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Lipid in Skeletal Muscle Myotubes is associated to the Donors' Insulin Sensitivity and Physical Activity Phenotypes

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Abstract

Objective—This study investigated the relationship between *in-vitro* lipid content in myotubes and *in-vivo* whole body phenotypes of the donors such as insulin sensitivity, intramyocellular lipids (IMCL), physical activity and oxidative capacity.

Design and Methods—Six physically active donors were compared to 6 sedentary lean and 6 T2DM. Lipid content was measured in tissues and myotubes by immunohistochemistry. Ceramides, triacylglycerols (TAGs) and diacylglycerols (DAGs) were measured by LC-MS-MS and GC-FID. Insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp (80mU/min/m^2), maximal mitochondrial capacity (ATPmax) by ^{31}P-MRS, physical fitness by VO_{2max} and physical activity level (PAL) by accelerometers.

Results—Myotubes cultured from physically active donors had higher lipid content (0.047±0.003 vs. 0.032±0.001 and 0.033±0.001AU; $p<0.001$) than myotubes from lean and T2DM donors. Lipid content in myotubes was not associated with IMCL in muscle tissue but importantly, correlated with *in-vivo* measures of ATPmax ($r=0.74$; $p<0.001$), insulin sensitivity ($r=0.54$; $p<0.05$), type-I fibers ($r=0.50$; $p<0.05$) and PAL ($r=0.92$; $p<0.0001$). DAGs and ceramides in myotubes were inversely associated with insulin sensitivity ($r=-0.55$, $r=-0.73$; $p<0.05$) and ATPmax ($r=-0.74$, $r=-0.85$; $p<0.01$).

Conclusions—These results indicate that cultured human myotubes can be used in mechanistic studies to study the *in vitro* impact of interventions on phenotypes such as mitochondrial capacity, insulin sensitivity, and physical activity.

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Keywords

lipid metabolism; IMCL; DAGs; Ceramides; skeletal muscle; insulin sensitivity; mitochondrial capacity; physical activity

Introduction

Obese non diabetic and obese diabetic individuals (T2DM) have elevated levels of intramyocellular lipid (IMCL) and are usually associated with insulin resistance and low skeletal muscle mitochondrial density and/or function[1-4]. Interestingly, endurance athletes who are insulin-sensitive and have high oxidative capacity also have high IMCL content, an observation referred to as the "Athlete's Paradox"[4]. Together, published data suggest that the relationship between IMCL and insulin action/muscle oxidative capacity is not linear but rather "U" shaped[5]. Increased IMCL content can result from elevated free fatty acid (FFA) and triacylglycerol (TAG) levels, hyperinsulinemia, increased dietary lipid consumption, decreased physical activity and impaired muscle lipid oxidation[6, 7]. It has been postulated that an impaired mitochondrial density and/or function may be causal for decreased lipid oxidation, increased IMCL content and hence insulin resistance[8, 9]. The fact that physical activity improves insulin sensitivity and skeletal muscle oxidative capacity[10, 11] also indicates that environmental factors such as diet and exercise probably have a large impact on IMCL levels.

Lipid metabolites such as diacylglycerols (DAGs) and ceramides, rather than total lipid content, have recently been proposed to be the major culprit in the development of insulin resistance[12, 13] and are elevated in obese and T2DM. Also, a decrease in DAGs and ceramides has been reported after exercise training.[14].

Since skeletal muscle primary cell culture can be studied without the long-term and acute impacts of the *in vivo* physiological milieu, they represent a powerful model to study genetic and epigenetic determinants of carbohydrate and lipid metabolism independent of environmental factors. Therefore, the purpose of this study was to determine the relevance of lipid and lipid metabolites, measured *in vitro* in myotubes in relation to the *in vivo* clinical characteristics of the donors such as insulin sensitivity, oxidative capacity and physical activity in physically active, sedentary healthy lean, and individuals with T2DM. All donors were extensively phenotyped for skeletal muscle oxidative functions (ATPmax measured by ^{31}P-MRS), insulin sensitivity (euglycemic hyperinsulinemic clamp at 80 mU/min/m^2), oxidative fiber type-I composition (immunohistochemistry), and physical activity (accelerometers).

Methods

Study population

After signing the informed written consent approved by the Pennington Biomedical Research Center (PBRC) ethical review board, volunteers were enrolled in TAKE TIME (Clinicaltrials.gov NCT00402012) for those with T2DM or ACTIV (NCT00401791) for those non-diabetic, age 20-40 years and BMI 20-30 kg/m^2. Physical activity level was

assessed from a 7-day physical activity questionnaire recall and a tri-axial accelerometer worn for at least 4 days. Physical activity level (PAL; total daily energy expenditure/resting metabolic rate) was calculated and donors were classified as sedentary healthy controls for a PAL < 1.4 or active donors for those with a PAL > 1.6. Another additional inclusion criterion for the sedentary donors was a VO_{2max} < 40 ml/kg/min and for the active donors a VO_{2max} > 40 ml/kg/min.

