Transient Responses of the Hepatic Lipidome to Acute Exercise Bouts in Mice

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Abstract

BACKGROUND: The content of triacylglycerol (TAG) in the liver is known to rapidly increase after a single bout of exercise followed by recovery to sedentary levels. The response of other hepatic lipids, and acyl chain composition of lipid classes, would provide a deeper understanding of the response of hepatic lipid metabolism to acute exercise.

METHODS: Female mice performed a single bout of continuous exercise (CE), high-intensity interval exercise (HIIE), or no exercise (CON). The total content of various lipids in the liver, and fatty acids within lipid classes, were measured in tissues collected 3 hours after exercise (Day 1) and the day following exercise (Day 2).

RESULTS: The total concentration of TAG rose on Day 1 after exercise ($P < 0.05$), with a greater elevation in HIIE than CE ($P < 0.05$), followed by a decline toward CON levels on Day 2. The total concentration of other measured lipid classes was not significantly altered by exercise. However, n-6 polyunsaturated fatty acid relative abundance in diacylglycerol (DAG) was increased by HIIE ($P < 0.05$). In CON liver, TAG content was positively correlated with DAG and phosphatidylethanolamine ($P < 0.05$), while these statistical associations were disrupted in exercised mice on Day 1.

CONCLUSIONS: Overall, the results characterize flexibility of the hepatic TAG pool size in the liver, and the relationship between TAG and other lipid abundances is altered during the transient TAG pool expansion after exercise. The transient expansion of the hepatic TAG pool and remodeling of the DAG pool may be fundamental components of the physiological response to intense exercise.

Background

The lipid that accumulates in the body during positive energy balance is primarily as triacylglycerol (TAG) in adipose tissue. However, TAG and other lipids can accumulate elsewhere as well at lower concentrations, and this ectopic lipid deposition is common during weight gain and other states of metabolic dysfunction (1–6). This lipid accumulation has serious ramifications for metabolism; excess TAG accumulation in liver and muscle typically presents alongside elevated levels of lipotoxic intermediates such as diacylglycerol (DAG) (1–5). DAG and even perhaps other lipotoxic intermediates in these insulin-responsive tissues can lead to the development of insulin resistance (2, 7, 8). Structural membrane lipids, such as phospholipid, are less likely to be responsive to lifestyle factors that drive weight gain or weight loss. However, phospholipids do coat intracellular lipid droplets and therefore could play a role in TAG accumulation within various tissues. The responses of tissue TAG concentrations to lifestyle factors that alter lipid metabolism have been studied in depth, such as the net utilization of intramuscular TAG with acute exercise (9–13) and the transient accumulation of hepatic TAG in response to an acute exercise bout (11, 13–17). In contrast to acute effects of exercise, chronic exercise training appears to elevate intramuscular TAG (6, 18) and can potentially reduce hepatic TAG to a modest extent in certain instances (6, 19). However, it must be noted that chronically training individuals could

potentially experience acute effects of each exercise bout. Thus, in individuals undertaking an exercise training program, meaningful fluctuations in the content of these lipid depots are expected to occur during the course of each day, as a result of their exercise participation. We are specifically interested in the liver's ability to transiently expand its TAG content during and after exercise, as this is a more recently appreciated component of the exercise response. In order to develop a better understanding of this phenomenon, a greater level of knowledge is needed regarding the complexity of the lipid profile in the liver and its acute response to exercise bouts.

While lipid content in the liver and other peripheral tissue is of great importance for metabolic health, the largest lipid-enriched fuel depot in the body is adipose tissue. TAG mobilization through lipolysis in adipose tissue is enhanced during exercise and post-exercise recovery (20–22). This lipolysis response, driven by catecholamines and other endocrine stress responses, likely evolved to match the mobilization of free fatty acids (FFA) to the fuel needs of exercised skeletal muscle. While FFA transport protein expression is expected to play some role in tissue FFA uptake (23), FFA concentration gradients and effects of mass action would still play critical roles in the control of FFA uptake into tissues. Plasma FFA concentration is elevated during exercise to drive FFA from adipose tissue to working skeletal muscle, yet the increased circulating FFA will also drive this lipid to other organs by mass action. The ability to cope with enhanced FFA supply to non-muscle tissues during exercise is metabolically critical but has not been addressed extensively in metabolic research. Mobilization of FFA during exercise appears to be approximately matched to skeletal muscle's needs, but to achieve this appropriate supply of FFA to skeletal muscle, the FFA supply to other tissues may exceed their needs. This could explain why hepatic TAG acutely accumulates during and after exercise in laboratory animals (9, 17, 24), followed by a spontaneous return of hepatic TAG back to near sedentary levels by the day after exercise as recovery from the stressor takes place (17). Furthermore, blocking lipolysis with nicotinic acid administration prevents this exercise-induced hepatic lipid accumulation in rats (24), further supporting the notion that exercise-induced lipolysis leads to the accumulation of lipid outside of exercised muscle. In human subjects studied by magnetic resonance spectroscopy (MRS) before and after exercise (11, 13–16), the accumulation of hepatic lipid has been observed, in agreement with the work on both rats and mice (9, 17, 24). Providing the appropriate amount of FFA to contracting muscle through accentuated lipolysis in adipose tissue even leads to an apparent excess of FFA supply to the non-exercised resting muscle, as resting muscle during exercise accumulates TAG (12). Clearly there is a need to develop a more elaborate understanding of the metabolic stress that is placed upon various organs during and after exercise, including those tissues that must cope with a net accumulation of TAG.

While clinically it is of interest to devise approaches to chronically reduce the level of TAG accumulation in the liver, it should not be ignored that substantial day-to-day and hour-to-hour fluctuations in hepatic TAG and other lipids may also be very important for support of metabolic integration and overall health. The ability to rapidly increase lipid storage at ectopic lipid deposition sites, when metabolically appropriate, is a physiological parameter that deserves increased attention; we propose the term "lipogenic flexibility" for this phenomenon in stress physiology. While lipogenic flexibility is depicted primarily by transient changes in tissue TAG, broadly testing the lipid profile in liver tissue within the

context of this phenomenon is vital. We tested a broad range of lipid classes and their fatty acyl chain composition to further characterize the response of hepatic lipids to an acute exercise bout in mice.

