

Postexercise changes in myocellular lipid droplet characteristics of young lean individuals are affected by circulatory nonesterified fatty acids

Citation for published version (APA):

Daemen, S., van Polanen, N., Bilet, L., Phielix, E., Moonen-Kornips, E., Schrauwen-Hinderling, V. B., Schrauwen, P., & Hesselink, M. K. C. (2021). Postexercise changes in myocellular lipid droplet characteristics of young lean individuals are affected by circulatory nonesterified fatty acids. *American Journal of Physiology: Endocrinology and Metabolism*, 321(4), E453-E463. <https://doi.org/10.1152/ajpendo.00654.2020>

Document status and date:

Published: 01/10/2021

DOI:

[10.1152/ajpendo.00654.2020](https://doi.org/10.1152/ajpendo.00654.2020)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 06 Oct. 2022

RESEARCH ARTICLE

Postexercise changes in myocellular lipid droplet characteristics of young lean individuals are affected by circulatory nonesterified fatty acids

Sabine Daemen,^{1*}  Nynke van Polanen,^{1*} Lena Bilet,¹  Esther Phielix,¹ Esther Moonen-Kornips,¹ Vera B. Schrauwen-Hinderling,^{1,2} Patrick Schrauwen,¹ and  Matthijs K. C. Hesselink¹

¹Department of Nutrition and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre +, Maastricht, The Netherlands and ²Department of Radiology, Maastricht University Medical Center +, Maastricht, The Netherlands

Abstract

Intramyocellular lipid (IMCL) content is an energy source during acute exercise. Nonesterified fatty acid (NEFA) levels can compete with IMCL utilization during exercise. IMCL content is stored as lipid droplets (LDs) that vary in size, number, subcellular distribution, and in coating with LD protein PLIN5. Little is known about how these factors are affected during exercise and recovery. Here, we aimed to investigate the effects of acute exercise with and without elevated NEFA levels on intramyocellular LD size and number, intracellular distribution and PLIN5 coating, using high-resolution confocal microscopy. In a crossover study, 9 healthy lean young men performed a 2-h moderate intensity cycling protocol in the fasted (high NEFA levels) and glucose-fed state (low NEFA levels). IMCL and LD parameters were measured at baseline, directly after exercise and 4 h postexercise. We found that total IMCL content was not changed directly after exercise (irrespectively of condition), but IMCL increased 4 h postexercise in the fasting condition, which was due to an increased number of LDs rather than changes in size. The effects were predominantly detected in type I muscle fibers and in LDs coated with PLIN5. Interestingly, subsarcolemmal, but not intermyofibrillar IMCL content, was decreased directly after exercise in the fasting condition and was replenished during the 4 h recovery period. In conclusion, acute exercise affects IMCL storage during exercise and recovery, particularly in type I muscle fibers, in the subsarcolemmal region and in the presence of PLIN5. Moreover, the effects of exercise on IMCL content are affected by plasma NEFA levels.

NEW & NOTEWORTHY Skeletal muscle stores lipids in lipid droplets (LDs) that can vary in size, number, and location and are a source of energy during exercise. Specifically, subsarcolemmal LDs were used during exercise when fasted. Exercising in the fasted state leads to postrecovery elevation in IMCL levels due to an increase in LD number in type I muscle fibers, in subsarcolemmal region and decorated with PLIN5. These effects are blunted by glucose ingestion during exercise and recovery.

exercise; lipid droplets; muscle

INTRODUCTION

One of the earliest hallmarks of type 2 diabetes (T2DM) is resistance of the peripheral tissues to the action of insulin, generally referred to as insulin resistance. Obesity is the major risk factor for insulin resistance and is characterized by excessive accumulation of fat in the body. Limited expandability of adipose tissue results in spill-over of fatty acids (FAs) from the adipose tissue into the circulation with accumulation of lipids in non-adipose tissue like muscle as a consequence (1). Intramyocellular lipid (IMCL) content is associated with the development of insulin resistance (2).

Although IMCL content can be detrimental for insulin sensitivity, it also serves as an energy source during muscle contraction. Prolonged exercise training results in elevated IMCL content (3). Likewise, endurance-trained athletes are

characterized by elevated IMCL levels (4, 5), probably serving as an increased intracellular pool of FAs to fuel oxidation during prolonged exercise. Using palmitate-labeled stable isotope tracers, it has been demonstrated that IMCL is indeed used during exercise (6–11). Some studies have shown that utilization of IMCL during acute exercise results in reduced IMCL content as measured directly after exercise by proton magnetic resonance spectroscopy (¹H-MRS) or in muscle biopsies (biochemically or histochemically; 9, 12–17). Other acute exercise studies, however, failed to detect a drop in IMCL content (18–21). These seemingly contrasting results may originate from differences in the methodology used (biochemically, histologically or non-invasively via MRS) as well as from differences in nonesterified fatty acid (NEFA) concentrations in the circulation. Upon exercise, stimulation of adipose tissue lipolysis occurs and plasma NEFA levels

* S. Daemen and N. van Polanen contributed equally to this work.

Correspondence: M. K. C. Hesselink (matthijs.hesselink@maastrichtuniversity.nl).

Submitted 24 December 2020 / Revised 6 August 2021 / Accepted 8 August 2021

increase. Plasma derived NEFAs compete with IMCL-derived FAs for oxidation but may also refuel IMCL stores during exercise and during the postexercise period. In inactive muscle, it has indeed been reported that elevated plasma NEFA levels augment IMCL content (15, 22). This indicates that elevated plasma NEFA levels promote incorporation of FAs in IMCL, independent of the need of FAs for oxidation.

Many studies examining the effect of exercise on IMCL content have used ¹H-MRS or biochemical triglyceride extraction to determine IMCL content. However, these methodologies do not take lipid droplet (LD) distribution and morphology into account. IMCL is largely stored in LDs of which the pattern of storage is affected by training status and disease state. Interestingly, we have recently shown that in endurance athletes, IMCL is dispersed in numerous small LDs, specifically in the intermyofibrillar (IMF) region of type I fibers whereas in patients with T2DM most IMCL is stored in large LDs, predominantly in the subsarcolemmal (SS) region of type II muscle fibers (5). This pattern fits with IMCL being an important source of fuel during exercise, as hypothetically, LDs in the IMF region may primarily serve to deliver FAs to mitochondria for oxidation. Hence, it can be hypothesized that IMF LDs would reduce in number and/or size upon exercise. On the other hand, SS LDs are less likely to fuel contraction during exercise but might be prone to grow in size and/or number due to their vicinity to the capillary bed and the resulting exposure to high circulating NEFA levels, such as may be the case in T2DM (which is characterized by high NEFA levels). These putative region and fiber type specific effects may also blunt the overall effect of exercise on total IMCL content. As of now, only one study has examined region specific effects of exercise and recovery on IMCL content and LD size and number (23). This study showed a more profound lowering effect of exercise on IMF LDs than on SS LDs upon 4 h of exercise at 56% VO₂Max in trained athletes (23), however in this study NEFA levels were not reported.

Perilipin 5 (PLIN5) is a LD coat protein involved in LD dynamics by affecting uptake, retention, and release of FAs from LDs to mitochondria for oxidation, depending on demand, nutrition, or training status (24–26). We previously observed that fasting-induced increases in plasma NEFA levels resulted in preferential storage of FAs in PLIN5 coated LDs (27). Others have shown that during moderate intensity exercise, PLIN5 coated LDs are more prone to reduce size compared with LDs devoid of PLIN5 (17). Together, this suggests a role for PLIN5 postexercise and during high NEFA levels. Here, we examined the effect of acute exercise (moderate intensity, that is, 50% of predetermined maximal power output) on LD size and number while taking muscle fiber type and subcellular LD distribution into account, and making the distinction between LDs coated with PLIN5 (PLIN5+) and those devoid of PLIN5 (PLIN5-). Furthermore, to investigate the effects of circulatory NEFA levels on exercise mediated oxidation and postexercise replenishment of myocellular LDs, the study was performed in both the glucose-fed (low NEFA levels) and the fasted state (high NEFA levels), along with measurements of glucose and fat oxidation.

