methods

A simple and rapid method to assay triacylglycerol in cells and tissues

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Abstract We have developed a reliable, rapid, and economical assay for the quantification of triacylglycerol (TG) in cells and animal tissues. In a few hours, this assay quantifies microgram amounts of TG from tens or even hundreds of samples. The protocol includes an organic extraction to partition TG away from proteins and other hydrophilic molecules found in cells and tissues that may interfere with the colorimetric enzyme-linked TG detection method. In addition, this assay is economical, as no expensive reagents, supplies, or equipment are needed. Another benefit of this assay is that it does not require environmentally unfriendly halogenated solvents.—Schwartz, D. M., and N. E. Wolins. **A simple and rapid method to assay triacylglycerol in cells and tissues. *J. Lipid Res.* 2007.** 48: **2514–2520.**

Supplementary key words fatty acids • fat • lipid • screen • acyl-glycerol

Excessive intracellular triacylglycerol (TG) storage is the hallmark of obesity. Complications of obesity include type 2 diabetes, vascular disease, nonalcoholic steatohepatitis, and cardiomyopathy (1, 2). These significant health problems have resulted in an increased interest in understanding TG metabolism, including the enzymes that catalyze TG synthesis, the proteins that package TG for storage and secretion, and the regulators of TG levels in cells or tissues. These studies often require TG quantification in cells and in animal tissues. In fact, a high-throughput assay that measures the cellular uptake and oxidation of fatty acids, which are the main components of TG, was developed recently (3).

Several strategies have been used to quantify TG in biological samples over many decades. The most rigorous methods resolve organically extracted lipids and compare the signal from TG in the samples with known amounts of similarly resolved TG (4–6). Each of these measurements requires extraction and resolution of the samples, making these measurements involved and tedious; thus, these methods are economically constraining when large data sets are needed.

More recently, basic researchers have used clinical kits designed to quantify TG emulsified in lipoproteins in the sera of patients. These kits use lipoprotein lipase (LPL) to hydrolyze the fatty acids from TG, releasing glycerol. Then, through a series of reactions, electrons from glycerol reduce a dye, increasing the dye's absorbance. This absorbance change is proportional to the moles of TG in the sample (**Fig. 1**). These kits are simple to use and sensitive. However, LPL's biological role is to target specific nutrients to the appropriate tissue by hydrolyzing a defined set of lipids from lipoproteins. Adapting these kits to measure intracellular TG requires care and thought. Intracellular TG is enclosed in protein-coated droplets that control lipase's access to the TG (4, 7–10). Thus, it is unclear whether the LPL used in these kits can hydrolyze TG in intact intracellular lipid droplets. To further complicate the situation, experimental manipulation can alter lipid droplet coat proteins (11–16), and changing the lipid droplet coat proteins likely changes TG vulnerability to lipases. These lipid droplet coat proteins may block the quantitative measurement of TG, making the results reflective of changes in TG accessibility and not TG quantity. Solvent extraction of TG can eliminate interference by lipid droplet coat proteins and other molecules that are insoluble in organic solvents.

Lipoprotein lipase effectively hydrolyzes the acyl chains from the glycerol backbone of TG packaged in lipoproteins; however, it may not be so effective at hydrolyzing the acyl chains from the glycerol backbone of TG stored within cells. Neither evolutionary nor regulatory pressure ensures that these clinical reagents detect TG with acyl chains that differ from those found in lipoproteins. (Title 21, Federal Code of Regulation 862, Clinical Chemistry and Clinical Toxicology Devices, requires testing of medical diagnostics devices.) Unfortunately, we and likely others have used these kits to quantify TG in cells and animal tissues without rigorous controls. These suboptimal controls and conditions include using a TG standard that differs in both chain length and saturation

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Abbreviations: IHW, isopropanol-hexane-water; LPL, lipoprotein lipase; TG, triacylglycerol; TO, Tet-On.