Methods

Animals. This protocol was approved by the Rutgers University Institutional Animal Care and Use Committee. Initial results from the study were published previously (17). Following initial study execution, lipidomics analysis was performed on liver samples from the female mice in the study, and those results are presented here and have not been published previously. C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in the animal facility at Rutgers University. The mice were maintained on a 12-hour light/dark photoperiod with all mice allowed ad libitum access to food and water. All mice consumed the Labdiet 5K52 diet (Purina Mills, Richmond, IN) and were acclimated to the facility for at least 5 days prior to exercise.

Exercise protocols. Mice were exercised between the ages of 14–16 weeks on a treadmill (Exer-3/6, Columbus Instruments, Columbus, OH) with a shock grid set at a low intensity (on a scale of 0–10, set at 1). On the day before exercise, all mice were acclimated to the treadmill for 5 minutes at a speed of 5 m/min with no incline (0°). Subsequently, mice were then assigned to sedentary control (CON), continuous exercise (CE), or high-intensity interval exercise (HIIE) groups. Food was withdrawn at 7:00AM on the day of exercise. For mice assigned to CE or HIIE, the exercise session began between 11:45AM and 12:30PM. CON was a time-of-day matched condition in which mice remained in their cages with water bottles withdrawn for the amount of time that CE and HIIE mice would spend away from their cages during the exercise session (approximately 35 minutes). For HIIE, following a 5 minute warmup at a slow walking speed (5 m/min), mice ran for 30 second intervals with 60 second walking rest periods (5 m/min) interspersed between intervals. The exercise session included 20 running intervals, the first at 15 m/min, next at 20 m/min, then at 25 m/min, followed by all remaining sprint intervals at a final speed of 30 m/min. Acceleration up to 15, 20, 25 and 30 m/min was performed within a 5 second ramping duration and deceleration back to 5 m/min within a 2 second duration. Both the warmup and the exercise session were performed at an incline of 25°. CE consisted of an incline-matched, duration-matched, and distance-matched continuous running session (13.8 m/min for 30 min) following the same 5 minute warmup phase. These HIIE and CE protocols have been confirmed previously to also be matched for energy expenditure in mice (25). To avoid repeated shocks and to maintain running speed, mice were gently prodded by hand when they closely approached the shock grid.

Tissue collection. On one set of mice, euthanasia followed by liver tissue collection was performed on the day of exercise (Day 1) while on a separate set of mice the euthanasia and tissue collection were performed on the day following exercise (Day 2). For the mice assigned to tissue collection on Day 1, they remained fasted for 8 hours until euthanasia at 3:00PM. For mice assigned to tissue collection on Day 2, on the day of exercise they were returned to their cages and given food at 7:00PM for overnight free food access; the following morning, food was again withdrawn at 7:00AM and then euthanasia was performed at 3:00PM following an 8-hour fast. Once collected, liver tissues were immediately frozen in liquid

nitrogen then stored at -80 °C until analysis. Liver tissue from a total of 36 female mice was collected analyzed (n = 6 per condition on Day 1, and n = 6 per condition on Day 2)

Lipid analysis. Liver samples from 6 mice per group were sent to Metabolon (Lipomics Inc, Sacramento, CA, USA) for lipid profile analysis. Lipids were extracted from liver tissue, fractionated into specific lipid classes, followed by derivatization of the acyl chains to fatty acid methyl esters (FAME). The derivatized fatty acids from each lipid class were then analyzed by gas chromatography by a previously published method (26). The total content of each lipid class was calculated (µmol per gram of liver wet weight) as well as the fatty acid composition (mol %) for each of the following lipid classes: TAG, DAG, cholesterol ester (ChE), cardiolipin (CL), lysophosphatidylcholine (LyPC), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and sphingomyelin (SM).

Statistical analysis

Data are presented as means ± SE. The total content of each lipid class in the liver was analyzed by 2-way ANOVA (time-by-trial). Within a lipid class, the relative abundance of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) were tested with the same ANOVA structure. The time level in ANOVAs had 2 factors (Day 1 vs Day 2) and the trial level had 3 factors (CON, CE, HIIE). Main effect of trial and trial-by-time interactions were tested. ANOVA was followed by Fisher's Least Significant Difference (LSD) *post hoc* test. Pearson's correlation coefficient was used to quantify the relationships between the total content of hepatic TAG and other lipid classes. Correlations were performed for sedentary controls alone (Day 1 and 2 data pooled); if there was a significant correlation in CON mice, next we tested for a correlation in post-exercise Day 1 samples (CE and HIIE groups pooled) to ascertain if the association was maintained during the expansion of the hepatic TAG pool after exercise. We also tested for a correlation in post-exercise Day 2 samples (CE and HIIE groups pooled) to ascertain if the association was maintained a day after exercise. For ANOVA and linear regression, the statistical analyses were performed with JMP version 8 (SAS Institute Inc., Cary, NC) and $P < 0.05$ was considered statistically significant. Principal component analysis (PCA) was performed on the relative fatty acid composition of lipid classes using Metabo-Analyst 4.0 software. Fatty acids with a relative abundance greater than or equal to 0.1% and a VIP score greater than 1 were included in the PCA.

Results

Hepatic TAG content from the same mice studied here, measured by an enzyme-linked colorimetric assay, was reported previously (17). For the lipidomics analysis that is presented in this report, a new piece of tissue was cut from each of these original liver samples, and underwent lipid extraction and then analysis by chromatographic techniques that are distinct from our previous colorimetric approach. Previously, the only lipid measured was TAG, while now we report results from a broad lipid profile. These independently performed analyses of TAG from the same mouse livers provide an opportunity for method comparison. The present results for group differences in TAG (Table 1) are in close agreement with our previously reported findings (17), despite the fact that the analyses were performed in different laboratories, by

different methods, and by different analysts. To further explore the method comparison, we performed linear regression to compare results for each liver sample from the two methods (total of 36 samples). The correlation was highly significant ($P < 0.0001$, $R^2 = 0.86$, slope = 0.99).

