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Effects of weight-loss and exercise on insulin resistance, intramyocellular triacylglycerol, diacylglycerol and ceramide

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ABSTRACT

Aims/hypothesis: Intramyocellular lipids, including diacylglycerol (DAG) and ceramides, have been linked to insulin resistance. This randomized repeated measures study examined the effects of diet-induced weight-loss (DIWL) or aerobic exercise (EX) on insulin sensitivity and intramyocellular triacylglycerol (IMTG), diacylglycerol (DAG) and ceramide.

Methods: Sixteen overweight to obese (BMI 30.6±0.8) adults (67.2±4.0 years) with either impaired fasting glucose, or impaired glucose tolerance completed one of two lifestyle interventions: DIWL (n=8) or EX (n=8). Insulin sensitivity was determined using hyperinsulinemic-euglycemic clamps. Intramyocellular lipids were measured in muscle biopsies using histochemistry and tandem mass spectrometry.

Results: Insulin sensitivity was improved with DIWL (20.6±4.7%) and EX (19.2±12.9%). Body weight and body fat were decreased by both interventions with greater decreases in DIWL compared to EX. IMTG content, muscle glycogen and oxidative capacity were all significantly (p<0.05) decreased with DIWL and increased with EX. DAG was decreased with DIWL (-12.4±14.6%) and EX (-40.9±12.0%). Ceramide decreased with EX (-33.7±11.2%), but not with DIWL. Dihydroceramide was decreased with both interventions. Sphingosine was only decreased with EX. Changes in total DAG, total ceramides and other sphingolipids did not correlate with changes in glucose disposal. SCD1 protein content was decreased with DIWL (-19.5±8.5,p<0.05), but increased with EX (19.6±7.4,p<0.05). DGAT1 was unchanged with the interventions.

Conclusions/interpretation: Diet-induced weight-loss and exercise training both improve insulin resistance and decrease DAG, while only exercise decreased ceramides despite having different effects on intramyocellular triacylglycerols and ceramide. These alterations may be mediated through differential changes in skeletal muscle capacity for oxidation and triacylglycerol synthesis.

Keywords: Insulin resistance, skeletal muscle, diacylglycerol, ceramide

Abbreviations: DAG, diacylglycerol. DGAT1, diacylglycerol acyltransferase 1. DXA, dual-energy X-ray absorptiometry. IMCL, intramyocellular lipids. IMTG, intramyocellular triacylglycerols. SCD1, stearoyl-Coenzyme A desaturase 1.

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INTRODUCTION

Skeletal muscle insulin resistance is a key feature in type 2 diabetes mellitus and in individuals at high risk for the development of diabetes [1]. Obesity and physical inactivity are major contributors to insulin resistance [2], although the exact mechanisms by which they cause insulin resistance are unknown. Over the last decade there has been considerable interest in the role of intramyocellular lipids (IMCL) in insulin resistance. Early evidence from cell culture [3], animal models [4] and human studies [5] strongly suggested that accumulation of intramyocellular triacylglycerols (IMTG) were responsible for insulin resistance of obesity and type 2 diabetes. We found that diet-induced weight-loss decreased IMCL in obese subjects with and without type 2 diabetes [6] in conjunction with enhanced insulin sensitivity [7]. We subsequently reported an 'athletes paradox' in which endurance-trained athletes are markedly insulin sensitive despite having high IMCL [8], a phenomenon we [9, 10] and others [11, 12] have confirmed with exercise training interventions. Accumulation of IMCL within the context of improved insulin sensitivity may be mediated through the lipogenic enzymes stearoyl-Coenzyme A desaturase 1 (SCD1) [13, 14] and diacylglycerol acyltransferase 1 (DGAT1) [15]. Thus, although weight-loss and exercise can both improve insulin sensitivity [7, 16] they may have distinctly different effects on IMCL [6, 9]. This hypothesis has not been directly tested in a randomized trial.

IMCL may represent a surrogate for other potentially lipotoxic metabolites such as diacylglycerol (DAG) and/or ceramide, which, in cell culture systems and animal models, have been demonstrated to directly mediate skeletal muscle insulin action [17]. The mechanisms linking skeletal muscle DAG and ceramide accumulation and insulin resistance involve activation of various isoforms of protein kinase C [18, 19] or inhibition of Akt signaling [20]. Hoy *et al*. [21] has recently challenged the notion that either DAG or ceramide within skeletal muscle is linked with insulin resistance. Following 5 hours of lipid oversupply in rodents, there were no changes in either DAG or ceramide content, yet several components of the insulin-signaling cascade were repressed. Data linking DAG or ceramide to human skeletal muscle insulin resistance are conflicting; while some studies have shown that DAG [22] and ceramide [18] are related to obesity or insulin resistance [23], others have failed to observe these associations [24, 25]. The complexity of these lipids, including their fatty acid composition, is one potentially key aspect underlying these inconsistencies that has not been adequately explored.

Human intervention studies designed to improve insulin sensitivity could provide the next level of evidence concerning whether DAG or ceramide play a role in skeletal muscle insulin resistance. Few such studies have been conducted [26]. While we [9] and others [26] have recently reported that exercise training improves insulin sensitivity and decreased both total ceramide and DAG in humans, direct comparisons of the effects of exercise and diet-induced weight-loss on insulin sensitivity, IMCL, DAG, ceramide and other sphingolipids have not been conducted. Determining intervention effects on the subspecies profile of these complex lipids using quantitative mass spectrometry could yield novel information about their role in insulin resistance. We tested the primary hypothesis that, despite opposing effects on IMCL, exercise and diet-induced weight-loss would both decrease DAG and ceramide content within skeletal muscle concomitant with improved insulin sensitivity in overweight sedentary adults. This study provides novel insight into the potential role of these complex lipids in human insulin resistance, and whether they may be modifiable targets to prevent or treat insulin resistance associated with obesity, aging or type 2 diabetes.

METHODS

Study subjects. Men and women aged 60-75 years were recruited though advertisements in the Pittsburgh area. Eligibility for participation included volunteers who were sedentary by self-report (structured exercise ≤1-day per week), were weight stable (<±3 kg in the previous 6 months), overweight to obese (BMI,25.0-38.0kg/m^2) and a non-smoker. Upon further medical screening at the Clinical Translational Research Center (CTRC), volunteers with uncontrolled hypertension (blood pressure>150 mmHg (20 kPa) systolic and >95 mmHg (12.7 kPa) diastolic), anemia (Hct<34%), elevated liver enzymes (25% above normal), proteinuria or hypothyroidism (sTSH>8) were excluded. Subjects taking chronic medications known to adversely affect glucose homeostasis were excluded. Individuals on anti-

hypertensive (n=7) and lipid lowering medications (n=6) were included and continued their pharmacotherapy throughout the study. Subjects with EKG abnormalities observed during the maximal aerobic capacity test or history of unstable coronary artery disease were excluded.

