

# Weight Loss Reduces IMAT and Liver Fat Compared to Exercise: Implications for Muscle Quality and Metabolic Health

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Running Title: Weight Loss Improves Muscle Quality and Liver Steatosis

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## FUNDING ACKNOWLEDGEMENTS

This work was partially supported by the National Institutes of Health General Clinical Research Center grant RR-00036, National Institutes of Health R01 grant R01DK111559 to Bryan C. Bergman

**ClinicalTrials.gov** (NCT03077360)

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**Disclosure Summary:** The authors declare no competing interests.

**Keywords:** Intermuscular adipose tissue, lipidomics, liver steatosis, muscle quality, obesity

ACCEPTED MANUSCRIPT

## ABSTRACT

**Background.** Weight loss improves insulin sensitivity and liver fat but reduces lean mass. Whether reductions in intermuscular adipose tissue (IMAT) exceed muscle loss and how these effects compare with endurance exercise training without weight loss remains unclear.

**Methods.** In a 12-week randomized intervention, forty-six individuals with obesity were assigned to weight loss (WL - age  $39.7 \pm 1.3$ ; BMI  $35.2 \pm 0.9$ ; W/M 8/9), endurance exercise training without weight loss (EX - age  $39.7 \pm 1.6$ ; BMI  $36.0 \pm 1.1$ ; W/M 8/8), or delayed-control intervention groups (age  $39.8 \pm 1.4$ ; BMI  $36.8 \pm 1.3$ ; W/M 6/7). Changes in IMAT volume, skeletal muscle mass, and liver fat measured by MRI; insulin sensitivity assessed by hyperinsulinemic–euglycemic clamp; and plasma lipidomics and metabolomics by LC-MS based methods.

**Results.** WL reduced body weight (-10.5%;  $p < 0.001$ ), liver steatosis (-33.1%;  $p < 0.0001$ ), and IMAT volume (-12.7%;  $p < 0.0001$ ), while improving insulin sensitivity (42%;  $p = 0.004$ ). IMAT decreased significantly more than skeletal muscle mass (-4.2%,  $p = 0.17$ ), indicating improved muscle quality. EX increased insulin sensitivity (23%;  $P = 0.04$ ) and  $VO_{2peak}$  (7.6%;  $p < 0.001$ ) but did not significantly change IMAT or liver fat. DXA overestimated muscle loss compared with MRI. WL decreased plasma sphingolipids and diacylglycerols, whereas EX reduced acylcarnitines. Plasma triacylglycerols and branched-chain amino acids were strongly correlated with liver fat, and triacylglycerols showed the strongest association with IMAT volume.

**Conclusions.** Weight loss is more effective than endurance exercise training without weight loss for reducing IMAT and liver steatosis, with IMAT loss exceeding muscle loss. Plasma lipids and metabolites signatures are associated with liver fat and IMAT, supporting their potential utility as non-invasive biomarkers.

## INTRODUCTION

Loss of muscle mass during weight loss is attracting considerable attention with new anti-obesity medications, and there is concern whether the loss of muscle mass is appropriate or more dramatic than expected for the amount of weight lost (1). Weight loss is known to decrease muscle mass, and several reports also showed decreased intermuscular adipose tissue (IMAT) content (2-4). However, the relative change in IMAT compared to muscle mass is less clear. Weight loss may improve muscle quality, if the decrease in IMAT outpaces the loss of muscle mass (3), yet this is not a consistent observation (5). The most effective lifestyle intervention to decrease IMAT is also not clear. Caloric restriction with or without exercise training resulted in similar decreases in IMAT content (5,6). Yet weight loss and exercise training have also been reported not to change IMAT content (7). Exercise training alone has also been reported to decrease IMAT (8,9), prevent its accumulation over time (10-12), or not change IMAT content (13). Differences between these reports, such as duration and amount of weight loss, duration and intensity of exercise training, as well as population differences between studies may help explain variable responses in the literature. Combined, the independent effects of weight loss and exercise training on IMAT content are unclear.

In patients with Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), weight loss and exercise training decrease hepatic fat and are the foundation for lifestyle interventions (14,15). Weight loss alone consistently decreases hepatic fat in individuals without existing liver disease with the amount of weight lost predicting the decrease in hepatic fat fraction (16,17). In individuals without MASLD, exercise training can decrease hepatic fat independent of weight loss (18,19), yet this is not a universal finding (20). However, in individuals with obesity without documented liver disease, direct comparisons of the change in hepatic fat with weight loss and exercise training are less common. Several studies found that adding exercise to a weight loss intervention does not result in further reductions in hepatic fat and that weight loss plays a more important role (21,22). Further, there is considerable interest in developing plasma biomarkers that can predict changes in liver fat content without the need for expensive MRI test or invasive liver biopsies (23).

We hypothesized that weight loss, compared with exercise training alone or control, would lead to greater reductions in IMAT and hepatic fat and that these changes would be reflected in plasma lipidomic and metabolomic profiles. Accordingly, we measured changes in IMAT, muscle mass, and hepatic fat fraction before and after insulin-sensitizing lifestyle interventions in individuals with obesity and identified circulating lipids and metabolites associated with IMAT and liver steatosis.

## **MATERIALS AND METHODS**

### *Participants*

Forty-six men and women with obesity, with and without pre-diabetes or type 2 diabetes, completed this study (Table 1). Participants gave written informed consent and exclusion criteria included a body mass index (BMI) <30 or >40 kg/m<sup>2</sup>, fasting triglycerides >150 mg/dL, or liver, kidney, thyroid, or lung disease. Participants were 29 to 50 years old, sedentary (<1 hour/week planned physical activity), and weight stable (<5 lb weight change) for at least 6 months prior to enrollment. Medication use was stable for participants in this study and included: SSRI/NDRI – 15, ACE inhibitors – 2, Thiazide diuretics – 1, Statins – 3, metformin – 1, Beta blockers – 2, proton pump inhibitors – 4. This study was approved by the Colorado Multiple Institution Review Board at the University of Colorado Denver and is registered on ClinicalTrials.gov (NCT03077360).

### *Overall Study Design*

Participants were randomized into one of three groups including a 12-week weight loss only (WL), exercise training intervention without weight loss (EX), or delayed intervention control. Preliminary testing included MRI for upper leg muscle mass, IMAT content, and hepatic fat fraction, as well as upper leg muscle strength testing. After preliminary testing, a standardized diet was provided for 3 days prior to completing a standard 3-hour hyperinsulinemic/euglycemic clamp. After the 12-week intervention, all pre-intervention testing was repeated. Prior to the post-intervention testing, individuals in the exercise training intervention refrained from exercise for 48 hours and individuals in the weight loss intervention were weight stabilized for 2 weeks.

### *Preliminary Testing*

Screening occurred at the University of Colorado Anschutz Clinical Translational Research Center (CTRC) following a 12-hour overnight fast. Participants underwent a review of medical history, physical examination, fasting blood draw, and body composition assessment by DXA analysis (Lunar DPX-IQ, Lunar Corporation, Madison, WI). A 3-day dietary history was collected using the DHQII validated dietary questionnaire.