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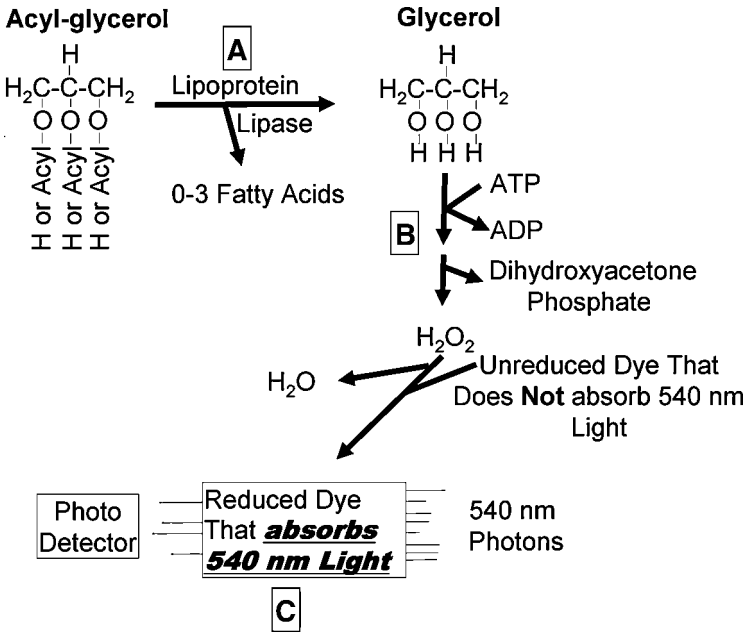

Fig. 1. Detection of acyl-glycerols by an enzyme-linked assay. A: Solvent-extracted acyl-glycerols are hydrolyzed by LPL, freeing glycerol. B: Through a series of reactions, the glycerol is oxidized and the dye is reduced. Reducing the dye increases its 540 nm light absorbance. C: Moles of triacylglycerol (TG) in standards and samples are determined by measuring the light absorbance at 540 nm.

from the TG found in our samples and quantifying TG found in lipid droplets with lipase-resistant coat proteins. The latter is particularly bothersome in our studies, given that we addressed experimentally induced heterogeneities in TG droplet coat proteins. It is likely that our results support our conclusions; however, the measurements were probably not quantitative (12, 17).

The clinical reagents used for the quantification of TG have several limitations. First, these assays do not distinguish between monoacylglycerols, diacylglycerols, and triacylglycerols. In fact, these reagents detect free glycerol, which is a metabolic intermediate and a product of lipolysis. Furthermore, these reagents measure moles of glycerol. The molecular weight of acyl-glycerols varies with length and the number of acyl chains; thus, these kits can only be used to estimate the mass of acyl-glycerol oil if the composition of the acyl-glycerols is known. In addition, LPL must be able to hydrolyze the acyl groups from the glycerol backbone (Fig. 1). These colorimetric methods lack cues seen in chromatographic methods, such as migration rates and peak shapes, that make the authenticity of the TG evident. When using these colorimetric reagents, any substance that absorbs light, reduces the dye, or blocks the reduction of the dye by the acyl-glycerol-driven reaction series (Fig. 1) will result in flawed measurements. Thus, the possibility of being misled is significant and rigorous controls are essential.

Using a rapid, simple, organic extraction and a 96-well format for solvent evaporation and absorbance reading, we have devised a simple and economical TG assay.

EXPERIMENTAL PROCEDURES

Materials

The following phospholipids and acyl-glycerols were purchased from Sigma (St. Louis, MO): phosphatidylcholine (catalog number P3556), triolein (catalog number T7140), diolein, 85% 1,3- and 15% 1,2-isomer (catalog number D8894), 1-monoolein (catalog number M7765), dipalmitin, a 1:1 mixture of 50% 1,3- and 50% 1,2-isomer (catalog number D2636), tripalmitin (catalog number T5888), and 1-monopalmitin (catalog number M1640). Other materials used included a 96-well rack with 1 ml glass vials (catalog number 9600E; Biotechsolutions, Vineland, NJ), a 96-well format manifold (catalog number 4100FB-930VL; Biotechsolutions), Infinity TG reagent (catalog number 2780-400H; Thermo Electron Corp.), a plastic 96-well microplate (catalog number 9017; Corning, Corning, NY), a 1.0 ml pump dispenser (catalog number 3001A; Barnstead International), and a 2.5 ml pump dispenser (catalog number 03-692-177; Fisher Scientific, Inc.).

Biological oils

Diacylglycerol rich oil Enova™ (Archer Daniels Midland Co., Decatur, IL), butter, lard, or coconut oil was dissolved in chloroform at 2 mg/ml. Subcutaneous bovine adipose tissue was rendered, and the fat was dissolved in chloroform at 2 mg/ml.

