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RESEARCH PAPER

Fasting enriches liver triacylglycerol with n-3 polyunsaturated fatty acids: implications for understanding the adipose–liver axis in serum docosahexaenoic acid regulation

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Abstract We investigated the effect of short-term fasting on coordinate changes in the fatty acid composition of adipose triacylglycerol (TAG), serum non-esterified fatty acids (NEFA), liver TAG, and serum TAG and phospholipids in mice fed ad libitum or fasted for 16 h overnight. In contrast to previous reports under conditions of maximal lipolysis, adipose tissue TAG was not preferentially depleted of n-3 PUFA or any specific fatty acids, nor were there any striking changes in the serum NEFA composition. Short-term fasting did, however, increase the hepatic proportion of n-3 PUFA, and almost all individual species of n-3 PUFA showed relative and absolute increases. The relative proportion of n-6 PUFA in liver TAG also increased but to a lesser extent, resulting in a significant decrease in the n-6:n-3 PUFA ratio (from 14.3 ± 2.54 to 9.6 ± 1.20), while the proportion of MUFA decreased significantly and SFA proportion did not change. Examination of genes involved in PUFA synthesis suggested that hepatic changes in the elongation and desaturation of precursor lipids could not explain this effect. Rather, an increase in the expression of fatty acid transporters specific for 22:6n-3 and other long-chain n-3 and n-6 PUFA likely mediated the observed hepatic enrichment. Analysis of serum phospholipids indicated a specific increase in the concentration of 22:6n-3 and 16:0, suggesting increased specific synthesis of DHA-enriched phospholipid by the liver for recirculation. Given the importance of blood phospholipid in distributing DHA to neural tissue, these findings have implications for understanding the adipose–liver–brain axis in n-3 PUFA metabolism.

Keywords n-3 Polyunsaturated fatty acids · Triacylglycerol · Adipose · Non-esterified fatty acids · Liver · Phospholipids · Fatty acid desaturases · Fatty acid elongases · Fatty acid transport proteins · Fatty acid binding proteins

Introduction

Adipose tissue lipolysis is activated during fasting [reviewed in (Raclot 2003; Duncan et al. 2007)]. The triacylglycerol (TAG) stored in adipose tissue is hydrolyzed at an increased rate by the lipases adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) (Jaworski et al. 2007; Duncan et al. 2010; Ahmadian et al. 2009), releasing more non-esterified fatty acids (NEFA) into the circulation to provide lipid substrates for the body. HSL has been shown to display a preference for TAG containing long-chain polyunsaturated fatty acids (PUFA) (Raclot et al. 2001), and there is some evidence of selective mobilization of more unsaturated fatty acids from adipose tissue. Connor and colleagues maximally induced lipolysis in rabbits by injecting adrenocorticotropic hormone after an overnight fast and then calculated the relative mobilization of individual fatty acid species as a ratio of the percent abundance in plasma NEFA relative to the percent

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in adipose tissue TAG plus free fatty acid fractions combined. Under these conditions, the calculated relative mobilization tended to increase as the degree of unsaturation increased for fatty acids of a specific chain length (Conner et al. 1996). Although this finding may have been confounded by effects of selective uptake on serum NEFA concentrations, Raclot et al. also observed a highly similar effect using isolated human mammary adipocytes maximally stimulated to undergo lipolysis with isoprenaline and adenosine deaminase (Raclot et al. 1997). Both studies also agreed on the finding that 20:5n-3 and 20:4n-6 were proportionately the most highly mobilized fatty acyl species.

Despite these reports, and others (Yli-Jama et al. 2001; Hellmuth et al. 2013), no studies have yet characterized the fatty acid composition of adipose TAG and serum NEFA in ad libitum fed animals versus animals undergoing a short-term fast, when lipolysis is not maximally stimulated. Furthermore, downstream effects of fasting-mediated changes in adipocyte and serum fatty acids have also yet to be characterized. For example, it is unknown whether short-term fasting leads to selective changes in the composition of stored fatty acids in liver TAG, or to changes in liver-derived circulating complex lipids. Understanding the metabolic journey of adipose-derived fatty acids, and PUFA in particular, has a variety of implications for health. Most PUFA are either essential or conditionally essential for cellular processes (Cunnane 2000). Evidence of the selective mobilization of PUFA from adipose tissue, particularly under relatively common conditions such as an extended overnight fast, would constitute a "second chance" mechanism ensuring that essential fatty acids remain bioavailable for use by tissues, rather than locked in the core of adipocyte lipid droplets. Additionally, it has recently been found that the brain, which requires a constant supply of the very long chain n-3 PUFA docosahexaenoic acid (DHA, 22:6n-3) (Rahman et al. 2010; Polozova and Salem Jr 2007), can also uptake DHA as lysophosphatidylcholine via a Mfsd2a receptor (Nguyen et al. 2014) in addition to crossing as a NEFA (Domenichiello et al. 2015), indicating a potentially significant role for the adipose–liver axis in brain health.