METHODS

Participants

Nine healthy lean young men participated in this study. Participation in competitive sports (>2 h/wk), an unstable body weight (>3 kg change in preceding 6 mo), medication use or any medical condition requiring treatment were exclusion criteria. The institutional medical ethics committee approved the study in accordance with the Declaration of Helsinki, and all participants gave their informed written consent. The study is registered as NCT01906333 at <https://clinicaltrials.gov>. Participant characteristics are shown in Table 1.

Study Protocol

At the beginning of the study, body composition (hydrostatic weighing) and maximal aerobic capacity ($\dot{V}O_{2max}$) were determined in all participants. The experimental trial comprised two test days separated by at least 1 wk and were performed in random order. Participants refrained from physical activity 2 days before the test days and consumed a standardized meal the evening before the test days. On the test days, participants reported to the laboratory after an overnight fast (no caloric intake after 10 PM the preceding day). A muscle biopsy was taken from the m. vastus lateralis under local anesthesia (2% lidocaine), according to the Bergstrom technique (28). The biopsy was immediately frozen in melting isopentane and stored at $-80^{\circ}C$ for later immunohistochemical analysis. Thereafter, a Teflon cannula was inserted into an antecubital forearm vein for sampling of blood and participants rested for 30 min. At baseline ($t = -60$), the participants ingested either 1.4 g/kg body wt of glucose (dissolved in water to a 20% solution and flavored with 1 mL lemon juice) or the same amount of plain water. After this, participants started exercising on a stationary bike at 50% of their predetermined maximal power output (W_{max}) ($t = 0$ min) for 2 h. During exercise, blood samples were drawn and substrate oxidation was measured by indirect calorimetry (Omnical, Maastricht, The Netherlands) every 30 min for 15 min (at $t = 30, 60, 90,$ and 120 min) with continuous heart rate monitoring. Participants consumed the glucose or water drink every 30 min during exercise. Immediately upon cessation of exercise, a second muscle biopsy was taken. Subsequently, participants bed rested for 4 h, followed by a third muscle biopsy 4 h postexercise. During the 4 h postexercise period, drinks (glucose or plain water) were consumed hourly and blood samples were drawn (before the drinks) and measurements of substrate oxidation were performed by indirect calorimetry for 30 min (at $t = 180, 240, 300,$ and 360 min).

Table 1. Participant characteristics

Characteristics	
Age, yr	23.2 ± 2.2
Height, m	1.85 ± 0.09
Weight, kg	77.9 ± 9.5
BMI, kg/m ²	22.7 ± 1.8
Fat mass, %	14.4 ± 5.5
$\dot{V}O_{2max}$ (mL O ₂ /kg/min)	43.3 ± 6.1

Data are means ± SD ($n = 9$); BMI, body mass index; $\dot{V}O_{2max}$, maximal aerobic capacity.

Fat oxidation and substrate oxidation was calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ using the formulas of Peronnet (29). The experimental design is depicted in Fig. 1.

Measurements Before Test Days

A routine incremental cycling test on a stationary bike until exhaustion was used to determine maximal exercise capacity as described previously (30) and hydrostatic weighing with simultaneous measurement of lung volume was performed to determine body composition. The equation of Siri (31) was used to calculate fat percentage, fat mass and fat-free mass.

Blood Sample Analysis

Blood samples were collected in EDTA-containing tubes and immediately spun at high speed and frozen in liquid nitrogen and subsequently stored at -80°C until assayed. Plasma NEFAs and glucose were measured with enzymatic assays automated on a Cobas Fara/Mira (NEFA: Wako Nefa C test kit; Wako Chemicals, Neuss, Germany) (glucose: hexokinase method; Roche, Basel, Switzerland).

Histochemical Analysis

From the muscle biopsies, 5- μm thick sections were cut and mounted on glass slides. To exclude effects of staining variability, all samples from one participant were mounted on the same glass slide. Immunohistochemical stainings were performed, as previously described (5). Primary antibodies against laminin (L9393; Sigma-Aldrich, Zwijndrecht, The Netherlands; 1:25), myosin heavy chain type I (A4.840; Developmental Studies Hybridoma Bank, Iowa, USA; 1:25), and PLIN5 (GP31; Progen Biotechnik, Heidelberg, Germany; 1:25) were used. Appropriate secondary antibodies were conjugated with Alexa Fluor 405, Alexa Fluor 555, or Alexa Fluor 647. LDs were stained with BODIPY 493/503 (D3922; Molecular Probes, Thermo Fisher Scientific, Landsmeer, the Netherlands; 1:100).

Image Acquisition and Analysis

Images were taken with a Leica TCS SP8 STED microscope (Leica Microsystems, Amsterdam, The Netherlands), in confocal mode with similar settings as described previously (5). For each time point of each participant, images of twenty fibers in cross-sectional view were taken with appropriate type I to type II fiber ratio, determined as previously described (5). Images were deconvolved using Huygens Professional software (Scientific Volume Imaging B.V., Hilversum, The Netherlands). IMCL, LD size, number and distribution were analyzed with ImageJ (NIH, Bethesda, MD) and MATLAB (The MathWorks Inc., Natick, MA). IMCL was determined by total lipid area (BODIPY staining)/cell area (% lipid area fraction), LD number by total LD number/cell area ($\#/\mu\text{m}^2$) and LD size by total lipid area/total LD count (μm^2). This was calculated for all fibers combined (taking into account the predetermined type I to type II fiber ratio) and in type I and type II fibers separately. As previously explained, the SS region was defined as the outer 8% of the cell and IMF region as the remaining 92% using a custom made MATLAB script (5). Within each region, IMCL, LD number and size was determined for all fibers combined. Finally, LDs were subdivided into LDs coated with PLIN5 (PLIN5+) and LDs devoid of PLIN5 (PLIN5-) using a custom written MATLAB script (32). For both PLIN5+ and PLIN5- LDs, number and size was computed for all fibers combined.

Statistics

Data are reported as means \pm SE. Statistical analyses were performed using SPSS v. 25 (IBM SPSS Inc., NC). A repeated-measures ANOVA was performed with time and treatment (fasting or glucose) as within-subject factors for energy expenditure, substrate oxidation, and plasma values of NEFAs and glucose. Thereafter a pairwise comparison was performed with a Bonferroni correction for multiple comparisons. A mixed model was used to compare IMCL content, LD size, number, and PLIN5 coating. In case of a time effect, paired *t* tests were performed between time points for both the glucose and fasting condition. If there was a significant treatment effect, glucose and fasting were compared at each time point using paired *t* tests. Statistical significance was set a priori at $P < 0.05$.

RESULTS

Plasma Nonesterified Fatty Acids and Glucose

There was a significant time ($P < 0.001$), treatment ($P < 0.001$), and time \times treatment interaction ($P < 0.001$) effect for plasma NEFA concentrations. Plasma NEFA concentrations were substantially higher postexercise ($P < 0.001$) and 4 h postexercise in the fasted state ($P = 0.001$) compared with the glucose-fed state (Fig. 2A). Moreover, plasma NEFA concentrations were increased at the end of exercise ($P < 0.001$) and 4 h postexercise ($P = 0.025$) in the fasted condition compared with baseline, whereas plasma NEFA concentrations were decreased 4 h postexercise ($t = 360$) in the glucose-fed state compared with baseline ($P = 0.047$, Fig. 2A).

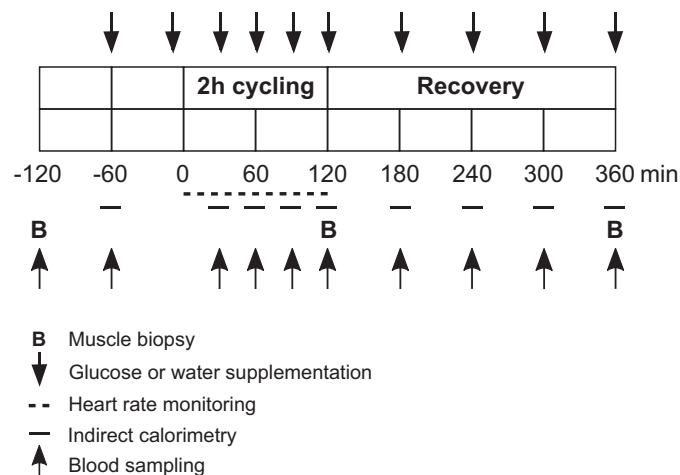


Figure 1. Experimental design. The experimental design of the study. All participants performed the protocol twice, once in the fasted state consuming water and once with glucose supplementation, with at least 1 wk in between.

