Determination of triglyceride in the human myocardium by magnetic resonance spectroscopy: reproducibility and sensitivity of the method

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IN THE FACE OF AN OBESITY EPIDEMIC, the development of new technologies to predict the progression of obesity-related chronic disease has advanced dramatically in the past decade. Studies in animals reveal that an excessive deposition of triglyceride (TG) droplets in metabolically active tissues, such as the liver (8), skeletal muscle (10), and myocardium (3, 6, 25), impairs cellular function, predisposing animals to type 2 diabetes (9) and heart failure (3, 6, 25). An attempt to translate these studies into a clinical setting, researchers have relied on biopsy-derived quantification of TG or in vivo magnetic resonance spectroscopy (MRS) imaging to study the role of intracellular TG accumulation in the human liver (15, 21) and skeletal muscle (7, 12, 19) in the development of metabolic diseases. Impressively, results from studies using MRS of hepatic and muscle tissue mirror results obtained in animals (11, 13, 14, 24), suggesting that intracellular TG may be a valuable biomarker for metabolic disease in humans. Unfortunately, the technology for measuring TG in the human myocardium is still not completely resolved.

Until recently, the evaluation of myocardial TG in humans was limited to invasive biopsies, limiting the usefulness of intracellular TG as a biomarker for chronic disease in humans (16). We (20) and others (4, 5) have developed a proton MRS (1H-MRS) technique similar to that used for skeletal muscle and hepatic tissue that quantifies intracellular TG in vivo in the beating human heart. Initial cross-sectional studies revealed that myocardial TG accumulated with increasing body mass and was related to cardiac structure and function in humans (20); however, the long-term reliability and sensitivity of the 1H-MRS technique was not determined. To address this limitation, the primary aim of this investigation was to assess the reliability of the 1H-MRS method for quantifying myocardial TG content over a prolonged period of time and its ability to detect changes in TG content following metabolic perturbations, including a high-fat meal and prolonged fasting.

METHODS

Study Subjects

The Institutional Review Board at the University of Texas Southwestern Medical Center approved all experimental protocols, and participants provided written informed consent before their participation in the study. Most of the participants were recruited from the Dallas Heart Study (DHS) database, which is a large, multi-ethnic, probability-based population sample of Dallas County (23). Participants were excluded if they met any of the following criteria: 1) were medicated for or diagnosed with a clinical metabolic or cardiovascular disorder, 2) were borderline or overtly hypertensive (>140/90 mmHg), 3) had fasting glucose >110 mg/dl, 4) were pregnant, and/or 5) had implanted devices that would limit imaging and spectroscopy. Twenty-five people were approved to participate; however, six volunteers withdrew before or during data collection for the following reasons: 1) had an abdominal circumference beyond the limits of the magnet, 2) had an inability to remain motionless during tissue imaging, and/or 3) displayed inefficient respiratory gating.

Experimental Protocols

Three experimental protocols were used to determine the stability of myocardial TG measurements using 1H-MRS in humans: 1) test-retest reliability of the measurement of myocardial TG collected 90 days apart; 2) changes in myocardial TG following consumption of a single high-fat meal, and 3) changes in myocardial TG following a 48-h fast.

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Experiment 1: Test-Retest Reproducibility over a 90-Day Period. Reproducibility experiments were performed in six subjects. All experiments were conducted in the evening between 4:00 PM and 7:00 PM, with subjects having consumed a meal in the mid afternoon and refraining from food within the 4-h period leading up to the experiment. All blood samples were collected in the afternoon, before the MRI imaging at the General Clinical Research Center (GCRC). Myocardial TG content was determined using cardiac imaging and spectroscopy methods (described in Myocardial Imaging and Spectroscopy) in a fed state on two separate occasions: at baseline and 90 days later. Ninety days was chosen because it is the time frame associated with typical interventional studies. We have previously reported short-term (<1 h) reproducibility in humans (20).

Experiment 2: Single High-Fat Meal. Six individuals completed the meal experiment. The experimental protocol included evening admission of study participants to the GCRC at University of Texas Southwestern Medical Center for a supervised overnight fast. The following morning, at 7:00 AM, we tested fasting serum glucose and lipids. Subsequently, at 8:00 AM, we assessed the levels of myocardial TG triglyceride using \(^{1}H\)-MRS. At 10:30 AM, study participants consumed a meal containing 50 g of fat and consisting of 25 g of grits, 20 g of butter, 100 g of scrambled eggs, 15 g of cheese, and 240 g of milk. Four hours after completion of the meal, phlebotomy, MRI, and \(^{1}H\)-MRS protocols were repeated.