Table 1
Total content of lipid classes in the liver

		TAG^&	DAG	ChE	CL	LyPC	PC	PE	PS	SM
Day 1										
	CON	16.7 ± 2.1	3.9 ± 0.4	4.0 ± 0.3	3.2 ± 0.3	1.6 ± 0.1	27.2 ± 3.0	15.0 ± 0.3	3.3 ± 0.3	1.5 ± 0.04
	CE	24.1 ± 2.2*	4.7 ± 0.4	4.7 ± 0.4	3.2 ± 0.4	1.5 ± 0.1	21.9 ± 2.0	14.4 ± 0.3	3.2 ± 0.2	1.4 ± 0.1
	HIIE	32.5 ± 3.9*#	4.8 ± 0.4	4.2 ± 0.2	3.0 ± 0.2	1.6 ± 0.2	22.6 ± 1.6	15.0 ± 0.4	3.2 ± 0.1	1.4 ± 0.03
Day 2										
	CON	6.7 ± 0.8	2.2 ± 0.2	3.5 ± 0.1	3.6 ± 0.5	1.3 ± 0.1	25.4 ± 2.5	13.4 ± 0.4	2.7 ± 0.2	1.3 ± 0.03
	CE	6.2 ± 0.9	1.8 ± 0.1	3.5 ± 0.2	3.0 ± 0.1	1.4 ± 0.1	25.1 ± 2.5	13.5 ± 0.2	3.0 ± 0.4	1.4 ± 0.1
	HIIE	8.5 ± 1.2	2.7 ± 0.4	4.1 ± 0.3	2.9 ± 0.04	1.4 ± 0.1	25.6 ± 0.9	14.0 ± 0.6	2.4 ± 0.1	1.3 ± 0.1

Values are total content, expressed as μmol of esterified lipid per gram of liver wet weight. N = 6 per group. Statistics by ANOVA. ^Main effect of trial, $p < 0.05$. &Trial-by-time interaction, $p < 0.05$. *Significantly different from CON within Day 1, $p < 0.05$. #Significantly different from CE within Day 1, $p < 0.05$.

The total content (μmol / gram liver wet weight) for each lipid class is reported in Table 1. For hepatic TAG, we observed a significant main effect of trial ($P < 0.05$) and significant trial-by-time interaction ($P < 0.05$). Post hoc testing indicated that on the day of exercise (Day 1) during post-exercise recovery both CE and HIIE groups exhibit elevated TAG compared to the CON group ($P < 0.05$) and the HIIE group exhibited higher hepatic TAG than the CE group ($P < 0.05$). There were no significant effects of exercise on hepatic TAG content on the day following exercise (Day 2). This intensity-dependent and transient effect of prior exercise on hepatic TAG content in female mice was previously reported (17) using a different TAG analysis method. There were no significant effects of exercise on the total content of other lipid classes (Table 1). However, novel discoveries about correlations between the content of TAG with other lipid classes are discussed below, as well as observations related to acyl chain compositions.

To develop a better understanding of the lipidomic context for hepatic TAG content under sedentary conditions and after exercise, we performed linear regression to test association between total content of hepatic TAG and the other lipid classes in the liver. Hepatic TAG content was significantly correlated with hepatic DAG content in CON mouse livers ($P < 0.05$, Fig. 1A). However, this positive association was transiently disrupted following exercise when hepatic TAG was elevated on Day 1, as indicated by analysis of exercised mouse livers (NSD, Fig. 1B). On the day following exercise, there was a trend for the association between hepatic TAG and DAG in exercised mice to correlate with one another (Fig. 1C, $P < 0.1$), suggesting a return to the phenotype of sedentary mice in exercised mice after a full day to recover. Hepatic TAG was also significantly correlated with hepatic PE in CON mouse livers ($P < 0.05$, Fig. 2A). However, this positive association was transiently disrupted following exercise on Day 1, as indicated by analysis of exercised mouse livers (NSD, Fig. 2B). On the day following exercise, there was a trend for the association between hepatic TAG and PE in exercised mice to correlate with one another (Fig. 2C, $P < 0.1$). This correlation analysis workflow revealed no other significant correlations between hepatic TAG and other lipid classes.

Although the total content of TAG was altered on Day 1 after exercise, there were no significant differences between any groups for the relative abundance of SFA, MUFA, and PUFA in the hepatic TAG pool (data not shown). In contrast, the fatty acid class distribution within hepatic DAG was altered by exercise; ANOVA indicated a main effect of trial for SFA and PUFA ($P < 0.05$) with no significant time-by-trial interactions. Post hoc testing indicated that SFA was significantly lower in HIIE than CE and CON, with no difference between CE and CON. Post hoc testing also indicated that total PUFA and n-6 PUFA in the DAG pool were significantly higher in HIIE than CE and CON, with no significant difference between CE and CON. The relative content of n-6 PUFA in DAG on Day 1 (Con, 42.2 ± 1.6%; CE, 43.8 ± 1.6%; HIIE, 45.8 ± 1.2) and Day 2 (Con, 38.4.±1.2%; CE, 39.0 ± 0.5%; HIIE, 42.6 ± 0.7) indicated a modest but sustained effect of a single bout of exercise on DAG acyl chain composition. No other lipid classes showed a change in the relative abundance of SFA, MUFA, and PUFA during the transient TAG accumulation after exercise.