The aim of the present study, therefore, was to better understand the metabolism of adipose-derived NEFA by comparing fatty acid profiles in overnight fasted versus ad libitum fed mice, in the following pools: (1) adipose TAG, (2) blood NEFA that are primarily derived from adipose TAG, (3) hepatic TAG, which are synthesized primarily from circulating NEFA in the post-absorptive state, and (4) serum phospholipids and (5) serum TAG, which in fasting are derived predominantly from hepatic rather than intestinal synthesis. To better understand our findings in liver, we also determined the relative hepatic gene expression of desaturase and elongase genes involved in PUFA biosynthesis, and the expression of genes involved in NEFA uptake.

Methods

Animals

All animal procedures were approved by the University of Waterloo Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care. Female C57Bl/6J mice, aged 12–16 weeks, were housed at a temperature of 21 ± 1 °C in the Central Animal Housing Facility at the University of Waterloo, on a 12:12-h light–dark cycle. Mice were maintained on a low-fat diet (fatty acid composition in Table 1) and were given ad libitum access to food and water during this time. Prior to euthanasia, animals were either fasted overnight for 16 h ($n = 4$ per group) or continued to have ad libitum access to food ($n = 4$ per group). Animals were killed by cervical dislocation. Livers and retroperitoneal adipose tissue were collected and immediately frozen in liquid nitrogen, and stored at −80 °C prior to mRNA and lipid analysis. Blood was collected and spun at 3000 rpm to separate serum from red blood cells, and stored at −80 °C prior to analyses.

Fatty acid composition

Hepatic, adipose, and serum lipids were extracted according to the method of Folch, Lees, and Sloane Stanley using 2:1 chloroform:methanol (v:v) (Folch et al. 1957). TAG, non-esterified fatty acids, and phospholipids were collected after isolation by thin layer chromatography, using 20 × 20 cm plates with a 60 Å silica gel layer (Whatman International Ltd, Maidstone, England). The mobile phase was 60:40:2 heptane:diethyl ether: acetic acid (v:v:v) (Christie 1989). Bands were visualized under UV light with 2,7-dichlorofluorescein (Sigma-Aldrich, Oakville, ON), and identified by comparison with a reference standard. Lipids were extracted off the silica using 2:1 chloroform:methanol that included 22:3n-3 as an internal standard.

Fatty acid fractions were transesterified by 14 % BF_3 in methanol at 85 °C for 1 h to produce fatty acid methyl esters (Morrison and Smith 1964), which were separated by fast gas chromatography (Stark and Salem Jr 2005; Metherel et al. 2013). Peaks were identified by comparison with the retention time and a reference mixture of fatty acids (GLC-569, Nu-Chek Prep Inc). Fatty acids were quantitated by comparison with the internal standard and expressed as concentrations and as the percentage of individual fatty acids of the total fatty acids identified.

Table 1 Fatty acid composition of diet

Fatty acid	Chow diet
Diet fatty acid composition (mg fatty acid/g diet)	
C 10:0	0.02 ± 0.01
C 12:0	0.06 ± 0.01
C 14:0	0.39 ± 0.05
C 16:0	9.33 ± 0.78
C 17:0	0.14 ± 0.01
C 18:0	4.18 ± 0.37
C 20:0	0.14 ± 0.01
C 22:0	0.14 ± 0.01
C 23:0	0.02 ± 0.01
C 24:0	0.05 ± 0.01
Total SFAs	14.45 ± 1.25
C 14:1	0.04 ± 0.03
C 16:1	0.47 ± 0.05
C 18:1n-7	1.12 ± 0.11
C 18:1n-9	15.65 ± 1.25
C 20:1n-9	0.19 ± 0.01
C 22:1n-9	0.05 ± 0.02
C 24:1n-9	0.01 ± 0.01
Total MUFAs	17.53 ± 1.41
C 18:2n-6	21.82 ± 1.79
C 18:3n-6	0.01 ± 0.01
C 20:2n-6	0.17 ± 0.01
C 20:3n-6	0.02 ± 0.01
C 20:4n-6	0.05 ± 0.01
C 22:2n-6	0.01 ± 0.01
C 22:4n-6	0.02 ± 0.01
C 22:5n-6	0.01 ± 0.01
Total n-6	22.11 ± 1.81
C 18:3n-3	2.22 ± 0.14
C 20:3n-3	0.03 ± 0.01
C 20:5n-3	0.01 ± 0.01
C 22:5n-3	0.02 ± 0.01
C 22:6n-3	0.01 ± 0.01
Total n-3	2.27 ± 0.16
Total fatty acids	56.36 ± 4.60

Results are presented as mean ± SD after three determinations of fatty acid concentrations

SFAs saturated fatty acids, *MUFAs* monounsaturated fatty acids, *N-6* n-6 polyunsaturated fatty acids, *N-3* n-3 polyunsaturated fatty acids

Reverse-transcriptase real-time PCR

RNA was extracted from mouse livers as described previously (Marks et al. 2013; Kitson et al. 2012). Briefly, livers were homogenized in Trizol® reagent (Invitrogen Co, Frederick, MD). Phases were separated by the addition of chloroform. Purity was determined by using the 260/280 ratio on a Nanodrop c2000 (Thermo Scientific, Wilmington, DE). Samples with a 260/280 ratio of 1.90 or greater were used for cDNA synthesis, using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Streetsville, ON) with an MJ Mini Personal Thermal Cycler (Bio-Rad Laboratories, Mississauga, ON) programmed cycle of 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 s, and 4 °C until storage at −20 °C.

