The beneficial effects of medium-chain fatty acids on metabolism and oxidative stress

M.K. Montgomery, G.J. Maghzal, R. Stocker, G.J. Cooney, N. Turner
School of Medical Science, University of Sydney, NSW 2006, Australia
E-mail: m.montgomery@garvan.org.au

Dietary intake of long-chain fatty acids (LCFA, C>16) plays a causative role in insulin resistance and risk of diabetes. Whereas LCFA promote lipid accumulation and have detrimental effects on metabolic health, diets rich in medium-chain fatty acids (MCFA; C8–C14) have been associated with increased oxidative metabolism and reduced adiposity, with little effect on insulin action in vivo. The molecular mechanisms underlying these differences are poorly understood. To shed light into this question, we have treated L6 myotubes with specific MCFA (capric acid, 10:0 and lauric acid, 12:0) and LCFA (palmitic acid, 16:0 and oleic acid, 18:1) and determined the effect of fatty acid treatment on metabolic parameters including triglyceride accumulation and insulin-stimulated glycogen synthesis as a measure of insulin sensitivity. A potential mechanism linking lipid accumulation and insulin resistance is increased oxidative stress within the insulin-sensitive tissue. Therefore, these studies were extended by measuring reactive oxygen species (ROS) production and oxidative damage in muscle cells treated with different fatty acids.

Incubation of myotubes with LCFA led to lipid accumulation (+90% with palmitic acid vs. control BSA-treated cells, p<0.01), impaired glycogen synthesis (−40%, p<0.05) and decreased succinate dehydrogenase activity (−40–55% vs. control, p<0.05), however these deleterious effects were not observed in the MCFA-treated myotubes. Furthermore, ROS generation (both superoxide and hydrogen peroxide), measured using HPLC and spectrophotometry, was not significantly altered with MCFA, but was significantly greater in the LCFA-treated cells (+500% vs. control, p<0.001), suggesting that mitochondrial oxidative stress with LCFA, but not MCFA, was confirmed by measuring a marker of lipidoxidative damage, lipid hydroperoxides, which were increased by LCFA (+40% vs. control, p<0.01), but remained unchanged in the MCFA-treated cells.

These results show that treatment of muscle cells with MCFA does not produce the detrimental metabolic effects observed when cells are exposed to LCFA. A potential mechanism is the differential effects observed in ROS production and oxidative damage in the MCFA- and LCFA-treated L6 myotubes.

References

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Obesity generates metabolic alterations in the myocardium leading to changes in mitochondrial membrane permeability

Adriana Riojas, Flor E. Morales-Marroquin, Gerardo García-Rivas,
Noemí García
Cátedra de Cardiología y Medicina Vascular, Escuela de Medicina y Ciencias de la Salud, Instituto Tecnológico de Monterrey, Monterrey Nuevo León, Mexico
E-mail: garciain@itesm.mx

Obesity is a chronic disease of multifactorial origin, characterized by an excessive accumulation of fat that generates multiple metabolic disorders leading to cardiovascular diseases (concentric cardiac hypertrophy, hypertension, atherosclerosis and heat failure), diabetes mellitus type 2, among others. High lipid accumulation has been found in the heart of obese rats and humans, and in addition it has been described that the apoptotic signaling pathway is active in hearts from obese patients. Apoptotic cell death involves several mechanisms; one of them is the mitochondrial permeability transition (MPT), phenomenon that is activated by ionic deregulation and high production of reactive oxygen species (ROS). In this work we have evaluated the mechanism by which the MPT apoptotic pathway is activated in hearts from obese rats (mOb).

The results show that mitochondria from mOb were 2.5 and 3 times more susceptible to MPT than mitochondria from Lean rats (mLean) when the respiratory chain was supplemented with Glutamate-Malate (GM) or Glutamate-Succinate (GS). However, this effect was not observed in the presence of Succinate-rotenone, as substrate. In all experimental conditions, cyclosporine A (CSA) protected the mitochondria, therefore, suggesting that the main mechanism involved in the MPT is the high ROS production. Since the NADH dehydrogenase is one of the enzymes that generate ROS, and, under conditions of GS, the succinate dehydrogenase generates even more ROS, we analyzed the oxidative stress, finding higher production of H2O2 in mOb than in mLean, mainly when the substrate was GS. On the other hand, when the MPT was induced with carbocationictractyloside (CAT), an inhibitor of the adenine nucleotide translocase (ANT), the susceptibility to MPT was higher in mOb than mLean, using GM as substrate and in the presence of CAT 1.5 μM, and even more susceptible in the presence of GS and CAT 0.4 μM. Probably because ROS is higher in mOb, and they may induce conformational changes in mitochondrial proteins, increasing the binding of CAT and accelerating the MPT. Finally, mitochondrial integrity was evaluated by measuring respiration, and the results show that the respiratory control and oxygen consumption in state 3 was lower in mOb; the effect was observed only in the presence of GM. These results suggest that ATP synthesis might be impaired due to decreased membrane potential in mOb and contributes to MPT sensitivity. Conclusion: The mOb has a higher sensibility to MPT than mLean and the principal mechanism involved is the ROS production.

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Anti-mitochondrial therapy in human breast multi-cellular spheroids

S. Rodríguez-Enríquez, J.C. Gallardo-Perez, E.A. Mandujano-Tinoco, R. Moreno-Sánchez
Instituto Nacional de Cardiología, Departamento de Bioquímica, México, Mexico
Instituto Nacional de Cancerología, Laboratorio de Medicina Translacional, México, Mexico
E-mail: saren960104@hotmail.com

During growth of solid tumors, gradients of oxygen and nutrients develop inducing specific metabolic changes in the proliferative and quiescent cellular layers. An integral analysis of proteomics, metabolomics, kinetomics and fluxomics revealed that both enrich-proliferative (PRL) and –quiescent (QS) cellular layers of mature...
human breast tumor MCF-7 multi-cellular spheroids maintained similar glycolytic rates (3-5 nmol/min/10⁶ 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-1α 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 ~25 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.

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

George W. Rogers¹, Smitha P. Jogunoori¹, Christiane Munkholm¹, Anne N. Murphy², Susanna Petrosyan², Andy Neilson¹, David A. Ferrick¹
¹Seahorse Bioscience, North Billerica, MA 01862, USA
²Dept. of Pharmacology, University of California, San Diego, USA
E-mail: grogers@seahorsebio.com

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) 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 agree 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.

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Bioenergetic profiling of isolated white adipocyte mitochondria

Th. Schöttl, L. Kappler, V. Hirschberg, T. Fromme, M. Klingenspor
Molecular Nutritional Medicine, Technische Universität München, Else Kröner-Fresenius Center, Gregor-Mendel-Str. 2, 85350 Freising — Weihenstephan, Germany
E-mail: theresa.schoettl@mytum.de

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 so 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.

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

Ignacio Vega-Naredo¹, Rute Loureiro¹, Ludgero C. Tavares¹,², Ana F. Branco¹,², Ana Burgeiro³, Jenna R. Erickson⁴, Jon Holy⁵, Edward L. Perkins⁶, Rui A. Carvalho¹,², Paulo J. Oliveira¹
¹CNC — Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
²IMAR—Institute of Marine Research, University of Coimbra, Coimbra, Portugal
³Department of Life Sciences, University of Coimbra, Coimbra, Portugal
⁴Chicago Medical School, Rosalind Franklin University of Medicine and Science, North Chicago, IL, USA
⁵Department of Biomedical Sciences, University of Minnesota-Duluth, MN, USA
⁶School of Medicine, Mercer University, Savannah, GA, USA
E-mail: ruteloureiro817@gmail.com

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