As the main findings presented above are related to TAG, DAG, and PE, here we present PCA plots for the relative fatty acid composition of these lipid classes. For TAG, Fig. 3A indicates that CON and HIIE achieved nearly complete separation on Day 1, while the lower-intensity exercise group (CE) overlaid with both CON and HIIE. This observation is consistent with the concept of intensity-dependent responses of lipid metabolism. Day 2 showed no meaningful trends toward group separation for TAG (Fig. 3B). Figure 4A indicates that CON and HIIE achieved nearly complete separation for the DAG lipid class on Day 1, with the exception of a single mouse in the HIIE group that appeared to be an outlier, while the CE group appearing to overlay with both CON and HIIE. Day 2 showed no meaningful trends toward group separation for DAG (Fig. 4B). Figure 5A indicates that CON and HIIE achieved nearly complete separation on Day 1 for the PE lipid class, with the exception of a single mouse in the HIIE group that appeared to be an outlier, while the CE group overlaid with both CON and HIIE. Day 2 showed no meaningful trends toward group separation for PE (Fig. 5B); testing additional combinations of principal components (e.g., PC1 with PC2 or with PC3) did not lead to any meaningful group separation on day 2. Review of PCA results in MetaboAnalyst indicated that it was indeed the same HIIE mouse that appeared to be an outlier

on Day 1 for TAG, DAG, and PE; however, we show all data points in the PCA to display the full variability observed.

Discussion

The liver transiently accumulates TAG following a single exercise bout, particularly if the exercise bout is challenging. This ability to rapidly expand the hepatic TAG pool size, referred to here as lipogenic flexibility, has been discovered previously but remains poorly understood. We explored the phenomenon of lipogenic flexibility through lipidomics analysis of the liver following a moderate-intensity exercise bout (CE) and a high-intensity exercise bout (HIIE) in mice. Through the present work, we have rigorously confirmed the presence of transient liver TAG accumulation after exercise, and we have elaborated the findings through the analysis of various lipid classes in the liver. We discovered associations between liver TAG content and other lipids in sedentary mice that are transiently altered by a single exercise bout, and the implications of these findings are discussed below. We also discuss the issue of intensity-dependence of the lipogenic flexibility response, as indicated by robust responses particularly to HIIE for TAG content changes as well as additional metabolic impacts indicated by PCA plots of acyl chain compositions. Finally, below we discuss the overall implications of the present results for understanding the mammalian exercise response, and we consider the implication for metabolic health.

The exercise-responsive lipogenic flexibility in the liver is depicted primarily by a significant accumulation of hepatic TAG after exercise, which returns back toward baseline sedentary levels by the following day. Accurate measurement of TAG is truly essential as we work to characterize this phenomenon. We have observed this hepatic TAG phenomenon by two independent analytical procedures, including a colorimetric biochemical TAG assay published previously (17) and an analytical chemistry-based approach using thin layer chromatography followed by gas chromatography as reported in the present report (Table 1). Both approaches led to the observation of an intensity-dependent accumulation of TAG in the liver of female mice after exercise which resolved by the following day. In the biochemical work, published previously, both sexes were studied, while for the current lipidomics approach we selected a single sex for sample submission to a lipidomics analysis laboratory. In these female mouse liver samples, we observed a high degree of correlation between TAG results from the two methods, with a slope near unity; thus, one can be confident that previous observations are robust and confirmed by the present TAG analysis. What's more, in the present report a broad range of lipids are reported, leading to an elaboration of the lipogenic flexibility phenomenon beyond the measurement of TAG and its lipid droplet-related proteins that we reported previously (17).

The present results in Fig. 1A indicate that under sedentary conditions, there is a significant positive correlation between hepatic TAG and DAG content. This finding is consistent with previous knowledge that TAG accumulation in the liver is associated with DAG accumulation (1–5), which can then lead to impairments in metabolic health (2, 7, 8). However, when transient TAG accumulation is triggered in the liver by exercise, the correlation between TAG and DAG is disrupted (Fig. 1B); this indicates that the metabolically inert TAG pool can accumulate in this scenario without being strongly associated with

lipotoxic DAG accumulation. Furthermore, as the lipid content remodeling is transient in the acute response to a single exercise bout, there was a trend toward a correlation between hepatic TAG and DAG in the liver in exercised mice on the day after exercise. As the hepatic TAG content returned back toward CON levels during recovery from exercise, its association with DAG content was also nearly reestablished. We have previously reported that the transient enhancement of perilipin-2 (pln2) expression in the liver after exercise may stabilize lipid droplets as the TAG pool size expands (17), and this stability may limit DAG accumulation when TAG is rapidly accumulating in the liver during and after exercise.

Under sedentary conditions, hepatic TAG is also significantly correlated with hepatic PE (Fig. 2A), while this statistical association is disrupted during the rapid expansion of the hepatic TAG pool following a single exercise bout (Fig. 2B). This is followed by a trend toward the correlation between TAG and PE being reestablished in exercised mice on the day after the exercise bout (Fig. 2C). The positive association between TAG and PE in sedentary mice or exercise-recovered mice could be potentially expected, based upon the literature, because cellular PE content could affect lipid droplet biology. PE is an important component of the phospholipid monolayer that surrounds lipid droplets (27). At least in the other cell types that have been studied, PE is important for the formation of lipid droplets (28) or the fusion of lipid droplets to promote accumulation of large lipid droplets (29). Thus, a relationship between TAG accumulation and tissue PE content under basal physiological conditions seems understandable. However, during the transient TAG accumulation in the liver that occurs rapidly during and/or soon-after exercise, PE level is no longer correlated with TAG. The TAG pool expansion may outpace that of PE biosynthesis during induction of the lipogenic flexibility response to exercise in the liver, as a potential increase in lipid droplet size could reduce the surface-area-to-volume ratio and therefore weaken the relationship between the phospholipid monolayer and TAG contents within lipid droplets.

