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Models of lipid droplets growth and fission in adipocyte cells

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ABSTRACT

Lipid droplets (LD) are spherical cellular inclusion devoted to lipids storage. It is well known that excessive accumulation of lipids leads to several human worldwide diseases like obesity, type 2 diabetes, hepatic steatosis and atherosclerosis. LDs' size range from fraction to one hundred of micrometers in adipocytes and is related to the lipid content, but their growth is still a puzzling question. It has been suggested that LDs can grow in size due to the fusion process by which a larger LD is obtained by the merging of two smaller LDs, but these events seems to be rare and difficult to be observed. Many other processes are thought to be involved in the number and growth of LDs, like the *de novo* formation and the growth through additional neutral lipid deposition in pre-existing droplets. Moreover the number and size of LDs are influenced by the catabolism and the absorption or interaction with other organelles. The comprehension of these processes could help in the confinement of the pathologies related to lipid accumulation. In this study the LDs' size distribution, number and the total volume of immature ($n=12$), mature ($n=12$, 10-days differentiated) and lipolytic ($n=12$) 3T3-L1 adipocytes were considered. More than 11,000 LDs were measured in the 36 cells after Oil Red O staining. In a previous work Monte Carlo simulations were used to mimic the fusion process alone between LDs. We found that, considering the fusion as the only process acting on the LDs, the size distribution in mature adipocytes can be obtained with numerical simulation starting from the size distribution in immature cells provided a very high rate of fusion events. In this paper Monte Carlo simulations were developed to mimic the interaction between LDs taking into account many other processes in addition to fusion (*de novo* formation and the growth through additional neutral lipid deposition in pre-existing droplets) in order to reproduce the LDs growth and we also simulated the catabolism (fission and the decrease through neutral lipid exit from pre-existing droplets) to reproduce their size reduction observed in lipolytic conditions. The results suggest that each single process, considered alone, can not be considered the only responsible for the size variation observed, but more than one of them, playing together, can quite well reproduce the experimental data.

1. Introduction

Lipid droplets (LDs) are cellular organelles for neutral lipids accumulation. Abnormal lipid content is crucial for metabolic diseases such as obesity, diabetes and atherosclerosis [1]. LDs contain neutral lipids in their core (mainly triacylglycerols and sterol esters) that is surrounded by a monolayer of phospholipids [1–5]. LDs size can vary, depending on the cell type and metabolic conditions, from fraction to 100 μm in white adipocytes [6]. LDs are cellular dynamic structures capable of varying their number and size accordingly to the cellular needs [7–9]. It is believed that they form between two phospholipid leaflets at specific sites in the endoplasmic reticulum (ER) through the accumulation of neutral lipids [10], germinate to generate discrete organelles [11] that increase in size and finally aggregate into large clusters [12]. LDs can also interact with other organelles including ER, endosomes, mitochondria and peroxisomes [13] (heterotypic interaction) or among them (homotypic LD–LD interaction) by a fusion process dependent on microtubules and regulated by SNARE proteins [10,14]. The homotypic fusion is a rare process producing larger LDs and in which the volume of the two initial droplets are retained in the final fused droplet. However, LDs can also grow through the addition of neutral lipids to the pre-existing LDs [11,15,16] or by transfer of neutral lipids from a smaller (donor) to a larger (acceptor) LD, depending on the internal pressure difference and mediated by a LD-associated protein called Fsp27 [17]. In contrast to the processes of LDs growth, the fission process was observed in adipocytes after a strong lipolytic stimulation and is

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Table 1
List of processes involving variation of lipid content in cells. Their influence on number and total volume of the LDs in a cell are shown (arrow up=increase, arrow down=decrease).

Process	Number of LDs	Total volume of LDs
De novo formation	↑	↑
Growth through additional neutral lipid deposition in pre-existing droplets	=	↑
Fusion	↓	=
Fission	↑	=
Decrease through neutral lipid exit from pre-existing droplets	=	↓
Absorption/interaction with other organelles	↓	↓

characterized by fragmentation of LDs in a myriad of small dispersed LDs [18]. The increase of the number, and consequently the surface, of the LDs is necessary to allow access of the lipase to the neutral-lipid cores [1,19]. The lipolytic process also involves both the fission of LDs and the size decrease of the pre-existing LDs due to the exit of neutral lipids. These lipids can be used by the cell to form new LDs.