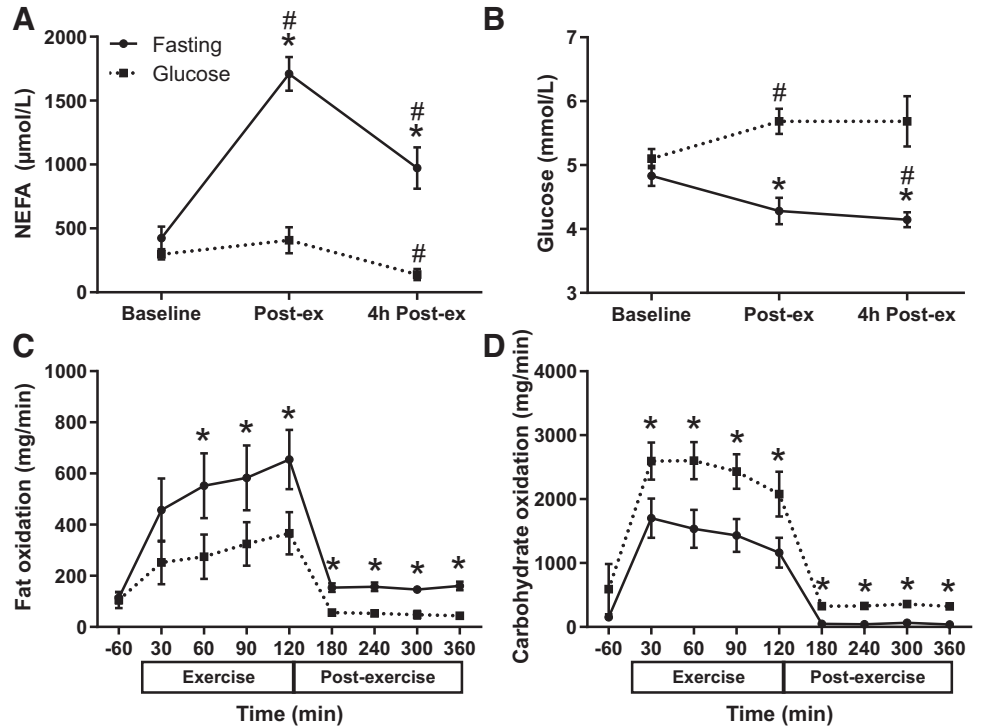


Figure 2. Differential substrate levels and usage with fasting vs. glucose supplementation. Plasma nonesterified fatty acids (NEFA) levels (A), plasma glucose levels (B), fat oxidation (C), and carbohydrate oxidation (D) before, during, and postexercise with (dotted line/squares) and without (solid line/circles) glucose supplementation. * $P < 0.05$ compared with glucose-fed state, # $P < 0.05$ compared with baseline ($t = -60$ min). Data are means \pm SE ($n = 9$).

During the entire experimental testing, plasma glucose concentrations remained within the normoglycemic range. Plasma glucose concentrations showed a significant treatment ($P = 0.002$) and time \times treatment interaction ($P = 0.008$) effect. Plasma glucose concentrations were higher directly postexercise ($P = 0.002$) and 4 h postexercise ($P = 0.008$) in the glucose-fed state compared with the fasted state (Fig. 2B). Moreover, plasma glucose concentrations were increased at the end of exercise ($t = 120$) compared with baseline ($t = -60$) in the glucose-fed state ($P = 0.029$), whereas plasma glucose concentrations were decreased 4 h postexercise in the fasted state compared with baseline ($P = 0.003$, Fig. 2B).

Substrate Oxidation

During exercise, there was a significant time ($P < 0.001$) and treatment ($P = 0.042$) effect for fat oxidation and a significant treatment ($P = 0.018$) effect for carbohydrate oxidation (and a tendency for a time effect ($P = 0.082$)). This was reflected in a higher carbohydrate oxidation and lower fat oxidation in the glucose-fed state compared with the fasted state ($P < 0.05$, Fig. 2, C and D). In the postexercise period, a significant treatment effect was observed for both fat ($P = 0.004$) and carbohydrate ($P = 0.011$) oxidation. Carbohydrate oxidation was higher and fat oxidation was lower in the glucose-fed state versus the fasting condition during all time points of the recovery period ($P < 0.05$, Fig. 2, C and D). Surprisingly, we observed that energy expenditure during exercise in the fasted state was modestly, but significantly, lower than in the glucose-fed state ($P < 0.05$, Table 2). Two individuals were unable to maintain the workload demanded throughout the full two hours, if fasted. Thus, workload was lowered to facilitate completion of the trial, which might explain the differences in EE during exercise. Postexercise, all

subjects rested and energy expenditure was similar between the two conditions ($P > 0.05$, Table 2).

Intramyocellular Lipid Content and Lipid Droplet Morphology

Total IMCL content (based on type I and II fibers together) displayed a significant time ($P = 0.013$) and treatment effect ($P = 0.007$). Irrespective of the fed or fasted condition, IMCL content was not significantly changed from baseline, directly postexercise (Fig. 3A). At the same time point, however, LD size appeared modestly reduced directly postexercise in the fasted state ($P = 0.004$, Fig. 3B) but not in the glucose-fed state. Four hours postexercise, however, IMCL content significantly ($P = 0.036$) increased in the fasting condition compared with directly after exercise, which resulted in a significantly higher IMCL content in the fasted state than in glucose-fed state 4 h postexercise ($P = 0.016$, Fig. 3A). LD number displayed a tendency ($P = 0.084$) for a time effect.

Table 2. Energy expenditure during and after exercise in glucose-fed and fasted state

Treatment	Time, Min	Glucose	Fasted
		EE, kJ/Min	EE, kJ/Min
Exercise	30	51.0 \pm 3.0	45.0 \pm 2.6*
	60	51.8 \pm 3.2	46.0 \pm 2.9*
	90	52.3 \pm 3.2	45.6 \pm 2.9*
	120	51.4 \pm 3.5	44.2 \pm 3.0*
Postexercise	180	7.5 \pm 0.5	6.9 \pm 0.4
	240	7.1 \pm 0.4	6.9 \pm 0.4
	300	7.3 \pm 0.4	6.8 \pm 0.5
	360	7.3 \pm 0.4	7.1 \pm 0.6

Data are means \pm SE, $n = 9$ during exercise, $n = 7$ during postexercise. EE, energy expenditure; * $P < 0.05$ compared with glucose-fed state.