Following the medical screen, volunteers completed a 2h, 75-gram oral glucose tolerance test (OGTT). Volunteers were classified as: normal fasting glucose (<5.5 mmol/l), impaired fasting glucose (≥5.5 mmol/l), normal glucose tolerance (NGT n=6;2h OGTT plasma glucose <7.0 mmol/l), or impaired glucose tolerance (IGT, n=15;2h OGTT plasma glucose >7.8 mmol/l). Subjects provided written consent to the protocol approved by the University of Pittsburgh's Institutional Review Board.

Study groups. Volunteers with normal or impaired fasting glucose, NGT or IGT were randomized into one of two 16-week interventions; diet-induced weight-loss (DIWL) or exercise (EX). Glucose tolerance status distribution across intervention groups was: DIWL (IGT n=8) and EX (IGT n=4 and NGT n=4). Data are reported for the 16 volunteers (DIWL=8 and EX=8) who completed the interventions. The dropout rate was DIWL=~10% and EX=~10% (Figure 1).

Diet-induced weight-loss (DIWL): The goal of this intervention was to achieve a 10% weight-loss; a reduction of 2093.4-4186.8 kJ/day (based on recent food records/history) and low-fat (<30% of calories from fat) diet. A registered dietician administered the weight-loss program individually to subjects and provided a weekly dietary prescription. Subjects were kept relatively weight stable for the last two of the 16-week period (-0.54±0.79kg) to account for potential effects acute negative energy balance on key outcome variables.

Exercise (EX): Subjects progressed to 4-5 days/week, 45-min/session (~180 min/week) of moderate intensity (determined by heart rate or perceived exertion [10]) supervised and unsupervised exercise (mostly stationary cycling and some walking) for 16-weeks. Heart rate was recorded (Polar Electro Oy, Finland) during their exercise sessions to monitor exercise intensity and total volume. Subjects were instructed to maintain current diet throughout their intervention. Subjects met with the registered dietician biweekly for body weight assessment and dietary intake review.

Insulin sensitivity. Subjects were admitted to the CTRC on the evening before the glucose clamp, fed a standardized meal, and remained fasted overnight. The next morning (~7A.M.), insulin sensitivity was measured as rates of peripheral insulin-stimulated glucose disposal (R_d) during a 4-hr hyperinsulinemic (40 mU m^{-2} min^{-1}), euglycemic clamp [7]. To measure R_d and residual endogenous glucose production (EGP), a primed (0.22 μM/kg), continuous (17.6 μM kg^{-1} min^{-1}) infusion of [6,6-$^2H_2$] glucose was given. [6,6-$^2H_2$] glucose enrichment in plasma was determined by gas chromatography/mass spectrometry (6890 Network/5973 Series; Agilent, Santa Clara, CA). Glucose clamps were performed 36-48h after any exercise bout to minimize the acute effects of exercise on insulin sensitivity. All subjects were instructed to follow similar Pre and Post intervention diets for three days prior to the glucose clamp.

Blood chemistry. Fasting blood samples were obtained and processed. Plasma and serum were stored at -70°C until assays were performed. Fasting glucose was measured by the glucose oxidase method (2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH). Fasting and clamp insulin were measured by ELISA (Milipore, Billerica, MA).

Body composition and maximal aerobic capacity (VO_2max). Total body fat and lean mass were assessed using DXA (Lunar, GE Lunar Prodigy and Encore 2005 software v9.30). A VO_2max test [10] was employed on a cycle ergometer before and after the intervention to determine changes in physical fitness and to determine the appropriate intensity for exercise prescription for those enrolled in EX.

Skeletal muscle biopsy and tissue analysis. *Biopsy:* ~30min prior to the glucose clamp, a fasting percutaneous muscle biopsy was performed [8]. From the total sample (~100-300mg), a portion was prepared for histochemical analysis as previously described [9] and ~30mg flash frozen for lipid (diacylglycerol and ceramide) analysis. *Tissue analysis: (i) Intramyocellular lipid*: IMCL content was assessed using Oil Red O staining [6], *(ii) Oxidative capacity*: succinate dehydrogenase staining [10] was used as a marker of oxidative capacity, *(iii) Fiber type*: the percentage of type I, slow oxidative, and type IIa, glycolytic, fibers were determined using immunohistochemistry [10] with antibodies specific to the myosin heavy chain isoforms (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), *(iv) Glycogen*: glycogen content was determined using a periodic acid:Schiff's reagent staining and *(v) Diacylglycerol (DAG) and*

ceramide: DAG species, individual species of ceramide and selective sphingolipids were quantified using tandem mass-spectrometry [27]. Total DAG and ceramide content was calculated by the addition of the individual species that represented >90% (diacylglycerol, C16:0/16:0, C16:0/18:0, C16:0/18:1, C18:0/18:0, C18:0/18:1 and C18:1/18:1; ceramide, C16:0, C18:0, C24:0 and C24:1) of the identifiable lipid species, as a few lipid species were consistently at levels below detectable or quantifiable limits (diacylglycerol, C14:0/14:0, C14:0/16:0, C14:018:0, C14:018:1, C16:1/18:0, C16:1/18:1 and C16:1/16:1; ceramide, C14:0 and C20:0). Pre and post intervention muscle samples from the same subject were analyzed in parallel to avoid potentially confounding inter-assay variability influence on the intervention effects. *Stearoyl-Coenzyme A desaturase 1 (SCD1) and diacylglycerol acyltransferase 1 (DGAT1) protein expression:* Protein extracts were prepared from ~30-40mg skeletal muscle. Total SCD1 and DGAT1 levels were determined using standard immunoblot protocols. Briefly, liquid nitrogen crushed tissue was homogenized in cell lysis buffer (Cell Signaling, Boston, MA) with protease inhibitors (Roche, Indianapolis, IN). Samples were rocked for 1h at 4°C and centrifuged for 20min at 14000*g*. Total protein content was determined by BSA and prepped in 5x Laemmli to provide 30-50µg protein. Samples were loaded on 10%-SDS Ready Gels (BioRad, Hercules, CA), transferred to ImmunoBlot PVDF membranes and blocked with 5% non-fat milk in PBS tween-20 (0.05% v/v). Proteins for SCD1 (Alpha Diagnostics, San Antonio, TX) and DGAT1 (Abcam, Cambridge, MA) were explored using manufacture's recommendations with appropriate secondary antibodies. Blots were visualized using Immun-Star WesternC chemiluminescence (BioRad, Hercules, CA) and imaged with ChemiDoc XRS+ (BioRad, Hercules, CA). Densitometry was completed using ImageJ software. β-actin was used as the control protein.

Statistical analysis. Baseline group differences were assessed using a one-way analysis of variance (ANOVA). A two-way repeated-measures ANOVA was used to determine main (group, treatment) and interaction effects for all outcome variables. Log transformations were used when ANOVA assumptions of normality were not met. Paired t-tests were used to examine intervention effects when repeated-measured ANOVA indicated an interaction effect. Pearson's correlation coefficient was used to relate alterations in muscle lipids to changes in insulin sensitivity. Statistical significance was assumed *a priori* at $p<0.05$. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Mac,v18).

