fish-oil feeding has anti-steatotic effects, the present work aimed to evaluate the effects of high-lard (mainly saturated fatty acids) and high-fish-oil (mainly omega-3 polyunsaturated fatty acids) diets on both hepatic mitochondrial bioenergetic and dynamic behaviour.

Methods: Hepatic lipid accumulation was monitored in rats fed a high-lard or high-fish-oil (40% J/J) diet for 6 weeks. Mitochondrial functions were assessed by evaluating $FADH_2$ linked respiratory rates, fatty acid oxidation rates, energetic efficiency (by measuring basal and fatty acid induced proton leak kinetics), and oxidative stress (by measuring H_2O_2 release and aconitase activity). Mitochondrial dynamic behaviour was assessed by analysing the proteins relevant to the processes of fusion (MFN2, OPA1) and fission (DRP1, Fis1) (by western blot and immunohistochemical analysis).

Results: Hepatic lipid accumulation, electron chain impairment and oxidative stress induced by high-lard diet were associated with both decreased fatty acid induced proton leak and a shift toward mitochondrial fission processes (decreased MFN2 content and increased DRP1 and Fis1 content). On the other hand, the anti-steatotic effect of high-fish oil feeding was associated with mild uncoupling (increases in both basal and fatty acid induced proton leak kinetics), oxidative stress prevention and increased mitochondrial fusion processes.

Conclusions: Fission phenotype and increased energetic efficiency are associated with the steatotic effect of high-lard diet, whereas fusion phenotype and decreased energetic efficiency contributed to the anti-steatotic effect of high-fish oil diet.

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8P3

Mitophagy mediated by Rheb and the maintenance of mitochondrial activity

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Cells constantly adapt the amount of mitochondria to supply energy in accordance to physiological demand. The amount of mitochondria depends on the fine balance between biogenesis and degradation processes. The relationship between mitochondrial biogenesis and bioenergetics has been broadly investigated but it is not clear how mitochondrial degradation and energetics may be linked.

Mitophagy is a conserved degradation process in which the autophagic machinery delivers mitochondria to the lysosomes. We found that increasing mitochondrial activity by supplying cells exclusively with oxidative substrates such as glutamine or dimethyl-alpha ketoglutarate triggered mitophagy.

Using gradient partition analysis and fluorescence microscopy we found that Ras-homologue enriched in brain (Rheb) can be recruited to mitochondria preferentially in cells with forced mitochondrial activity. Interestingly, silencing of Rheb induces mitochondrial outgrowth, while ectopic expression increases formation of autophagosomes and mitochondrial degradation. Furthermore, mitophagy induced by Rheb overexpression also triggered the enhancement of OXPHOS efficiency; respiration measured with a Seahorse XF 24 analyzer was increased in these cells despite a lower amount of mitochondria. In contrast, Rheb silencing decreased routine oxygen consumption and coupled mitochondrial respiration.

In mitochondria, Rheb is able to regulate mitophagy triggered by mitochondrial activity through physical interaction with the mitochondrial mitophagy receptor Nix and the autophagosome related protein LC3, as shown by immunoprecipitation and Blue Native-PAGE. Rheb likely promotes interaction between Nix and LC3 and therefore Nix silencing inhibits Rheb-induced mitophagy.

In summary, our findings indicate that mitochondrial degradation through mitophagy mediated by Rheb participates in the regulation of cellular energy homeostasis.

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8P4

Disrupted mitochondrial fusion affects oxidative phosphorylation by mtDNA depletion

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The mitofusin 2 gene (*MFN2*) encodes a mitochondrial outer membrane protein crucial for mitochondrial fusion machinery [1]. Mutations in *MFN2* are associated with Charcot–Marie–Tooth disease type 2A (CMT2A), which is an autosomal dominant axonal neuropathy [2].

It is still unclear how the mitochondrial fusion dynamics alter the mitochondrial function. Here, we investigated the mitochondrial function in skeletal muscle and cultured fibroblasts from four CMT2A patients, all carrying different *MFN2* mutations (p.Met376Val, p.Arg707Pro, p.Val226_Ser229del, and p.Gln74Arg).