### *MRI*

A multi-slice MRI was used to quantify IMAT and muscle content in both upper legs (~20 axial images, 10 mm thickness) with 10 mm spacing between images starting superior to the patella (Siemens, Skyra 3Tesla). Muscle volume was quantified using standard methods with anatomical cross-sectional area evaluated in each T1 weighted, high resolution, gradient echo profile scan, and multiplied by the length of the muscle as described previously (24). IMAT was quantified using the validated Sirlin 6-echo method and normalized to muscle volume (25,26). Hepatic fat fraction (HFF) was quantified with an abdominal MRI using an MRI-PDFF methodology based on the Dixon method (27). Briefly, hepatic imaging was acquired using a multi-breath-hold gradient echo sequence covering the whole liver. Fat fraction maps were generated in OsiriX using the Lipoquant plug-in and HFF was calculated as the weighted average of the mean fat fraction for all slices.

### *Muscle Strength Testing*

Upper leg muscle strength testing was performed using an isokinetic dynamometer (Cybex NORM, Computer Sports Medicine, Stoughton, MA, USA) with isokinetic, concentric knee extension and flexion measured at 60°/sec from 100° of knee flexion to 0°. After practice trials for familiarization, participants completed four maximal efforts through their full range of motion. Peak torque measurements were recorded to measure maximum voluntary strength of the knee extensors.

### *VO<sub>2</sub>peak Testing*

VO<sub>2</sub>peak was determined by indirect calorimetry during a Balke treadmill exercise test. The walking speed for the test was determined by finding the speed at 0% incline that elicits 70% of the age predicted

maximum heart rate. This speed was then fixed, with the grade increasing by 2% every 2 minutes until they could not continue.

*Single-Stage hyperinsulinemic euglycemic clamp with controlled activity and diet.*

Three days prior to the clamp, all participants consumed provided food for 3 days prior to the metabolic study to minimize the known confounding influence of alterations in dietary fat on plasma (28). Energy requirements were estimated based on DXA determined fat free mass according to the following equation (Daily energy intake =  $1.4 \times [372 + (23.9 \times \text{FFM})]$ ) with the macronutrient intake designed to match the individual's normal intake (29). Two days (48 hours) prior to the metabolic study participants were asked to refrain from planned physical activity, including the last exercise training bout, to determine the effects of chronic, not acute, endurance exercise training on plasma lipid content and insulin sensitivity. The night prior to the metabolic study, participants were admitted for an overnight stay to the inpatient CTRC to ensure compliance with the 12-hour overnight fast. The next morning, a percutaneous vastus lateralis muscle biopsy and hyperinsulinemic-euglycemic clamp (40 mIU/kg/min) with [6,6-<sup>2</sup>H<sub>2</sub>]glucose infusion (Cambridge Isotope Labs, Tewksbury, MA) were performed as previously described (8). During the last 30 minutes of the clamp, respiratory gas exchange was measured by indirect calorimetry, and arterialized blood was collected for hormone and substrate measurements. Insulin stimulated glucose Rd was averaged over the last 30 minutes of the clamp as the measurement of insulin sensitivity. When applicable, women were tested during the mid-follicular phase of the menstrual cycle to control for potential effects on insulin sensitivity.

*Weight loss intervention*

*Nutrition supervision and education:* The delivery of this weight loss program was supervised by the Clinical Core of the Nutrition and Obesity Research Center (NORC) at the Anschutz Health and Wellness Center. Participants attended weekly one-on-one sessions with a registered dietician for nutritional counseling. Each week a different topic was addressed to help educate participants on diet, exercise, healthy living, and weight loss. Individuals were asked not to change their activity level during the intervention period to isolate the impact of weight loss in the outcomes.

*Dietary intervention for Weight Loss:* Participants in the WL group received a low-calorie diet consisting of a meal replacement product that can be consumed as a liquid or made into a variety of food forms (Health Nutrition Technology Inc., Carmel California). Participants were provided powdered HealthOne formula and instructed to consume 4-5 portions per day based on sex, body weight, and tolerability, providing 890-1090 kcal/d, 75-90 g of protein, 15-16 g fat and 110-134 g of carbohydrate and 100% of the DRI of all vitamins, minerals and micronutrients along with 4-5 servings per day of vegetables. To promote gall bladder contraction and reduce the risk of gallstone formation participants were asked to consume 2 teaspoons (10g) per day of vegetable oil. Participants were allowed to consume non-caloric beverages but no other food intake was allowed.

*Dietary intervention for Weight Stabilization.* After the 3-month dietary weight loss intervention, participants underwent a 2-week weight stabilization period (<5 lb weight change) to avoid confounding effects of negative energy balance on insulin sensitivity. This 2-week diet consisted of 3 meal replacements and one conventional meal per day to stabilize weight. This phase was supervised by research dieticians who have extensive experience helping participants maintain a reduced state for metabolic studies at the University of Colorado Anschutz CTSC during which the average change in body weight was  $0.8 \pm 0.3\%$ .

#### *Endurance Training Intervention*

EX group participants underwent supervised endurance exercise training using well-described procedures used by the NORC Energy Balance Core (30-33). Volunteers were asked to attend 4 sessions per week, 60 min per session, which included a short warm-up of stretching exercises and walking, 40-50 min of endurance exercise, and a cool-down. The exercise program consisted primarily of brisk walking or jogging, and was supplemented with rowing, stepping, or elliptical exercise to provide variety and relieve joint discomfort when necessary. Individualized exercise prescriptions accounted for the fitness level of the participant, preferences regarding type of exercise, and any orthopedic limitations. The initial exercise prescription was 30 minutes at 65% of maximal HR, based on the highest HR attained during the baseline maximal exercise test, which was repeated after 6 weeks of training. During the first 2 to 3 weeks of training, exercise duration and intensity was gradually increased to 45 min at 80 to 85% of maximal HR. The exercise prescription was updated every 2 weeks and was carefully geared to the participant's exercise capacity.

The rate at which the intensity of the exercise was increased was determined by the magnitude of the training-induced increases in exercise capacity, and the participant's reaction to the exercise in terms of fatigue and musculoskeletal symptoms. Participants in the EX group completed 89% of the weekly exercise sessions. The average duration of each exercise session was 48.1 +/- 3 minutes, at 81.5 +/- 1.2 % of maximal heart rate. Individuals were asked to maintain body weight and instructed to weigh weekly and to increase energy intake with typical macronutrient content if fluctuations were observed.

#### *Delayed Intervention Control*

Individuals were asked maintain their baseline diet and exercise habits during the 12-week intervention. To monitor weight stability, they were contacted every 2 weeks and asked to report their body weight.

#### *Weight Stabilization*

After the 3-month intervention, participants transitioned to a 2-week weight maintenance diet consisting of 3 meal replacements and one conventional meal per day to stabilize weight. This phase was supervised by research dieticians who have extensive experience helping participants maintain a reduced state for metabolic studies at the University of Colorado Anschutz CTRC.

#### *Post-Intervention Testing*

After completing the weight stability period of the weight loss protocol, or at least 48 hours following their last exercise training session, or following the delayed intervention control, individuals repeated the DXA, MRI, muscle strength testing, as well as the 3-day dietary control prior to repeating the insulin clamp visit.

#### *Plasma Metabolomic and Lipidomic Analysis*

Metabolites from plasma were measured using high-performance liquid chromatography and high-resolution quadrupole orbitrap mass spectrometry by the Metabolomic Core Facility at the University of Colorado Anschutz Medical Campus, as previously described (34). Plasma lipids, triacylglycerols, diacylglycerols, sphingolipids, acylcarnitines, and phospholipids, were measured using previously established LC-MS/MS methods at the NORC Lipidomic Core at the University of Colorado Anschutz Medical Campus (35-37). Concentrations were

determined using stable isotope dilution with standard curves for saturated and unsaturated lipids for each class. Glucose isotopic enrichment was measured using gas chromatography-mass spectrometry (Thermo ISQ) as previously described (38).