The Primer-BLAST program on the NCBI Web site was used to design primers for mouse sequences of *Elovl2, Elovl5, Fads1, Fads2, Scd1, Elovl6*, and *18S ribosomal RNA* (Sigma-Aldrich, Oakville, ON, Canada) (sequences in Table 2). In addition, primers for fatty acid transport proteins were also designed (sequences in Table 2). Reverse-transcriptase real-time PCR was performed using SsoFast EvaGreen Supermix (Bio-Rad) on a CFX Connect Real-Time System (Bio-Rad). The program had an initial incubation of 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s and 65 °C for 45 s. Analysis of data was performed by normalizing the threshold cycle number (Ct) of the gene of interest to the Ct for the housekeeping gene, *18S ribosomal RNA*, and changes in gene expression in fasted livers were calculated relative to ad libitum fed mice using the $2^{-\Delta\Delta Ct}$ method.

Western blotting

Livers from fed and fasted mice were homogenized in a buffer containing 25 mM Tris, pH 7.4, 130 mM NaCl, 2.7 mM KCl, 5 mM EDTA, and 1 % Triton X-100. Protein content was determined using a bicinchoninic acid procedure. Seventy-five micrograms of protein were separated on a 12 % polyacrylamide TGX Stain-Free FastCast gel (Bio-Rad) and transferred to a polyvinylidene difluoride membrane. The membranes were blocked with 5 % skim milk in TBS with 0.5 % (v:v) Tween-20 at room temperature for 1 h, followed by incubation at 4 °C overnight with primary antibodies for FATP2 (1:1000 in skim milk in TBS-T, ab83763, Abcam), which demonstrated the largest single change in mRNA expression with fasting, or beta-actin (1:1000 in skim milk in TBS-T, sc130657, Santa Cruz Biotechnology). The membrane was washed, incubated with appropriate secondary antibodies for 1 h at room temperature, washed again, and detected by chemiluminescence using a Bio-Rad ChemiDoc system. Protein content was normalized to total protein loaded on the gel, as determined by gel activation and visualization prior to transfer using the ChemiDoc system.

Respiratory exchange ratio analysis

Average whole-body oxygen consumption and carbon dioxide production were measured in mice under fasted and non-fasted conditions using a Comprehensive Lab Animal Monitoring System (CLAMS) (Oxyman series;

Table 2 Mouse primer sequences for reverse-transcriptase real-time PCR

Gene name	Accession number	Sequence (5′–3′)
18S	NR_046233.2	F: GATCCATTGGAGGGCAAGTCT
		R: AACTCGAGCAACTTTAATATACGCTATT
Elovl2	NM_019423.2	F: CACACAGGCTCAGCTGGTGCA
		R: CCAAAGGGGAAGCCACAGGGC
Elovl5	NM_134255.3	F: CCATCCCGTCCATGCGTCCCTA
		R: CCCCGCAGGTCGTCTGGATGA
Fads1	NM_146094.2	F: GAAAACCCTGCGCGCGAACG
		R: CACGCCAGGCTCGCGAACTA
Fads2	NM_019699.1	F: AATGATCAGCCGCAGGGACTGG
		R: CTCCCAAGATGCCGTAGAAAGGGAT
Fabp1	NM_017399.4	F: AAGGGGGTGTCAGAAATCGT
		R: AGTCATGGTCTCCAGTTCGC
Fabp5	NM_010634.3	F: TGGCAACAACATCACGGTCA
		R: TCTGCCATCAGCTGTCGTTT
Fabp7	NM_021272.3	F: TGATCCGGACACAATGCACA
		R: CCATCCAACCGAACCACAGA
Slc27a2 (FATP2)	NM_011978.2	F: CATCGTGGTTGGGGCTACTT
		R: GGTACCGAAGCAGTTCACCA
Slc27a3 (FATP3)	NM_011988.2	F: TGGGACGATTGCCAGAAACA
		R: AAGCGCACCTTATGGTCACA
Slc27a4 (FATP4)	NM_011989.4	F: GGGGCCAATAAACTCTGCCT
		R: TCCCAAGGGCTAAGCGAAAG
Slc27a5 (FATP5)	NM_009512.2	F: TTGTTGCGAATGTACGACGC
		R: ACCAAGGCGGTCTTGAAAGT
Acsl6	NM_144823.4	F: TGCCGAGATTGCTCTCTCAC
		R: AAAACTGGCCCAAGTCCGAT

Columbus Instruments, Columbus, OH). The ratio of carbon dioxide to oxygen consumption was used to calculate the respiratory exchange ratio (RER), to provide an estimate of metabolic substrate usage. Animals were monitored for a 24-h period under consistent environmental temperature (22 °C). Measures were averaged over 10 h of the dark cycle.

