β-Adrenergic and cAMP-Stimulated Mitochondrial Uncoupling in Human Adipocytes

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Abstract

Metabolic syndrome is a major public health problem; however, options are limited in treating obesity and dyslipidemia. Coaxing white adipose tissue (WAT) to oxidize its fat stores during lipolysis would decrease fat mass without raising circulating fatty acid (FA). In brown adipocytes, FAs generated during lipolysis are consumed by uncoupled mitochondrial FA oxidation via uncoupling protein UCP1, however it is unclear to what extent liberated FA are oxidized in WAT. Catecholamines are the major stimulators of lipolysis by β-adrenergic receptors (βAR) and elevated cAMP. We examined the effect of βAR-agonist isoproterenol (ISO) and cAMP elevating agent forskolin (FSK) on mitochondrial respiration and uncoupling in human subcutaneous adipocytes. ISO or FSK led to a rapid oxidative and glycolytic respiration increase. The increase in oxygen consumption rate (OCR; XF24 Analyzer) was oligomycin-insensitive and was accompanied by mitochondrial depolarization (TMRM staining, confocal microscopy), implying mitochondrial uncoupling. OCR-increase was blocked by H89 (PKA inhibitor) or FA-free BSA, suggesting uncoupling role for FA released by PKA-induced lipolysis. Evidence exists in other tissues that FA induces mitochondrial uncoupling independent of an uncoupling protein *per se*. Our results suggest an ability of white adipocytes to acutely increase mitochondrial uncoupling via PKA-dependent lipolysis. Moreover, preliminary results show a decreased response to ISO or FSK-induced OCR with increasing body mass index (BMI); although ISO or FSK-induced glycerol release was actually increased with BMI and PKA activation was unchanged. These data suggest an impaired ability of WAT to oxidize FA and/or uncouple respiration in response to βAR stimulation with obesity. Defining the signals (e.g. calcium) and/or substrates (e.g. ANT, Bcl2, UCPs) mediating βAR-induced mitochondrial uncoupling/FA-oxidation in WAT may provide new routes to combat metabolic syndrome.

Introduction

Adipose tissue is a key player in whole-body energy regulation. It is characterized as white adipose tissue (WAT) and brown adipose tissue (BAT); both are able to store energy as triglyceride and as a result of catecholamines stimulation can break them down into fatty acids (FAs) and glycerol via lipolysis. BAT is rich in mitochondria, high amounts of uncoupling protein 1 (UCP1), and able to oxidize FA and dissipate the resultant energy as heat. In contrast, one of the primary functions of WAT is to store excess energy as triglyceride and provide FAs as a fuel for other tissues in times of need. Since WAT has lesser amounts of mitochondria than BAT and only a negligible amount of UCP1, the possibility that WAT itself can control FA release via lipolysis by increasing mitochondrial uncoupling and FA oxidation is less understood.

Mechanisms that stimulate energy dissipation within the white adipocytes will reduce adipocyte size without releasing FAs into the circulation and therefore can be beneficial in preventing/limiting obesity and the related complications.

OCR/PPR measurements

The Seahorse XF24 analyzer is a relatively new instrument for measuring oxygen consumption rate (OCR) and proton production rate (PPR) in very small amounts of cells/tissues, detecting shifts between aerobic respiration (OCR) and anaerobic respiration (PPR, glycolysis and its resultant lactic acid).

Cells are seeded in a 24-plate and inserted into the XF24 analyzer. A matching cartridge with inert optical biosensors for oxygen and pH is lowered into the wells, creating 7μl volume of media above the cells, which enables sensitive and fast (2-5 min) measurements. After each measurement, the small volume of media is re-equilibrated with the larger volume of media present in the well and another 2-5 min measurement is performed. This allows multiple samples to be measured at once, each has a repeated OCR/PPR measurements over time. Drugs can be injected to each well at user defined intervals via 4 ports located on the cartridge. (http://www.seahorsebio.com)

Major Results

FIGURE 1: Isoproterenol or Forskolin acutely increase oxygen consumption and proton production rates in human subcutaneous adipocytes.