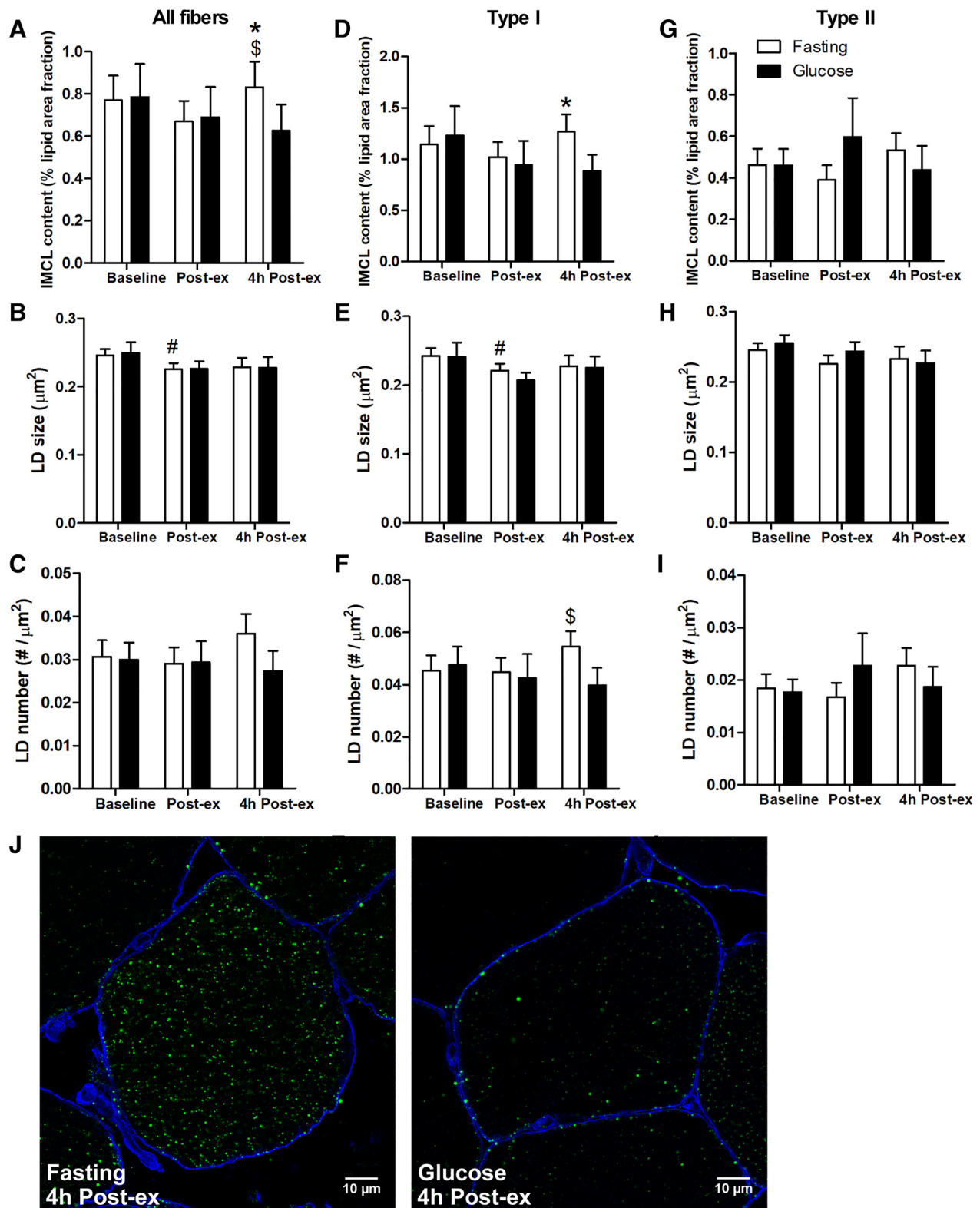


Figure 3. Increased intramyocellular lipid (IMCL) content 4 h postexercise with fasting. Intramyocellular lipid (IMCL) content (A, D, and G), lipid droplet (LD) size (B, E, and H) and LD number (C, F, and I) in all fibers, type I and type II fibers. Representative images of type I fibers of the same participant 4 h postexercise in fasting condition or with glucose-supplementation (J). * $P < 0.05$ compared with glucose-fed state; # $P < 0.05$ compared with baseline; \$Compared with postexercise. Data are means \pm SE ($n = 9$).

The increase in IMCL content in the fasted state during the recovery period appeared to result from an increased number of LDs (Fig. 3C) rather than an increase in LD size ($P = 0.754$, Fig. 3B). These observations are also visualized in Fig. 3J that displays type I fibers of the same participant 4 h post-exercise in both fasting and glucose-fed state.

When distinguishing between fiber types, the elevated IMCL content 4 h postexercise in the fasted condition compared with the glucose-fed state was only observed in the type I fibers ($p = 0.012$, Fig. 3D). Higher total IMCL content 4 h postexercise is more likely to originate from the near significant increase ($p = 0.058$) of IMCL directly postexercise than from the modest non-significant ($p = 0.841$) drop in the glucose-fed state. Higher IMCL content in type I fibers 4 h postexercise was paralleled by an increase in the number of LDs ($p = 0.010$, Fig. 3F) while LD size did not change ($p = 0.602$, Fig. 3E). Similar to the overall picture, exercise in the fasted condition reduced LD size in type I fibers ($p = 0.002$, Fig. 3E). No significant effects of exercise on IMCL content, LD number nor size were observed in type II fibers (Fig. 3G-I). Thus, the effects of exercise on IMCL seems to be mainly

due to effects in type I fibers. Of note, in these untrained participants, type I and type II fibers represented $42 \pm 3.1\%$ and $58 \pm 3.1\%$ of total fibers, respectively.

Cellular Distribution of Lipid Droplets

We next investigated if exercise differentially affected IMCL content, LD size and LD number in the SS versus the IMF region. Contrary to our expectations, and in the absence of significant changes in total IMCL content, we observed a significant decrease in IMCL content in the SS region directly after exercise in the fasting condition ($P = 0.012$, Fig. 4A). The decrease in SS IMCL content originated from a decrease in LD size ($P = 0.005$, Fig. 4B) as well as in LD number ($P = 0.040$, Fig. 4C). Four hours postexercise, SS IMCL content was replenished in the high NEFA (fasted) condition ($P = 0.028$, Fig. 4A), mainly due to an increase in LD number (Fig. 4C, $P = 0.040$). As a result, IMCL content in the SS region was significantly higher in the fasted versus the glucose-fed state 4 h postexercise ($P = 0.005$, Fig. 4A). In the IMF region, no significant differences in IMCL content or LD number and size in the IMF region were detected (Fig. 4, D-F).

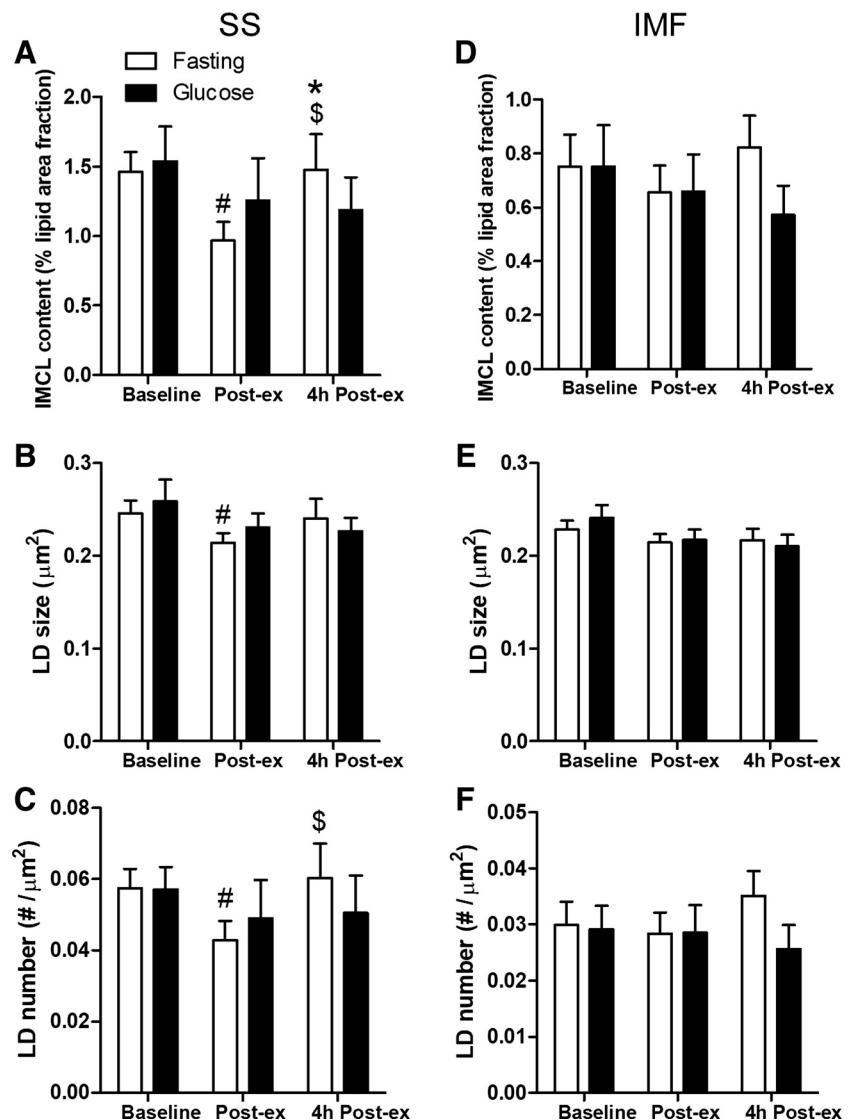


Figure 4. Subsarcolemmal intramyocellular lipid (IMCL) content transiently decreases directly postexercise and is replenished during recovery in the fasted state. IMCL content (A and D), lipid droplet (LD) size (B and E), and LD number (C and F) in all fibers in the subsarcolemmal (SS) or intermyofibrillar (IMF) region of the muscle fiber. * $P < 0.05$ compared with glucose-fed state; # $P < 0.05$ compared with baseline; \$ $P < 0.05$ compared with postexercise. Data are means \pm SE ($n = 9$).