RESULTS

Study subjects.

Approximately equal numbers of males and females were included in each intervention group (Table 1). All but four subjects had either impaired fasting glucose or IGT. Baseline 2h OGTT values were not different between intervention group.

Body composition and physical fitness. There were no baseline differences in body weight or composition between the intervention groups (Table 1). Each intervention decreased ($p<0.05$) body weight, BMI and fat-mass (Table 1). Diet-induced weight-loss (DIWL) reduced body weight (-8.5±1.5%) to a significantly greater extent ($p<0.05$) than exercise (-1.8±0.9%). A similar pattern was observed in fat mass. Fat-free mass (FFM) was significantly decreased with DIWL (-3.9±0.8%) and increased slightly in EX (1.0±0.7%, $p<0.05$).

Baseline aerobic fitness levels (VO_{2max}, Table 1) were similar among the groups. Aerobic capacity tended to increase with EX ($p=0.07$), but not with DIWL ($p=0.20$) (Table 1). Maximal power output was higher following EX (93.6±6.6 to 99.5±8.0 Watts,$p=0.05$), but not with DIWL (94.0±6.1 to 97.2±4.1 Watts,$p=0.30$). The EX group was adherent to the program as evidenced by average exercise intensity (72.9% VO_{2max}, range 65.0%-86.8%; 77.4% of maximal heart rate, range 70.0%-85%); confirmed from Polar heart rate recordings and exercise sessions per week (3.9 days, range 2.7-5.7 days).

Insulin Sensitivity. Baseline insulin sensitivity was similar among groups. Insulin-stimulated glucose disposal was improved ($p<0.05$) in DIWL (20.6±4.8%) and EX (19.2±12.9%) (Figure 2). Insulin suppression of EGP was improved with DIWL (-8.2±4.1%,$p=0.05$), but not altered with EX (-2.1±9.2%).

Fasting glucose and insulin values were not different among the groups (Table 1). DIWL reduced fasting glucose (-5.9±3.9%,p=0.07), while EX did not (1.5±3.6%,p=0.40). Fasting insulin was reduced in DIWL (-33.5±1.5%,p=0.04), but not with EX (10.4±10.7%,p=0.50). Steady-state insulin levels during the clamp were not different between the groups (DIWL, 373.1±21.6pmol/l and EX, 337.8±23.1pmol/l) and were unchanged in response to the intervention.

Intramyocellular lipids, oxidative capacity and glycogen content. No baseline differences were observed in intramyocellular lipids (IMCL), succinate dehydrogenase activity (SDH), or glycogen content. Baseline differences in the diacylglycerol (DAG) species Di-14:0 and Di-18:0, total ceramide content and ceramide species C20:0, C24:0 and C24:1 were observed. No other baseline differences were noted. Thus, subsequent analyses were weighted to the baseline measure.

DIWL reduced IMCL content (16.0±3.2%,p=0.01), while EX increased IMCL content (40.8±18.2%,p=0.03, Figure 3a). SDH was decreased by DIWL (-12.2±5.2%,p=0.04), but increased by EX (19.9±13.2%,p=0.05, Figure 3b). In order to examine whether changes in the proportion of type I muscle fibers, which contain more IMCL as triacylglycerols [23], influence changes in total IMCL content, we also examined changes in IMCL within specific fiber types. Although the changes in the proportion of type I, slow-oxidative fibers were not statistically significant (DIWL=-4.9±8.1% and EX=6.5±7.5%), EX tended (p=0.07, n=6) to increase IMCL content specific to type I fibers, while WL (n=5) tended (p=0.10) to decrease IMCL within type I fibers. Glycogen content (Figure 3c) was decreased with DIWL (-16.8±4.6%,p=0.04), but increased with EX (13.9±3.3%,p=0.05).

We have recently published quantitative DAG and sphingolipid profiles within human muscle [23]. Much of the DAG data did not meet the ANOVA assumption of normality, thus the data were log transformed and analyzed. Because the EX group did lose a minimal, albeit significant, amount of body weight, changes in body weight were used as a covariant in subsequent analyses. At baseline, no individual DAG species or total DAG content correlated with insulin sensitivity when all subjects were combined (n=16). Both DIWL and EX decreased total DAG content (Figure 4a). Both DIWL and EX decreased several of the DAG species, with EX decreasing C14:0/18:0, C16:1/18:0, C16:1/18:1, C18:0/18:0, DI-C16:1, DI-C18:0 and DI-C18:1 to a greater degree DIWL (Table 2). Although the data did not reach statistical significance, EX tended (p=0.10) to decrease total DAG to a greater degree than DIWL. (Absolute values for DAG species,Supplemental Table 1).

The sphingolipid data did not meet ANOVA assumptions, thus the data were log transformed and analyzed. Baseline total ceramide and several individual ceramide species were inversely ($p\leq0.05$) related to insulin sensitivity when all subjects were combined (n=16, total ceramide R=-0.54, C18:0 R=-0.43, C18:1 R=-0.39, C20:0 R=-0.45, C24:0 R=-0.53 and C24:1 R=-0.43). No relation between other sphingolipids and insulin sensitivity was observed at baseline. Total ceramide content was decreased in EX ($p<0.05$), but not with DIWL (Figure 4b). All ceramide species were decreased with EX, while only C14:0, C20:0 and C24:0 ceramides were decreased with DIWL (Table 3). Dihydroceramide was decreased with both interventions (Table 3, Figure 4c). Sphingosine was significantly decreased with EX ($p<0.05$), but not DIWL (Table 3, Figure 4d). Sphingosine 1-phosphate did not change (Figure 4e). (Absolute values for sphingolipid species,Supplemental Table 2). Decreases in both C16:0 and C24:1 ceramide were significantly ($p\leq0.05$) correlated with improved insulin-stimulated glucose disposal, while the change in total ceramide and other sphingolipids did not correlate to changes in insulin sensitivity.

Protein expression. No baseline differences were observed for protein expression of stearoyl-Coenzyme A desaturase 1 (SCD1) or diacylglycerol acyltransferase 1 (DGAT1). No relation between protein expression of SCD1 or DGAT1 and insulin sensitivity was observed. SCD1 expression was significantly decreased (-19.5±8.5,p=0.01) with DIWL, but increased with EX (19.6±7.4, Figure 5a). No differences were observed in the protein expression of DGAT1 (Figure 5b). The change in protein expression of SCD1 and DGAT1 did not predict improvements in insulin sensitivity. Change in SCD1 (delta *vs* delta) was positively correlated to IMCL content (R=0.56) and negatively correlated to ceramide C16:0 (R=-0.64), C24:0 (R=-0.52) and total ceramide (R=-0.55), all $p<0.05$. Change in DGAT1 was negatively correlated to ceramide C20:0 (R=-0.81), C24:1 (R=-0.76) and dihydroceramide C16:0 (R=-0.82).