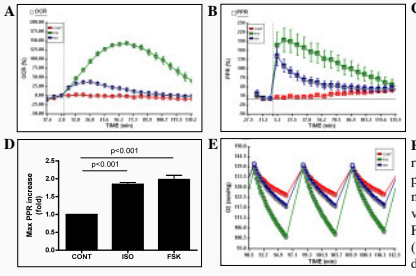


Fig. 1. A, Oxygen consumption rate (OCR) and **B**, proton production rate (PPR) were measured. At 0.0 min (dotted vertical line), ISO (1μM, blue), FSK (10μM, green), or media (CONT, red) were injected. Each data point is the mean OCR/PPR of 6-7 different wells. Each well contains adipocytes pooled from 5 human subjects. **C**, **D**, Maximum levels of OCR (**C**) or PPR (**D**) in response to ISO or FSK. Data is a mean of 5 experiments from 15 subjects (BMI range of 21.7-35.5). Respective OCR or PPR levels in the control cells were set as 1.0. **E**, Representative raw data of repeated oxygen consumption measurements from which OCR is calculated.

FIGURE 2: Isoproterenol or Forskolin induce mitochondrial uncoupling in human subcutaneous adipocytes.

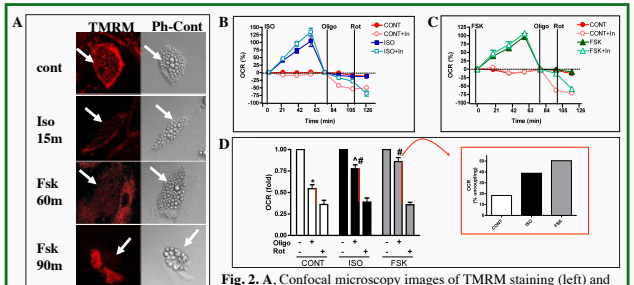


Fig. 2. A, Confocal microscopy images of TMRM staining (left) and phase-contrast (right) show a decreased-TMRM intensity after ISO or FSK treatment, indicating lower inner-mitochondrial membrane potential. **B**, **C**, Representative OCR measurements. As indicated, at 0 min, ISO (**B**, blue lines), FSK (**C**, green lines) or DMEM (**B**, **C**, red lines) were injected. Thereafter, some wells were injected with DMEM (filled symbols) and some with inhibitors (+In, open symbols): ATP synthase inhibitor oligomycin (Oligo) was injected first and then complex 1 inhibitor, rotenone (Rot). OCR measurements before ISO/FSK and Oligo injections were set as 1. **D**, Average OCR of 5-8 experiments. %OCR difference between Oligo to Rot can be attributed to uncoupling (red-border figure). Mitochondrial depolarization together with oligomycin-insensitive OCR increase, indicate mitochondrial uncoupling in response to increased cAMP.

FIGURE 3: Isoproterenol or Forskolin-induced OCR increase is inhibited by BSA fatty-acids free.

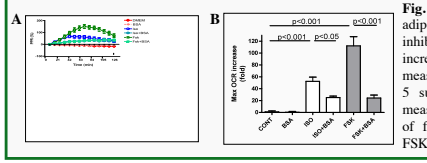


Fig. 3. Pre-treatment of human adipocytes with 5% fatty acid free BSA inhibits the ISO or FSK-induced OCR increase. **A**, representative OCR measurement of adipocytes pooled from 5 subjects. **B**, Average OCR of 10 measurements. This implies the role of fatty acids in mediating ISO and FSK-induced uncoupling.

FIGURE 4: Isoproterenol or Forskolin-induced OCR increase is inhibited by H89.