PLIN5 Coating of Lipid Droplets

LDs can be separated into pools of LDs decorated with PLIN5 (PLIN5+ LDs) and those devoid of PLIN5 (PLIN5-LDs) (Fig. 5H). Interestingly, we observed a time ($P = 0.007$)

and treatment effect ($P = 0.004$) for IMCL content in PLIN5+ LDs (Fig. 5A). Four hours postexercise, IMCL content in PLIN5+ LDs was significantly higher in the fasting state compared with the glucose-fed state ($P = 0.013$) (Fig. 5, A and G). This was predominately due to a tendency ($P =$

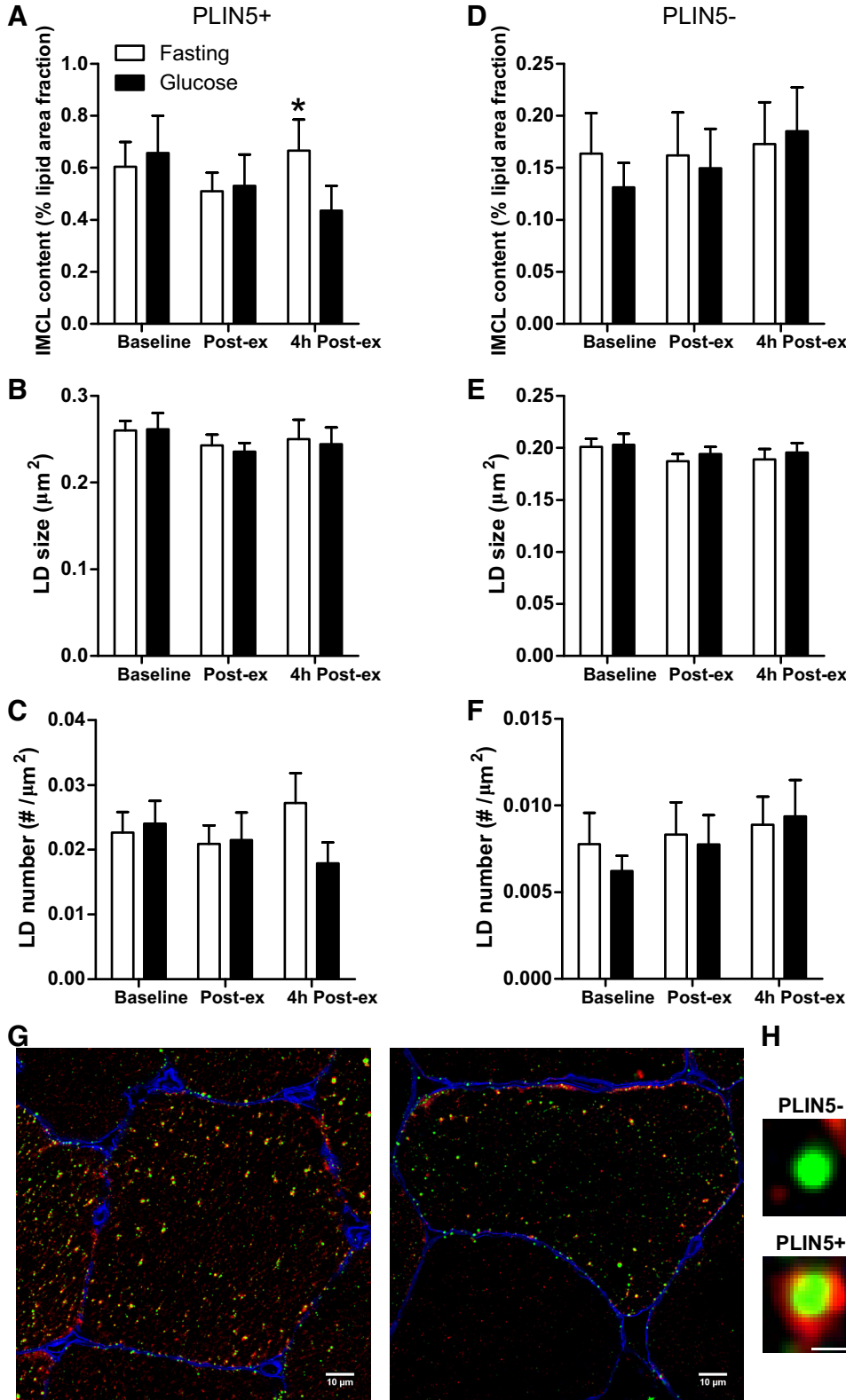


Figure 5. Increased intramyocellular lipid (IMCL) content 4 h postexercise in the fasting state was specifically in lipid droplets (LDs) coated with PLIN5. IMCL content (A and D), LD size (B and E), and LD number (C and F) in all fibers in LDs coated with PLIN5 (PLIN5+) or devoid of PLIN5 (PLIN5-). Representative images of type I fibers of the same participant 4 h postexercise in fasting condition or with glucose-supplementation in PLIN5+ LDs (G). Example of a PLIN5- and a PLIN5+ LD. Scale bar = 1 μm (H). * $P < 0.05$ compared with glucose-fed state. Data are means \pm SE ($n = 9$).

0.062) for an increase in lipid content in the fasting condition during the recovery period, rather than due to a drop in IMCL content in the glucose fed state (Fig. 5A, $P = 0.534$). The higher IMCL content in the fasted state, 4 h postexercise, seems to originate from a higher LD number (Fig. 5C), rather than from differences in LD size (Fig. 5B). For PLIN5- LDs, we could not detect any significant changes in IMCL content, LD size or number with exercise or during the recovery period (Fig. 5, D–F).

DISCUSSION

Data on the effect of acute exercise on IMCL content are ambiguous. The different and sometimes contradictory data on postexercise IMCL content may originate from a variety of factors, including muscle fiber typology, subcellular location of the stored fat and nutritional status, or more specifically, circulatory NEFA levels. Here, we examined the effect of an acute bout of exercise on IMCL content and distribution in healthy lean young men under a fasted and glucose-fed condition. It was observed that the effect of an acute bout of exercise on IMCL is affected by nutritional status during and after exercise, probably related to plasma NEFA levels. Changes in IMCL content upon exercise were muscle fiber type and region specific and dependent on the presence of PLIN5 on the LD. We observed that IMCL content decreased in the SS- but not in the IMF- region upon acute exercise and that 4 h following exercise, IMCL content was elevated in the fasted but not in the glucose-fed state. These significant changes were restricted to type I fibers, LDs decorated with PLIN5 and under fasted conditions, when plasma NEFA levels were highest.