Volunteers with chronic illnesses such as heart disease, hypothyroidism, renal, lung and liver diseases were excluded. All donors consumed a provided standard American diet (15% protein, 30-35% fat and 50-55% carbohydrate) for 3 days before admission to our inpatient clinic.

Six physically active donors were compared with 6 sedentary lean and 6 T2DM. Myotubess from one lean donor could not be successfully cultured for lipid measurements.

Physical activity level (PAL)

Physical activity level was calculated from a tri-axial accelerometer worn for at least 4 days in sedentary healthy and active donors but not in donors with T2DM. Physical activity level (total daily energy expenditure / resting metabolic rate) was calculated using daily activity level from the accelerometer data. Sedentary healthy donors and active donors were selected as having a PAL of less than 1.4 and greater than 1.6, respectively.

Maximum aerobic capacity (VO_{2max})

Cardiorespiratory testing was conducted a few days before admission using a standardized graded exercise testing protocol on a stationary bicycle ergometer. Unfortunately, VO_{2max} was not measured in T2DM donors.

Body composition

Body weight was measured in a gown after voiding; waist circumference in the standing position and height with a calibrated stadiometer using standardized protocols. Body fat mass and fat-free mass were calculated from metabolic weight and % body fat using Dual Energy X-ray Absorptiometer (QDR 4500A, Hologic, Inc, Waltham, MA).

Euglycemic hyperinsulinemic clamp

The clamp was performed as previously described[15]. Briefly, after an overnight fast and following the muscle biopsy procedure, intravenous catheters were inserted in an antecubital vein for infusions and in a vein on the dorsum of the contra-lateral hand for sampling of arterialized blood. After baseline blood samples were obtained, a primed low-dose infusion (for adipose tissue insulin sensitivity) of regular insulin was then initiated and continued for 60 min, followed by a high-dose rate of 80 mU/min/m^2 for 90 min (whole body insulin sensitivity). The steady state plasma glucose concentration was maintained at ~90 mg/dl (5 mmol/l) by a variable 20% glucose infusion. The mean steady state rate of exogenous glucose infusion was calculated for the last 30 minutes of the high dose insulin, corrected for changes in glycemia and normalized for estimated metabolic body size (EMBS; kg fat-free mass [FFM]+17.7) to assess insulin sensitivity[16].

Mitochondrial capacity: maximal ATP synthesis rate (ATP_{max})

ATP_{max} was determined as previously described[17] on a 3T GE Signa MNS magnet (GE, Milwaukee, WI) using a 4 or 6 cm ^{31}P-tuned surface coil positioned over the distal *vastus lateralis*. Following the acquisition of a fully relaxed spectrum, ^{31}P spectra were acquired every 6 seconds at rest (4 NEX) and continuously during a 24-, 30- or 36-sec ballistic exercise obtained by 'kicking' against Velcro straps positioned tight across the leg and thigh. Exercise time and intensity was targeted to drop PCr by 33 – 50% of basal PCr and to avoid a pH of < 6.8 since lower pH inhibits oxidative phosphorylation and results in an artificially low ATP_{max}. ATP_{max} was calculated using the PCr recovery time constant (tau) and [PCr] rest: ATP_{max} = [PCr] rest/tau.

Resting ATP turnover/flux (ATPase)

Resting ATP turnover, or flux, was measured on the same magnet by ^{31}P-MRS as previously described[18] using a 4, 6 or 8 cm (based on approximate muscle size) ^{31}P-tuned surface coil positioned over the *vastus lateralis*. Spectra were acquired during a baseline rest period and during cuff inflation. The breakdown of PCr under anoxic conditions represents net ATP demand, i.e., cellular ATP use minus glycolytic ATP supply[19]. Spectra were analyzed using the Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES) algorithm in the jMRUI software. Using this method, repeated measures of muscle ATP_{flux} on the same donor agree to within ±11.0% .

Biopsy and immunohistochemistry

After an overnight fast and local anesthesia (lidocaine/bupivicaine), skeletal muscle samples were collected from the *vastus lateralis* using the Bergstrom technique with suction. At the bedside, samples were rapidly cleaned and blotted dry prior to mounting in a mixture of optimal cutting temperature (OCT) compound and tragacanth powder for immunohistochemistry. IMCL and fiber type was measured by immunohistochemistry performed on 12 micron sections using bodipy green 493/503 (Invitrogen molecular probe, CA) along with mouse monoclonal antibody specific for slow muscle (MAB1628; Chemicon, Temecula, CA) and a monoclonal antibody to laminin (AB2500, Abcam Inc, Cambridge, MA). Type-I fibers were counted to determine fiber type. Lipid was measured in myotubes cultured from the *vastus lateralis* muscle (see below), using the exact same immunohistochemisty technique. Images were taken using confocal microscope (Leica SP5, Leica, Bannockburn, USA). Lipid content in skeletal muscle was quantified by carefully identifying area inside the muscle fibers excluding extramyocellular lipid (EMCL). IMCL was quantified using the Sigma Scan Pro 5.0 software.