DISCUSSION

Both diacylglycerol (DAG) and ceramide have been implicated in the etiology of insulin resistance. While cell systems and animal studies have demonstrated that these lipids can cause insulin resistance in particular situations [17, 19], studies in humans have only provided circumstantial, and contradictory, evidence regarding their potential role in skeletal muscle insulin resistance [24, 28]. Therefore, the main purpose of this study was to examine whether or not changes in both the content and fatty acid profile of DAG, ceramide species and certain other sphingolipids correspond to improvements in insulin sensitivity with either diet-induced weight-loss or exercise in humans.

The primary novel findings of our study were that both diet-induced weight-loss and exercise training decreased intramyocellular DAG content, while exercise decreased many individual subspecies and total ceramide content to a greater degree than diet-induced weight-loss. These decreases in DAG and ceramide with the interventions did not parallel changes in total intramyocellular lipid (IMCL) content; diet-induced weight-loss decreased IMCL, while exercise training increased IMCL. Our study provides the first evidence concerning the separate effects of weight-loss and exercise on these toxic lipids in human skeletal muscle, offering potential mechanisms by which lifestyle interventions may improve insulin sensitivity.

The decrease in skeletal muscle DAG by both exercise and weight-loss corroborates a link between skeletal muscle DAG and insulin resistance [19, 29]. However, the lack of correlation between changes in DAG and improvements in insulin sensitivity suggests that DAG is not a sole mediator insulin action [30]. Although we [9] and others [26] have previously reported exercise-induced decreases in DAG, this is the first study to directly compare the independent effects of weight-loss and exercise on total and various species of DAG in human skeletal muscle. Diet-induced weight-loss decreased DAG in parallel with IMCL, while exercise decreased DAG, but increased IMCL. These findings are supported by the observation that exercise decreases DAG [9, 26] but increases IMCL [9, 10]. It is not clear why exercise induced greater reductions in specific subspecies of DAG than those observed with diet-induced weight-loss. Although the exercise group did lose weight, the data do not support a combined effect of weight-loss and exercise on IMCL or insulin sensitivity. DAG within skeletal muscle serves as precursors for IMTG synthesis, is a substrate molecule, are components of cell membranes and act as lipid-signaling second messengers [31]. Our data do not discern the subcellular localization of DAG, their source or the specific roles they have within the cell. Nevertheless, our data suggest a repartitioning of free fatty acids away from DAG, which is likely deleterious to insulin signaling, into neutral IMCL stores with exercise training or a decrease in total lipid content (i.e. DAG and IMCL) with diet-induced weight-loss.

Another key finding in our study was that exercise, but not diet-induced weight-loss, significantly decreased ceramide content within muscle concomitant with improved insulin sensitivity. This is in agreement previous observations [9, 26] that exercise training decreases skeletal muscle ceramide content. These data suggest that exercise training may decrease ceramide content through a shift in fatty acid partitioning away from *de novo* ceramide synthesis toward triacylglycerol formation and/or increased fatty acid oxidation within muscle. The lack of significant correlations between changes in ceramide and improved insulin sensitivity suggest that alterations in ceramide, like DAG, are not solely required to improve insulin action. The quantification ceramide subspecies and other sphingolipids is another novel aspect of this study. We observed that exercise resulted in significant decreases in both dihydroceramide and sphingosine. Since dihydroceramide may not be as bioactive as ceramide and may only serve only as a ceramide precursor [32], this decrease may not have a functional consequence. Alternatively, because sphingosine, an intermediate between ceramide and sphingosine-1-phosphate [32], activates protein kinase C (PKC) [33], increases in this lipid may impair insulin action. In fact, we observed significant positive correlations between changes in DAG C14:0/16:0, C14:0/18:1, C16:1/18:0, C16:1/18:1, DI-C14:0, DI-C16:1 and DI-C18:1 and changes in sphingosine in support of the sphingosine-DAG-PKC pathway. It is unclear why sphingosine or ceramide did not decrease with weight-loss. Perhaps exercise induces additional or independent effects on insulin sensitivity compared to weight-loss through reductions in ceramides or other sphingolipids within muscle. The effect of exercise, but not diet-induced

weight-loss, to decrease specific sphingolipids and enhance the oxidative capacity in muscle is consistent with our report that ceramide content is associated with both insulin resistance and lower oxidative capacity of muscle [23].

Repartitioning of skeletal muscle lipids with lifestyle interventions concomitant with improved insulin sensitivity may be related to changes in key lipogenic enzymes. We report significant decreases in stearoyl-Coenzyme A desaturase 1 (SCD1) with diet-induced weight-loss, but higher levels following exercise. This rate-limiting enzyme that converts saturated fatty acids to monounsaturated fatty acids has been implicated in the development of obesity [34]. Overexpression of SCD1 in muscle cells results in a protection from lipid-induced insulin resistance [14, 35] and muscle protein expression is increased following exercise training [13]. These studies, coupled with our observation of a significant correlation between SCD1 expression and IMCL suggest that SCD1 is a key regulator of lipid partitioning within human skeletal muscle. Further, our data suggest that SCD1 likely contributes to decreases in several ceramide subspecies, supported by evidence of decreased ceramide content following acute exercise concomitant with increased IMCL and insulin sensitivity [13]. In accord with previous exercise studies [36, 37], we report no changes in diacylglycerol acyltransferase 1 (DGAT1) protein expression, which catalyzes the final step in triacylglycerol synthesis. Increased DGAT1 protein expression following acute exercise [13] suggests tightly coupled protein regulation in response to lipolytic stimuli. Finally, our observation of a negative correlation between DGAT1 and several ceramide species suggests an influence of DGAT1 in the repartitioning of fatty acids from deleterious lipid pools to more neutral IMCL stores.

Despite our novel observations there were study limitations. We did not have a control group; each subject acted as their own control in a randomized, repeated-measures design. Low statistical power prevented a prospective gender-specific analysis. The exercise-training stimulus may not have been sufficient to induce significant changes in fitness [38], yet still robust enough to elicit changes in oxidative capacity of muscle, IMCL and insulin sensitivity. The intervention effects on IMCL and insulin sensitivity were possibly mediated or modulated through changes in glycogen content or muscle fiber type, which were differentially affected by weight-loss and exercise. The fatty acid composition of the diets may have affected the composition of DAGs or sphingolipids [39]. Thus, future studies are warranted to elucidate the potential compartmental differences in lipid intermediates, the role of individual DAG/sphingolipid subspecies, and optimal weight-loss and/or exercise regimens to influence lipid partitioning within the context of improved insulin sensitivity in humans.