In contrast to the glucose-fed state, moderate intensity exercise in the fasted state profoundly elevated plasma NEFA levels and increased fat oxidation, similar to what we reported previously using the same study design (33, 34). We previously showed that the increase in plasma NEFA content and fat oxidation in the fasted state upon exercise was paralleled by increased cardiac (34) and hepatic (33) fat content 4 h postexercise. This increase in ectopic fat accumulation was completely blunted upon consumption of a glucose drink during and after exercise, which resulted in blunted NEFA levels and fat oxidation rates. These observations in heart and liver are in line with the results of the current study, revealing restoration of IMCL 4 h postexercise in the fasted, but not the glucose-fed state. This was observed as well in trained athletes who were fasted during and after exercise (23). We suggest that these findings may be related to higher plasma NEFA levels (and lower fat oxidation), which we report in parallel with the changes in IMCL. However, we cannot exclude that other factors, such as higher glucose flux or higher insulin levels in the glucose-fed condition may be involved in the differential effects of exercise in the fed or fasted state on IMCL content 4 h postexercise. In addition, timing of the measurements could be a factor in the results we obtained, as we have recently shown that IMCL displays 24 h rhythmicity under resting conditions in healthy volunteers (35). It should be noted that in the current study, the timespan of the entire protocol, i.e., exercise and recovery, was ~8 h and that we used a crossover design where volunteers were their own controls and both

arms were carried out at the same time points. Therefore, although we cannot exclude a small effect of the diurnal rhythm of IMCL content on our findings, such an effect likely is insufficient to explain the difference we observe between the glucose-fed and fasted state.

In the fasted state, we observed a transient non-significant drop in IMCL content during exercise with an increase in IMCL in the recovery phase, resulting in higher IMCL levels in the fasted than in the glucose fed state. The higher IMCL levels originated from an increase in number (rather than size) of LDs, similar to what has been reported by others (23). In line with previous studies (17, 23), the overall changes in IMCL content were detected in type I muscle fibers (that store most IMCL and are predominantly recruited at exercise with this low intensity) but not in type II fibers. These findings are in line with previous observations that trained individuals store a higher number of LDs in type I muscle fibers compared with sedentary individuals (5). Tracer studies revealed that FAs derived from IMCL contribute to total fat oxidation during exercise (6–8, 10, 11). The increased IMCL-derived fat oxidation can result in a drop in IMCL content (36) and consequently changes in LD size and number (23). Directly postexercise, we did not observe a significant drop in total IMCL content in the present study. In the fasted state, fat oxidation rates and plasma NEFA levels were higher throughout exercise and recovery compared with the glucose-fed state. While the methodology used in the present study does not permit statements on FA flux through the LD, it is conceivable that profound elevation of plasma NEFAs in the fasted condition (and the concomitant increased NEFA delivery to the muscle) may refuel the LDs during exercise, hence preventing a significant drop in IMCL. Indeed, re-esterification of fat (37) and increased IMCL synthesis (19) in exercising muscle have been shown. As expected, glucose levels and carbohydrate oxidation levels were much higher in the glucose-fed state, indicating that carbohydrates were the main energy source in the glucose-fed condition.

In that respect, it is interesting to note that exercise in the fasted state resulted in a transient drop in IMCL content in the SS region, followed by restoration 4 h postexercise, whereas no significant effects were observed in the IMF region. LDs in the IMF region and in vicinity of mitochondria supposedly fuel muscle contraction (38). Thus, our observation in lean untrained individuals does not support our hypothesis that IMF LDs would contribute more to lipid oxidation during exercise than SS LDs, and therefore would reduce in size and/or number more profoundly. Our findings also do not match with recent observations in trained athletes (23) and young active individuals (36), where it was found that exercise resulted in a drop in IMCL in the IMF region, which was restored 4 h postexercise (23). On the other hand, our results are in line with previous findings where the number of SS LDs was reduced in the leg muscle of elite skiers after 1 h cross-country skiing (39), however they found a decrease in IMF LD number in the arms. It should be noted that prolonged exercise training programs reduce SS lipid content (40–42) and trained individuals display a lower SS lipid compared with sedentary individuals (5). The untrained participants in the current study may have higher SS lipid storage compared with athletes, possibly explaining this discrepancy. Another potential explanation for the

observation that exercise in the fasted state seems to specifically affect SS LDs may include FA trafficking from SS to IMF LDs; a high IMF LD-derived fat oxidation (with IMF lipid content quantitatively being the larger pool) may trigger FA flux from SS LDs toward IMF LDs. FA transport from the SS to the IMF region is more likely than LD motility in the skeletal muscle due to its organized structure (43). As both LD number and size decreased in the present study, a transfer of FAs is expected rather than a transfer of LDs, as the latter would result in a decrease of primarily LD number. However, the importance of lipid oxidation in the SS mitochondria themselves during exercise should also be considered, as exercise training more profoundly affects mitochondrial density and oxidative capacity of SS mitochondria compared with IMF mitochondria (44, 45). Regardless the exact mechanism, our data suggest a yet unidentified role for SS LDs in acute exercise and recovery of exercise.

The higher and elevated IMCL levels in fasted state after 4 h of recovery were detected when plasma NEFA levels were still profoundly elevated, while rates of fat oxidation had dropped substantially. Jointly, these data suggest that IMCL is restored and LDs serve as a sink to store excess FAs, an observation that we showed previously when comparing active and nonactive muscle during exercise (15) or upon prolonged fasting (27). The storage of excess fat upon prolonged fasting was accounted for specifically by LDs decorated with PLIN5 (PLIN5+ LDs) (27). PLIN5 is a LD coat protein involved in lipid storage and release of fat for oxidation (for review see Ref. 46). Others have shown that PLIN5 coated LDs are preferentially used for lipolysis during exercise (17). The increase in IMCL content after 4 h of recovery in the fasting state, was completely accounted for by PLIN5+ LDs in the present study, suggesting preferential storage of FAs into PLIN5+ LDs postexercise, which has previously been observed by others as well (23). PLIN5 interacts with enzymes such as ATGL (47–49) and these enzymes may be involved in this regulation of LD turnover as well. In that context, it is interesting to note that we have previously shown that acute exercise leads to a rapid upregulation of PGC1 and PPAR gene expression (50), which are transcription factors regulating lipid metabolism gene expression. Interestingly, we previously showed that glucose ingestion during exercise lead to blunted expression of lipid metabolism genes (51), suggesting that transcriptional regulation may be involved in the regulation of LD turnover. Moreover, it has been observed that HSL preferentially recruits to PLIN5+ LDs after acute exercise (52). Jointly, these data indicate involvement of LD coating proteins such as PLIN5, ATGL, HSL, DGAT and other regulation proteins in LD turnover during fasted conditions, and show that PLIN5+ LDs can serve as a sink for excess FAs postexercise, but also serve to fuel oxidation during exercise (17, 23). These observations match the dual role that has been postulated for PLIN5 in skeletal muscle; regulating FA flux from LD to mitochondria upon metabolic demand (25) as well as protecting against lipotoxicity under basal conditions by stimulating lipid storage (27, 53).

Our study comes with some limitations especially when it comes to generalizability. Thus volunteers in our study were lean, young men and it is possible that other results will be

found when studying women. Furthermore, our volunteers were relatively untrained, which might explain the discrepancy with observations reported in endurance-trained athletes. Furthermore, different types of exercise, for example higher intensity exercise, may result in different conclusions regarding the role of SS and IMF lipids in exercise.

In summary, we have shown here that acute exercise affects IMCL storage and LD morphology during exercise and during recovery from exercise, and that the effects of exercise on IMCL content are affected by nutritional status, reflected in higher plasma NEFA levels and fat oxidation rates. Remarkably, we observed a decrease in lipids stored in the SS region of the muscle fiber acutely after exercise in the fasting condition. During recovery from exercise in the fasting state, IMCL levels increased specifically in type I fibers and in PLIN5+ LDs, predominantly via an increased number of LDs in the SS region. Future studies should aim at unraveling intracellular FAs fluxes during exercise to establish a greater understanding of IMCL metabolism.

GRANTS

This work was funded by Diabetes Fonds (Dutch Diabetes Research Foundation) (Grant DF 2014.00.1756; to S.D.), European Research Council (ERC) (759161 “MRS in diabetes”; to V.B. and S.-H.), Hartstichting (Heart Foundation) (CVON2014-02 ENERGISE; to N.v.P.).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.D., L.B., E.P., V.B.S.-H., P.S., and M.K.C.H. conceived and designed research; S.D., N.v.P., L.B., and E.M.-K. performed experiments; S.D., N.v.P., L.B., and E.M.-K. analyzed data; S.D., N.v.P., L.B., E.P., E.M., V.B.S.-H., P.S., and M.K.C.H. interpreted results of experiments; S.D., N.v.P., and L.B. prepared figures; S.D., N.v.P., L.B., P.S., and M.K.C.H. drafted manuscript; S.D., N.v.P., L.B., E.P., V.B.S.-H., P.S., and M.K.C.H. edited and revised manuscript; S.D., N.v.P., L.B., E.P., E.M.-K., V.B.S.-H., P.S., and M.K.C.H. approved final version of manuscript.