Methods

Here, we combine the 96-well format, a simple organic extraction, and a clinical colorimetric kit to efficiently, precisely, and accurately measure TG.

TG assay general considerations. The two major strategies we used to streamline this assay are the adaptation of an organic extraction (18), in which the organic phase floats on the aqueous phase without centrifugation, and the 96-sample format. For the organic extraction step, we use racks in an 8 × 12 format to hold the 100 mm × 13 mm disposable extraction tubes. We then transfer the organic phase to a glass shell vial in the corresponding position of a 96-well microplate format vial holder. The organic solvent is evaporated for all 96 samples simultaneously using a 96-pin microplate format gas manifold. After completion of the colorimetric assay, we then read the sample absorbance in a 96-well plate reader.

Simple protocol for TG quantification

1. Add 2 ml of isopropanol-hexane-water (IHW) (80:20:2, v/v/v) to glass tubes (13 mm × 100 mm) in an 8 × 12 format rack using a solvent pump dispenser.

2. Add sample or standard in a total volume of 200 μl of PBS/10 mM EDTA, pH 7.4, with 0.1% green food coloring (GPBS-EDTA) to each 13 mm × 100 mm glass tube. Mix by vortexing. (The organic phase will hold at least 400 μl of water. As the assay is described, 200 μl of water is added with the isopropanol-hexane and the sample is added with 200 μl of aqueous buffer. Omitting water from the isopropanol-hexane allows 400 μl of sample in aqueous buffer to be added to the isopropanol-hexane without phase separation.)
3. Incubate covered with aluminum foil for 30 min at room temperature.
4. Add 500 μl of hexane-diethyl ether (1:1) to each tube using a solvent pump dispenser. Mix by vortexing.
5. Incubate covered with aluminum foil for 10 min at room temperature.
6. Add 1 ml of water to separate phases using a solvent pump dispenser. Mix by vortexing.
7. Incubate covered with aluminum foil until phases separate (~20 min) at room temperature.
8. Pipette 900 μl of the organic phase into the corresponding glass vial of a 96-well plate. Polyethylene pipette tips are sufficiently solvent-resistant for this transfer.
9. Evaporate the organic phase with nitrogen at 100 kPa/14.50 p.s.i. using a 96-well format manifold at <100°C.
10. Add 400 μl of Infinity TG reagent to each vial using a 12-channel pipette. Cover vials with Parafilm.
11. Incubate for 90 min at 37°C with shaking at 360 rpm.
12. Transfer 360 μl of Infinity TG reagent plus sample from each glass vial to a flat-bottom 96-well plastic microplate.
13. Determine absorbance at 540 nm using a microplate reader.
14. Generate a standard line and interpolate to determine the mass of the TG in samples.

Note well. Solvents used in this assay are flammable and volatile at low temperature. Thus, storage in non-explosion-proof refrigerators or freezers may result in explosion and fire. We recommend that when the samples are collected at different times, but within a few days, samples be added to IHW and stored covered (e.g., by a glass plate) in a cooler on dry ice.

Cell culture. HeLa Tet-On (TO) cells from Clontech (catalog number 630901; Mountain View, CA) were grown in Dulbecco's modified Eagle's medium supplemented with 10% bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, 200 μg/ml geneticin, and 100 μg/ml streptomycin.

Protein measurements. Protein concentration was determined using the BCA™ Kit (catalog number 23225; Pierce, Rockford, IL) with serum albumin standards (catalog number 23210; Pierce).

Chloroform-methanol extraction. Chloroform-methanol extraction is based on a previously described protocol (19). Briefly, add sample or standard to 1.5 ml of methanol in a 100 mm × 13 mm glass tube, vortex, add 625 μl of chloroform, vortex, add 1 ml of water, vortex, incubate at room temperature for at least 2 h, remove 1 ml of the organic phase (bottom layer), and process as described for the basic protocol starting at step 9.

Preparation of fatty acid stock. Oleic acid was neutralized with sodium hydroxide and bound to BSA at a molar ratio of 5.5:1 at a final concentration of 18 mM (12).

Rate of color development driven by various acyl-glycerols. We added 20 μl of the indicated acyl-glycerol dissolved in chloroform at 2 μg/μl to the glass drying vials containing 200 μl of hexane-diethyl ether (1:1) and then continued from step 9 of the protocol. At the times indicated in **Fig. 2C**, we removed the Infinity TG reagent from the glass drying vials and read the absorbance at 540 nm.