In summary, corresponding with improved insulin sensitivity, both diet-induced weight-loss and exercise decrease DAG content within skeletal muscle, while only exercise training appears to decrease ceramide and other sphingolipids. Despite similar improvements in insulin sensitivity, weight-loss and exercise have opposing effects on intramyocellular triacylglycerols and the oxidative capacity of skeletal muscle. Taken together, this study supports the concept that DAG and ceramide within skeletal muscle may be modifiable targets for interventions designed to improve insulin sensitivity in humans. Moreover, that lifestyle interventions repartition these deleterious lipids through alterations in fatty acid oxidation and triacylglycerol synthesis. However, given a lack correlation between alterations in these bioactive lipids and insulin sensitivity supports the concept multiple causal pathways and subsequently multiple targets for improving insulin action in human muscle. Additional investigations are needed to address whether these diet-induced weight-loss and exercise-induced changes in specific species of these complex lipids underlie or are merely coincidental with improved insulin action.

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Table 1. Subject characteristics, body composition, glucose homeostasis and fitness.

	DIWL		EX	
	Pre	Post	Pre	Post
N (male/female)	8 (3 / 5)		8 (4 / 4)	
Age (years)	66.9 ± 1.7		68.4 ± 1.5	
Body weight (kg)	86.3 ± 3.5	78.9 ± 3.1*	84.4 ± 2.8	82.7 ± 2.3*†
BMI (kg/m^2)	31.2 ± 1.2	28.4 ± 1.2*	30.0 ± 1.0	29.5 ± 0.9*†
Fat mass (kg)	37.4 ± 2.1	32.1 ± 2.4*	33.5 ± 3.1	31.8 ± 2.9*†
Fat-free mass (kg)	45.2 ± 2.8	43.4 ± 2.6	47.4 ± 2.4	48.5 ± 2.3†
Fasting glucose (mmol/L)	5.8 ± 0.2	5.4 ± 0.1	5.2 ± 0.2	5.3 ± 0.1
OGTT 2-hr glucose (mmol/L)	8.3 ± 0.6		7.8 ± 0.7	
Fasting insulin (pmol/L)	52.5 ± 18.7	30.8 ± 8.8*	39.4 ± 6.8	39.7 ± 6.1†
VO_2max (mL kg_{FFM}^{-1} min^{-1})	30.7 ± 2.1	32.1 ± 1.9	31.5 ± 2.1	32.8 ± 2.3

Data are mean±SEM. DIWL, diet-induced weight-loss. EX, exercise. BMI, body mass index. OGTT, Oral glucose tolerance test. A two-way (group x time) repeated measures analysis of variance was used to assess differences. *$p<0.05$ within-group intervention effect. † $p<0.05$ between-groups difference.

Table 2: Percent changes in individual and total diacylglycerol by intervention group

	DIWL	EX
N (male/female)	8 (3/5)	8 (4/4)
Diacylglycerol species		
C14:0/16:0	-31.6±12.6*	-41.3±16.0*
C14:0/18:0	-17.1±20.9*	-42.9±12.7*
C14:0/18:1	-22.2±13.1*	-57.6±12.9*†
C16:0/18:0	-21.4±10.6*	-34.2±12.7*
C16:0/18:1	-8.5±13.1*	-51.8±11.1*
C16:1/18:0	-16.9±11.1	-63.9±8.9*†
C16:1/18:1	-27.8±14.4*	-66.5±10.7*†
C18:0/18:1	-15.3±5.8*	-57.8±9.9*†
DI-C14:0	-41.2±8.6*	-58.1±10.9*
DI-C16:0	-26.7±10.8*	-52.9±14.7*
DI-C16:1	-41.3±15.3	-55.0±16.2* †
DI-C18:0	-8.8±18.4	-48.4±13.9*†
DI-C18:1	-22.8±8.8 *	-64.1±11.2 *†
Total DAG	-12.4±14.7*	-40.9±12.0*

Data are mean±SEM. DIWL, diet-induced weight-loss. EX, exercise. A two-way (group x time) repeated measures analysis of variance was used to assess differences. Data were log transformed when ANOVA assumption of normality was not met. *$p<0.05$ within-group intervention. † $p<0.05$ between-groups difference.

Table 3: Percent changes in individual and total ceramide and other sphingolipids by intervention group

	DIWL	EX
N (male/female)	8 (3/5)	8 (4/4)
Ceramide species		
C14:0	-14.8±19.3*	-33.1±15.2*
C16:0	40.6±26.1	-46.4±18.8
C18:0	20.8±19.2	-45.1±12.3*†
C18:1	37.9±20.8	-44.1±13.6
C20:0	-7.4±19.6*	-44.7±12.8*
C24:0	-20.1±12.6*	-40.3±11.1*
C24:1	15.1±19.3*	-44.3±12.3*†
Total ceramide	1.6±13.6	-36.1±11.8*†
Sphingolipids		
Dihydroceramide	-11.8±17.4*	-44.0±11.3*
Sphingosine	135.2±110.0	-19.1±16.1*†
Sphingosine 1-phosphate	36.7±38.0	-28.1±30.5

Data are mean ± SEM. DIWL, diet-induced weight-loss. EX, exercise. A two-way (group x time) repeated measures analysis of variance was used to assess differences. Data were log transformed when ANOVA assumption of normality was not met. *$p<0.05$ within-group intervention. † $p<0.05$ between-groups difference.

FIGURE LEGENDS.
Figure 1. Patient flow diagram.

Figure 2. Insulin sensitivity Pre (white bars) and Post (black bars) a 16-week lifestyle intervention of diet-induced weight-loss (DIWL) or aerobic exercise (EX). DIWL n=8, EX n=8. The rate of insulin-stimulated glucose disposal (R_d) was determined using as described in *Methods* and normalized to fat-free mass (FFM). Data are mean±SEM. Group and intervention effects were explored using a 2-way (group x time) repeated measures analysis of variance. **p*<0.05 within-group intervention effect.

Figure 3. Skeletal muscle substrate storage and capacity for oxidation Pre (white bars) and Post (black bars) a 16-week lifestyle intervention of diet-induced weight-loss (DIWL) or aerobic exercise (EX). DIWL n=8, EX n=8. All measures were performed on biopsy samples of the vastus lateralis. Intramyocellular lipid (IMCL), as determined by Oil Red O staining, content (*Panel a*), succinate dehydrogenous (SDH) activity staining (*Panel b*) and glycogen content (*Panel c*) were determined using histochemical analyses as described in *Methods*. Data are mean±SEM. Group and intervention effects were explored using a 2-way (group x time) repeated measures analysis of variance. **p*<0.05 within-group intervention effect. †*p*<0.05 between-groups difference.

Figure 4. Skeletal muscle diacylglycerol (DAG) and ceramide Pre (white bars) and Post (black bars) a 16-week lifestyle intervention of diet-induced weight-loss (DIWL) or aerobic exercise (EX). DIWL n=8, EX n=8. Data for DAG (*Panel a*), ceramide (*Panel b*), dihydroceramide (*Panel c*), sphingosine (*Panel d*) and sphingosine 1-phosphate (*Panel e*) were analyzed using tandem mass-spectrometry as described in *Methods*. Data are normalized to the "Pre" intervention values of each group. Group and intervention effects were explored using a 2-way (group x time) repeated measures analysis of variance. Data were log transformed when ANOVA assumption of normality was not met. **p*<0.05 within-group intervention effect. †*p*<0.05 between-groups difference.