Lipid content in cultured myotubes

Sorted muscle cells were grown as described before[20]. In brief, same number of cells (~20,000 per well) were seeded and grown in DMEM (changed every other day) supplemented with 10% fetal bovine serum (FBS) and incubated in a humidified chamber with 95% air/5% CO_2 at 37°C. When myoblasts reached ~70% confluence, differentiation into multinucleated myotubes was induced by incubation in αMEM supplemented with 2% FBS. After ~5 days of differentiation, cells were harvested to measure lipid content.

Myotubes were stained as previously described[21]. Lipid droplets and nucleus were stained using bodipy 493/503 (Invitrogen Molecular Probes) followed by DAPI (Sigma-Aldrich, St. Louis, MO). Lipid content was quantified using Softamax PRO5 FLEX station (Molecular devices, USA) and was adjusted for any variability in nuclear content, measured by DAPI staining (not different among groups; data not shown).

Extraction of Lipids for Lipid metabolite quantitation

Cells were collected into microtubes with 200ul ice-cold DI water. After sonication, 2ul was removed for protein determination. An aliquot equivalent to 300ug protein for each sample was extracted: for samples aimed for ceramide analysis only, 4ul of a 10ug/ml ceramide C17 was added to the remaining cells as the extraction standard. Lipids were extracted using Folch's partition (300ul of methanol/chloroform (1:2). The lower chloroformic layer was used for subsequent analysis.

Quantitative Analysis of intracellular Triglycerides and Diglycerides

Lipid extracts were dried under nitrogen gas at room temperature, reconstituted with 100ul chloroform and silylated by adding 100ul N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA). Acylglycerides (monoglycerides, diglycerides and triglycerides) were analyzed simultaneously by gas chromatography coupled with a flame ionization detector (GC-FID) according the procedure shown in Obanda et al[22].

Quantitative Analysis of intracellular Ceramides

Lipid extracts were dried under nitrogen gas at room temperature and reconstituted with 200ul acetonitrile. Liquid chromatography-electrospray ionization tandem-mass spectrometry (LC-ESI-MS/MS) was used to measure intracellular levels of ceramides C16, C18, C18:1, C20, C22, C24 and C24:1, and C17 the extraction standard. Ceramide C2 was included as the internal standard. LC was performed using a Waters Acquity UPLC. The detector used was a Waters Aquity TQD triple quadropole MS/MS with ion source ESI operated in the positive mode. Procedures and parameters were according to the method shown in Obanda et al[23]. According to the retention times of standards, the individual long-chain fatty acids were identified for each ceramide species and quantified.

Mitochondrial content

Mitotracker Green probe preferentially accumulates in mitochondria regardless of the mitochondrial membrane potential and thus provides an accurate assessment of mitochondrial mass. Mitochondrial mass was determined by measuring fluorescence intensity of the cells after incubation with Mitotracker Green (Molecular Probes, Invitrogen, Eugene, OR) using a spectrometer model LS50B (PerkinElmer, East Lyme CT, USA) with excitation and emission wavelengths of 490 and 516 nm, respectively.

Statistical analysis

All statistical analyses were performed using GraphPad Prism, version 5.0 (*GraphPad* Software Inc, La Jolla, California). All values are presented as means ± SE. One-way ANOVA with Tukey post hoc test was used to determine any group difference (active,

sedentary and T2DM). Myotubes characteristics and clinical phenotypes were compared by Pearson correlation analysis and nonparametric Spearman's rho analysis, when applicable. A $P<0.05$ was considered significant.

Results

Donors' characteristics

Donors in all groups were males except for two females in the T2DM group (Table 1). Diabetic donors had a significantly higher body weight, body mass index (BMI) and were older compared to active and lean sedentary donors. Insulin sensitivity (clamp), physical fitness (VO_{2max}), and maximal mitochondrial capacity (ATP_{max}) were significantly higher in active compared to lean sedentary and T2DM individuals. Resting ATP flux (ATPase) was significantly higher in active compared to T2DM donors.

Lipid content in myotubes vs. muscle tissue

The lipid content measured in myotubes was significantly higher (~30%) in cells from physically active donors compared to sedentary lean and T2DM donors (Figure 1A). There was no significant difference in lipid content between myotubes from lean and T2DM donors.

Lipid measured directly in *vastus lateralis* muscle tissue (IMCL) only tended to be lower in lean sedentary donor but was not different across groups (Figure 1B; $p=0.1$). There was no association between IMCL content measured in muscle tissue vs. that measured in myotubes ($r=0.03$, $P=0.90$; Figure 1C).

Mitochondrial Mass

The mitochondrial mass, measured by Mitotracker Green, in myotubes was significantly higher in cells from physically active donors compared to sedentary lean donors ($p<0.05$; Figure 2A). In myotubes, the mitochondrial mass positively correlated to the lipid content in myotubes (Figure 2B; $r=0.60$; $p=0.02$).