Detection of biological oils. We added 12.5 μl of the indicate-dacyl-glycerol dissolved in chloroform at 2 μg/μl or 2.6 μl of 1 μg/μl glycerol dissolved in water to the glass drying vials containing 200 μl of IHW and then continued from step 9 of the protocol (Fig. 2D).

Detection of TG in cells and liver. To generate cells and liver with easily detectable amounts of TG, we incubated TO-HeLa cells in 1 mM oleate for 16 h and fasted a 13 week old male C57BL/6 mouse for 18 h. We released TO-HeLa cells from the dishes with GPBS-EDTA. Liver was homogenized in 1 ml of PBS-EDTA with a motor-driven Teflon pestle tissue homogenizer. We added the amounts indicated in **Fig. 3** of TO-HeLa cells or of liver homogenate to either IHW or methanol.

Stability of TG in freezer, solvent, or 4° C. We generated TG-laden TO-HeLa cells by incubating them in 1 mM oleate for 14 h. We released TO-HeLa cells from the dishes with GPBS-EDTA. We then divided the TO-HeLa cell suspension into aliquots containing 20 μg of cellular protein. We stored the aliquots as indicated in **Fig. 4**. At the end of 38 h, we simultaneously processed the samples.

Measuring oleate-driven TG accumulation in TO-HeLa cells. We grew TO-HeLa cells to 80% confluence. Depending on the time incubated in oleate, we plated TO-HeLa cells in the following formats: no time in oleate on 100 mm plates; 2 h on 6-well plates; 8 h on 24-well plates; 24 h on 48-well plates. We released TO-HeLa cells from the dishes with GPBS-EDTA. We pelleted and resuspended the no oleate sample in 250 μl of GPBS-EDTA. All other samples were washed with PBS and released into 400 μl of GPBS-EDTA. We added 200 μl of the TO-HeLa cell suspensions to IHW and stored it on dry ice. We processed the samples simultaneously (**Fig. 5**). ANOVA was performed to determine whether the differences between treatments were significant.

RESULTS

Using an enzyme-linked colorimetric clinical reagent (Fig. 1), we optimized a protocol to measure solvent-extracted TG from cells and tissue. We maximized throughput by evaporating the solvent and reading optical absorbance in a 96-well format.

Acyl-glycerols drive a precise linear increase in 540 nm absorbance

To validate this new method, we determined the 540 nm absorbance increase driven by increasing masses of the indicated acyl-glycerols (Fig. 2A). This assay system detects the increase in absorbance driven by glycerol molecules (Fig. 1). Hence, we expect this assay to detect any molecule that partitions into the organic phase and that LPL can hydrolyze to free glycerol. The molecular weights of acyl-glycerols can vary by severalfold as a result of differences in acyl chain length and number (Fig. 2A–C). As expected, because the assay measures moles of glycerol, the absorbance change of the acyl-glycerols is different