Figure 5. Skeletal muscle stearoyl-Coenzyme A desaturase 1 (SCD1) and diacylglycerol acyltransferase 1 (DGAT1) Pre (white bars) and Post (black bars) a 16-week lifestyle intervention of diet-induced weight-loss (DIWL) or aerobic exercise (EX). DIWL n=8, EX n=8. Skeletal muscle protein expression for SCD1 (*Panel a*) and DGAT1 (*Panel b*) were determined by Western blot analysis as described in Methods. Data are normalized to the "Pre" intervention values of each group. Group and intervention effects were explored using a 2-way (group x time) repeated measures analysis of variance. **p*<0.05 within-group intervention effect. †*p*<0.05 between-groups difference.

1

Figure 1

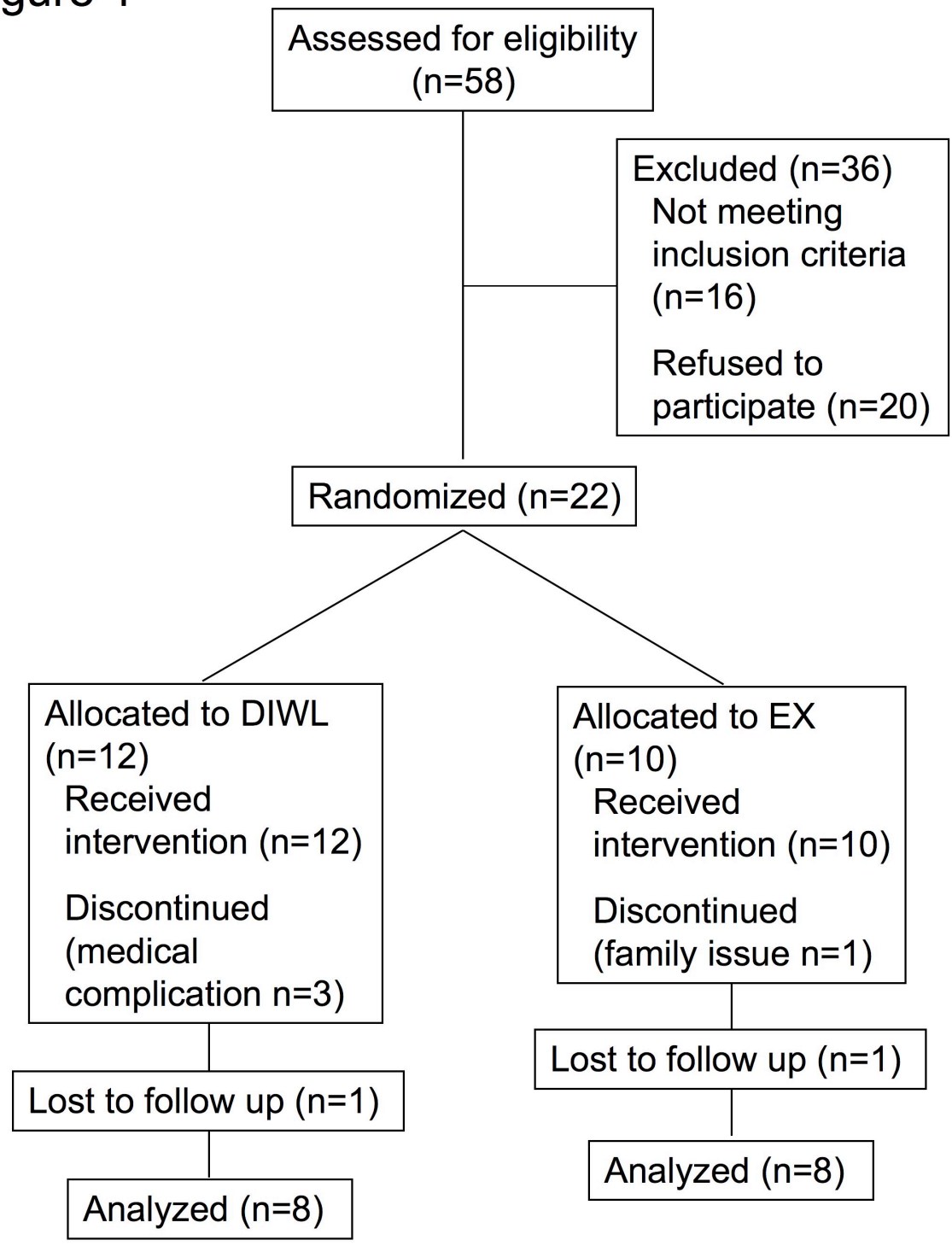

Assessed for eligibility (n=58)
Excluded (n=36)
Not meeting inclusion criteria (n=16)
Refused to participate (n=20)
Randomized (n=22)
Allocated to DIWL (n=12)
Received intervention (n=12)
Discontinued (medical complication n=3)
Allocated to EX (n=10)
Received intervention (n=10)
Discontinued (family issue n=1)
Lost to follow up (n=1)
Lost to follow up (n=1)
Analyzed (n=8)
Analyzed (n=8)