### *Statistical Analysis*

Data are presented as mean  $\pm$  SEM. Differences in clinical variables before and after the interventions were calculated using a 2-way ANOVA. When differences after the intervention were found ( $p < 0.05$ ), changes by group were calculated using the Sidak method which corrects for multiple comparisons and adjusted p-values reported. Differences in mean changes in normally distributed data between groups were analyzed using a 1-way ANOVA (GraphPad Prism, Boston, MA). When significant differences were detected using a 1-way ANOVA, changes in the weight loss and exercise training groups were compared to the control group while adjusting for multiple comparisons using Dunnett's multiple comparisons test with adjusted p-values reported. Significant relationships between plasma lipids or metabolites and hepatic steatosis or IMAT content were determined using Pearson's correlation coefficient for normally distributed data and Spearman's correlation coefficient for non-normally distributed data. For analyses involving multiple comparisons, p-values were adjusted using the Benjamini-Hochberg false discovery rate procedure. Statistical significance was defined as FDR-adjusted  $p < 0.05$ . For lipids, a hierarchical multiple testing correction was applied, with p-values first adjusted for total lipid class-level comparisons and then for individual lipid species within each significant class. A p-value of less than 0.05 was considered significant.

### *Data and Resource Availability*

The data sets generated in this study are available upon request from the corresponding author. No applicable resources were generated during the study.

## **RESULTS**

Table 1 summarizes the demographic data for this study, which was completed by 17 participants (8 women/9 men) in the WL group, 16 (8 women/8 men) in the EX group, and 13 (6 women/7 men) in the control group. Initial BMI, body fat,  $VO_2$ peak, and insulin sensitivity did not differ between groups. After the WL intervention, body weight decreased by 10.5% ( $p < 0.001$ ) and body fat by 5.0% ( $p = 0.009$ ). This rate of weight loss over 12 weeks is similar to the weight loss achieved using Tirzepatide and Retatrutide (39,40). BMI or body fat did not significantly change in the EX or control groups, however  $VO_2$ peak increased by 7.6% ( $p < 0.001$ ) with exercise training. Insulin sensitivity, measured by insulin stimulated glucose Rd, improved significantly in both the WL ( $p = 0.004$ ) and EX interventions ( $p = 0.04$ ), with a greater increase after weight loss (42%) compared to exercise training (23%). Some muscle strength measurements from this study have been previously published by our group (41). Strength measured as peak torque at 60°/sec tended to decrease after weight loss but was not significantly different from the control group ( $p = 0.076$ ). There were no other significant differences observed for exercise training or control groups.

#### *Changes in IMAT Volume, Muscle Mass, and Liver Steatosis After Intervention*

Weight loss resulted in a significant decrease in IMAT (Figure 1A). The percent change in IMAT was significantly greater following weight loss compared to both the EX ( $p < 0.0001$ ) and control groups ( $p < 0.0001$ ). Notably, in the WL group the decrease in IMAT was significantly greater than the loss in muscle mass ( $p < 0.0001$ ) (Figure 1A). Before the intervention, men had greater IMAT content than women ( $p = 0.04$ , Supplemental Figure S1) (42), and the reduction in IMAT after weight loss was significantly greater in men compared to women with or without co-varying for initial IMAT content (Figure 1B). As expected, muscle mass also decreased with weight loss, but the average change of -4.2% did not significantly differ from the EX ( $p = 0.12$ ) or control groups ( $p = 0.17$ ), and there were no differences in the change in muscle mass by sex.

Consistent with the MRI results, DXA measurements showed that weight loss caused significant decreases in both leg fat mass ( $p < 0.0001$ ) and lean mass ( $p < 0.0001$ ) (Figure 1C). The reduction in leg fat mass was significantly greater compared to both the EX ( $p < 0.0001$ ) and control groups ( $p < 0.0001$ ). Similarly, the decrease in lean mass in the WL group was significantly greater than the EX ( $p < 0.0001$ ) and control groups ( $p = 0.0017$ ).

Interestingly, the relative decrease in leg lean mass by DXA was significantly greater than the relative decrease in quadriceps muscle mass by MRI ( $p=0.006$ ) (Figure 1D). After combining pre- and post-intervention data, IMAT volume and insulin sensitivity measured by hyperinsulinaemic–euglycaemic clamp glucose rate of disappearance,  $R_d$ , were inversely correlated ( $p=0.004$ , Figure 1E). In addition, the intervention induced change in IMAT volume was inversely associated with the change in insulin sensitivity ( $p=0.037$ , Figure 1F). These data reinforce the literature showing a strong relationship between IMAT content and insulin sensitivity (43).

Hepatic fat fraction assessed by MRI also decreased significantly after weight loss ( $p<0.0001$ ), whereas no significant changes were observed in the EX or control groups (Figure 2A). The percent change in hepatic fat fraction did not differ by sex (Figure 2B).

#### *Plasma Lipidomics and Metabolomics*

Plasma lipidomic and metabolomic profiles were significantly altered following the WL and EX interventions compared to the control group (Figure 3). In the WL group, changes in the total lipid classes and metabolites pre- and post-intervention compared to control are shown in Figures 3A and B. Using cutoffs of adjusted  $p$ -value $<0.05$  and a  $\log_2FC>2$ , three lipid classes (deoxydihydroceramides, 1,2-diacylglycerols, and phosphatidylglycerol) decreased while total lysophosphatidylcholine increased after WL intervention (Figure 3A). Three metabolites, sphingosine-1-phosphate, sphinganine-1-phosphate, and C18:1 carnitine, were significantly reduced in the WL compared to control group (Figure 3B). In contrast, no significant changes in total lipid classes in the plasma of the EX group compared to control were observed (Figure 3C). However, six metabolites were significantly reduced in the EX compared to control group including docosahexaenoic acid, docosapentaenoic acid, (5-L-glutamyl)-L-glutamine, C14:1 carnitine, C14:0 carnitine and C18:1 carnitine (Figure 3D).

After combining pre- and post-intervention data liver steatosis and plasma lipidomics and metabolomics data, several plasma lipid classes and metabolites were correlated to liver steatosis measured using MRI. As shown in Figures 4A-K, total triacylglycerol ( $p=0.003$ ), phosphatidylinositol ( $p=0.005$ ), dihydroceramide ( $p=0.008$ ), deoxydihydroceramide ( $p=0.01$ ), phosphatidylglycerol ( $p=0.012$ ), 1,2-diacylglycerol ( $p=0.015$ ), acylcarnitine ( $p=0.018$ ), lysophosphatidylinositol ( $p=0.02$ ), 1,3-diacylglycerol ( $p=0.022$ ), ceramide ( $p=0.025$ ), and

phosphatidylcholine ( $p=0.028$ ) were positively correlated to liver steatosis after adjustment for multiple comparisons. The lipid species within each class that were significantly related to hepatic steatosis are listed in Supplemental Table S1 (42). After adjusting for multiple comparisons, twenty-two metabolites, including branched-chain amino acids, amino acids, and acylcarnitines, were significantly correlated with hepatic steatosis measured from MRI (Table 2). Additionally, several plasma lipid classes were also predictive of IMAT content (Figures 5A-F). Significant positive relationships were observed for plasma total triacylglycerol ( $p=0.001$ ), phosphatidylglycerol ( $p=0.003$ ), lysophosphatidylinositol ( $p=0.005$ ), 1,2-diacylglycerol ( $p=0.009$ ), total phosphatidylethanolamine ( $p=0.011$ ), and phosphatidylserine ( $p=0.015$ ). The individual lipid species significantly related to IMAT content within each of these lipid classes are listed in Supplemental Table S2 (42). There were no plasma metabolites that were significantly correlated with IMAT content after correcting for multiple comparisons. After adjusting for multiple comparisons, we also found no significant relationships between the change in these lipidomic and metabolic variables and the change in liver fat or IMAT volume.