Lipid content in myotubes is associated with mitochondrial function, insulin sensitivity, oxidative fibers and physical activity of the donors

Lipid content measured *in vitro* in myotubes was strongly associated with *in vivo* measures of maximal mitochondrial capacity (ATPmax) (Figure 3A; $r=0.74$, $p<0.001$), insulin sensitivity (clamp; Figure 3B; $r=0.54$, $p<0.05$), the proportion of type-I fibers of the donor's tissue (Figure 3C; $r=0.50$, $P<0.05$) and physical activity level (accelerometers; Figure 3D; $r=0.92$, $p<0.0001$). Additionally, lipid in myotubes tended to correlate with resting ATP flux (ATPase) ($r=0.43$, $p=0.09$) and physical fitness measured by VO_{2max} ($r=0.26$, $p=0.1$). Together, the data indicate that higher lipid content in cultured myotubes is associated with higher mitochondrial function/capacity, higher insulin sensitivity, more oxidative skeletal muscle fiber morphology and greater physical activity and fitness level of the donor.

Diacylglycerols and ceramides in myotubes are inversely associated with insulin sensitivity and mitochondrial function of the donors

TAGs measured in myotubes of active donors, was significantly higher than that in lean donors (Figure 4A) whereas DAGs and ceramides in active donors were significantly lower compared to that in T2D donors (Figure 4B-C). Several ceramide species were also significantly higher in myotubes cultured from T2DM donors compared to active (C16, C18, and C24; all $p<0.05$) as well as lean (C18; $p<0.05$) donors. Both DAGs and ceramides in myotubes showed a strong inverse association with *in vivo* measures of insulin sensitivity (Figure 5A-B; DAG $r=-0.55$, $P<0.05$; Ceramides $r=-0.73$, $P<0.01$) and maximal mitochondrial capacity (Figure 5C-D; DAG $r=-0.74$, $P<0.01$; Ceramides $r=-0.85$, $P<0.001$).

Discussion

Our results demonstrate for the first time that lipid content measured in cultured myotubes established from human volunteers is strongly associated with clinical phenotypes of the donor including maximal ATP production capacity (ATPmax), insulin sensitivity, proportion of oxidative type-I fibers and physical activity/fitness levels. Moreover, we show that lipid metabolites – DAGs and ceramides – are negatively associated with insulin sensitivity and ATPmax. Such results demonstrate that independent of the ambient milieu, skeletal muscle lipids retain important phenotypes from the donors and therefore myotubes can be used as a model for more mechanistic studies, such as exercise mimetics including electrical pulse stimulation, activation of cAMP/PKA and Ca^{2+} signaling pathways with pharmacological treatment[24], energy substrate availability or hormonal modulation.

One of the aims of this study was to compare the skeletal muscle lipid content measured *in vivo* in the *vastus lateralis* muscle vs. that measured *in vitro* in myotubes grown from the same donors. Differentiated human myotubes represent the best available alternative system to intact human skeletal muscle[25]. Since myotubes are now used extensively as *in vitro* models for the study of glucose/lipid metabolism and insulin sensitivity, it is important to know other potential phenotypes retained in these cultured cells. Overall, human myotubes seem to retain most of the genetic background of the donor since they often display the morphological, metabolic and biochemical properties of the donor's skeletal muscle[25].

As observed *in vivo*, lipid content in cultured myotubes was significantly higher in active donors compared to healthy sedentary lean individuals but was surprisingly not different between sedentary individuals and patients with T2DM (Figure 1A). The often reported linear relationship between IMCL content and insulin sensitivity in sedentary individuals[1-3] disappears with the inclusion of endurance trained athletes in the study population because both athletes and patients with T2DM have high levels of IMCL[4]. Here, we show that lipid measured in myotubes established from T2DM donors have actually significantly lower IMCL compared to myotubes from active donors. The lipid content of the myotubes was positively related to the mitochondrial mass measured in myotubes indicating higher energy content in cells with higher oxidative capacity.

In contrast to several studies including our own[4, 26, 27] IMCL content in T2DM donors (Figure 1B) were not elevated although there was clearly a trend of higher IMCL in active

and T2DM donors (p=0.1). However, this finding is consistent with those of Schrauwen-Hinderling et al who reported comparable level of IMCL in lean and T2DM subjects[28]. The lipid content of the myotubes did not correlate with the lipid content of the donor's muscle (Figure 1C). This lack or correlation may be related to the absence of excess fatty acids in the culture medium and/or may indicate that epigenetic influences may override genetic factors. Lifestyle factors such as diet and physical activity are indeed known to play an important role in the level of triacylglycerols found in skeletal muscle. For example, high IMCL levels in obese and T2DM population are often associated with consumption of hypercaloric high-fat diets and/or a defect in lipid oxidation capacity[29, 30]. The resulting accumulation of lipid metabolites such as DAGs and ceramides[13, 29] are known to impair the insulin signaling pathway and cause insulin resistance[31, 32] in synergy to visceral adiposity[33].