Histology of the muscle biopsies showed altered mitochondrial distribution in type 2A skeletal muscle fibers, gathering of

subsarcolemmal mitochondria and rarefaction of intermyofibrillar mitochondria. Although the maximal activities of respiration of saponin-permeabilized muscle fibers and digitonin-permeabilized fibroblasts were only slightly affected by the *MFN2* mutations, their sensitivity to the cytochrome *c* oxidase (COX) inhibitor azide was increased, which indicates a decrease of *in vivo* activity of COX.

In comparison to controls, the *MFN2* fibroblast samples showed a decrease in the mitochondrial DNA copy number, which explains the observed mitochondrial respiratory chain dysfunction. Additionally, an increased amount of deletions was observed. However, the deletions are unlikely to contribute significantly to the detected respiratory impairment, because of their minor overall amounts in these patients.

Our findings support the viewpoint that impairment of mitochondrial fusion causes mild respiratory chain dysfunction through defective mitochondrial DNA replication.

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8P5

A lung cancer model linking apoptotic resistance and metastatic potential via defects in mitochondrial fission protein Dynamin-related protein 1

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Resistance to apoptosis is a hallmark of cancer. Evasion of apoptosis is implicated in almost all aspects of cancer progression, as well as treatment resistance. Apoptosis is regulated in part by mitochondria, which control tissue homeostasis by eliminating damaged cells. In this study, resistance to apoptosis was identified in lung epithelial (A549) cells as a consequence of defects in mitochondrial and autophagic function.

Mitochondrial function is determined in part by mitochondrial morphology, a process regulated by mitochondrial dynamics whereby the joining of two mitochondria, fusion, inhibits apoptosis while fission, the division of a mitochondrion, initiates apoptosis. Mitochondrial length correlated with metastatic potential; lung epithelial cells with increased metastatic potential had mitochondria with an elongated phenotype—mimicking cells deficient in mitochondrial fission protein, Dynamin-related protein 1 (Drp1). A549 cells had impaired Drp1 mitochondrial recruitment and decreased Drp1-dependent fission. Cytochrome *c* release, caspase-3 and PARP cleavage were impaired both basally and with apoptotic stimuli in A549 cells.

Metastatic potential positively correlated with mitochondrial mass, suggesting defects in mitophagy (mitochondrial selective autophagy). A549 cells had decreased LC3-II lipidation and lysosomal inhibition suggesting that defects in autophagy occur upstream of lysosomal degradation. Immunostaining also indicated that mitochondrial localized LC3 punctae in A549 cells increased after mitochondrial uncoupling or with a combination of mitochondrial depolarization and ectopic Drp1 expression. Increased inhibition of apoptosis in A549 cells is correlated with impeded mitochondrial fission and mitophagy. We suggest that mitochondrial fission defects contribute to apoptotic resistance in lung cancer cells with a high propensity for metastasis.

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8P6

Mitochondrial fusion/fission proteins in NARP and Rho0 human osteosarcoma cells

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Dysfunctions of mitochondria are usually associated with numerous diseases like metabolic disorders, cancer and neurodegenerative diseases.

Changes caused by the chronic mitochondrial stress include defects in respiratory chain complexes, morphology and organization of the mitochondria, mitochondrial membrane potential ($\Delta\psi$), cytosolic Ca^{2+} concentration, ATP and ROS levels. These parameters are involved in the retrograde signaling from mitochondria to nucleus that triggers mitochondrial stress response (MSR) of the cell and its subsequent adaptation to altered mitochondrial functions [1,2].

Although knowledge about components involved in the mitochondrial retrograde signal transduction is still incomplete, it is likely that mitochondrial morphology and positioning within the cell can play an important role in mitochondrial–nuclear communication. Proteins implicated in dynamics of mitochondria were investigated in cells with chronic mitochondrial stress:

1) Rho0 human osteosarcoma cells, lacking mitochondrial DNA,
2) Cybrid NARP human osteosarcoma cells with point mutation T8993G in subunit 6 of ATP synthase (98% of heteroplasmy).

We have previously shown that many aspects of physiology (calcium homeostasis, ROS metabolism) as well as mitochondrial network and cytoskeleton organization in cells with chronic mitochondrial stress (NARP and Rho0) differs from that in WT cells [3]. Our new results indicate that the profile of proteins responsible for the dynamics of mitochondria (Drp1, Opa1, Mfn1 and Fis1) is different in investigated cell lines. The observations carried out in the confocal microscope show changes in the organization of mitochondria within these cells.

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8P7

The MINOS complex: Keeper of mitochondrial membrane architecture

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