## DISCUSSION

The most effective lifestyle intervention to decrease IMAT is uncertain as both weight loss and exercise training have been shown to decrease IMAT in diverse populations. Weight loss also decreases lean muscle mass. However, if the reduction of IMAT exceeds the loss of muscle mass, overall muscle quality could still improve. Additionally, weight loss decreases hepatic fat, and exercise training has been shown to lower steatosis in individuals with MASLD, yet the impact of exercise training on individuals without liver disease is less clear. This study was designed to compare the impact of weight loss only and exercise training without weight loss against a delayed intervention control group on changes in IMAT, muscle mass, and hepatic fat measured by MRI. Plasma lipidomic and metabolomic analysis was performed to identify circulating markers that are predictive of liver steatosis and IMAT in individuals with obesity.

Lifestyle interventions (weight loss and/or exercise training) reveal that weight loss is the most effective strategy to reduce IMAT, with decreases reported in both men and women (2-5,44,45), although not all studies

agree (46). Adding aerobic or resistance exercise training to weight loss typically does not result in a further reduction in IMAT volume beyond weight loss alone (5,6,44,47). These previous studies included nutrition intervention strategies such as ours, as well as bariatric surgical procedures, and ranged from 3 months to 18 months in duration. Together, they show that weight loss reliably decreases IMAT content when measured by MRI or CT as the only negative study reported changes in IMAT content by histological evaluation. The impact of exercise only without weight loss in humans is less clear, with reports of either decreased (8,9,48) or unchanged IMAT volume (13,47). Other studies suggest exercise training primarily prevents the accumulation of IMAT over time in older adults (7,10,12), which is consistent with lifelong exercisers having less IMAT than age-matched sedentary peers (11). IMAT also increases after detraining, further supporting the idea that chronic exercise dampens its accumulation over time (49). In addition to our study, the two other studies that did not report a change in IMAT content with exercise training were 12 weeks in duration, so it is possible that more prolonged exercise training is required to observe a reduction in IMAT. Our data contribute to the literature and reinforce the concept that exercise training without weight loss does not decrease IMAT, though it is possible that longer exercise training periods may be required (8).

Weight loss causes IMAT to decline more dramatically than muscle mass, resulting in improvements in muscle quality (4,5,44,45). Our data parallel the literature with a greater reduction in IMAT volume than in skeletal muscle mass in the weight loss cohort. IMAT is negatively related to muscle strength in both cross-sectional and reduced physical activity studies after normalizing to cross-sectional area, suggesting that IMAT itself could negatively impact muscle strength (50-52). While mechanistic evidence is lacking, the IMAT secretome decreases muscle insulin sensitivity (53), raising the possibility that secreted factors could also impact strength. Prior research also demonstrates lower density and smaller cross-sectional area of contractile muscle fibers in wild type mice compared to those with blocked IMAT formation, suggesting that IMAT may decrease skeletal muscle strength through the inhibition of muscle fiber regeneration and atrophy of fibers (54). Improved muscle quality after weight loss suggests reduced IMAT may benefit muscle strength. Consistent with this, weight loss has been associated with improvement in muscle strength (3) and decreases in IMAT often correlate more strongly with improvements in functional strength than changes in muscle cross-sectional area (55), though not all studies agree (10). Cross-sectional studies have also reported significant relationships between IMAT content and

functional strength (56,57). Overall, these findings suggest that when IMAT loss exceeds muscle mass loss, muscle quality, strength, and function are maintained or improved.

Sex differences in IMAT have been reported inconsistently with studies showing more IMAT in men (58,59), in Asian men specifically (60), in women (8,61), or no difference between sexes (5,7,62-64). Our data add to the literature showing greater IMAT content in men compared to women before the intervention. Sex differences in IMAT are impacted by age, with most studies showing no difference between sexes performed in post-menopausal women (7,62,63). Differences may also be muscle group specific, as greater IMAT volume in women compared to men was only found in the adductor magnus muscle while no changes were observed in other leg muscle groups (65). However, sex differences in IMAT with weight loss or exercise training have received less attention, with one report showing similar loss of IMAT after 8-9 months of exercise training in men and women (8). In contrast, we found a greater proportional reduction in IMAT volume in men compared to women after the WL intervention. This may be due to the greater starting concentration in men, or it may suggest that weight loss is more efficacious to decrease IMAT in men compared to women.

Direct comparisons of DXA and MRI-based measurements of lean mass in individuals with obesity after a weight loss intervention are not common. Our findings are similar to the only other direct study in the literature, which found that DXA overestimated loss of lean mass compared to MRI measurements of muscle mass (66). In our study, DXA measured nearly twice the loss of lean muscle mass compared to MRI. Similar discrepancies have been found in exercise training studies, where DXA overestimated gains in lean body mass while underestimated losses compared to MRI (67), and overall was considered imprecise to measure changes in muscle mass in response to exercise (68). These findings have significant public health implications, particularly when interpreting composition of weight loss for the new GLP1 receptor agonists (69,70), underscoring the need for caution when relying solely on DXA to assess skeletal muscle mass.

The changes in plasma lipidomic and metabolomic profiles following WL and EX interventions are consistent with distinct intervention-specific metabolic alterations. Following the WL intervention, decreased plasma deoxydihydroceramides and 1,2-diacylglycerols along with increased lysophosphatidylcholine are observed and have previously been associated with enhanced insulin sensitivity (71-73). Additionally,

decreased sphingosine-1-phosphate is noteworthy as higher circulating concentrations are linked to obesity and metabolic dysfunction (74). In contrast, the EX group exhibited no significant changes in total lipid classes but showed consistent reductions in several metabolites, including docosahexaenoic acid, docosapentaenoic acid, and some acylcarnitines, consistent with enhanced mitochondrial fatty acid oxidation, a hallmark of exercise adaptation (75). Together, circulating lipidomic and metabolomic profiles reveal intervention-specific signatures of increased insulin sensitivity.

Weight loss decreases hepatic fat content progressively, with greater weight loss resulting in greater loss of hepatic fat (17,76). Our data are very similar by showing that weight loss decreased hepatic fat in individuals with obesity but without known MASLD/MASH. A meta-analysis on exercise training found strong evidence that exercise decreases hepatic steatosis in individuals with MASLD (14), which parallels decreases in hepatic steatosis after exercise training in individuals without known MASLD (19,77). However, others report no changes in hepatic fat after training (20), which is similar to results from the current study. It is not obvious why our study did not find reductions in hepatic fat, other than our protocol prevented changes in body weight. Change in body weight with exercise training explains our divergent results in some but not all studies (19). Independent of changes in hepatic steatosis, previous publications reported exercise training can change triacylglycerol saturation (78) and alter hepatokine secretion, including FGF21 and fetuin-A (79), which could contribute to exercise-induced insulin sensitization. Our data suggests that weight loss is more effective at decreasing hepatic fat compared to endurance exercise training.