To understand the clinical relevance of lipid and lipid metabolites measured *in vitro* in myotubes, we compared these lipids to independently measured clinical phenotypes of the donors. The most important and novel finding from our study is that *in vitro* IMCL is strongly associated with mitochondrial function, insulin sensitivity, the proportion of type-I fibers and physical activity level of the donors (Figures 3A-D). These findings are of significance since *in vitro* lipid content in human muscle cells seems not to be a marker of lipid measured *in vivo* but still retain an important determinant of cellular insulin sensitivity and "cellular fitness" as well as whole body fitness.

The non-linear relationship between IMCL content and insulin sensitivity in skeletal muscle, known as the "athlete's paradox", reflects the effect of diet, hormone concentration and exercise training. Lipid measured in myotubes using a cell culture model eliminates these confounding factors. Here we demonstrate that the lipid in myotubes holds a linear relationship with insulin sensitivity measured by the euglycemic hyperinsulinemic clamp (Figure 3B) whereas this relationship does not exist when lipid is measured directly in the *vastus lateralis* muscle[4, 5]. Emerging evidences suggest that lipid metabolites such as diacylglycerols (DAG) and ceramides are more directly associated with the etiology of insulin resistance rather than the lipid content[12, 13]. Indeed, DAGs and ceramides content in myotubes were significantly higher in T2DM donors compared to physically active donors. C16, C18, C24 ceramide species were significantly elevated in myotubes of T2DM donors. (Figure 4A-D). Moreover, both DAG and ceramide content in myotubes were negatively associated with *in vivo* measures of insulin sensitivity and mitochondrial function (Figure 5A-D), confirming the role of these lipid metabolites in insulin resistance and mitochondrial function.

While the *in vivo* system is greatly influenced by environmental factor such as diet and physical activity, the *in vitro* cell culture model is independent of such influences. Insulin resistance and type 2 diabetes are often associated with consumption of high fat diet and lack of physical activity[34] whereas athletes have high levels of physical activity and generally low fat diets. In a mechanistic way, future research should investigate the impact of *in vitro* "overfeeding" (fatty acid and/or glucose concentration) and "physical activity" on lipid content and lipid metabolites such as DAG and ceramides in myotubes, insulin sensitivity and mitochondrial capacity. Our results clearly demonstrate, for the first time,

that skeletal muscle lipid content and lipid metabolites (DAGs and ceramides), measured *in vitro*, are associated to some important *in vivo* clinical phenotypes measured independently in human donors.

Exercise training (aerobic) is associated with improvement in insulin sensitivity, VO_{2max} and mitochondrial respiration[35]. In the present study, lipid content in myotubes was associated with physical fitness and physical activity level of the donors in non-diabetics (Figure 2D). Several studies suggest that IMCL is an important source of energy for skeletal muscle during endurance exercising[36, 37]. The fact that we found a similar association in a cell culture model suggests that similarly to *in vivo* conditions, high lipid content in myotubes may be indicative of a better physical fitness and high physical activity level.

One of the limitations of our study is that our T2DM donors were older compared to the active and sedentary groups. However, when a covariate analysis was performed, age was not a significant factor in the model[17]. Moreover, Amati et al. also suggested that insulin resistance was not associated with age, but rather associated with obesity and physical inactivity[38]. Unfortunately, our study does not offer mechanistic insight for the disparity in lipid content among donors. However, a defect in lipid metabolism has been reported in vitro, using obese and type 2 diabetic donors[39, 40] suggesting possible defect in lipid uptake and/or oxidation in myotubes cultured from T2DM donors.

In summary, myotubes from active individuals had higher amount of lipids, but lower amount of DAGs and ceramides, compared to myotubes cultured from sedentary lean and T2DM donors. Lipid content measured in myotubes was not associated with IMCL measured directly in skeletal muscle tissue but correlated positively to *in vivo* mitochondrial function, insulin sensitivity, fiber type-I, physical fitness and activity level of the donor. Lipid metabolites – DAGs and ceramides correlated negatively to *in vivo* insulin sensitivity and mitochondrial function. Together our results indicate that lipids and lipid metabolites in human myotubes reflect several *in vivo* phenotypes and human myotubes can be used in mechanistic studies of the effect of energy substrate availability and exercise.

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What is already known about this project?

- Skeletal muscle primary cell culture model represent a powerful model to study genetic and epigenetic determinants of carbohydrate and lipid metabolism independent of environmental factors. However, the relevance of lipid and lipid metabolites measured *in vitro,* in myotubes, in relation to the *in vivo* clinical characteristics are not known.

What does this study add?