When considering the variety of lipid classes analyzed in this study, it is clear that TAG was the most responsive to exercise. This indicates that the lipidomic response to exercise in the liver may be primarily related to fuel metabolism rather than changes in structural lipids within the cell. While TAG was the only lipid class showing a change in concentration, DAG was the lipid class that showed a change in the distribution of fatty acid classes within the esterified lipid pool, with exercise-induced increases in total PUFA and n-6 PUFA alongside the corresponding reduction in SFA content. While these statistically significant changes in DAG composition may be reasonably modest in magnitude, it is noteworthy that they occurred in response to only a single bout of exercise, and they were sustained even the day following exercise. Humans with non-alcoholic fatty liver disease exhibit reduced total PUFA and n-6 PUFA in hepatic DAG and TAG (1), and we show presently in a mouse model that even a single bout of exercise may act to correct this pathology-related reduction of PUFA in liver DAG. The mice in our study consumed the 5K52 diet which we previously reported to contain a reasonably substantial n-6 PUFA abundance (46% of fatty acids) (30). After HIIE the PUFA content in liver DAG rose to approach this value of PUFA expected from the diet, which may be caused by an exercise-induced release of dietary fatty acids that were stored in adipose tissue. Furthermore, while discussing the impacts of exercise on hepatic lipids, it should be noted that the most substantial impacts were following HIIE, which is an exercise approach that exhibits particularly potent impacts upon health and metabolism (17, 25, 31–36). The

remodeling of PUFA content in DAG occurred after HIIE but not CE, and the response of TAG concentration was enhanced following HIIE in comparison to CE. Furthermore, principal component analysis indicated that the separation between HIIE and CON to be more notable than separation between CE and CON, suggesting potentially a more potent impact upon turnover of cellular lipids in the liver. While PCA plots, even with an individual mouse as an outlier, indicated a general separation between HIIE and CON for the lipid classes reported (TAG, DAG, PE), the CE data points were broadly dispersed and overlapped with the CON group. As a whole, the data are supportive of a biologically distinct impact of HIIE in comparison to CE, even when these exercise types are matched for distance, duration, and energy expenditure. Thus, it appears that intermittently challenging exercise is more metabolically potent in the liver than sustained mild exertion.

In order to understand the metabolic events that lead to exercise-induced changes in hepatic lipids, the timing of the changes in TAG accumulation could be considered. In this work we collected liver tissue 3 hours after exercise and the following day. The accumulation of hepatic TAG seen at 3 hours after exercise hypothetically could have occurred during exercise, during the first few hours of post-exercise recovery, or during both time periods. There have been some reports indicating that TAG has already accumulated in the liver at the end of the exercise bout in humans (11, 13, 15), mice (37), and rats (24). In another study on human subjects, TAG accumulation did not occur during exercise but subsequently accumulated during four hours of recovery (15); a similar observation was made studying mice, in which hepatic TAG did not accumulate during exercise but subsequent accumulated during three hours of recovery (38). In contrast, in a study on rats hepatic TAG accumulated during exercise but began to recover soon after, substantially returning toward baseline even within an hour of recover (9). Alternatively, in mice the hepatic TAG that accumulated during exercise was fully maintained 3 hours after exercise (37). Overall, it appears that TAG can potentially accumulate in the liver both during exercise and during hours following exercise, with a sustained elevation typically lasting for hours, but with recovery time ranging from 1 hour to perhaps approximately 24 hours. Nutritional status likely plays a role, and investigation of the effects of food/beverage intake after exercise deserves attention in the future. If accumulation of TAG in the liver is driven by plasma FFA concentration, then accumulation could be promoted both during and after exercise; exercise indeed leads to increased plasma FFA turnover and concentration both during exercise and during hours of post-exercise recovery (20). As discussed in the Introduction, control of lipolysis during and after exercise may have evolved based upon the fuel supply needs of skeletal muscle. However, as enhanced lipolysis drives an elevated FFA concentration in plasma and thus increased FFA uptake down concentration gradients into working muscle (39–41), this response places a metabolic burden upon non-contracting muscle and other organs such as the liver that will be presented with circulating FFA levels that are beyond their needs. It appears that enhanced circulating FFA, though useful for fuel trafficking from storage sites to sites of use, can place a burden and enhanced lipotoxicity risk upon peripheral tissues. Ideally, for preservation of metabolic health, this elevated FFA would be buffered into the TAG pool intracellularly, which is metabolically inert, rather than being stored in lipotoxic intermediate pools such as DAG.

It is understood that the liver after exercise is able to exhibit a lipogenic flexibility, supported by pln2 expression, that allows rapid expansion of the TAG pool and buffering of FFA into this inert pool (17). Next, it would be useful to consider this phenomenon exhibited by the liver in the context of lipid changes occurring in other tissues in response to exercise and related stressors. During exercise, the amount of intramuscular TAG declines in the exercised muscles, while TAG tends to accumulate elsewhere. TAG accumulates in the liver (present results) and even in skeletal muscle that was not actively recruited for the exercise bout (12). During fasting, which also stimulates lipolysis but is not associated with vigorous muscle contraction, skeletal muscle actually accumulates TAG as seen in laboratory animals by biochemical analysis (42) and in human subjects by measuring intramyocellular lipid by MRS (43–47). As with exercise, fasting leads to accumulation of TAG in the liver as observed in rodent studies (48–51) and accumulation of intrahepatocellular lipid (presumably mostly TAG) as observed by non-invasive MRS in human subjects research (52). It is important to keep in mind that the acute response to each bout of a stressor is not necessarily qualitatively similar to the chronic stress response. Specifically, chronic caloric restriction typically reduces hepatic TAG concentration (53) while acute fasting leads to elevation of hepatic TAG (52). Similarly, in some cases chronic exercise training modestly reduces hepatic TAG (6, 19); however, each acute bout of exercise transiently raises hepatic TAG (11, 13–16), even when pre-exercise hepatic TAG is high as in NAFLD patients (14). Specifically, the exercise modalities reported here acutely raise hepatic TAG on the day of exercise, but in mice that were chronically trained by CE or HIIE, with liver tissue collection on the day following the last exercise bout, hepatic TAG content in exercised mice was not elevated above CON (25). While chronic adaptations are certainly meaningful, the ability to buffer excess plasma FFA into the hepatic TAG pool is likely to be metabolically critical when a stressor is acutely applied that increases circulating FFA.