Statistical analyses

Statistical analyses were performed using GraphPad Prism v4.0. Independent samples t test was used to determine differences between the fasting and feeding groups. Significance was inferred when $p < 0.05$.

Results

Fasting decreased adiposity and lowered the respiratory exchange ratio in mice

Fasting significantly reduced the weight of the renal white adipose tissue depot in fasted mice compared to mice provided with ad libitum access to food (Fig. 1a). The relative degree of weight loss was larger than would typically be observed in humans but was within the range expected for mice (Ayala et al. 2006), which are nocturnal feeders and small animals that have a relatively high metabolic rate (Agouni et al. 2010; Pacini et al. 2013). Fasting also induced a significant reduction in the respiratory exchange ratio (RER), (Fig. 1b), indicating as expected that there was a shift toward the use of stored fat as the main source of energy when nutrients were withdrawn.

Fatty acid composition of adipose TAG in fed and fasted mice

As expected, total adipose TAG fatty acid concentration, and the concentrations of all major fatty acid classes and almost all individual fatty acid species in TAG, decreased in fasted mice as compared with ad libitum fed mice (Supplementary Table 1). However, this reduction was highly proportionately similar across all major classes and individual species of fatty acids (Table 3). As a result, there were no significant changes in the relative proportion

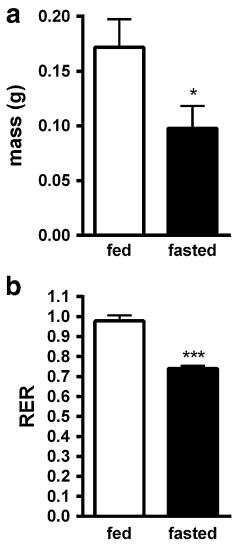

Fig. 1 Short-term fasting decreases adipose tissue mass and increases lipid oxidation in mice. **a** Retroperitoneal white adipose tissue mass in mice provided with food ad libitum or fasted for 16 h overnight. **b** Respiratory exchange ratio (RER). Data are mean ± SEM; $n = 4$–6; *$p < 0.05$, ***$p < 0.001$

of any major fatty acid class (i.e., saturates (SFA), monounsaturates (MUFA), or n-6 or n-3 PUFA) in adipose tissue, and only two significant changes in the relative proportion of any specific fatty acid species (i.e., an increase in the proportion of 12:0 and 20:3n-3) (Table 3). Thus, these data do not support a selective liberation of PUFA from adipose TAG with short-term fasting.

Fatty acid composition of serum NEFA in fed and fasted mice

No significant changes occurred with overnight fasting in the relative abundance of any major class of fatty acid species in the serum NEFA pool (Table 4). Furthermore, only three individual species of NEFA changed significantly with fasting. There was an increase in 20:1n-9 and decrease in 22:5n-6 and 20:3n-3 (Table 4). Absolute concentration changes in serum NEFA were modest with the current protocol, and no significant increase was observed for total serum NEFA, or for any major class or individual species of fatty acid (Supplementary Table 2).

Fatty acid composition of hepatic TAG in fed and fasted mice

As expected (Ryu et al. 2005; Leone et al. 1999; Narayan et al. 2014), fasting more than tripled the total hepatic content of TAG from 13.41 ± 3.42 μg fatty acid/mg liver in ad libitum fed mice to 44.53 ± 9.52 μg fatty acid/mg liver in fasted mice (Supplementary Table 3). As a result, the total concentration of all major fatty acid classes and most individual fatty acid species also increased. However, unlike effects observed in adipose and serum, differences in the relative proportion of different classes and individual species of fatty acids were evident. In terms of major classes of fatty acids, there was a significant increase in the relative percent of n-6 and n-3 PUFA, but a decrease in the relative proportion of MUFA in liver TAG, with no significant change in the proportion of SFA (Table 5). Specifically, the relative percentage of total hepatic TAG n-3 PUFA increased nearly by fourfold, from 0.72 ± 0.29 % in the ad libitum fed state to 2.82 ± 0.31 % in the fasted state. Notably, 22:6n-3 increased by over

Table 3 Relative percentage of fatty acids in TAG from perirenal white adipose tissue