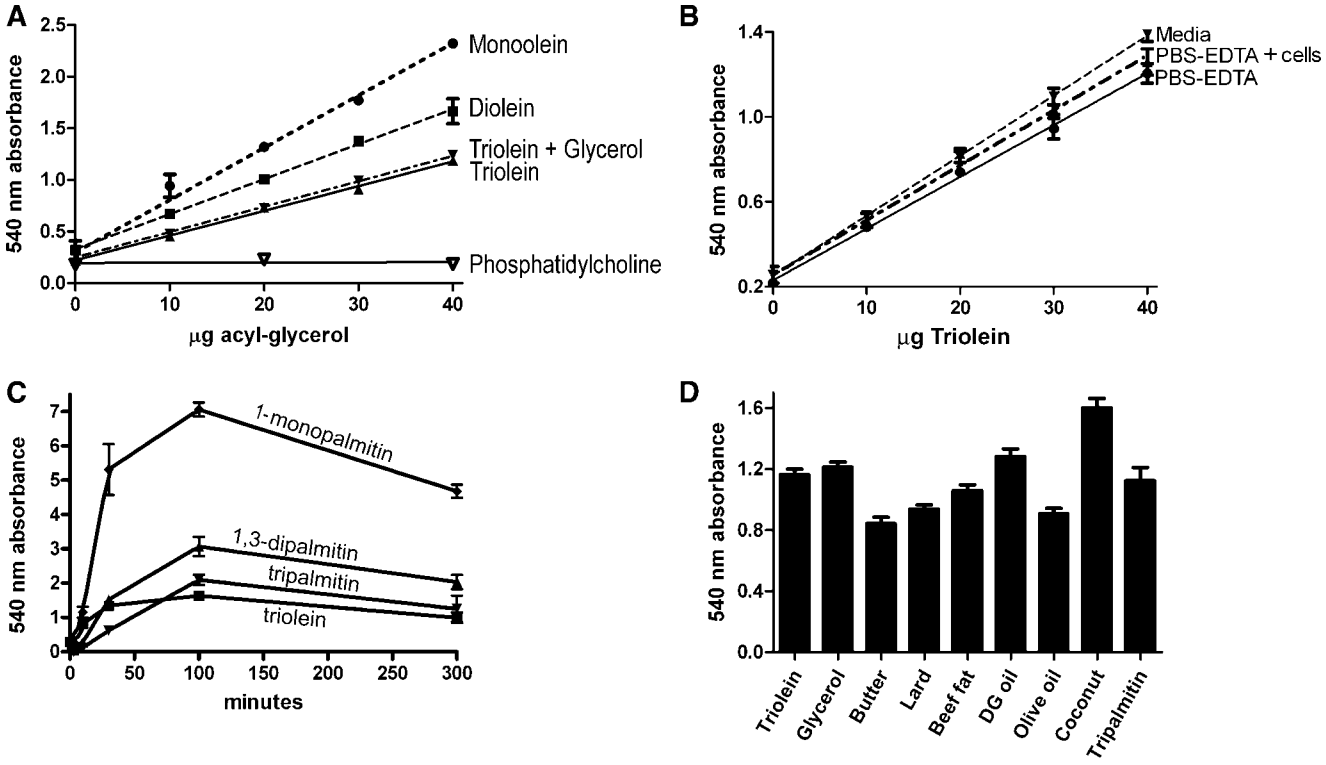

Fig. 2. Detection of various acyl-glycerols. A: We determined the 540 nm absorbance change driven by the indicated masses of monoolein, diolein, triolein, triolein + 20 μg of glycerol, and phosphatidylcholine in this assay system (n = 4). We dissolved free glycerol in water and added it to the isopropanol-hexane-water (IHW). B: As indicated, we used as the aqueous component 200 μl of GPBS-EDTA, tissue culture medium, or a suspension of Tet-On (TO)-HeLa cells at a concentration of 1.4 μg/μl protein in PBS-EDTA. C: We added the indicated acyl-glycerol and measured the 540 nm absorbance at the times indicated. D: We assayed 25 μg of the indicated oils or 2.6 μg of glycerol as described in Experimental Procedures. DG oil indicates the diacylglycerol rich oil Enova™. Error bars show SEM.

when normalized to mass ($P < 0.0001$); however, when normalized to moles, triolein and diolein increase 540 nm absorbance to the same extent, 21.22 and 21.08 absorbance units/μmol, respectively (Fig. 2A). Monoolein increases the 540 nm absorbance by slightly less, 18.03 absorbance units/μmol. This is likely because monoolein is more polar and does not completely partition into the organic phase.

Neither free glycerol nor glycerol backbone phospholipid confounds this assay

Because the colorimetric reagent we use detects glycerol (Fig. 1) and glycerol is a product of lipolysis and a metabolic intermediate, free glycerol may confound these measurements. To determine how much free glycerol partitions into the organic phase, in addition to the indicated amounts of triolein, 20 μg of free glycerol (the