Conclusions

In summary, the hepatic lipidome responds to exercise in an intensity-dependent manner. Furthermore, we have reported associations between the fluctuations in hepatic TAG storage after exercise and the relationship between TAG content and that of other lipid classes. It may be useful in the future to further explore the implications of exercise-induced lipogenic flexibility in the liver. For example, it could be tested if there are populations that are unable to rapidly expand tissue TAG storage at ectopic deposition sites when plasma FFA rise. Another future direction could be to test the impacts of inhibiting hepatic TAG accumulation after exercise on the systemic impacts of exercise. As the exercise-induced transient changes in the hepatic lipidome become well-appreciated and characterized by the scientific community, it will become critical to explore the implications for human health.

List Of Abbreviations

Analysis of variance, ANOVA; Cardiolipin, CL; Cholesterol ester, ChE; Continuous exercise, CE; Control, CON; Diacylglycerol, DAG; Free fatty acid, FFA; High-intensity interval exercise, HIIE; Lysophosphatidylcholine, LyPC; Magnetic resonance spectroscopy, MRS; Monounsaturated fatty acid,

MUFA; Phosphatidylcholine, PC; Phosphatidylethanolamine, PE; Phosphatidylserine, PS; Polyunsaturated fatty acid, PUFA; Principal component analysis, PCA; Sphingomyelin, SM; Standard error, SE; Saturated fatty acid (SFA); Triacylglycerol, TAG; Variable importance in the projection, VIP

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable

Availability of data and materials*:* The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests

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Figures

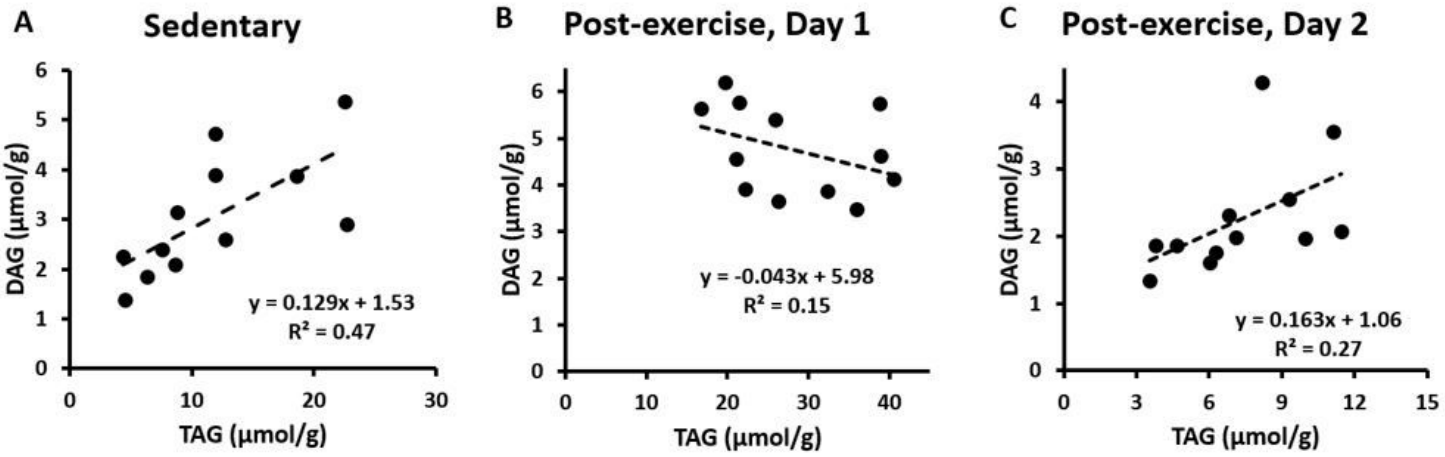

Figure 1

Correlation between total hepatic TAG and total hepatic DAG. A) CON mice ($P < 0.05$), B) 3 hours after exercise ($P = 0.22$, NSD), C) the day following exercise ($P = 0.08$). Analyses by linear regression indicate a statistically significant association between hepatic TAG and DAG under sedentary conditions, a disruption of this association soon after an exercise bout, and a trend toward resuming the association between TAG and DAG on the day following exercise.

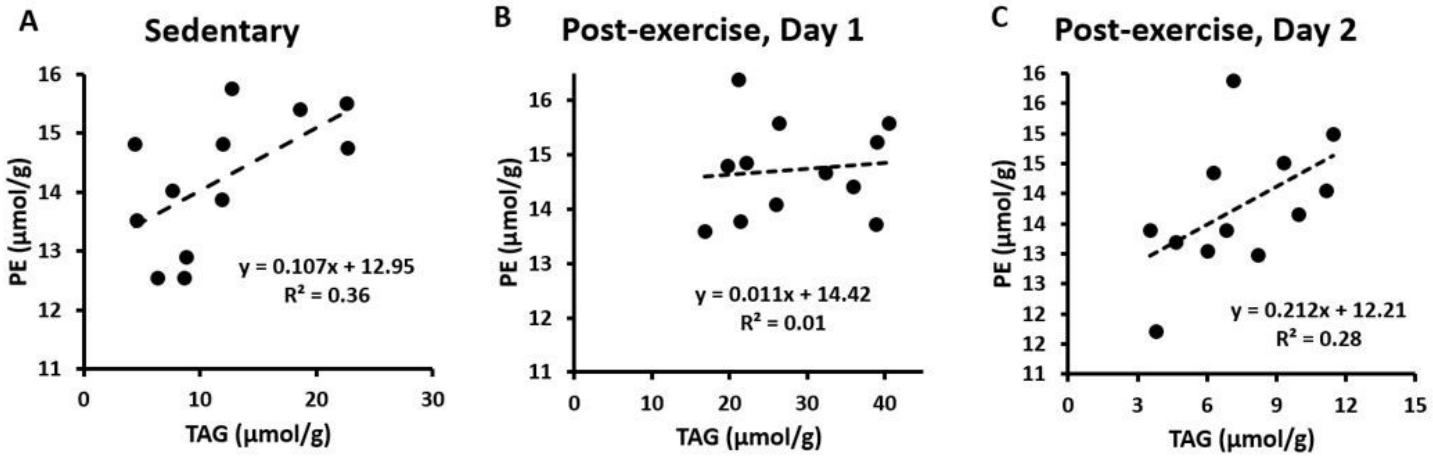

Figure 2

Correlation between total hepatic TAG and total hepatic PE. A) CON mice ($P < 0.05$), B) 3 hours after exercise (NSD, $P = 0.74$), C) the day following exercise ($P = 0.08$). Analyses by linear regression indicate a statistically significant association between hepatic TAG and PE under sedentary conditions, a disruption of this association soon after an exercise bout, and a trend toward resuming the association between TAG and PE on the day following exercise.