- Our results demonstrate for the first time that lipid content measured in cultured myotubes established from human volunteers is strongly associated with clinical phenotypes of the donor including maximal ATP production capacity (ATPmax), insulin sensitivity, proportion of oxidative type-I fibers and physical activity/fitness levels.
- Lipid metabolites – DAGs and ceramides are negatively associated with insulin sensitivity and ATPmax.
- Such results demonstrate that independent of the ambient milieu, skeletal muscle lipid retain important phenotypes from the donors and therefore myotubes can be used as a model for more mechanistic studies

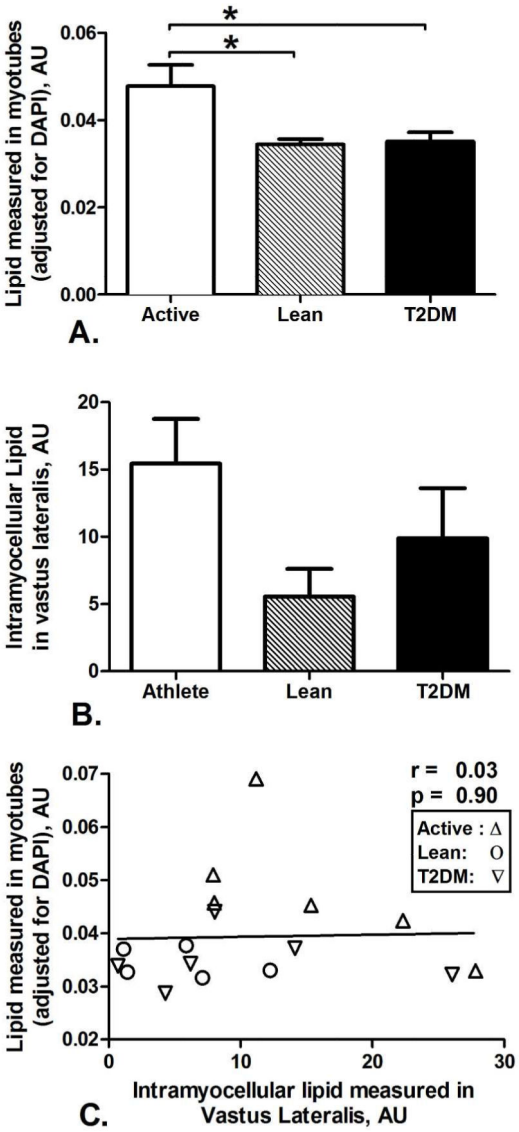

Figure 1.

Lipid measured in myotubes from sedentary lean (n=5) and donors with type 2 diabetes (n=6) is significantly lower compared to active (n=6) donors (Figure 1A). Intramyocellular lipid measured in *vastus lateralis* muscle (Figure 1B) did is not correlate with lipids measured in myotubes (Figure 1C). *p<0.05.

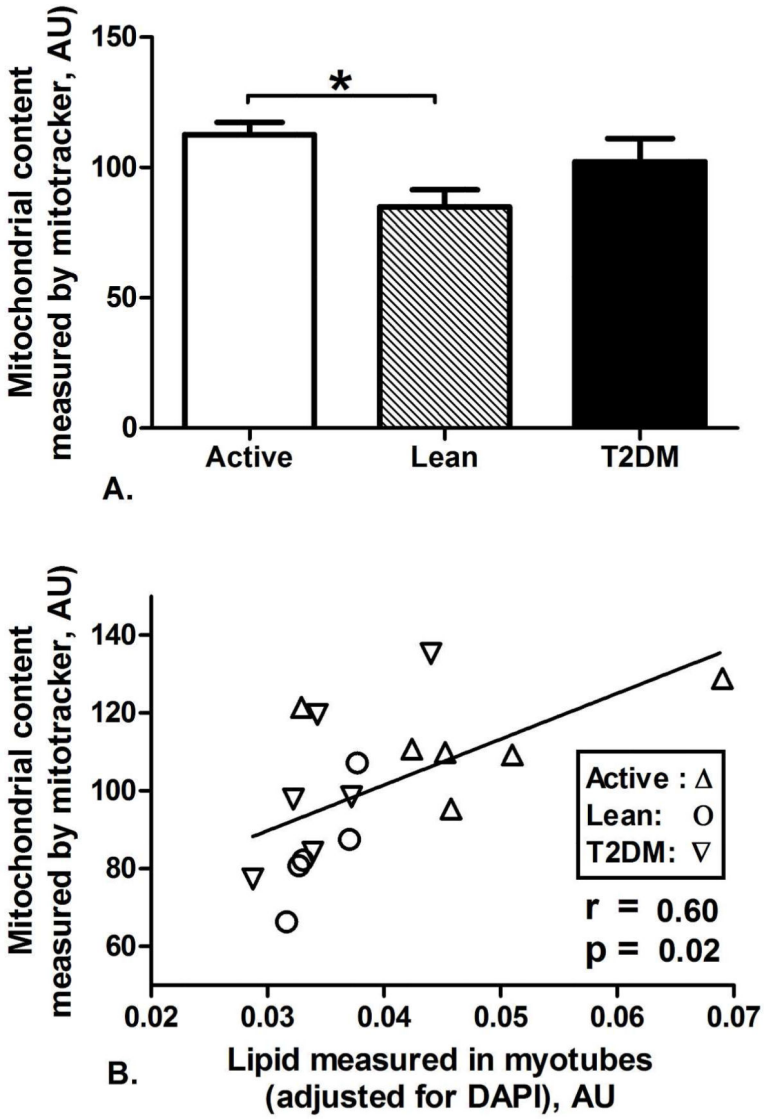

Figure 2.