The high cost and limited availability of imaging methods underscore the need for non-invasive plasma biomarkers that predict the presence and severity of MASLD, MASH, and liver fibrosis. Clinical panels have demonstrated predictive power for detecting MASH with significant or advanced fibrosis (23) with less focus on hepatic steatosis. Newer methods of imaging, primarily Fibroscan, are also more accurate for staging fibrosis, rather than assessing liver steatosis (80). Most existing biomarker panels combine clinical lab values, demographic variables, and plasma proteomics. However, plasma metabolomic and lipidomic profiling can provide a broad spectrum of candidate biomarkers for non-invasive diagnosis and stratification of MASLD and MASH (81). Elevated branched-chain and aromatic amino acids, glutamate, and related metabolites are consistently associated to

MASH and correlate with insulin resistance, fibrosis, and liver steatosis, while reduced glycine, serine, and betaine levels are linked to more severe steatosis (82-86). Lipidomic profiling has further identified triacylglycerol and diacylglycerol species as markers of disease stage (86-88). Phospholipids and sphingolipids provide additional diagnostic value, with reductions in lysophosphatidylcholines and ether lipids and elevations in ceramides and sphingomyelins consistently reported, which correlate with MRI-measured liver steatosis in type 2 diabetes (89-91). These advances have enabled the development of predictive panels such as oxNASH (92), OWLiver (86-88), Lipid Triplet (93), and NASH ClinLipMet (82), which integrate clinical, metabolomic, and lipidomic data and achieve predictive power of 0.71–0.95. Most lipidomic predictive panels distinguish types of MASLD with less ability to discriminate simple steatosis (81). Our findings reveal multiple plasma lipid classes (Figure 4) and a broad set of plasma metabolites (Table 2) that are strongly correlated with MRI-measured liver steatosis in individuals with obesity without known liver disease. These results provide further evidence that circulating lipids and metabolites can be used to predict liver steatosis and validate prior cross-sectional observations. By linking specific lipid and metabolite signatures to hepatic steatosis, our study highlights the potential of plasma metabolomic and lipidomic profiling as a non-invasive strategy for detecting the development of MASLD and discriminating MASLD from MASH, which could result in more accurate predictive biomarker panels.

In contrast, efforts to identify plasma predictors of IMAT are limited. One proteomic study reported 722 proteins associated with IMAT content (94). To develop plasma biomarker panels that predict IMAT volume, broad measurement of the predictive power of the metabolome and lipidome are warranted but not yet published. Our work is the first to show plasma lipids, but not metabolites, correlate with IMAT content. While this data could simply reflect co-linearity of plasma lipids with BMI and adiposity, they also suggest that IMAT expansion may be driven in part by circulating lipids.

This study has several limitations. MRI based measurements of muscle mass were limited to the dominant quadriceps muscle, whereas DXA assessed the entire limb, raising the possibility that the quadriceps muscle mass does not represent the entire limb. The exercise intervention did not include a resistance training component, which could have differential impacts on IMAT. Although several plasma lipid classes and metabolites correlated with hepatic fat and IMAT, these associations are cross-sectional and do not establish causality. Finally, while our

lipidomic and metabolomic results highlight potential plasma predictors of hepatic fat and IMAT, a larger cross-sectional study across the spectrum of MASLD/MASH severity is needed to validate these observations.

In summary, weight loss is more effective than aerobic exercise training to reduce IMAT volume and hepatic fat in humans. DXA overestimated loss of muscle mass compared to MRI by roughly twofold, highlighting the need for careful evaluation of body composition data. Further, several classes of plasma lipids and metabolites revealed were strong correlations to hepatic fat and IMAT, supporting the potential utility in developing non-invasive biomarker panels to estimate hepatic steatosis and intermuscular adipose tissue content.

ACCEPTED MANUSCRIPT

## ACKNOWLEDGMENTS

**Acknowledgments.** The authors thank the University of Colorado School of Medicine Metabolomics Core, CTRC staff and NORC nutrition team for contributions to this article.

**Grants.** This work was supported by the American Diabetes Association grant 1-14-CE-05 to B.C.B and the Colorado Nutrition Obesity Research Center grant P30DK048520. The Metabolomics Core Facility is supported by the Colorado Cancer Center support grant P30CA046934.

**Disclosures.** The authors declare no competing interests.

**Author Contributions.** KZB performed plasma lipidomic analysis, metabolomic data analysis, helped with data interpretation, and write/edit the manuscript, AG helped with participant testing, EM helped recruit and complete testing on all individuals in this study, SZ helped with participant recruitment and testing, plasma lipidomic analysis, data interpretation and manuscript preparation, SB derivatized plasma samples for glucose kinetics analysis, MGC provided medical oversight, performed all biopsies, and edited the manuscript, JSB helped with the statistical analysis for this study, SB analyzed the muscle strength data and helped write/edit the manuscript, BCB designed the study, performed participant testing, analyzed data, and wrote the manuscript. BCB is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Data Availability Statement.** The data sets generated in this study are available upon request from the corresponding author. No applicable resources were generated during the study.

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## Figure Legends

**Figure 1.** Changes in IMAT and muscle following a weight loss, exercise training or control 12-week intervention. (A) IMAT and muscle volume measured by MRI. (B) IMAT by sex. (C) Leg fat mass and lean mass measured by DXA. (D) Comparison of MRI versus DXA lean mass change in the weight loss group. Values are mean  $\pm$  SEM; n = 13-17 participants /group. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . WL – weight loss, EX – exercise training. Pearson correlation between (E) insulin sensitivity measured by hyperinsulinaemic–euglycaemic clamp rate of disappearance, Rd, and IMAT volume and (F) change in insulin sensitivity measured by hyperinsulinaemic–euglycaemic clamp and change in IMAT volume in pre- vs post-intervention samples. WL – gray circles, EX – yellow squares, CTRL – blue triangles; pre-intervention – filled, post-intervention – open.

**Figure 2.** Change in liver steatosis following a 12-week weight loss, exercise training, or control intervention. (A) Percent liver fat pre- and post-intervention (B) Change in liver fat by sex. Values are means  $\pm$  SEM; n = 13-17 participants /group. \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ . WL – weight loss, EX – exercise training.

**Figure 3.** Volcano plots illustrating differential changes in plasma lipid and metabolite profiles following a 12-week weight loss or exercise intervention. Plots show  $-\log_{10}(\text{p-value})$  versus  $\log_2(\text{fold change})$  for total lipid classes and metabolites for weight loss versus control (A, B) and exercise training versus control (C, D). Red indicates a decrease and blue indicates an increase relative to control; n = 13-17 participants /group. WL – weight loss, EX – exercise training, ctrl – control, dDHCer – deoxydihydroceramide, PG – phosphatidylglycerol, 1,2-DAG – diacylglycerol, S1P – sphingosine-1-phosphate, Sa1P – sphinganine-1-phosphate, DPA – docosapentaenoic acid, DHA – docosahexaenoic acid.

**Figure 4.** Correlations between plasma lipids and percent liver fat in pre- and post-intervention samples. Pearson correlation coefficients are shown for total (A) triacylglycerol (TAG), (B) phosphatidylinositol (PI), (C) dihydroceramide (dHCer), (D) deoxydihydroceramide (dDHCer), (E) phosphatidylglycerol (PG), (F) 1,2-diacylglycerol (1,2-DAG), (G) acylcarnitine (AC), (H) lysophosphatidylinositol (LPI), (I) 1,3-diacylglycerol (1,3-DAG), (J) ceramide (Cer), and (K) phosphatidylcholine (PC), measured by LC-MS/MS. n = 13-17 participants /group. WL – gray circles, EX – yellow squares, CTRL – blue triangles; pre-intervention – filled, post-intervention – open. P-values are corrected for multiple comparisons.