Experiment 3: 48 h of Fasting. To elicit a physiological elevation of serum lipids from endogenous sources, seven participants performed a 48-h fast. Forty-eight hours was chosen because it is has been used in animal studies to elicit a significant increase in hepatic and skeletal muscle lipid content. One subject was studied at a time, and the fasting was not supervised. On the initial day of testing, participants reported to the GCRC at 8:00 AM, following an overnight fast, for baseline blood sampling of serum TG and glucose. On that same day, participants were allowed to have a light lunch. At 5:00 PM, baseline tissue imaging and spectroscopy were performed. Volunteers were asked to avoid exercise and to consume only water for the entire 48-h period. On the third day of the experiment, participants reported to the GCRC at 4:30 PM for final blood sampling and to Roger’s MRI facility for follow-up \(^{1}H\)-MRS at 5:00 PM.

Myocardial Imaging and Spectroscopy

Myocardial morphology and TG content were determined using a 1.5 Tesla Gyroscan INTERA whole body system (Philips Medical Systems, Best, The Netherlands) equipped with spectroscopy and cardiac packages. The imaging of the myocardium for the placement of spectroscopic volume of interest was performed with a cardiac synergy coil (20). Patients were instructed to hold their breath at end-exhalation during imaging. A 17-cm surface coil was used for myocardial spectroscopy. To confirm the accurate position of the surface coil relative to the heart, coronal and axial scout images were obtained. Additional high-resolution cardiac images in the vertical, horizontal, and short-axis views were collected to locate precisely the biventricular septum. End-systolic images in the aforesaid three planes were used to position the spectroscopic volume of interest (6 cm\(^3\) to 10 \(\times\) 20 \(\times\) 30 mm\(^3\)) within the interventricular septum (20) (Fig. 1). Patients were instructed to breathe normally during spectroscopy; however, the spectroscopic data acquisition was triggered by electrocardiograph-derived R wave and synchronized with respiratory cycle at end-exhalation. Spectra were collected using a body coil for radio frequency transmission and a 17-cm surface coil for a signal reception. PRESS sequence (PointRESolvedSpectroscopy) was used for spatial localization and signal acquisition. The interpulse delay \(T_{R}\) was defined by the length of a respiratory cycle (~4 s). Spin echo time \(T_{E}\) was 25 ms. Data points (1,024) were collected over a 1,000-Hz spectral width and averaged \(>64\) acquisitions. Areas of resonances for water and methylene groups in fatty acid chains were evaluated with line-fit procedure and commercial software (NUTS-ACORNMR, Freemont, CA). Signal decay due to a spin-spin relaxation was calculated from a monoexponential relaxation equation (19, 20) using mean \(T_{2}\) relaxation time of 40 ms for water and 78 ms for myocardial TG.

Biochemical Assays

Assays to determine serum concentrations of standard clinical chemistry analyses were performed on a Beckman CX9AXL chemical analyzer (Beckman-Coulter, Fullerton, CA) as previously described (21).

Statistics

All statistics were performed using SPSS for Windows version 12.0 software. Mean tissue TG values were compared using paired t-tests for repeated measures in the same individual. Reliability data were determined using a Student’s paired t-test to test for differences between the groups, and relations were tested using partial Spearman correlation. A value of \(P < 0.05\) was considered statistically significant.

RESULTS

Experiment 1: Test-Retest Reliability

Participant demographics are presented in Table 1, top. To determine the reliability of the measurement of myocardial TG content in vivo, spectra of myocardial tissue were measured in six individuals at baseline and 90 days later. Measurements were highly correlated (\(r = 0.987\)) with the coefficient of variation 5% (Fig. 1). Additionally, a significant positive relationship (\(r = 0.58\)) was observed between myocardial TG content and body mass index (BMI).

Experiment 2: Effects of a Single High-Fat Meal

Participant demographic information is provided in Table 1, middle. The serum glucose levels measured 4 h after a high-fat meal were not any different from values obtained after an
overnight fast. In contrast to glucose, serum TG levels were increased over twofold following consumption of a high-fat meal (P < 0.05). Despite a twofold increase in serum TG content, myocardial TG remained unchanged from baseline (Fig. 2A). No changes were observed in blood pressure following the high-fat meal. When these experiments were repeated in one participant (BMI = 23 kg/m²), baseline and postprandial myocardial TG contents were similar to values observed during the initial experiment (data not shown).

Experiment 3: Effects of 48 h of Fasting

Demographic information is provided in Table 1, bottom. The 48-h fast elicited a decline in fasting glucose uniformly in all subjects, whereas serum TG decreased 37% from baseline values, suggesting that all subjects had complied with the fasting protocol. Although serum TG values decreased after the 48-h fast, the average increase in TG content in myocardial tissue was 311 ± 48 mmol/l (Fig. 2A). One person (BMI = 29 kg/m²) repeated this experiment, and the relative increase in the myocardial TG content was nearly identical in both experiments (>2-fold). A significant negative relationship was also observed between the magnitude of change in myocardial tissue TG content following a prolonged fast and BMI (r² = 0.60; Fig. 3).