Figure 2

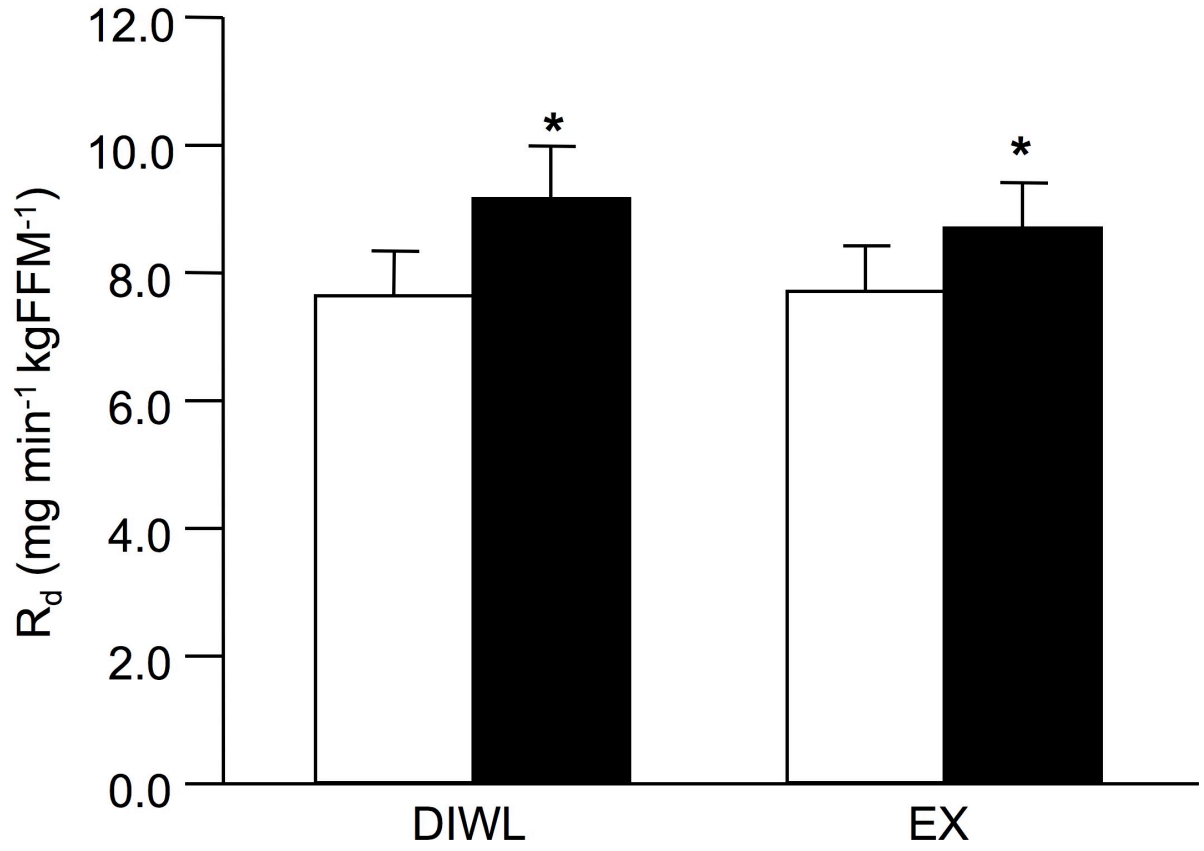


Figure 3

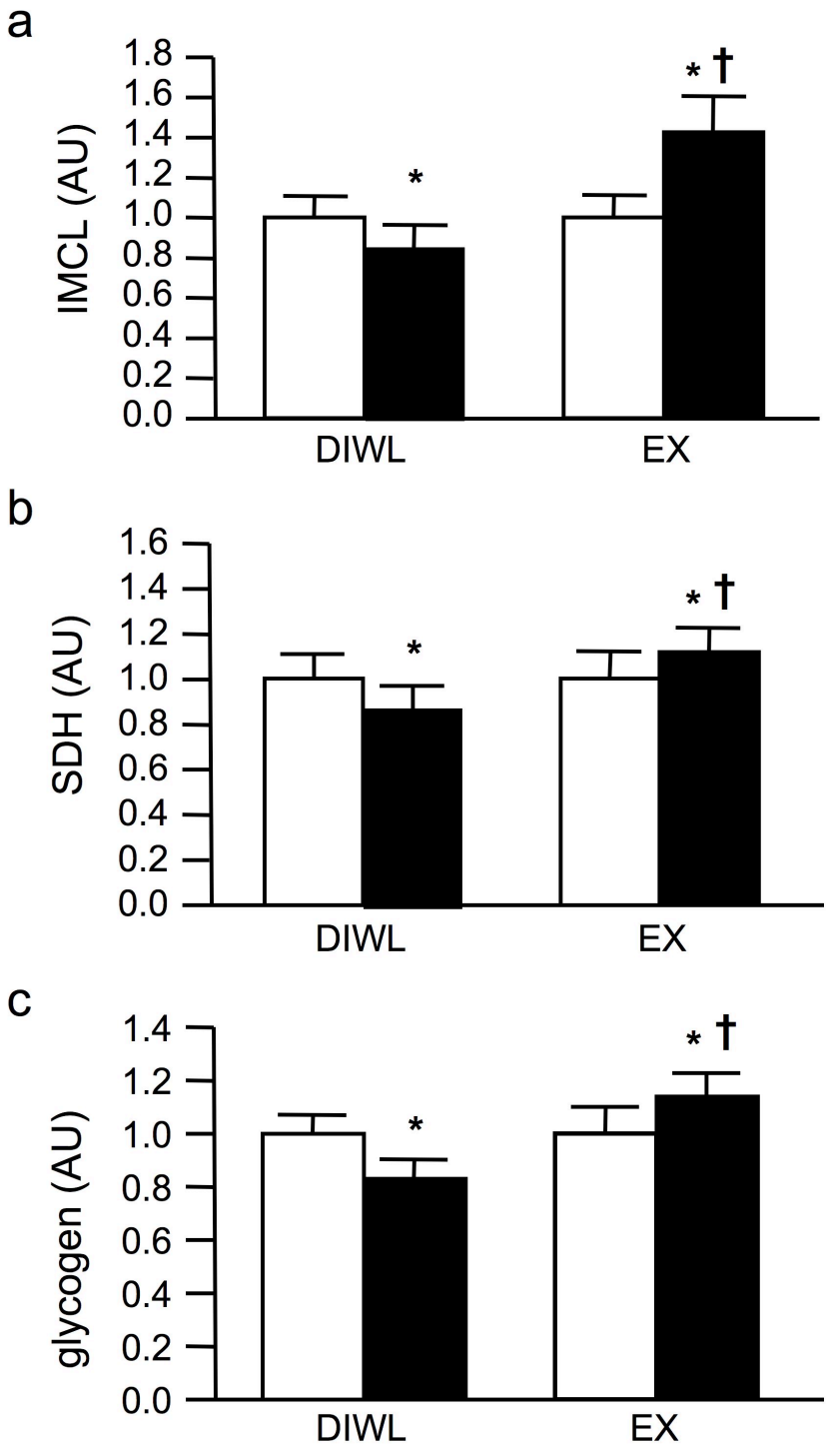

a
IMCL (AU)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
* †
DIWL
EX
b
SDH (AU)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
* †
DIWL
EX
c
glycogen (AU)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
* †
DIWL
EX