**Figure 5.** Correlations between plasma lipids and IMAT volume in pre- and post-intervention samples. Pearson correlation coefficients are shown for total (A) triacylglycerol (TAG), (B) phosphatidylglycerol (PG), (C) lysophosphatidylinositol (LPI), (D) 1,2-diacylglycerol (1,2-DAG), (E) phosphatidylethanolamine (PE), and (F) phosphatidylserine (PS), measured by LC-MS/MS. n = 13-17 participants /group. WL – gray circles, EX – yellow squares, CTRL – blue triangles; pre-intervention – filled, post-intervention – open. P-values are corrected for multiple comparisons.

Table 1. Participant Demographics

Variable	Weight Loss Only		Exercise Training Only		Control	
	Pre	Post	Pre	Post	Pre	Post
N (W/M)	17 (8/9)		16 (8/8)		13 (6/7)	
Race (Caucasian/Black/Asian)	14/1/2		13/3/0		11/2/0	
NGT/Pre-diabetes/T2D	10/6/1		12/4/0		6/7/0	
Age (yrs)	39.7±1.3		39.7±1.6		39.8±1.4	
BMI (kg/m <sup>2</sup> )	35.2±0.9	31.5±1.0§	36.0±1.1	35.8±1.1	36.8±1.3	37.0±1.4
Body Weight (kg)	103.8±3.1	92.8±2.8§	107.1±4.1	106.6±4.1	104.5±5.0	105.1±5.1
Body Fat (%)	40.7±1.4	38.6±1.7§	41.6±1.5	41.7±1.3	40.6±1.2	40.9±1.6
DXA Lean Mass (kg)	57.2±2.0	52.8±1.9§	58.6±3.2	58.5±3.0	58.6±3.4	58.2±3.6
DXA Fat Mass (kg)	43.4±3.0	35.3±2.0§	43.0±1.6	43.4±1.7	41.6±2.1	41.7±2.1
MRI IMAT (g/cm <sup>3</sup> )	4.5±0.3	3.9±0.3§	4.5±0.3	4.5±0.3	5.2±0.4	5.3±0.5
MRI Skeletal Muscle (g/cm <sup>3</sup> )	75.6±4.1	72.4±4.1§	76.9±4.5	78.3±4.9	81.4±5.4	81.4±5.1
HbA1c (%)	5.6±0.1		5.5±0.1		5.5±0.1	
Fasting Glucose (mg/dL)	94.1±5	89.5±3	95.2±2	92.1±2	93.6±2	90.0±2
Fasting Insulin (uU/mL)	7.8±1	5.8±0.6§	9.2±0.8	8.7±0.6	9.0±1.3	9.0±1.0
Fasting FFA (uEq/L)	632±44	554±37	617±38	557±46	549±31	526±48
Fasting TG (mg/dL)	129.6±13.9	98.5±9.3§	122.6±12.3	124.4±14.3	113.5±12.8	119.3±15.3
VO <sub>2</sub> peak (L/min)	2.7±0.1	2.6±0.1	2.7±0.2	2.9±0.2§	2.6±0.2	2.6±0.2
Glucose Rd during insulin clamp (mg/kg/min)	3.9±0.3	5.3±0.5§	3.7±0.3	4.4±0.4§	3.9±0.5	3.7±0.5
Glucose Ra (mg/kg/min)	2.2±0.1	2.3±0.1	2.2±0.1	2.1±0.1	2.2±0.1	2.0±0.1
Peak Torque (Newton*meters)	183±14	167±10	166±15	173±19	125±20	137±14

Values are mean ± SEM. § = significantly different than pre, p<0.05.

Table 2. Plasma metabolites significantly related to hepatic steatosis

<i>Metabolite</i>	<i>Pearson r</i>	<i>P (two-tailed)</i>	<i>Benjamini-Hochberg adjusted p-value</i>
L-Leucine/isoleucine	0.4765	<0.0001	0.0004
L-Lysine	0.4028	<0.0001	0.0008
L-Tyrosine	0.4264	<0.0001	0.0012
Kynurenine	0.3982	<0.0001	0.0015
L-Palmitoylcarnitine	0.4216	<0.0001	0.0019
O-Dodecenoyl-carnitine	0.3763	0.0002	0.0023
Xanthine	0.3735	0.0003	0.0027
L-Glutamate	0.3581	0.0005	0.0031
L-Phenylalanine	0.3545	0.0006	0.0035
L-Tryptophan	0.3479	0.0007	0.0038
D-Ribose	0.3262	0.0016	0.0042
L-Valine	0.3216	0.0019	0.0046
Urate	0.3195	0.002	0.0050
5-Oxoproline	0.317	0.0022	0.0054
Dehydroascorbate	0.3015	0.0037	0.0058
Allantoate	0.3001	0.0039	0.0062
(5-L-Glutamyl)-L-glutamine	0.2982	0.0041	0.0065
Hypoxanthine	0.2971	0.0042	0.0069
Tetrahydrofolate	0.2889	0.0055	0.0073
L-Proline	0.2883	0.0056	0.0077
O-Dodecanoyl-carnitine	0.2863	0.0059	0.0081
L-Cystine	0.2777	0.0077	0.0085