REFERENCES

1. Unger RH, Clark GO, Scherer PE, Orci L. Lipid homeostasis, lipotoxicity and the metabolic syndrome. *Biochim Biophys Acta* 1801: 209–214, 2010. doi:10.1016/j.bbali.2009.10.006.
2. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42: 113–116, 1999. [Erratum in *Diabetologia* 42: 386, 1999]. doi:10.1007/s001250051123.
3. Schrauwen-Hinderling VB, Schrauwen P, Hesselink MK, van Engelshoven JM, Nicolay K, Saris WH, Kessels AG, Kooi ME. The increase in intramyocellular lipid content is a very early response to training. *J Clin Endocrinol Metab* 88: 1610–1616, 2003. doi:10.1210/jc.2002-021464.
4. Goodpaster BH, He J, Watkins S, Kelley DE. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86: 5755–5761, 2001. doi:10.1210/jcem.86.12.8075.
5. Daemen S, Gemmink A, Brouwers B, Meex RCR, Huntjens PR, Schaart G, Moonen-Kornips E, Jörgensen J, Hoeks J, Schrauwen P, Hesselink MKC. Distinct lipid droplet characteristics and

- distribution unmask the apparent contradiction of the athlete's paradox. *Mol Metab* 17: 71–81, 2018. doi:10.1016/j.molmet.2018.08.004.
6. Coyle EF, Jeukendrup AE, Wagenmakers AJ, Saris WH. Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. *Am J Physiol Endocrinol Physiol* 273: E268–E275, 1997. doi:10.1152/ajpendo.1997.273.2.E268.
 7. Martin WH 3rd, Dalsky GP, Hurley BF, Matthews DE, Bier DM, Hagberg JM, Rogers MA, King DS, Holloszy JO. Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *Am J Physiol Endocrinol Physiol* 265: E708–E714, 1993. doi:10.1152/ajpendo.1993.265.5.E708.
 8. Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GF, Hill RE, Grant SM. Effects of training duration on substrate turnover and oxidation during exercise. *J Appl Physiol (1985)* 81: 2182–2191, 1996. doi:10.1152/jap.1996.81.5.2182.
 9. Stellingwerff T, Boon H, Jonkers RA, Senden JM, Spriet LL, Koopman R, van Loon LJ. Significant intramyocellular lipid use during prolonged cycling in endurance-trained males as assessed by three different methodologies. *Am J Physiol Endocrinol Metab* 292: E1715–E1723, 2007. doi:10.1152/ajpendo.00678.2006.
 10. van Loon LJC, Koopman R, Stegen JHCH, Wagenmakers AJM, Keizer HA, Saris WHM. Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state. *J Physiol* 553: 611–625, 2003. doi:10.1113/jphysiol.2003.052431.
 11. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, Wolfe RR. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Physiol* 265: E380–E391, 1993. doi:10.1152/ajpendo.1993.265.3.E380.
 12. Brechtel K, Niess AM, Machann J, Rett K, Schick F, Claussen CD, Dickhuth HH, Haering HU, Jacob S. Utilisation of intramyocellular lipids (IMCLs) during exercise as assessed by proton magnetic resonance spectroscopy (1H-MRS). *Horm Metab Res* 33: 63–66, 2001. doi:10.1055/s-2001-12407.
 13. Bucher J, Krüsi M, Zueger T, Ith M, Stettler C, Diem P, Boesch C, Kreis R, Christ E. The effect of a single 2 h bout of aerobic exercise on ectopic lipids in skeletal muscle, liver and the myocardium. *Diabetologia* 57: 1001–1005, 2014. doi:10.1007/s00125-014-3193-0.
 14. Egger A, Kreis R, Allemann S, Stettler C, Diem P, Buehler T, Boesch C, Christ ER. The effect of aerobic exercise on intrahepato-cellular and intramyocellular lipids in healthy subjects. *PLoS One* 8: e70865, 2013. doi:10.1371/journal.pone.0070865.
 15. Schrauwen-Hinderling VB, van Loon LJ, Koopman R, Nicolay K, Saris WH, Kooi ME. Intramyocellular lipid content is increased after exercise in nonexercising human skeletal muscle. *J Appl Physiol (1985)* 95: 2328–2332, 2003. doi:10.1152/jap.2003.00304.2003.
 16. Vermathen P, Saillen P, Boss A, Zehnder M, Boesch C. Skeletal muscle (1)H MRSI before and after prolonged exercise. I. muscle specific depletion of intramyocellular lipids. *Magn Reson Med* 68: 1357–1367, 2012. doi:10.1002/mrm.24168.
 17. Shepherd SO, Cocks M, Tipton KD, Ranasinghe AM, Barker TA, Burniston JG, Wagenmakers AJ, Shaw CS. Sprint interval and traditional endurance training increase net intramuscular triglyceride breakdown and expression of perilipin 2 and 5. *J Physiol* 591: 657–675, 2013. doi:10.1113/jphysiol.2012.240952.
 18. Rico-Sanz J, Hajnal JV, Thomas EL, Mierisova S, Ala-Korpela M, Bell JD. Intracellular and extracellular skeletal muscle triglyceride metabolism during alternating intensity exercise in humans. *J Physiol* 510: 615–622, 1998. doi:10.1111/j.1469-7793.1998.615bk.x.
 19. Bergman BC, Butterfield GE, Wolfel EE, Casazza GA, Lopaschuk GD, Brooks GA. Evaluation of exercise and training on muscle lipid metabolism. *Am J Physiol Endocrinol Physiol* 276: E106–E117, 1999. doi:10.1152/ajpendo.1999.276.1.E106.
 20. Kiens B, Richter EA. Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am J Physiol Endocrinol Physiol* 275: E332–E337, 1998. doi:10.1152/ajpendo.1998.275.2.E332.
 21. Roepstorff C, Steffensen CH, Madsen M, Stallknecht B, Kanstrup IL, Richter EA, Kiens B. Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. *Am J Physiol Endocrinol Metab* 282: E435–E447, 2002. doi:10.1152/ajpendo.00266.2001.
 22. Hoeks J, van Herpen NA, Mensink M, Moonen-Kornips E, van Beurden D, Hesselink MK, Schrauwen P. Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. *Diabetes* 59: 2117–2125, 2010. doi:10.2337/db10-0519.
 23. Jevons EFP, Gejl KD, Strauss JA, Ørtenblad N, Shepherd SO. Skeletal muscle lipid droplets are resynthesized before being coated with perilipin proteins following prolonged exercise in elite male triathletes. *Am J Physiol Endocrinol Metab* 318: E357–E370, 2020. doi:10.1152/ajpendo.00399.2019.
 24. Bosma M, Sparks LM, Hooiveld GJ, Jorgensen JA, Houten SM, Schrauwen P, Kersten S, Hesselink MK. Overexpression of PLIN5 in skeletal muscle promotes oxidative gene expression and intramyocellular lipid content without compromising insulin sensitivity. *Biochim Biophys Acta* 1831: 844–852, 2013. doi:10.1016/j.bbali.2013.01.007.
 25. Gemmink A, Daemen S, Kuijpers HJH, Schaart G, Duimel H, López-Iglesias C, van Zandvoort M, Knoop K, Hesselink MKC. Super-resolution microscopy localizes perilipin 5 at lipid droplet-mitochondria interaction sites and at lipid droplets juxtaposing to perilipin 2. *Biochim Biophys Acta Mol Cell Biol Lipids* 1863: 1423–1432, 2018. doi:10.1016/j.bbali.2018.08.016.
 26. Shepherd SO, Strauss JA, Wang Q, Dube JJ, Goodpaster B, Mashek DG, Chow LS. Training alters the distribution of perilipin proteins in muscle following acute free fatty acid exposure. *J Physiol* 595: 5587–5601, 2017. doi:10.1113/JP274374.
 27. Gemmink A, Bosma M, Kuijpers HJ, Hoeks J, Schaart G, van Zandvoort MA, Schrauwen P, Hesselink MK. Decoration of intramyocellular lipid droplets with PLIN5 modulates fasting-induced insulin resistance and lipotoxicity in humans. *Diabetologia* 59: 1040–1048, 2016. doi:10.1007/s00125-016-3865-z.
 28. Bergström J, Hermansen L, Hultman E, Saltin B. Diet, muscle glyco-gen and physical performance. *Acta Physiol Scand* 71: 140–150, 1967. doi:10.1111/j.1748-1716.1967.tb03720.x.
 29. Péronnet F, Massicotte D. Table of nonprotein respiratory quotient: an update. *Can J Sport Sci* 16: 23–29, 1991.
 30. Kuipers H, Verstappen FT, Keizer HA, Geurten P, van Kranenburg G. Variability of aerobic performance in the laboratory and its physiologic correlates. *Int J Sports Med* 6: 197–201, 1985. doi:10.1055/s-2008-1025839.
 31. Siri WE. The gross composition of the body. *Adv Biol Med Phys* 4: 239–280, 1956. doi:10.1016/b978-1-4832-3110-5.50011-x.
 32. Gemmink A, Daemen S, Brouwers B, Huntjens PR, Schaart G, Moonen-Kornips E, Jörgensen J, Hoeks J, Schrauwen P, Hesselink MKC. Dissociation of intramyocellular lipid storage and insulin resistance in trained athletes and type 2 diabetes patients; involvement of perilipin 5? *J Physiol* 596: 857–868, 2018. doi:10.1113/JP275182.
 33. Bilet L, Brouwers B, van Ewijk PA, Hesselink MK, Kooi ME, Schrauwen P, Schrauwen-Hinderling VB. Acute exercise does not decrease liver fat in men with overweight or NAFLD. *Sci Rep* 5: 9709, 2015. doi:10.1038/srep09709.
 34. Bilet L, van de Weijer T, Hesselink MKC, Glatz JFC, Lamb HJ, Wildberger J, Kooi ME, Schrauwen P, Schrauwen-Hinderling VB. Exercise-induced modulation of cardiac lipid content in healthy lean young men. *Basic Res Cardiol* 106: 307–315, 2011. doi:10.1007/s00395-010-0144-x.
 35. Held NM, Wefers J, van Weeghel M, Daemen S, Hansen J, Vaz FM, van Moorsel D, Hesselink MKC, Houtkooper RH, Schrauwen P. Skeletal muscle in healthy humans exhibits a day-night rhythm in lipid metabolism. *Mol Metab* 37: 100989, 2020. doi:10.1016/j.molmet.2020.100989.
 36. Chee C, Shannon CE, Burns A, Selby AL, Wilkinson D, Smith K, Greenhaff PL, Stephens FB. Relative contribution of intramyocellular lipid to whole-body fat oxidation is reduced with age but subsarcolemmal lipid accumulation and insulin resistance are only associated with overweight individuals. *Diabetes* 65: 840–850, 2016. doi:10.2337/db15-1383.
 37. Sacchetti M, Saltin B, Osada T, van Hall G. Intramuscular fatty acid metabolism in contracting and non-contracting human skeletal muscle. *J Physiol* 540: 387–395, 2002. doi:10.1113/jphysiol.2001.013912.
 38. Hood DA. Invited Review: contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol (1985)* 90: 1137–1157, 2001. doi:10.1152/jap.2001.90.3.1137.

