human breast tumor MCF-7 multi-cellular spheroids maintained similar glycolytic rates (3-5 nmol/min/10^6 cells), which correlated with similar protein contents (GLUT1, GLUT3, HKII, and LDH-A) and enzyme activities (HK and LDH). Enhanced glycolytic fluxes in both cell layer fractions also correlated with higher expression of the transcriptional factors HIF-1a and TIGAR compared to MCF-7 monolayer cultures. On the contrary, the contents of the mitochondrial proteins ND1, SDH, COXIV, PDH, 2-OGDH, glutaminase, and ATP synthase (3-20 times) as well as the respiratory chain enzyme activities (COX, SDH) and the oxidative phosphorylation (OxPhos) flux (2-times) were higher in PRL vs. QS. Enhanced mitochondrial metabolism in the PRL layers correlated with an increase in the oncogenes h-Ras and c-Myc, and transcription factors p32 and PGC-1α involved in the OxPhos activation. On the other hand, the lower mitochondrial function in the QS layers was associated with an increase in Atg7, Beclin, LC3B, Bnip3 and LAMP protein levels indicating active mitophagy and lysosome biosynthesis. Although a substantial increase in glycolysis was developed, OxPhos was the main ATP supplier in both QS and PRL cell layers. Therefore, anti-mitochondrial therapy by using oligomycin or Casiopeina II-gly was effective to arrest MCF-7 spheroid growth (IC50 20 nM) without apparent effect on normal epithelial breast tissue at similar doses; canonical anti-neoplastic drugs such as cisplatin and tamoxifen were significantly less potent.

doi:10.1016/j.bbabio.2012.06.329

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Simultaneous measurements of the TCA cycle and respiration in isolated mitochondria and intact cells with the XF24-3 Analyzer

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Measuring mitochondrial dysfunction is increasingly important in the study of neurodegenerative and cardiovascular diseases, metabolic syndrome, diabetes, cancer, and aging. Mitochondrial function is usually measured by oxygen consumption rates using respirometry methods. However, a critical aspect of mitochondrial function may be overlooked when only O2 consumption is employed: the tricarboxylic acid (TCA) or Krebs cycle, a central metabolic pathway. Measuring the TCA cycle function requires monitoring the flux of an additional analyte, such as carbon dioxide (CO2). The reported method describes simultaneous monitoring of carbon dioxide evolution rates (CDER) and oxygen consumption rates (OCR) using isolated mitochondria and intact cells, in real-time, using the XF24-3 Analyzer. This technology employs fluorescent sensors specific for CO2 and O2, which operate reversibly, and reveal details of mitochondrial respiration and the TCA cycle. Results indicate that O2 consumption and CO2 evolution may be monitored simultaneously, and that data agrees with attributes of TCA cycle and mitochondrial function obtained by other methods. Differential rates of O2 and CO2 flux can be identified, relative to substrate utilization and interdependency among the TCA cycle, electron transport, and oxidative phosphorylation systems.

doi:10.1016/j.bbabio.2012.06.330

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Bioenergetic profiling of isolated white adipocyte mitochondria

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The still rising prevalence of obesity stimulates intensive research on white adipose tissue biology in diet-induced or genetically obese mice as model organisms. Studies on obesity and its common co-morbidities indicate a far underestimated relevance of mitochondria for both white adipocyte function as well as whole body energy balance regulation. Protocols for the bioenergetic analysis of mitochondria isolated from white adipocytes using state of the art respirometry technology are lacking. White adipose tissue is only equipped with a low respiratory capacity and it is a complex and dynamic tissue consisting of multiple cell types such as endothelial cells, pericytes, fibroblasts, preadipocytes, mature adipocytes and macrophages. The main challenge is to isolate intact mitochondria from mature adipocytes in quantities sufficient for comprehensive bioenergetic analyses.

We established a new protocol for the isolation of intact mitochondria from murine white adipocytes suitable for high throughput 96-well microplate respirometry. Therewith substrate specific bioenergetic profiles including basal respiration, ATP turnover, proton leak and maximal respiration rates as the key parameters of mitochondrial function can be measured. This new technology will be applicable to A) elucidate regional differences in mitochondrial function in adipocytes isolated from different adipose tissue depots, B) to study the potential influence of genetic and dietary manipulations of body adiposity on mitochondrial function in adipocytes and C) to identify adipocyte specific mitochondrial proteins.

doi:10.1016/j.bbabio.2012.06.331

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Mitochondrial behavior on cancer stem cells and differentiated cancer cells: A key element for metabolic remodeling and regulation of chemoresistance

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Recent data supports the cancer stem cell (CSC) theory accounting for their ability to evade chemotherapy resulting in tumor regrowth [1]. Our objective is to find mitochondrial and overall metabolic differences which can explain selective CSC survival. P19 embryonal CSC and retinoic acid-differentiated cells (dCC) were used. Metabolic profiles were evaluated by 13C isotopomer analysis using 1H nuclear magnetic resonance showing increased lactate production on CSC. dCC NMR spectra show a more complex metabolic profile. Mitochondrial