Mitochondrial content in myotubes from active donors were significantly higher compared to sedentary lean donors (Figure 2A). Mitochondrial content was associated with lipid measured in myotubes (Figure 2B). *p<0.05.

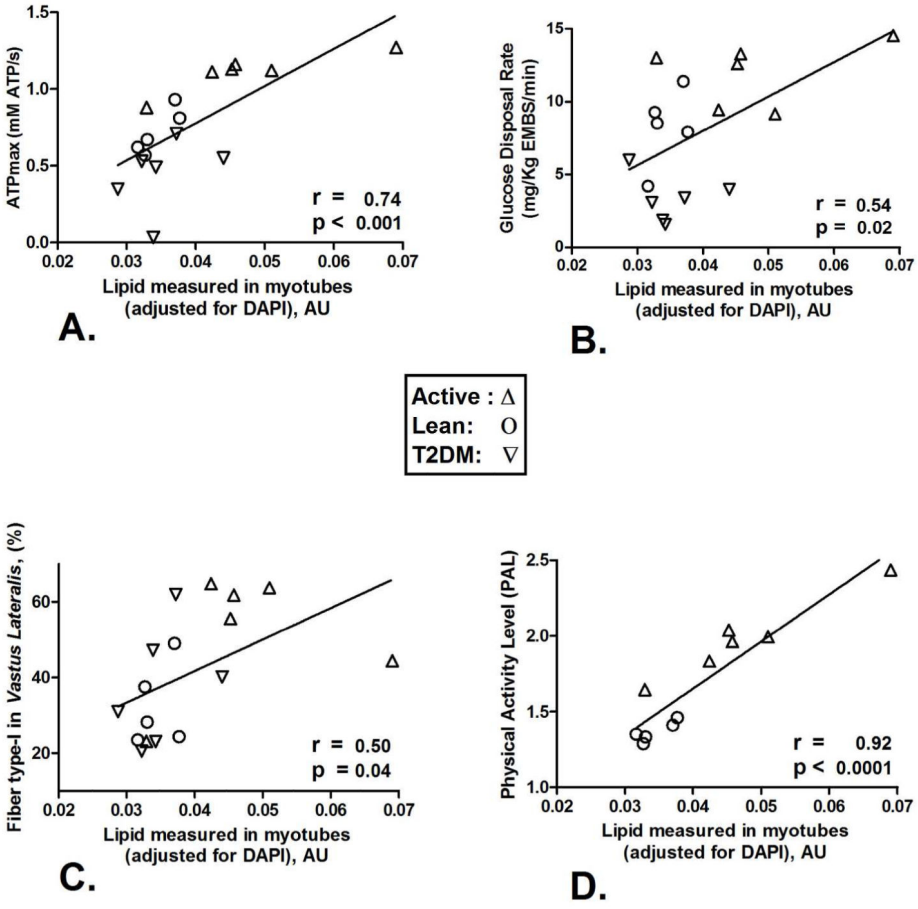

Figure 3.

Lipid measured in myotubes is correlated with donor's phenotypes such as maximal ATP synthesis rate (ATPmax) (A), glucose disposal rate (B), percent of type-I fiber in *vastus lateralis* muscle (C) and physical activity level (PAL) (D).

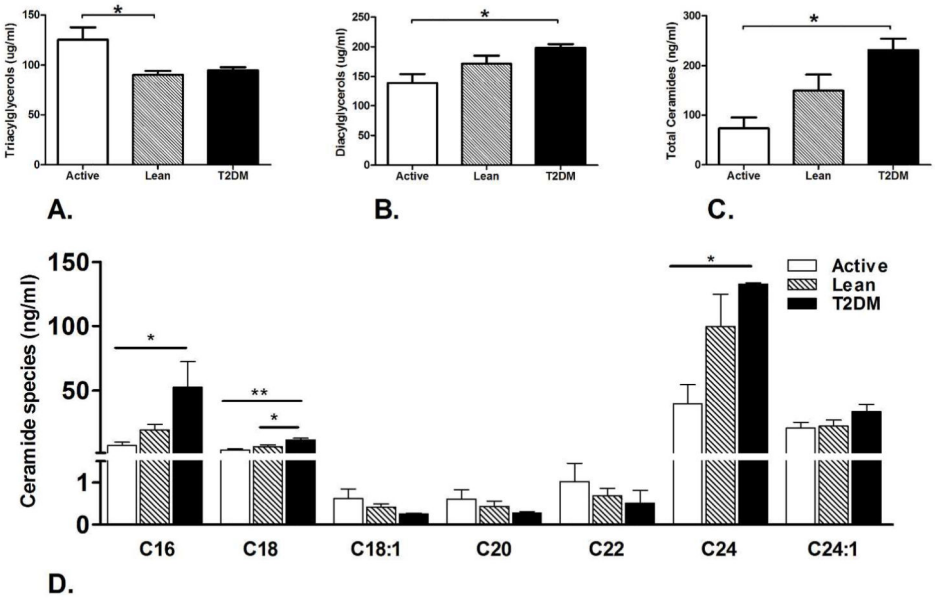

Figure 4.