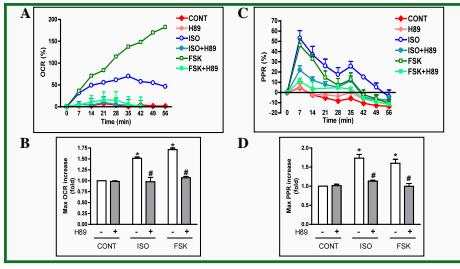


Fig. 4. Pre-treatment of human adipocytes with H89 (1 hour, 10μM) inhibits the increase in ISO or FSK-induced OCR and PPR. **A**, **C**, Representative OCR measurements of adipocytes pooled from 5 subjects. **B**, **D**, Average of 10 OCR/PPR measurements.

This implies the possible role of PKA in mediating ISO and FSK-induced uncoupling.

FIGURE 5: Isoproterenol or Forskolin-induced OCR/PPR increase is lowered by BMI.

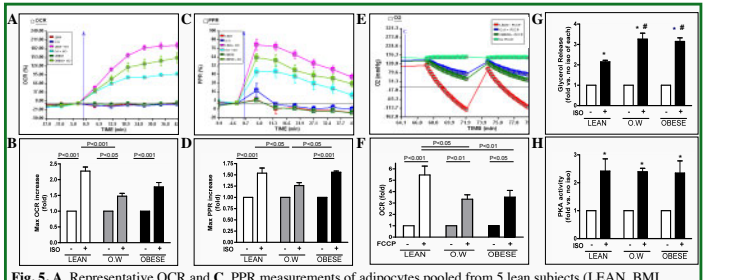
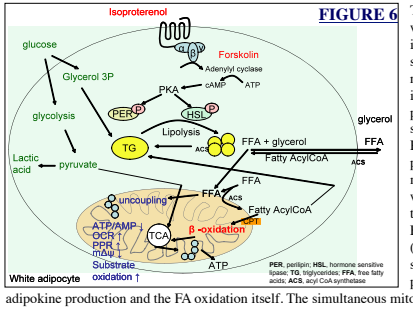


Fig. 5. A, Representative OCR and **C**, PPR measurements of adipocytes pooled from 5 lean subjects (LEAN, BMI 21.7-24.6, red/pink), 5 over-weight (O.W, BMI 25.7-28.9, blue/light-blue) or 5 obese (OBESE, BMI 30-35.5, green/light-green). **B**, **D**, Average OCR/PPR of 4 experiments. The O.W and obese show a relative decrease in ISO or FSK-induced OCR and PPR (**A**-**D**). Maximum uncoupling levels, evoked by FCCP (**E**, **F**) were also reduced in O.W and obese adipocytes vs. lean. However, with increasing BMI there is higher ISO-induced lipolysis (**G**) and no change in ISO-induced PKA activity (**H**). This may imply a reduced ability of white adipocytes to oxidize FA and/or uncouple respiration with obesity.

Conclusions and Significance

FIGURE 6



The results together support a model (Fig. 6) in which βARs and cAMP elevating agents not only increase lipolysis in adipocytes, but simultaneously increase oxidative and glycolytic respiration. A portion of this oxidative respiration is FA-induced mitochondrial uncoupling. The physiological purpose of this uncoupling and the source of substrate oxidized are not clear. However, work in rodent adipocytes has previously observed βAR-dependent mitochondrial depolarization (Davis RJ, 1982), as well as FA oxidation (Cho SY, 2009). We propose that during βARs and cAMP stimulation, some FAs are oxidized to increase cellular ATP synthesis (that is partly lost by mitochondrial uncoupling) in support of subsequent energy-consuming processes as triglyceride synthesis, adipokine production and the FA oxidation itself. The simultaneous mitochondrial membrane depolarization might allow this FA oxidation and ATP production with minimal generation of ROS. Thus the FA liberated from lipolysis may serve as both substrate and uncoupler. In states of increasing BMI a progressive loss in this ability of adipocytes to promote uncoupled mitochondrial respiration and FA oxidation could contribute to increasing adipocyte size, plasma free fatty acid levels and oxidative stress: all of which exist in obesity and its metabolic complications.

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