Summarizing, the LDs can undergo many different processes like growth through additional neutral lipids deposition in existing droplets, *de novo* formation of LDs, fusion, fission, decrease through neutral lipids exit from pre-existing LDs and interaction with other organelles. The processes are organized in Table1 and their effects on the number of LDs and on the total LDs volume are highlighted.

The *de novo formation* increases the total number of LDs and increases the total volume of the LDs. The *growth through additional neutral lipid deposition in pre-existing droplets* maintains the number of LDs, increases the volume of the existing LDs, thus increasing their total volume. The *fusion* reduces the number of LDs and maintains the total volume of the LDs. The *fission* increases the number of LDs maintaining the total volume. The *decrease through neutral lipid exit from pre-existing droplets* maintains the number of LDs, deceases the volume of existing LDs and decreases the total volume of lipids in the LDs. The *absorption* or *interaction with other organelles* reduces the number of LDs, maintains the volume of surviving LDs and reduces their total volume.

In previous works we introduced the analysis of the size distribution of the LDs [20] and the use of the Monte Carlo simulation in order to mimic the fusion process alone between LDs [21]. This preliminary study prompted us to write a more complete algorithm in which many more processes are considered beside fusion, like *de novo* formation of LDs and neutral lipid deposition in pre-existing LDs in order to explain the growth of the LDs and the fission, the exit of neutral lipids from pre-existing LDs and the absorption (or interaction with other organelles) in order to explain the LDs' size decrease. Here we would like to use the power of computational algorithms to get a shine on the entire bulk of processes undergoing the LDs' size regulation. For this purpose not only the size, but also the number and the volume of the LDs in immature, mature and mature in lipolytic condition 3T3-L1 cells were considered. Different scenarios are here simulated in order to clarify the effects on the LDs size of one single process considered alone or the combination of two or more of them acting together.

2. Materials and methods

2.1. Cell culture

The 3T3-L1 cells (ECACC Sigma-Aldrich) were cultured at 37 °C in a 5% CO2 atmosphere in 250 ml polystyrene sterilized flasks with 0.2 μm vented plug seal cap (Becton Dickinson, Franklin Lakes, New Jersey, USA); as a culture medium DMEM/GlutaMAX was used containing 10% of FBS and 1% of Antibiotic Antimycotic Solution. After reaching a concentration of $2.5–3.0 \times 10^6$ cells (confluence 85–90%), 3T3-L1 cells were detached by trypsin-EDTA and seeded in 4-wells Bio Coat™ CultureSlides (Becton Dickinson). Two days after confluence in wells, the cells were washed three times with PBS and induced to differentiate in DMEM/F12 containing 10% FBS, 1% Antibiotic Antimycotic Solution, 0.2 mM IBMX, 10 μM rosiglitazone, 1 μM dexamethasone, 10 μg/ml insulin for three days. After 72 h, the cells were washed three times with PBS, and the medium was replaced with the adipocytes maintaining medium (AMM: DMEM/F12 enriched with 10% FBS, 1% Antibiotic Antimycotic Solution, 10 μg/ml insulin) in which the cells were cultured for 2 days. The cell cultures were washed with 0.1 M PBS pH 7.4 and fixed for 20 min with 4% formalin in 0.05 M PBS; after washing with sterile double distilled water and 60% isopropanol for 2 min, the cells were stained with 0.35% Oil Red O solution in 60% isopropanol for 10 min at room temperature. Then the cells were washed with sterile double distilled water and stained with Mayer's Hematoxylin (Bio-Optica) for 1 min at room temperature, washed with sterile double distilled water, and finally mounted in Dako faramount aqueous mounting medium.

After 10-days differentiation, a large number of 3T3-L1 immature (fibroblastic-like, PID1) and mature (adipocyte-like, PID10) cells coexist in the same culture. The 10-days time point was chosen to better study the effects of the mechanisms regulating the growth of the LDs in the mature adipocytes and the consequences of the lipolytic conditions with respect the 5-days time point selected for our previous study [21]. The lipolytic condition was obtained by treating the 3T3-L1 PID10 cells with 1 μg/ml of