Quantification of fatty acid uptake in HepG2 cells and adipocytes using the QBT™ Fatty Acid Uptake Assay

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#12

Abstract

Regulators of fatty acid uptake have emerged as important therapeutic targets due to their potential involvement in metabolic diseases such as diabetes and cardiovascular disease. Recently, a long-chain fatty acid (LCFA) uptake assay using a fluorescently labeled fatty acid and a cell impermeable quenching agent was developed in order to facilitate real-time and high-throughput analysis of fatty acid transport. The QBT Fatty Acid Uptake Assay from Molecular Devices has been used successfully to study LCFA uptake in adipocytes. Here we demonstrate that it also well suited to the analysis of LCFA uptake in other mammalian cell types as well. Fatty acid uptake in HepG2 cells is analyzed using the SpectraMax M5 microplate reader and fluorescence imaging. This versatile assay is amenable to both high-throughput and more detailed examination of fatty acid uptake, and its functionality in different mammalian cell types can improve our understanding of fatty acid transport processes in a variety of physiological settings.

Introduction

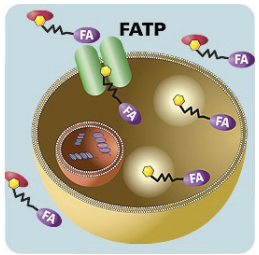
In order to develop fatty acid uptake modifiers as drugs, robust, HTS-compatible assays are essential. Radioactive assays are often time-consuming and demand special waste disposal procedures, while some fluorescence-based assays require FACS and/or wash steps that may damage delicate cell types such as adipocytes. The QBT Fatty Acid Uptake Assay is a homogeneous, HTS-friendly assay that utilizes a BODIPY-dodecanoic acid fluorescent fatty acid analog and proprietary quencher-based technology. Used in conjunction with the SpectraMax M5, a multimode microplate reader with bottom-reading capability, this assay gives rapid results and can be used to obtain kinetic or endpoint data.

Originally developed using 3T3-L1 adipocytes[1], we show that the assay is also useful for examining fatty acid uptake in another mammalian cell type, HepG2 human hepatoma cells. Fatty acid uptake in HepG2 cells has been shown to occur through a novel caveolae-mediated pathway and can be inhibited using the sterol-binding reagent filipin III[2]. We demonstrate that the QBT Fatty Acid Uptake Assay provides a convenient way to study fatty acid uptake in multiple cell types that use distinctly different transport pathways.

Materials and methods

Materials

QBT Fatty Acid Uptake Assay Kit (Molecular Devices Corp., P/N R8132)
HepG2 liver cancer cells (ATCC P/N HB-8065)
3T3-L1 fibroblasts (ATCC P/N CL-173)
Insulin (Sigma P/N I-5500)
BD BioCoat™ Poly-D-Lysine assay plates (BD Biosciences P/N 354640 and 354663)



Methods

I. Cell preparation

A. 3T3-L1 fibroblasts were grown 2 days post-confluence in DMEM/FBS, then for 2 days in DMEM/FBS supplemented with 0.83 µM insulin, 0.25 µM dexamethasone, and 0.25 mM isobutylmethylxanthine. Medium was then changed to DMEM/FBS with 0.83 µM insulin for 2 days, after which the cells were maintained in DMEM/FBS alone for a further 3-5 days prior to assay

B. HepG2 human hepatoma cells were grown in EMEM/FBS and plated at 80,000 cells/well (96-well plate) or 20,000 cells/well (384-well plate). They were incubated in serum-free medium overnight prior to assay. Cells were incubated in 0, 2.5, 5, or 10 ug/mL filipin III for 30 minutes before assay.

C. Adipocytes were plated on the day of the assay at ~80,000 cells/well in 96-well plates. They were first incubated for 4-5 hours in DMEM/FBS, then serum deprived for one hour, followed by ± insulin treatment for 30 minutes.

II. Fatty acid uptake assay

A. For 1X Loading Buffer, 10 mLs of 1X Hank's Balanced Salt Solution (HBSS)/20 mM Hepes/0.2% fatty acid-free BSA was added to Component A of the Fatty Acid Uptake Assay Kit.

B. 100 µL (96-well) or 25 µL (384-well) of Loading Buffer was added to each assay well. Loading Buffer was also added to a set of cell-free wells for background controls.

C. Plates were read in the SpectraMax M5 microplate reader at 485ex/515em with bottom read. Kinetic assays were read every 20-28 seconds for one hour, and endpoint assays were read after a one-hour incubation with Loading Buffer.

Results

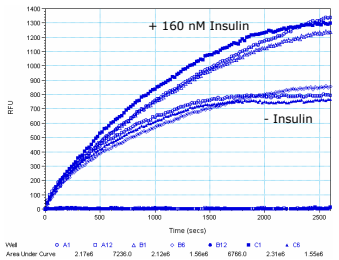


Figure 1. Kinetic FAU assay in adipocytes ±insulin. Background is Loading Buffer without cells.

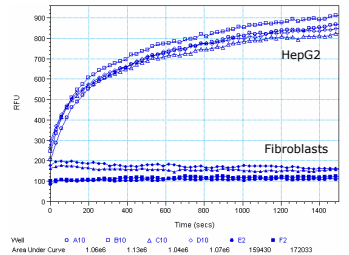


Figure 2. Kinetic FAU assay in HepG2 vs. 3T3-L1 fibroblasts.

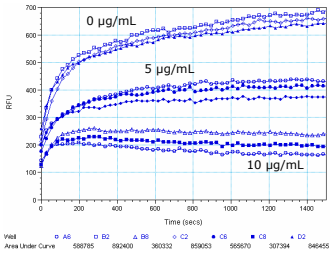


Figure 3. Inhibition of FAU in HepG2 by filipin III.

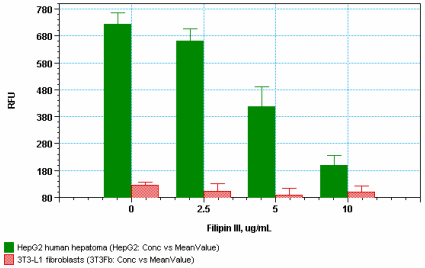
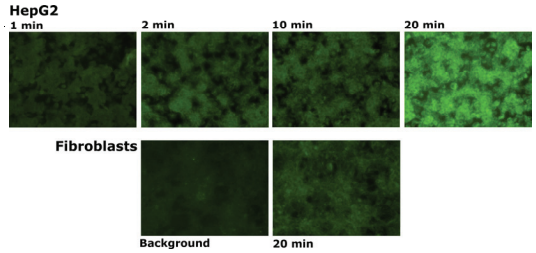


Figure 4. Endpoint bottom read of FAU assay in HepG2 and 3T3-L1 fibroblasts.

Figure 5. Imaging of FAU assay in HepG2 and 3T3-L1 fibroblasts using the Discovery-1 imaging system from Molecular Devices.



Instrument settings

Instrument settings for running the QBT Fatty Acid Uptake assay on SpectraMax M5:

Read type: Fluorescence intensity
Plate must be read from the bottom
Excitation: 485 nm
Emission: 525 nm, with 515 nm cutoff filter
Readings/well: 10
PMT: Medium

Summary

Understanding FAU mechanisms is critical

Need a fast, convenient assay with HTS capability

QBT Fatty Acid Uptake Assay is easily run on microplate readers without pipettors/injectors: SpectraMax M5, kinetic or endpoint

Can be used to study FAU in different cell types

Microplate-based screens can be complimented with imaging: Discovery-1

Molecular Devices