Triacylglycerols measured in myotubes, were higher in active donors compared to sedentary lean donors (Figure 4A). Whereas, diacylglycerols (Figure 4B) and ceramides (Figure 4C), measured in myotubes, were significantly higher in donors with type 2 diabetes, compared to active. Ceramide species: C16, C18 and C24 were significantly higher in mytubes from donors with type 2 diabetes (Figure 4D). *p<0.05; **p<0.01

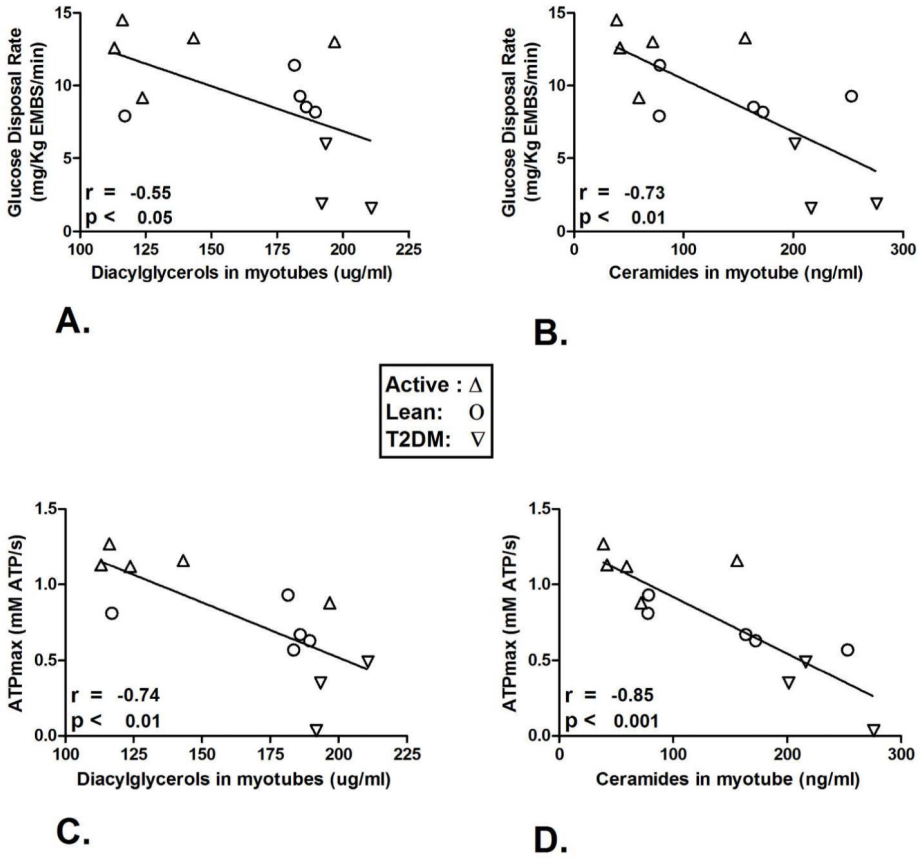

Figure 5.

Diacylglycerols and ceramides content in myotubes were negatively correlated with donor's ATP synthesis rate (Figure 5A-B) and glucose disposal rate (Figure 5C-D).

Table 1

Subject Characteristics.

	Active	Lean	T2DM
N (M/F)	6 (6/0)	6 (6/0)	6 (4/2)
Age (years)	23 ± 1	25 ± 1	43 ± 4*$
Weight (kg)	77.6 ± 3.2	71.4 ± 4.2	110 ± 6.5*$
BMI (kg/m^2)	24.4 ± 0.9	22.9 ± 0.9	40.2 ± 2.2*$
VO_{2max} (ml kg^{-1} min^{-1})	49.8 ± 1.4	35.3 ± 1.7	Not measured
GDR (mg/kg EMBS/min)	12.0 ± 0.9	8.2 ± 1.0*	3.3 ± 0.7*
ATPmax (mM/sec)	1.1 ± 0.1	0.7 ± 0.1*	0.5 ± 0.1*
ATPase (μM/sec)	7.2 ± 0.8	5.3 ± 0.4	3.1 ± 0.6*
Intramyocellular Lipid content (*Vastus Lateralis*, AU)	15.4 ± 3.3	5.6 ± 2.1	9.9 ± 3.7
Body fat (%)	13.6 ± 1.2	19.7 ± 1.4	36.7 ± 3.4*$
Fasting glucose (mmol/l)	4.8 ± 0.1	4.7 ± 0.2	6.9 ± 0.5*$
Fasting insulin (μU/ml)	3.5 ± 0.9	8.1 ± 2.1	29.4 ± 3.3*$

Data are presented as mean ± SE and analyzed using ANOVA.

*Values with are significantly different from active subjects.

$Values with are significantly different from lean. P<0.05 is considered significant. BMI, body mass index; VO_{2max}, maximal oxygen consumption; GDR, glucose disposal rate; EMBS, estimated metabolic body size.