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Metabolic studies in human skeletal muscle cells

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Outline

Cell model	Fuel handling methods	SPA	CO_2 trapping
Human myotubes	Radiolabeled substrates	Uptake and lipolysis of oleic acid	Dose-response etomoxir
Mouse skeletal muscle cell models	Cellular energy metabolism		PPARδ agonist
			Galactose (remodeling)
			Hyperglycemia and metabolic modulators
			Cellular metabolic flexibility

Myotubes and energy metabolism

"Exercise" (EPS)
Hyperglycemia
Lactate, galactose
Mild hypothermia

Fatty acids: PA, OA, LA, EPA, synthetic FAs

Metabolic nuclear receptors
LXR modulators
PPAR modulators

Human Myotubes
Lean, Obese, T2D, Athlete

Lipid accumulation
Lipid distribution

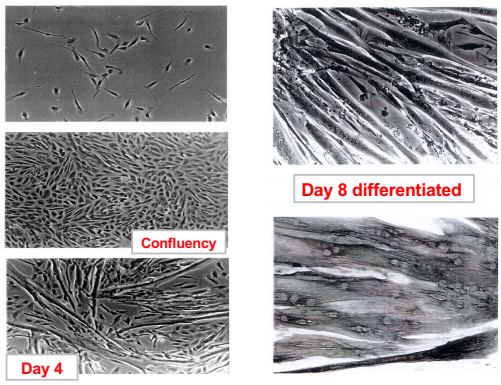
Lipid oxidation

Glucose uptake
Glucose oxidation

Metabolic "flexibility"

Human satellite cell culture

morphological appearance (phase contrast microscopy)



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Mouse skeletal muscle cells available

- AMPKα2 knockout (KO) (A/J and B6/J mice)
- Plin2 KO
- LXRα, LXRβ KO
- Plin2,3 doKO

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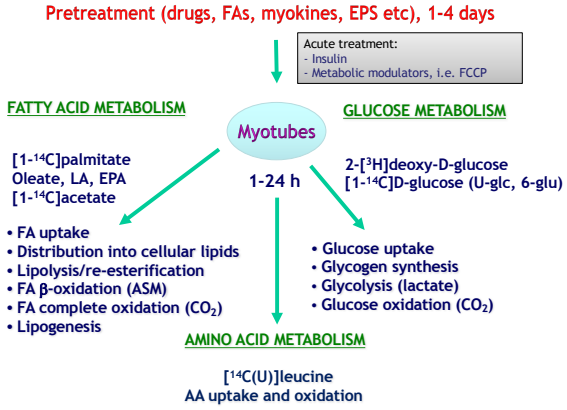
"Fuel handling" methods

- Method 1. Scintillation proximity assay (SPA) for real-time measurement of influx or efflux of β-emitting energy substrates non-invasively in 96-well plates
- Method 2. 96-well "plate press" method for capture and quantification of $^{14}CO_2$ released from cultured cells
- Method 1 and 2 combined

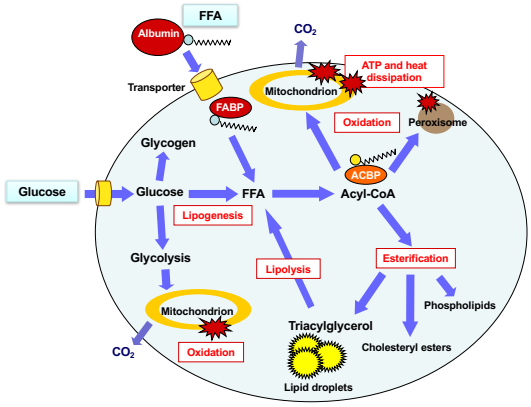
Additional measurements

- Cell-associated radioactivity (substrate accumulation)
- Total cellular lipids (by filtration) + lipid distribution (by TLC)
- Lipolysis and re-esterification (SPA)
- Fatty acid β-oxidation (acid soluble metabolites)
- Glycogen synthesis ± insulin
- Lactate (glycolysis)

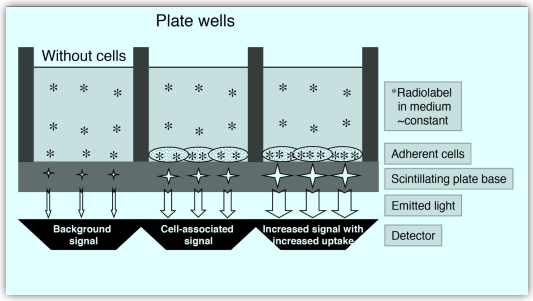
Strategy example human myotubes



Cellular energy metabolism



Method 1: SPA assay (96 w SPA plate)