Fatty acid	Ad libitum	Fasted
Adipose TAG (% weight total fatty acids)		
C 10:0	0.01 ± 0.01	0.01 ± 0.01
C 12:0	0.09 ± 0.02	0.16 ± 0.03*
C 14:0	1.10 ± 0.08	1.16 ± 0.08
C 16:0	22.21 ± 0.51	22.28 ± 0.23
C 18:0	4.80 ± 1.25	6.29 ± 1.31
C 20:0	0.18 ± 0.05	0.26 ± 0.06
C 22:0	0.07 ± 0.02	0.07 ± 0.01
C 24:0	0.01 ± 0.01	0.05 ± 0.03
Total SFAs	28.47 ± 1.61	30.27 ± 1.61
C 12:1	0.01 ± 0.01	0.01 ± 0.01
C 14:1	0.05 ± 0.01	0.05 ± 0.01
C 16:1	3.90 ± 0.33	3.31 ± 0.46
C 18:1n-7	2.26 ± 0.12	2.14 ± 0.11
C 18:1n-9	32.25 ± 1.07	31.18 ± 1.05
C 20:1n-9	0.69 ± 0.10	0.75 ± 0.05
C 22:1n-9	0.06 ± 0.02	0.07 ± 0.01
C 24:1n-9	0.03 ± 0.01	0.06 ± 0.08
Total MUFAs	39.24 ± 1.05	37.55 ± 1.36
C 18:2n-6	28.82 ± 2.25	28.25 ± 2.33
C 18:3n-6	0.08 ± 0.01	0.08 ± 0.01
C 20:2n-6	0.17 ± 0.01	0.19 ± 0.01
C 20:3n-6	0.16 ± 0.02	0.15 ± 0.01
C 20:4n-6	0.33 ± 0.06	0.3 ± 0.06
C 22:2n-6	0.01 ± 0.01	0.02 ± 0.02
C 22:4n-6	0.06 ± 0.01	0.06 ± 0.02
C 22:5n-6	0.01 ± 0.01	n.d.
Total n-6	29.63 ± 2.33	29.07 ± 2.38
C 18:3n-3	1.60 ± 0.21	1.45 ± 0.20
C 20:3n-3	0.02 ± 0.01	0.04 ± 0.01*
C 20:5n-3	0.04 ± 0.01	0.03 ± 0.01
C 22:5n-3	0.06 ± 0.01	0.07 ± 0.02
C 22:6n-3	0.21 ± 0.05	0.26 ± 0.02
Total n-3	1.93 ± 0.28	1.85 ± 0.17
n-6/n-3	15.45 ± 1.14	15.74 ± 0.15
Total (μg/mg tissue)	15.36 ± 1.43	6.36 ± 1.47*

Data are mean ± SD ($n = 3$–4)

Significant differences between ad libitum fed and fasted mice are denoted by * $p < 0.05$

Table 4 Relative percentage of NEFA in serum

Fatty acid	Ad libitum	Fasted
Serum NEFA (% weight total fatty acids)		
C 10:0	0.01 ± 0.02	0.01 ± 0.01
C 12:0	0.39 ± 0.35	0.30 ± 0.24
C 14:0	2.15 ± 0.62	1.98 ± 0.40
C 16:0	26.6 ± 1.77	24.5 ± 3.01
C 18:0	12.45 ± 0.93	10.51 ± 3.43
C 20:0	0.29 ± 0.08	0.28 ± 0.15
C 22:0	0.16 ± 0.03	0.15 ± 0.10
C 23:0	0.05 ± 0.04	0.03 ± 0.02
C 24:0	0.10 ± 0.04	0.15 ± 0.12
Total SFAs	42.2 ± 1.86	37.9 ± 5.87
C 14:1	0.16 ± 0.09	0.22 ± 0.20
C 16:1	6.22 ± 2.96	5.07 ± 1.57
C 18:1n-7	1.99 ± 0.34	1.91 ± 0.26
C 18:1n-9	21.1 ± 2.10	25.14 ± 3.40
C 20:1n-9	0.43 ± 0.05	0.51 ± 0.05*
C 22:1n-9	0.43 ± 0.13	0.41 ± 0.19
C 24:1n-9	0.11 ± 0.10	0.05 ± 0.03
Total MUFAs	30.44 ± 5.12	33.3 ± 4.87
C 18:2n-6	14.26 ± 4.08	17.12 ± 5.26
C 18:3n-6	0.17 ± 0.13	0.21 ± 0.04
C 20:2n-6	0.26 ± 0.08	0.22 ± 0.09
C 20:3n-6	0.38 ± 0.13	0.29 ± 0.24
C 20:4n-6	2.88 ± 1.29	2.63 ± 0.53
C 22:2n-6	0.14 ± 0.04	0.11 ± 0.06
C 22:4n-6	0.20 ± 0.09	0.22 ± 0.05
C 22:5n-6	0.36 ± 0.05	0.17 ± 0.10*
Total n-6	18.65 ± 5.53	20.98 ± 4.84
C 18:3n-3	0.97 ± 0.40	0.87 ± 0.43
C 20:3n-3	0.10 ± 0.02	0.04 ± 0.04*
C 20:5n-3	0.24 ± 0.14	0.14 ± 0.03
C 22:5n-3	0.22 ± 0.17	0.16 ± 0.04
C 22:6n-3	1.71 ± 0.84	1.50 ± 0.17
Total n-3	3.24 ± 1.55	2.71 ± 0.50
n-6/n-3	6.33 ± 1.60	7.75 ± 1.23
Total (μg/mg tissue)	47.24 ± 16.3	55.43 ± 12.59

Data are mean ± SD ($n = 3$–4)