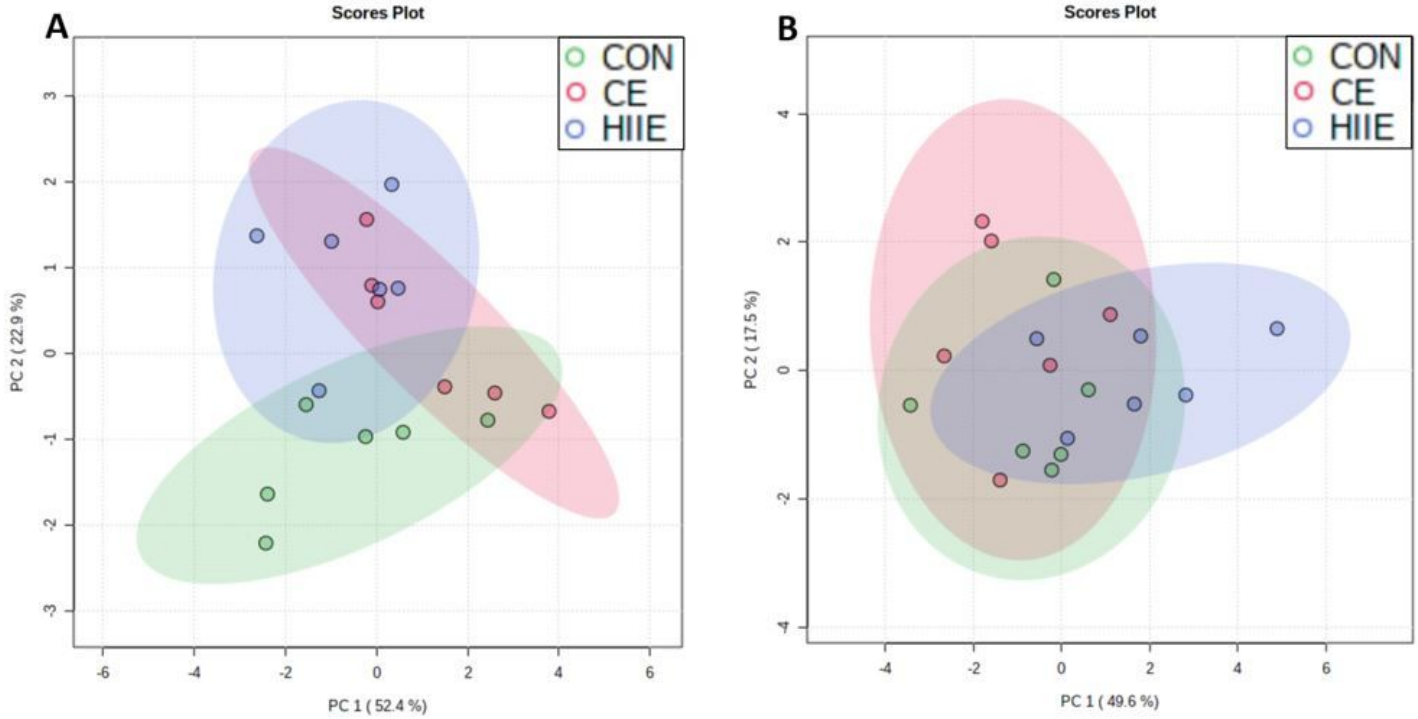

Figure 3

Principal Component Analysis of Hepatic Triacylglycerol. A) The day of exercise (Day 1), B) the day after exercise (Day 2). The relative fatty acid composition of triacylglycerol (TAG) was analyzed by PCA to

assess remodelling of the acyl chain composition as a means of identifying the group assignment of each mouse liver sample. CON, green; CE, red; HIIE, blue.