DISCUSSION

The present study was designed to assess the reliability and sensitivity of ¹H-MRS-derived measurements of myocardial TG content over time and in response to various metabolic stressors. The primary novel findings are that 1) test-retest reproducibility over a prolonged period of time is exceptional within subjects despite considerable between-subject variability; 2) this is a stable method for the determination of myocardial TG content, being unaffected by a single high-fat meal, despite a significant postprandial elevation of serum TG; and finally, 3) this method is sufficiently sensitive to detect significant changes in myocardial TG content, such as those that occur following a prolonged fast. The observed, remarkable, long-term reproducibility of the method confirms our previous findings (19, 21) that demonstrated excellent reproducibility and extends the day-to-day reproducibility from days to months.

Previous studies in skeletal muscle and hepatic tissues have revealed that ¹H-MRS is a valid and reliable technique for the determination of TG content in humans (1, 2, 4, 5, 16, 17, 22). Since that time, several interventional and cross-sectional studies, using this technology, have revealed that hepatic TG is a very plastic intracellular substrate that appears to be elevated in...
states of insulin resistance (11, 15), and that is reduced with thiazolidinedione therapy (11, 14, 22). Similarly, skeletal muscle TG is also increased in states of insulin resistance (13, 24) and is negatively associated with insulin sensitivity (17) but remains unchanged following various therapeutic interventions (11). The data presented here extend previous studies in liver (21) and muscle tissue (19) by demonstrating that 1H-MRS is a reliable method for quantifying TG in the human myocardium and is sufficiently sensitive to detect changes in TG content after metabolic perturbations. Future studies are required to evaluate the clinical significance of this measurement and whether or not it is sensitive to antidiabetic therapy.

Acutely, 1H-MRS-derived measurements of skeletal muscle TG have proved to be sensitive enough to detect changes in TG content following various interventions, including Intralipid infusion (1), 72 h of fasting (18), and multiple consecutive high-fat meals (1). Of these three metabolic stressors, prolonged fasting elicited the greatest relative change in skeletal muscle TG, with the response being nearly sevenfold greater than changes observed following Intralipid or consecutive high-fat meals (18). It was for this reason that we chose to use a prolonged fast to study the sensitivity of this method for detecting changes in myocardial TG following a metabolic stress. The data presented here support previous findings in muscle and extend them to myocardial tissue by demonstrating an average threefold increase in TG content following a 48-h fast in otherwise healthy humans. Interestingly, hepatic TG increased significantly after the 48-h fast (147 ± 77%) as well, whereas no change in skeletal muscle TG was observed (data not shown). Furthermore, it appears that the magnitude of change in myocardial TG content following a prolonged fast is dependent on BMI, such that lean individuals elicited far greater elevations in myocardial TG than the obese participants ($r^2 = 0.60$; Fig. 3). In relation to the aim of this study, 1H-MRS was able to detect the significant changes in myocardial TG following the prolonged fast despite a reduction in plasma TG.

Limitations

One of the potential concerns associated with this and other studies of in vivo human metabolism relates to the validity of the method. Validation studies in animals suggest that 1H-MRS-derived measurements of intracellular TG closely approximate values obtained directly with biochemical assays in both skeletal muscle and myocardial tissue (19, 20). This study complements reproducibility experiments with our earlier studies of the 1H-MRS as a method for determining hepatic and myocardial TG content (19, 21). As studies performed here are limited to a small sample of healthy lean and obese individuals free from cardiovascular or metabolic disease, the reliability of these methods in individuals with type 2 diabetes mellitus or coronary artery disease may be different. Finally, the relatively small sample of individuals studied limits the ability to determine whether the response to fasting or feeding may be altered by obesity, sex, and ethnicity.

Conclusions

First, 1H-MRS-technology is a reliable method for the determination of TG within the human myocardium, over a prolonged fast.
prolonged period of time, similar to what would be used for a typical intervention trial. Second, myocardial TG does not change significantly following the consumption of one high-fat meal, despite a twofold increase in serum TG. Finally, this method is sufficiently sensitive to detect changes in myocardial TG that occur following a moderate physiological stress, such as prolonged fasting, irrespective of serum TG. Thus the H-MRS method is a reliable and robust method for the quantification of TG in the beating human myocardium and reflects changes in tissue TG content that occur independently of serum TG. The present data extend previous H-MRS studies to suggest that noninvasive quantification of TG content in cardiac muscle, as well as in skeletal muscle and liver, constitutes a valuable research tool for translational research in humans.

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