Significant differences between ad libitum fed and fasted mice are denoted by * $p < 0.05$

fivefold, from 0.26 ± 0.10 % in the fed state to 1.35 ± 0.15 % in the fasted state, although all species of n-3 PUFA analyzed demonstrated significant increases, including 18:3n-3, which increased by threefold, and 20:5n-3, which increased by sevenfold. While n-6 PUFA also increased from 10.75 ± 5.73 % in liver TAG from ad libitum fed mice to 28.85 ± 2.71 % in liver TAG from fasted mice, this represented a smaller proportionate increase than that observed for n-3 PUFA, with the result that the n-6/n-3 ratio was significantly lower in fasted mice (9.60 ± 1.20 versus 14.3 ± 2.54 in ad libitum fed mice). Significant increases in 18:2n-6, 18:3n-6, and 20:4n-6 contributed to the rise in n-6 PUFA that was evident in liver TAG of fasted mice. Within SFA species, there was a 40 % decrease in 18:0, but no significant change in the relative proportion of liver TAG 16:0 with fasting. Within MUFA species, significantly lower levels of 18-carbon fatty acids were also evident, including 18:1n-7 and 18:1n-

Table 5 Relative percentage of fatty acids in liver TAG

Fatty acid	Ad libitum	Fasted
Liver TAG (% weight total fatty acids)		
C 10:0	0.07 ± 0.04	0.05 ± 0.02
C 12:0	0.10 ± 0.08	0.02 ± 0.02
C 14:0	0.06 ± 0.03	0.05 ± 0.03
C 16:0	25.76 ± 4.65	22.63 ± 2.76
C 18:0	3.50 ± 0.66	1.49 ± 0.35*
C 20:0	0.12 ± 0.07	0.04 ± 0.01
C 22:0	0.05 ± 0.05	0.02 ± 0.02
C 24:0	0.04 ± 0.03	0.01 ± 0.01
Total SFAs	34.25 ± 5.79	27.54 ± 1.55
C 12:1	0.10 ± 0.07	0.05 ± 0.01
C 14:1	0.21 ± 0.18	0.16 ± 0.03
C 16:1	4.37 ± 2.37	4.12 ± 1.31
C 18:1n-7	4.46 ± 1.16	2.54 ± 0.58*
C 18:1n-9	44.04 ± 4.92	35.35 ± 3.41*
C 20:1n-9	0.75 ± 0.11	0.38 ± 0.04*
C 22:1n-9	0.30 ± 0.10	0.16 ± 0.07
C 24:1n-9	0.05 ± 0.03	0.04 ± 0.05
Total MUFAs	54.28 ± 6.68	42.79 ± 4.29*
C 18:2n-6	9.37 ± 5.11	24.15 ± 2.74*
C 18:3n-6	0.27 ± 0.06	0.78 ± 0.07*
C 20:2n-6	0.19 ± 0.09	0.19 ± 0.05
C 20:3n-6	0.25 ± 0.13	0.29 ± 0.08
C 20:4n-6	0.43 ± 0.27	1.14 ± 0.13*
C 22:2n-6	0.07 ± 0.05	0.01 ± 0.01
C 22:4n-6	0.11 ± 0.04	0.15 ± 0.03
C 22:5n-6	0.06 ± 0.04	0.12 ± 0.03*
Total n-6	10.75 ± 5.73	26.85 ± 2.71*
C 18:3n-3	0.32 ± 0.17	0.90 ± 0.15*
C 20:3n-3	0.03 ± 0.02	0.02 ± 0.01
C 20:5n-3	0.04 ± 0.02	0.29 ± 0.08*
C 22:5n-3	0.07 ± 0.01	0.26 ± 0.05*
C 22:6n-3	0.26 ± 0.10	1.35 ± 0.15*
Total n-3	0.72 ± 0.29	2.82 ± 0.31*
n-6/n-3	14.3 ± 2.54	9.60 ± 1.20*
Total (μg/mg tissue)	13.41 ± 3.42	44.53 ± 9.52*

Data are mean ± SD ($n = 3$–4)

Significant differences between ad libitum fed and fasted mice are denoted by * $p < 0.05$

9, while 16-carbon monounsaturated fatty acyl species did not change.

Serum phospholipid composition in fed and fasted mice

There were no changes with fasting in the relative proportion of any major class of fatty acids within serum phospholipids, and only minor changes in specific species (Table 6). The relative proportion of 16:0 in serum phospholipids increased by 1.2-fold in fasted mice compared with ad libitum fed mice, while the relative proportion of 20:5n-3 decreased by approximately 30 % with fasting. Of interest, however, there was a significant increase in the total concentration of 16:0 as well as 22:6n-3 in serum phospholipids when absolute levels are considered (Table 6). The concentration of 16:0 increased to 26.09 ± 2.63 μg/100 μl of serum during the fasted state, compared to 19.37 ± 5.16 μg/100 μl during the fed state, while the concentration of 22:6n-3 increased from 3.45 ± 0.73 μg fatty acid/100 μl serum during the fed state to 4.66 ± 0.60 μg fatty acid/100 μl during the fasted state.