39. Koh HE, Nielsen J, Saltin B, Holmberg HC, Ørtenblad N. Pronounced limb and fibre type differences in subcellular lipid droplet content and distribution in elite skiers before and after exhaustive exercise. *J Physiol* 595: 5781–5795, 2017. doi:10.1113/JP274462.
40. Li Y, Lee S, Langleite T, Norheim F, Pourteymour S, Jensen J, Stadheim HK, Storås TH, Davanger S, Gulseth HL, Birkeland KI, Drevon CA, Holen T. Subsarcolemmal lipid droplet responses to a combined endurance and strength exercise intervention. *Physiol Rep* 2: e12187, 2014. doi:10.14814/phy2.12187.
41. Devries MC, Samjoo IA, Hamadeh MJ, McCreedy C, Raha S, Watt MJ, Steinberg GR, Tarnopolsky MA. Endurance training modulates intramyocellular lipid compartmentalization and morphology in skeletal muscle of lean and obese women. *J Clin Endocrinol Metab* 98: 4852–4862, 2013. doi:10.1210/jc.2013-2044.
42. Nielsen J, Mogensen M, Vind BF, Sahlin K, Højlund K, Schrøder HD, Ortenblad N. Increased subsarcolemmal lipids in type 2 diabetes: effect of training on localization of lipids, mitochondria, and glycogen in sedentary human skeletal muscle. *Am J Physiol Endocrinol Metab* 298: E706–E713, 2010. doi:10.1152/ajpendo.00692.2009.
43. Bosma M. Lipid droplet dynamics in skeletal muscle. *Exp Cell Res* 340: 180–186, 2016. doi:10.1016/j.yexcr.2015.10.023.
44. Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, Cortright RN. Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. *Am J Physiol Cell Physiol* 288: C1074–C1082, 2005. doi:10.1152/ajpcell.00391.2004.
45. Bizeau ME, Willis WT, Hazel JR. Differential responses to endurance training in subsarcolemmal and intermyofibrillar mitochondria. *J Appl Physiol (1985)* 85: 1279–1284, 1998. doi:10.1152/jappl.1998.85.4.1279.
46. Gemmink A, Schrauwen P, Hesselink MKC. Exercising your fat (metabolism) into shape: a muscle-centred view. *Diabetologia* 63: 1453–1463, 2020. doi:10.1007/s00125-020-05170-z.
47. Granneman JG, Moore HP, Mottillo EP, Zhu Z, Zhou L. Interactions of perilipin-5 (Plin5) with adipose triglyceride lipase. *J Biol Chem* 286: 5126–5135, 2011. doi:10.1074/jbc.M110.180711.
48. MacPherson RE, Ramos SV, Vandenboom R, Roy BD, Peters SJ. Skeletal muscle PLIN proteins, ATGL and CGI-58, interactions at rest and following stimulated contraction. *Am J Physiol Regul Integr Comp Physiol* 304: R644–R650, 2013. doi:10.1152/ajpregu.00418.2012.
49. Keenan SN, De Nardo W, Lou J, Schittenhelm RB, Montgomery MK, Granneman JG, Hinde E, Watt MJ. Perilipin 5 S155 phosphorylation by PKA is required for the control of hepatic lipid metabolism and glycemic control. *J Lipid Res* 62: 100016, 2021. doi:10.1194/jlr.RA120001126.
50. Russell AP, Hesselink MK, Lo SK, Schrauwen P. Regulation of metabolic transcriptional co-activators and transcription factors with acute exercise. *FASEB J* 19: 986–988, 2005. doi:10.1096/fj.04-3168fje.
51. Civitarese AE, Hesselink MK, Russell AP, Ravussin E, Schrauwen P. Glucose ingestion during exercise blunts exercise-induced gene expression of skeletal muscle fat oxidative genes. *Am J Physiol Endocrinol Metab* 289: E1023–E1029, 2005. doi:10.1152/ajpendo.00193.2005.
52. Whytock KL, Shepherd SO, Wagenmakers AJM, Strauss JA. Hormone-sensitive lipase preferentially redistributes to lipid droplets associated with perilipin-5 in human skeletal muscle during moderate-intensity exercise. *J Physiol* 596: 2077–2090, 2018. doi:10.1113/JP275502.
53. Wang H, Bell M, Sreenivasan U, Sreenevasan U, Hu H, Liu J, Dalen K, Londos C, Yamaguchi T, Rizzo MA, Coleman R, Gong D, Brasaemle D, Sztalryd C. Unique regulation of adipose triglyceride lipase (ATGL) by perilipin 5, a lipid droplet-associated protein. *J Biol Chem* 286: 15707–15715, 2011. [Erratum in *J Biol Chem* 288: 10952, 2013]. doi:10.1074/jbc.M110.207779.