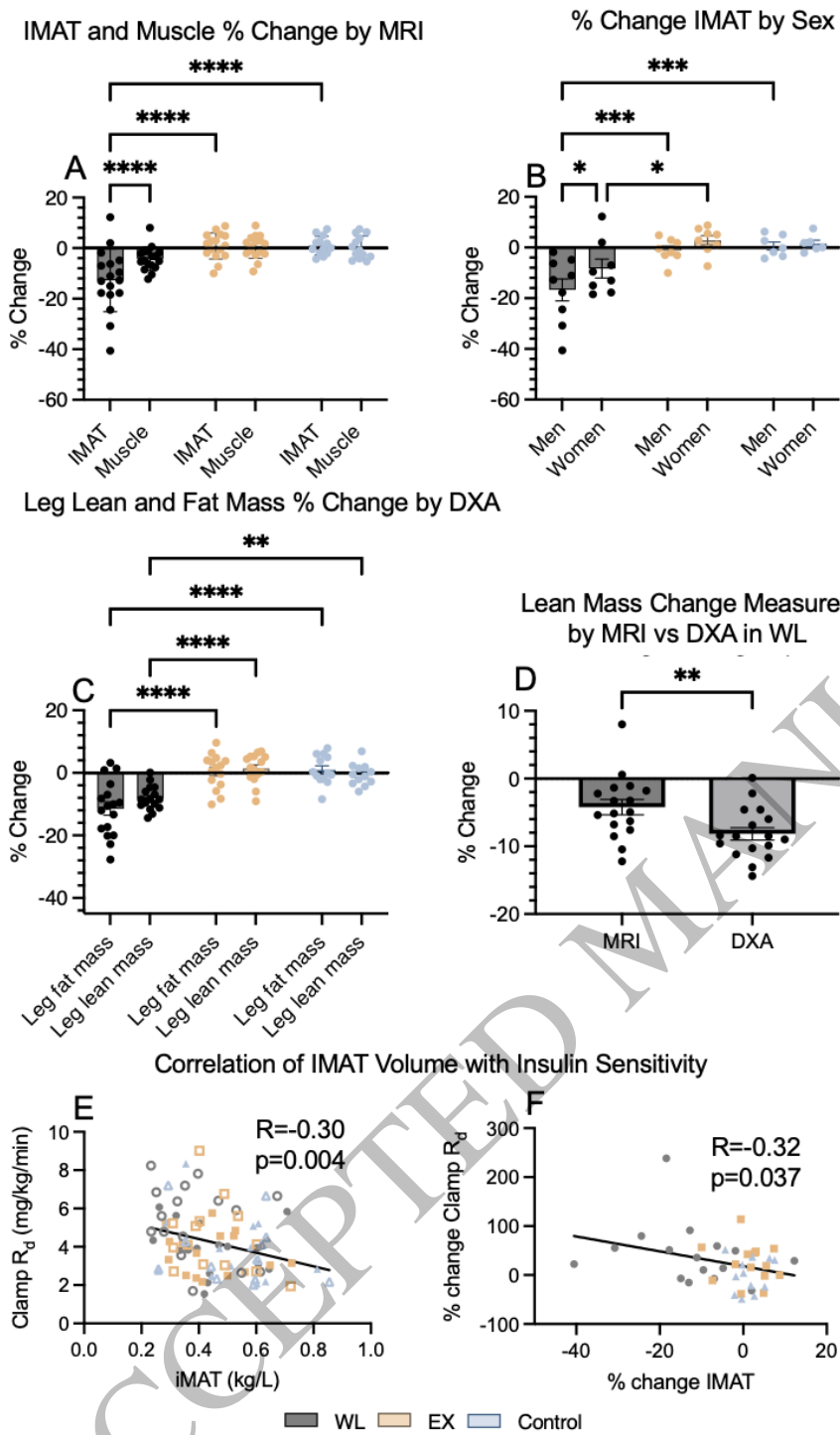


Figure 1

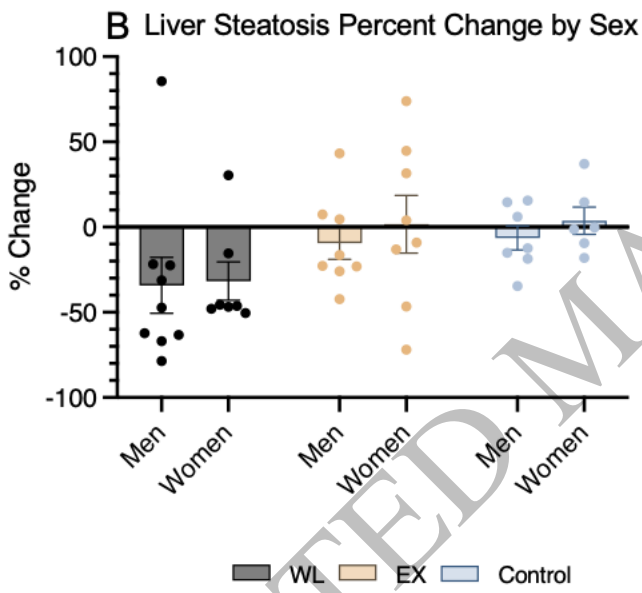
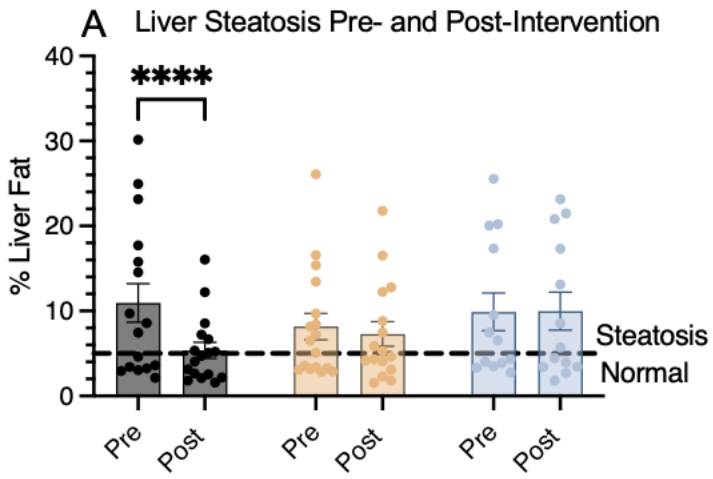