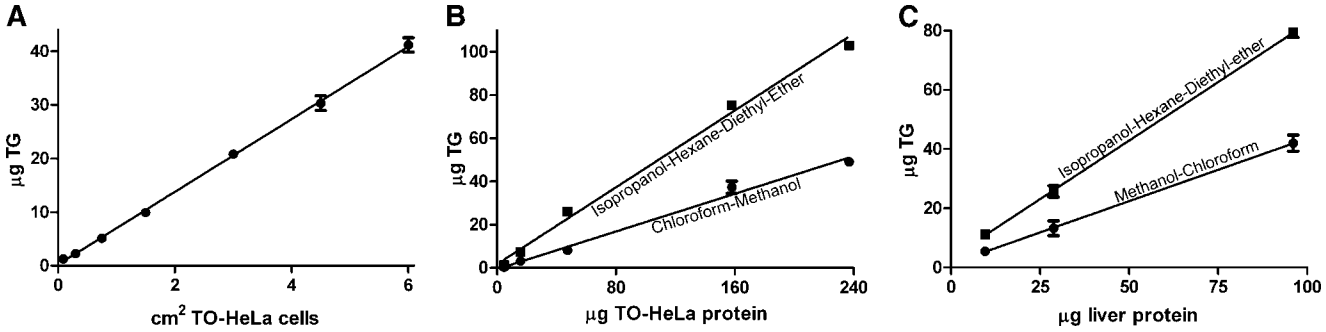

Fig. 3. Detection of TG in cells and tissues. We determined the TG mass in samples by interpolating against known masses of triolein processed in parallel. A: We released TG-laden TO-HeLa cells from a confluent cell monolayer. Then we assayed the amount of cells released from the indicated area of the monolayer. Next, we plotted TG masses versus monolayer area. B: We assayed protein concentration in a TG-laden TO-HeLa cell suspension. Then we extracted the cell suspension using IHW or chloroform-methanol (19). We estimated TG masses in these extracts against known triolein masses similarly extracted and processed in parallel. We then plotted TG mass versus protein mass. C: We compared the TG extraction efficiency of IHW and chloroform-methanol from liver as described for B.

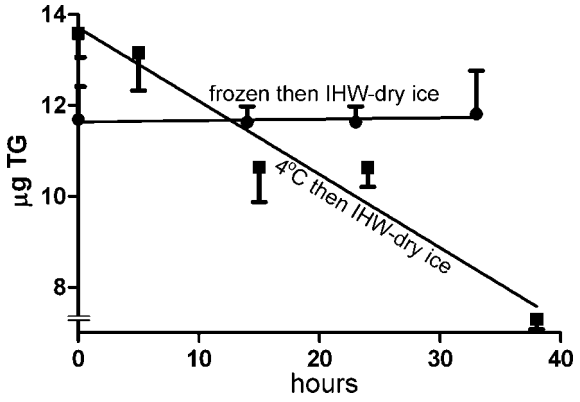

Fig. 4. TG stability in TO-HeLa cells at 4°C, in frozen TO-HeLa cells, or in IHW on dry ice. We released the TG-laden TO-HeLa cells from the tissue culture plate and divided the cell suspension into equal aliquots. The aliquots were either frozen (circles) or stored at 4°C (squares) and then added to IHW at the times indicated (n = 4). The samples in IHW were then stored on dry ice for the duration of the 38 h experiment. All samples were then processed simultaneously. For example, the 4 h (square) point represents the measurements obtained from samples frozen in PBS-EDTA for 4 h and then transferred to IHW for the remaining 34 h of the time course. We fit separate lines to data from the samples that were frozen and then dry ice-stored in IHW (circles) and samples that were kept at 4°C and then dry ice-stored in IHW (squares). Error bars show SEM.

moles of glycerol in 186 µg of triolein) was added in GPBS-EDTA. The glycerol only shifted the line up the *y* axis 0.0298 absorbance units, or the absorbance increase driven by 1.0 µg of triolein.

Glycerol is also found in the glycerol backbones of phospholipids; however, one such phospholipid, phosphatidylcholine, is not detected in this assay (Fig. 2A), nor does it interfere with the detection of triolein (data not shown).

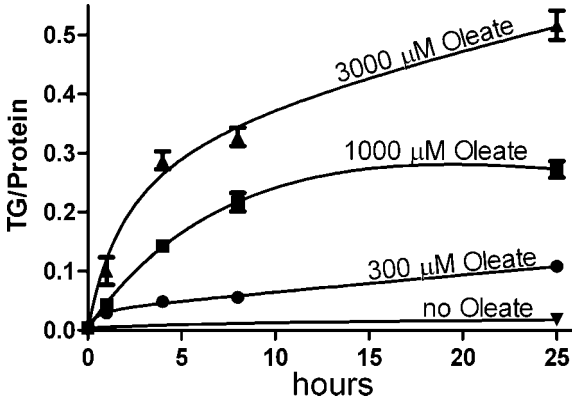

Fig. 5. Oleate-driven intracellular TG accumulation. We incubated TO-HeLa cells for the times and the oleate concentrations indicated (n = 6). We released cells from culture dishes and then assayed protein and TG as described in Experimental Procedures. Then we plotted the TG/protein ratio versus hours. Error bars show SEM.