Figure 4

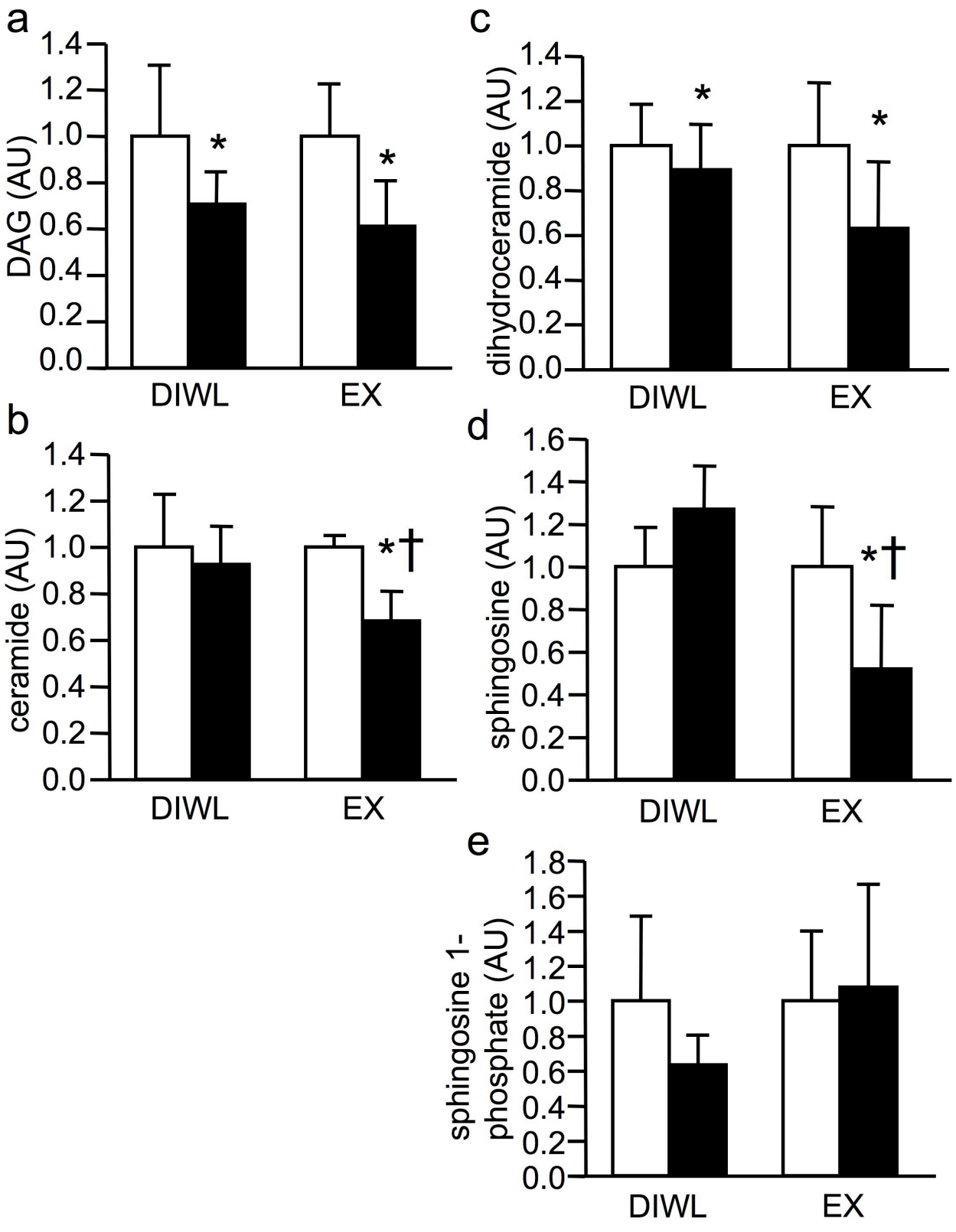
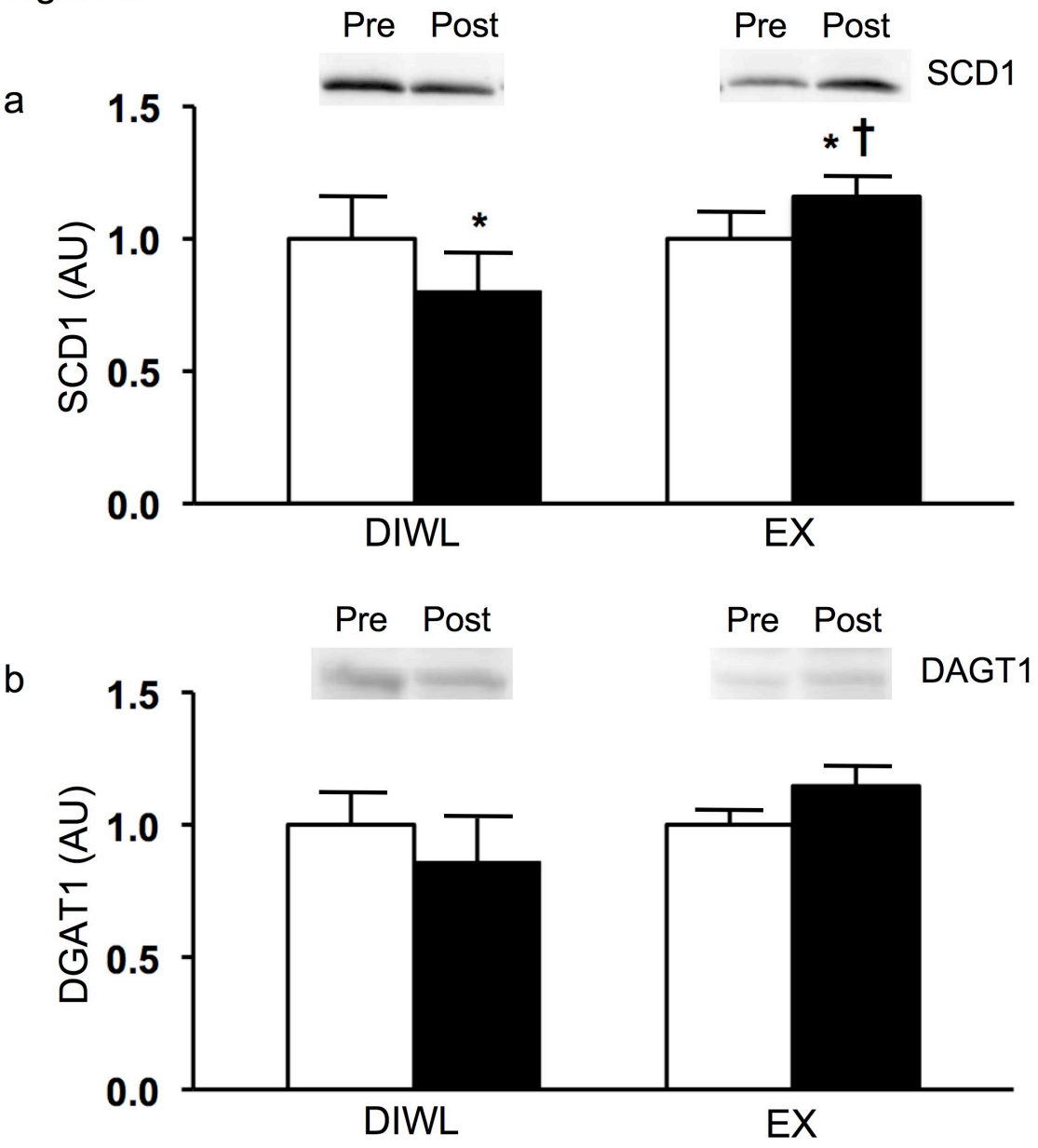

a
DAG (AU)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
DIWL
EX
c
dihydroceramide (AU)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
DIWL
EX
b
ceramide (AU)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*†
DIWL
EX
d
sphingosine (AU)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*†
DIWL
EX
e
sphingosine 1-phosphate (AU)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
DIWL
EX

Figure 5


a
Pre
Post
Pre
Post
SCD1
1.5
1.0
0.5
0.0
SCD1 (AU)
*
* †
DIWL
EX
b
Pre
Post
Pre
Post
DAGT1
1.5
1.0
0.5
0.0
DGAT1 (AU)
DIWL
EX