Figure 2

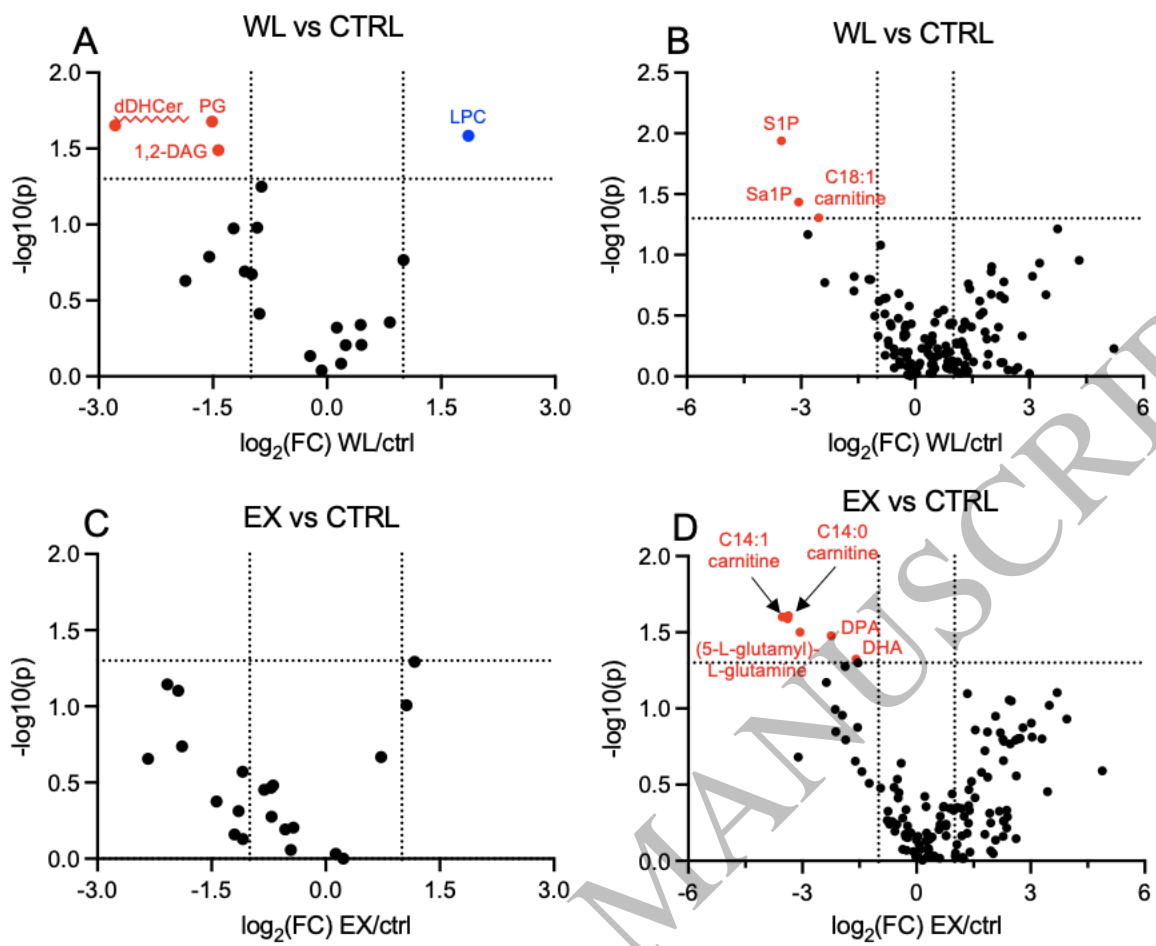


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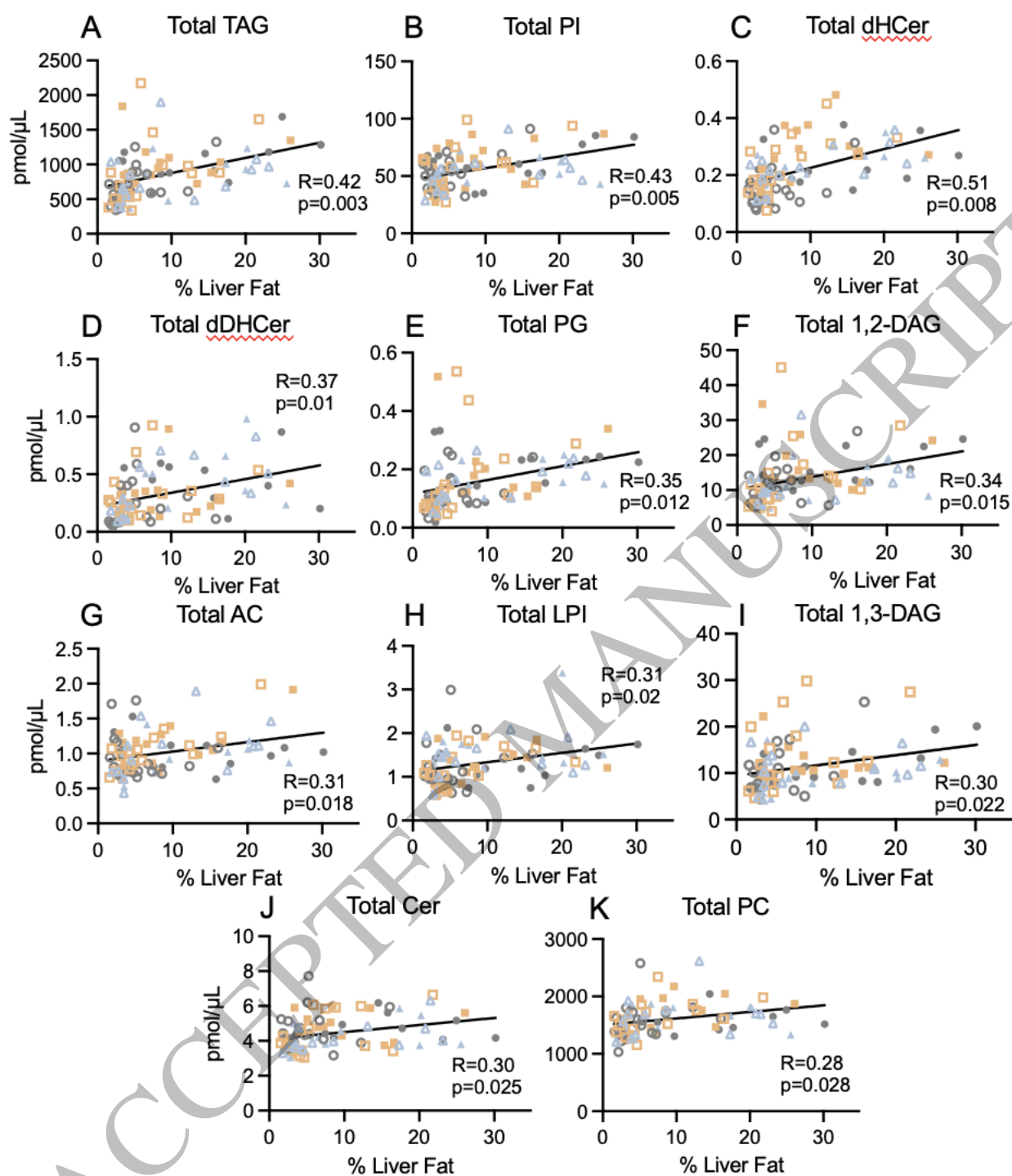


Figure 4

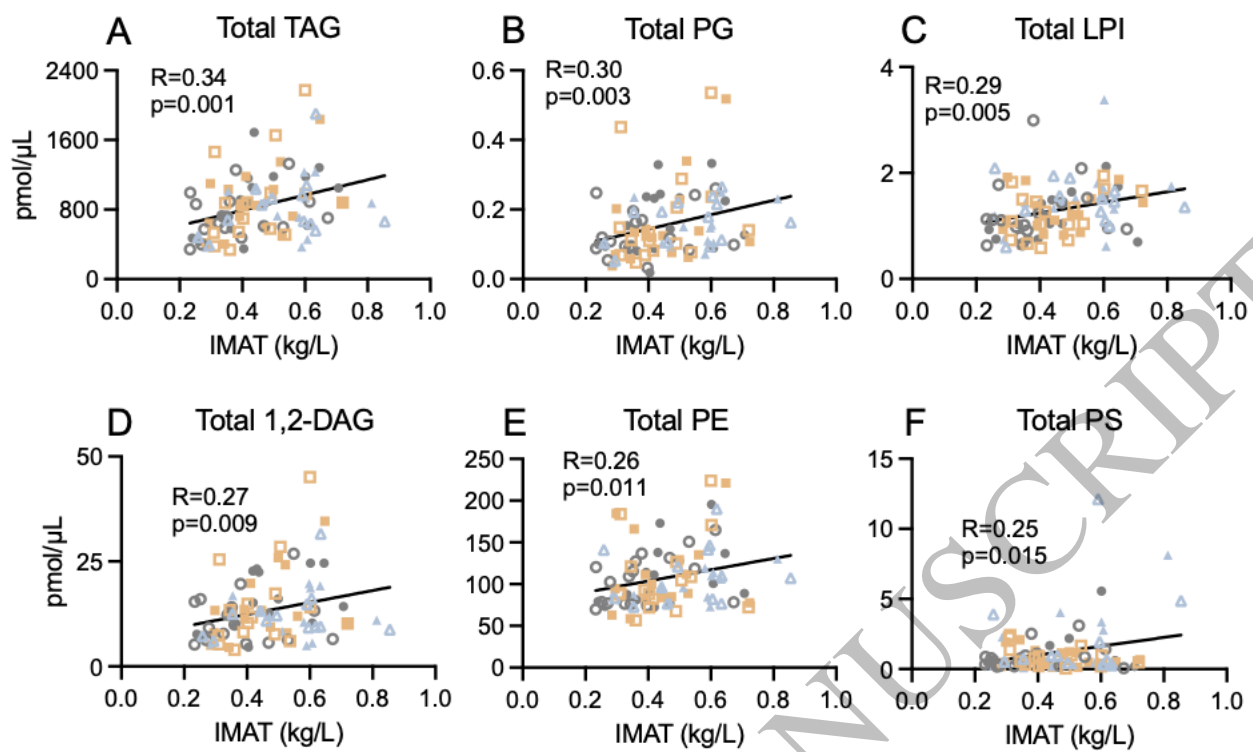


Figure 5