Large amounts of complex mixtures of biological molecules do not interfere with TG detection

Because samples in which TG levels are of interest are often complex mixtures of biological molecules, we tested whether TG is measurable in two such complex mixtures that contain lipids, proteins, carbohydrates, and nucleic acids. We added large amounts of either tissue culture medium or the HeLa cells to the assay. Both caused a small, but significant, 540 nm absorbance increase, compared with GPBS-EDTA ($P < 0.0001$) (Fig. 2B). Both serum in the medium and TO-HeLa cells contain TG; thus, TG in the samples likely caused the small 540 nm absorbance increase. Neither the medium nor the HeLa cells changed the slope of the line, showing that these two complex mixtures did not inhibit TG detection.

Each acyl-glycerol species drives the increase of 540 nm absorbance at a different rate

To determine an appropriate incubation time, we assessed how differences in saturation and number of the acyl chains affect the rate of increase in 540 nm absorbance. We found that the unsaturated triolein increased the 540 nm absorbance faster than the saturated tripalmitin and that the fewer palmitates attached to the glycerol, the faster the 540 nm absorbance increased. Even with these marked differences in the rates of 540 nm absorbance increase, we found that the four acyl-glycerols tested drove the reaction to near completion by 90 min (Fig. 2C).

This assay effectively quantifies a broad range of acyl-glycerol oils

To assess how broad a spectrum of oils this assay detects, we tested several different oils. We found that LPL released glycerol from a broad range of biological oils, including medium-saturated acyl chains (coconut oil) to long-unsaturated oils (olive oil) (Fig. 2D). We added equal masses of acyl-glycerols, and thus more moles of the lower molecular weight oils. As expected, the lower molecular weight oils, coconut oil and diacylglycerol, drove a larger 540 nm absorbance increase (Fig. 2D). Furthermore, to assess the extent of glycerol release by LPL, the same molar amount of glycerol found in 25 µg of triolein was added to the assay. We also found that glycerol added as free glycerol or as triolein was equally well detected, showing that in this assay LDL hydrolyzed essentially all of the triolein to glycerol and oleate (Fig. 2D).

Microgram amounts of TG are detectable from cells and tissue

The purpose of this assay is to measure the amount of TG in biological samples. To assess whether acyl-glycerols in cells and tissues drive a dose-dependent increase in 540 nm absorbance, we assayed increasing amounts of cells and liver homogenate. In addition, to assess the effectiveness of the IHW extraction, we compared it to a chloroform-methanol extraction (19). We found that this assay has a large linear response range to intracellular TG (Fig. 3A). We fitted measurements to a line, $r^2 = 0.99$,

slope = 6.78 ± 0.135, *y* intercept = 0.206, *x* intercept = 0.0304. This line passes very near the origin, showing that there is no significant threshold phenomenon; thus, the assay will be effective at quantifying low TG masses. Also, the IHW extraction recovers more TG than chloroform-methanol extraction over wide ranges of cell and tissue amounts ($P < 0.0001$) (Fig. 3B, C).

Samples are stable in cold solvent

Because it is not always possible to collect samples within a short period of time, we measured the stability of TG in TG-laden TO-HeLa cells under different storage conditions. The line fit to the TG in TO-HeLa cells stored at 4°C has a significant negative slope ($P < 0.001$). This demonstrates that TG in cells suspended at 4°C in aqueous buffer degrades measurably in tens of hours (Fig. 4). In samples that we added directly to IHW at the beginning of the time course, we detected ~14 μg of TG. In samples we froze and then added to IWH on dry ice, we detected ~12 μg of TG (Fig. 4). This demonstrates that freezing samples decreases the amount of detectable TG. However, the line fit to the TG in frozen samples does not have a significant slope ($P > 0.86$). This indicates that the TG in frozen samples is stable. Also, we detected very similar amounts of TG in all of the frozen samples. This shows that TG lost from freezing is uniform (Fig. 4). Finally, we detected similar amounts of TG in frozen samples stored for varying times in IHW on dry ice. This demonstrates that TG from samples is stable in IHW on dry ice.