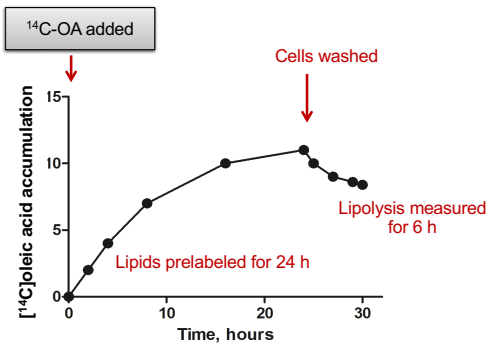
Requires the use of β-emitting isotopes, such as ^{14}C, ^{32}P, ^{35}S, ^{125}I or ^{3}H

MicroBeta TriLux (PerkinElmer)

MicroBeta^2 counter

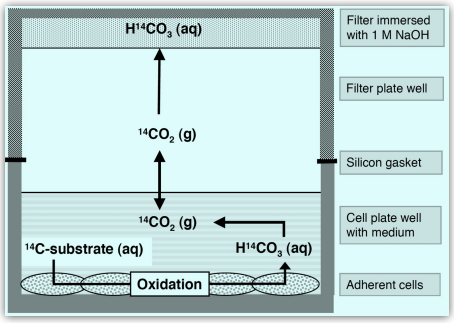
- MicroBeta TriLux 2450 is a multi-detector instrument designed for liquid scintillation or luminescence detection of samples in microtitration plates, tubes or on filters
- We have used a 6 detector instrument for the SPA measurements

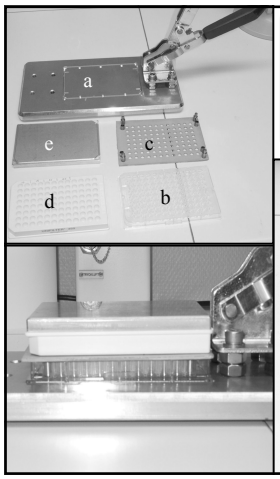
Measurement of uptake and lipolysis in myotubes by SPA



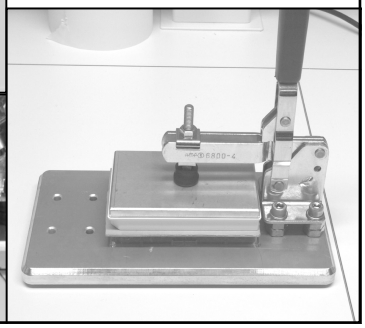
- Wensaas AJ et al. Lipid Res. 2007; 48:961-7.
- Kase ET et al. Biochim Biophys Acta. 2015;1851: 1194-201.

Method 2: Trapping and quantification of cell-derived $^{14}CO_2$



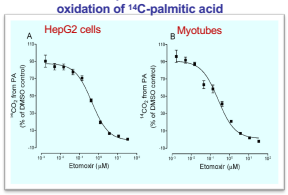


a) base device with the parts to be assembled; b) 96-wells tissue culture plate; c) silicone gasket with stabilizing knobs for each corner well; d) 96- wells glass fibre filter plate; e) metal plate for applying even pressure to the "sandwich"

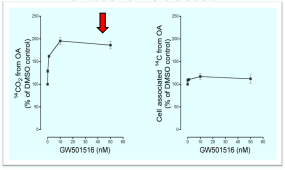


Examples from CO_2 trapping

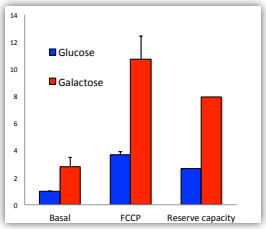
Dose-response etomoxir – oxidation of ^{14}C-palmitic acid



PPARδ activation human myotubes – oxidation of ^{14}C-oleic acid



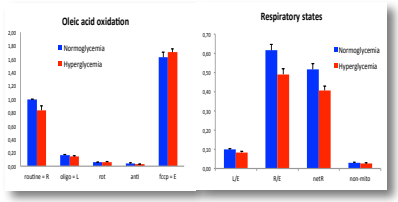
Energy remodeling of human myotubes – oxidation of ^{14}C-U-glucose

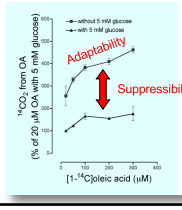


Mitochondrial uncoupling

Hyperglycemia and metabolic modulators

(20 mM glucose, 4 days during differentiation)





Oxidation of ^{14}C-oleate by human myotubes with increasing concentrations of OA and with or without 5 mM glucose acutely

Cellular "metabolic flexibility"

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Outcomes

- The scintillation proximity assay (SPA) and trapping of $^{14}CO_2$ in 96-well sealed microfilter plates can be successfully applied on different cell cultures as demonstrated in human myotubes, adipocytes and hepatocytes (HepG2 cells)
- Both methods are adaptable for medium to high capacity screening of compound collections, and at the same time provide easy-to-use and time saving research tools for *in vitro* studies of cellular nutrient handling
- The fuel handling technology extends transcriptome, genome or proteome analysis as it provides a functional, and therefore crucial, readout for changes that might have been revealed by 'omics' analysis but whose relevance for human physiology is uncertain