Serum TAG composition in fed and fasted mice

Both the absolute and relative content of total MUFA were decreased in serum TAG (Table 7). A decline in oleic acid was primarily responsible for this effect, although a significant decrease in total palmitoleic acid concentration was also evident (Table 7). While these changes tended to mirror effects observed in hepatic TAG, significant decreases in serum TAG linoleic acid total and relative contents were also evident, which was opposite to the relative enrichment seen in liver TAG. The relative, but not the absolute proportion of n-3 PUFA in serum TAG also increased. This was a result of multiple small but cumulative effects, rather than larger specific effects, since there were no significant differences between fasted and fed mice in the relative proportion of any specific n-3 PUFA species. Of interest, the total relative and absolute level of 22:6n3 in serum TAG was highly similar between fasted and fed mice. Our results indicate, as expected, that phospholipid was the major carrier of 22:6n3 in the blood, with absolute concentrations in this fraction containing 10- to 20-fold higher levels than those found in serum TAG.

Expression of elongase, desaturase, and transport genes in fasted and ad libitum fed mouse livers

The selective enrichment of liver TAG with n-3 and n-6 PUFA, coordinate with the selective reduction in MUFA, suggests a liver-specific effect that is independent of changes in adipose tissue fatty acid liberation or blood NEFA concentrations. To examine whether changes in hepatic synthesis may be a factor, we determined the expression of desaturase and elongase genes involved in the synthesis of these fatty acyl species, including *Fads1*, *Fads2*, *Elovl2*, *Elovl5, Elovl6, and Scd1* (Fig. 2a). As expected (Turyn et al. 2010), hepatic expression of both *Scd1* and *Elovl6* was decreased during fasting (Fig. 2a), suggesting that reduced synthesis of MUFA may have been a factor in the liver TAG fatty acid profile that was measured. However, no significant differences were evident in the expression of genes involved in PUFA synthesis (*Fads1*, *Fads2*, *Elovl2*, and *Elovl5*), suggesting that changes in hepatic PUFA synthesis were not likely factors in the fasting-mediated enrichment that was observed. We therefore next examined hepatic gene expression of fatty acid transporters that could mediate a selective and differential uptake of fatty acids by the liver. We found a variety of changes with fasting (Fig. 2b). These included significant increases in hepatic *Fatp2* (fourfold increase in livers of fasted compared to ad libitum fed mice), *Fatp5* (1.5-fold increase with fasting), *Acsl6*, (1.1-fold increase), *Fabp1* (1.5-fold increase), and *Fabp7* (3.7-fold increase). We confirmed the increase in protein levels of FATP2, which demonstrated the largest induction of mRNA, by immunoblotting (Fig. 3). Conversely, hepatic expression of several other fatty acid transporters was significantly lower in the fasted group compared with the ad libitum fed group. Transporters that were significantly reduced included *Fatp3* and *Fatp4,* which were 10 % lower in livers of fasted mice, *Fat/Cd36,* which was 5 % lower, and *Fabp5,* which was 90 % lower.

Discussion

In adults, synthesis of n-3 PUFA is limited (Burdge and Calder 2005; McCloy et al. 2004; Pawlosky et al. 2003), and diet is thus the major source of these essential fatty acids (Kris-Etherton et al. 2009). Adipose tissue, however, can constitute a substantial pool of stored n-3 PUFA, containing up to 70 mg/100 g adipose (Clifton et al. 2004) that can be released to the circulation when lipolysis is activated, thereby providing a significant endogenous source. Studies suggest that n-3 PUFA are preferentially released from adipose tissue compared with SFA, MUFA, or n-6 PUFA when lipolysis is maximally activated (Raclot 2003; Gavino and Gavino 1992; Conner et al. 1996; Yli-Jama et al. 2001; Nieminen et al. 2006; Raclot et al. 1997), which could increase the recirculation of these essential nutrients to extra-adipose tissues. To the best of our knowledge, however, this is the first study to coordinately examine changes in the fatty acid profile of adipose TAG, serum NEFA, liver TAG, and serum phospholipids in both