This assay allows precise, economical TG measurements

To demonstrate a large-scale application, we measured TG accumulation in TO-HeLa cells driven by adding different oleate concentrations to the culture medium. Oleate drives time-dependent TG accumulation ($P > 0.0001$). Furthermore, the rate and extent of TG accumulation increases with oleate concentration ($P > 0.0001$). To show that this assay can detect TG in cells grown in a format amenable to large-scale screening, we measured TG in TO-HeLa cells grown on 96-well plates. We found that TG extracted from oleate-treated TO-HeLa cells grown in 1 well of a 96-well plate is easily detectable (8.38 μg/well, with a standard deviation of 0.814 μg/well; n = 6).

We have found that exposing samples or standards to high heat during drying results in greatly reducing the 540 nm absorbance increase (data not shown). We also found that making the organic phase more polar by adding diethyl ether made TG extraction more reliable than using hexane alone, as prescribed in the original procedure (data not shown) (18).

DISCUSSION

We have devised a rapid and robust method to measure solvent-extracted TG from cells and tissues. Solvent extraction partitions potentially inhibitory TG coat proteins from obstructing LPL access to TG. Furthermore, partitioning hydrophilic molecules away from TG minimizes these molecules' interference in the assay. Large amounts of complex mixtures of hydrophilic molecules found in cells or media do not interfere with this assay (Fig. 2B). The intracellular TG droplet coat proteins control lipases' access to the TG they enclose (4, 9, 10, 20). The TG detection reagent requires the release of the glycerol from acyl-glycerols (Fig. 1), but TG coat proteins may block LPL access to the TG. The inaccessible TG will escape detection. This possibility is partially troublesome for those studying TG coat proteins, because the inability to distinguishing between changes in TG accessibility and TG mass make the data uninterpretable. The possibility that TG droplet coat proteins interfere with TG detection in this assay is negated, because proteins do not partition with the TG into the organic phase.

This assay is very economical. By using disposable tubes and parallel processing of sample, it saves laboratory workers' time. The assay uses small volumes of inexpensive solvents, and the only specialized piece of equipment is an inexpensive 96-pin drying manifold. Finally, this assay eliminates the use of chloroform, which is more toxic and environmentally destructive than the nonhalogenated solvents used previously (21).

The ability to efficiently generate data makes feasible at least two new uses for TG measurements: first, the simultaneous assessment of multiple parameters affecting TG storage (Fig. 5); second, to screen panels of compounds that affect TG storage, or other large-scale screens.

This assay detects a TG-driven, enzyme-linked increase in 540 nm absorbance of a dye (Fig. 1). This scheme has three inherent limitations. First, this assay does not reveal the nature or the number of acyl chains that constitute the glycerol oils. Acyl chain number has been revealing; ablating the hormone-sensitive lipase gene in mice increases the amount of diacyl-glycerol in adipose tissue, implicating the existence of a TG-specific lipase (22). This TG lipase was then quickly identified (23, 24). Hence, the decision to disregard the information gained by chromatographic quantification methods must be weighed against the rapid throughput of this system. Second, because the molecular weights of acyl-glycerol can vary manyfold depending on the number and length of the acyl chains, to accurately determine the mass of the acyl-glycerols the molecular weight of the acyl-glycerol must be known. Third, because the detection of TG requires LPL activity, the effects of lipase or protease inhibitors may be confounding. In fact, any molecule that interferes with the dye reduction, reduces the dye, or absorbs or diffracts 540 nm light will alter the reading and thus the results.

TG has no specific properties that make it amenable to this strategy. Hence, with only minor modifications to this protocol, it can be used to quantify other lipids that are extractable in a solvent that floats on the aqueous phase and are colorimetrically detectable. It is conceivable that one might measure multiple species of lipids in parallel by splitting the organic phase. Because colorimetric assays for cholesterol, cholesteryl esters, and fatty acids are already available, developing assays for these molecules will not be technically difficult.

We have developed a simple method that precisely measures TG extracted from cells and tissues. When appropriate standards are used, this assay is also accurate. This method will allow the simultaneous measurement of multiple parameters (Fig. 5) that affect TG levels. In addition, the throughput potential presents the possibility of using TG as an end point for screening compounds or genetic manipulations. Finally, because the extent and location of TG accumulation are factors in both the length and the quality of life, improving the ability to measure levels of TG will likely prove useful.

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