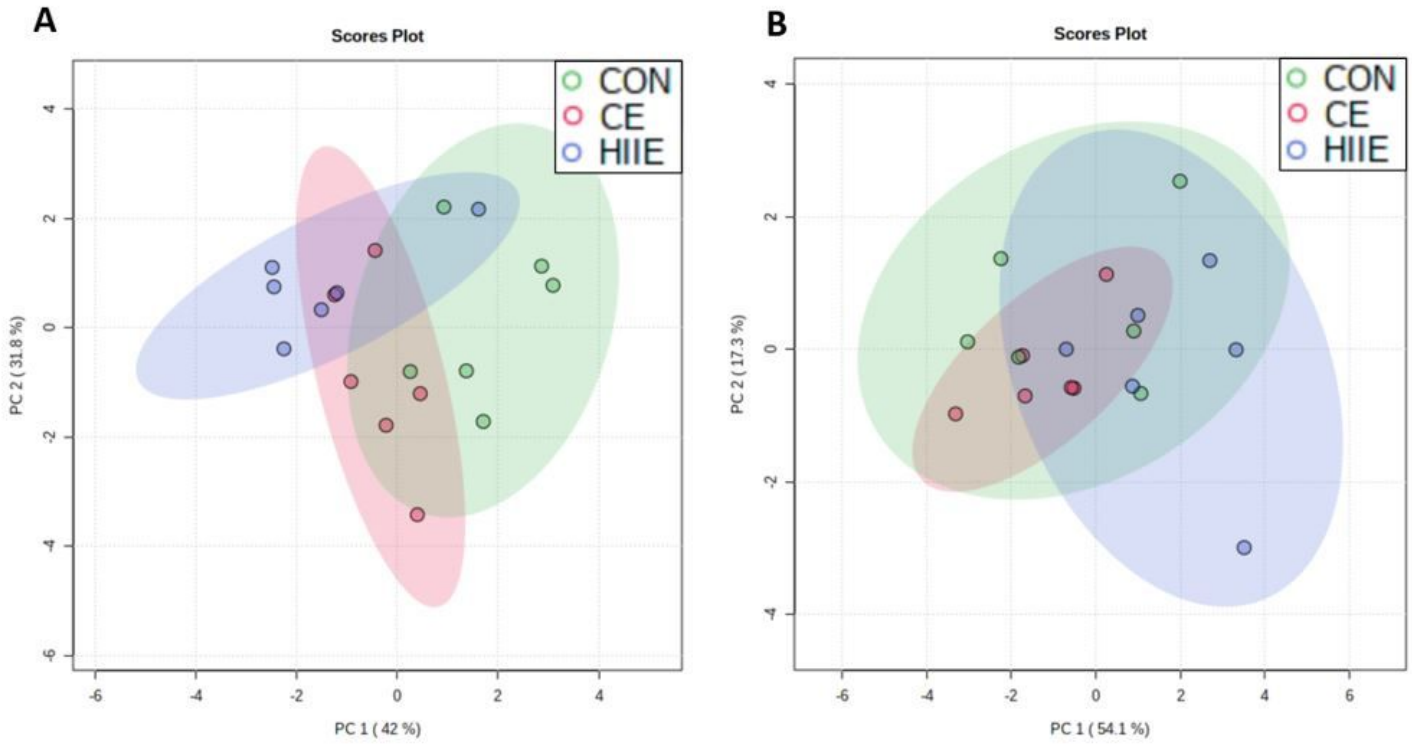

Figure 4

Principal Component Analysis of Hepatic Diacylglycerol. A) The day of exercise (Day 1), B) the day after exercise (Day 2). The relative fatty acid composition of diacylglycerol (DAG) was analyzed by PCA to assess remodelling of the acyl chain composition as a means of identifying the group assignment of each mouse liver sample. CON, green; CE, red; HIIE, blue.

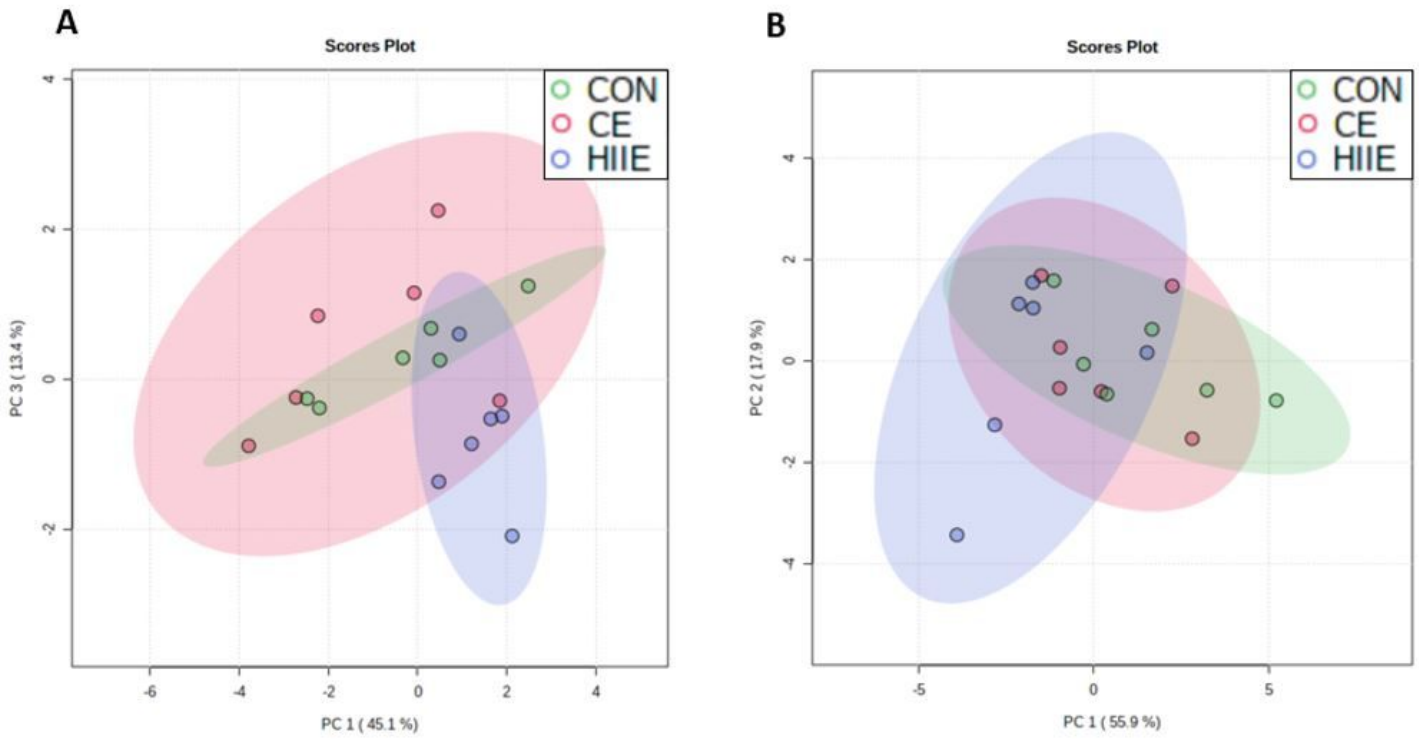

Figure 5

Principal Component Analysis of Hepatic Phosphatidylethanolamine. A) The day of exercise (Day 1), B) the day after exercise (Day 2). The relative fatty acid composition of phosphatidylethanolamine (PE) was analyzed by PCA to assess remodelling of the acyl chain composition as a means of identifying the group assignment of each mouse liver sample. CON, green; CE, red; HIIE, blue.