Table 6 Fatty acid composition of serum phospholipids

Fatty acid	μg fatty acid/100 μl serum		% weight total fatty acids	
	Ad libitum	Fasted	Ad libitum	Fasted
C 10:0	0.03 ± 0.04	0.01 ± 0.01	0.03 ± 0.04	0.02 ± 0.01
C 12:0	0.11 ± 0.06	0.11 ± 0.06	0.14 ± 0.07	0.13 ± 0.06
C 14:0	0.26 ± 0.10	0.38 ± 0.07*	0.34 ± 0.09	0.45 ± 0.09
C 16:0	19.37 ± 5.16	26.09 ± 2.63*	24.94 ± 2.67	30.32 ± 0.67*
C 18:0	17.29 ± 5.89	16.68 ± 2.91	22.07 ± 2.28	19.31 ± 2.19
C 20:0	0.17 ± 0.09	0.20 ± 0.06	0.21 ± 0.08	0.24 ± 0.07
C 22:0	0.25 ± 0.12	0.24 ± 0.04	0.31 ± 0.10	0.28 ± 0.06
C 23:0	0.18 ± 0.07	0.12 ± 0.02	0.23 ± 0.05	0.15 ± 0.05*
C 24:0	0.23 ± 0.06	0.21 ± 0.04	0.29 ± 0.04	0.25 ± 0.08
Total SFAs	37.95 ± 10.85	44.35 ± 5.23	48.64 ± 1.04	51.50 ± 2.32
C 14:1	0.09 ± 0.12	0.14 ± 0.07	0.10 ± 0.15	0.15 ± 0.07
C 16:1	0.48 ± 0.19	0.46 ± 0.10	0.63 ± 0.19	0.55 ± 0.15
C 18:1n-7	2.00 ± 0.80	1.40 ± 0.15	2.54 ± 0.55	1.63 ± 0.11
C 18:1n-9	7.64 ± 3.34	6.62 ± 0.87	9.67 ± 2.99	7.75 ± 1.22
C 20:1n-9	0.25 ± 0.10	0.23 ± 0.05	0.32 ± 0.06	0.27 ± 0.05
C 22:1n-9	0.22 ± 0.12	0.24 ± 0.09	0.28 ± 0.09	0.28 ± 0.10
C 24:1n-9	0.67 ± 0.19	0.69 ± 0.20	0.85 ± 0.07	0.81 ± 0.22
Total MUFAs	11.34 ± 4.48	9.79 ± 1.16	14.39 ± 3.51	11.44 ± 1.52
C 18:2n-6	11.44 ± 2.75	13.37 ± 3.09	14.85 ± 1.62	15.40 ± 2.26
C 18:3n-6	0.10 ± 0.04	0.12 ± 0.03	0.12 ± 0.04	0.14 ± 0.03
C 20:2n-6	0.26 ± 0.11	0.21 ± 0.04	0.33 ± 0.07	0.24 ± 0.03
C 20:3n-6	0.91 ± 0.66	0.68 ± 0.15	1.29 ± 0.87	0.79 ± 0.14
C 20:4n-6	7.85 ± 2.53	9.50 ± 1.97	10.34 ± 2.84	11.00 ± 1.59
C 22:2n-6	0.11 ± 0.04	0.09 ± 0.02	0.13 ± 0.02	0.11 ± 0.03
C 22:4n-6	0.15 ± 0.08	0.18 ± 0.03	0.19 ± 0.08	0.21 ± 0.02
C 22:5n-6	0.24 ± 0.12	0.19 ± 0.05	0.32 ± 0.14	0.23 ± 0.08
Total n-6	21.07 ± 4.54	24.34 ± 5.14	27.58 ± 3.62	28.11 ± 3.76
C 18:3n-3	0.14 ± 0.09	0.07 ± 0.04	0.16 ± 0.09	0.08 ± 0.05
C 20:3n-3	0.04 ± 0.07	0.04 ± 0.04	0.05 ± 0.06	0.05 ± 0.05
C 20:5n-3	0.21 ± 0.08	0.14 ± 0.02	0.26 ± 0.06	0.16 ± 0.01*
C 22:5n-3	0.20 ± 0.05	0.23 ± 0.04	0.26 ± 0.07	0.27 ± 0.02
C 22:6n-3	3.45 ± 0.73	4.66 ± 0.60*	4.60 ± 1.17	5.43 ± 0.53
Total n-3	4.03 ± 0.77	5.14 ± 0.64	5.34 ± 1.01	5.98 ± 0.49
n-6/n-3	5.21 ± 0.32	4.71 ± 0.58	5.21 ± 0.32	4.71 ± 0.58
Total	74.52 ± 19.14	83.72 ± 9.80		

Data are mean ± SD ($n = 3$–4)

Significant differences between ad libitum fed and fasted mice are denoted by * $p < 0.05$

the fasted and ad libitum fed states. It is also the first to perform a detailed characterization of adipose TAG and serum NEFA changes with a short-term fasting protocol, rather than a protocol that maximally stimulates lipolysis.

More than 90 % of adipose cell volume is comprised of TAG, which is hydrolyzed and released into the blood as NEFA during fasting. The enzymes HSL and ATGL are responsible for approximately 95 % of adipose tissue TAG lipolysis (Schweiger et al. 2006). HSL will use TAG containing many different fatty acyl species as substrates, but it has preference for TAG enriched in some n-3 PUFA such as 20:5n-3 (Raclot et al. 2001), while ATGL shows a preference for TAG containing long-chain fatty acids, but not n-3 PUFA specifically (Eichmann et al. 2012). In the present study, we did not observe substantial differences in the relative proportion of fatty acids remaining in adipose TAG when examining fasted compared to ad libitum fed mice. We also did not observe major class or species

Table 7 Fatty acid composition of serum TAG

Fatty acid	μg fatty acid/100 μl serum		% weight total fatty acids	
	Ad libitum	Fasted	Ad libitum	Fasted
C 10:0	0.12 ± 0.07	0.03 ± 0.03	0.28 ± 0.16	0.09 ± 0.08
C 12:0	1.43 ± 1.41	0.21 ± 0.10	3.40 ± 3.69	0.73 ± 0.20
C 14:0	1.73 ± 0.88	1.09 ± 0.94	4.02 ± 1.99	3.67 ± 2.24
C 16:0	13.83 ± 3.85	9.81 ± 2.38	32.58 ± 4.87	36.61 ± 2.42
C 18:0	6.19 ± 1.89	6.01 ± 3.07	14.44 ± 1.20	21.13 ± 6.06
C 20:0	0.32 ± 0.08	0.35 ± 0.18	0.76 ± 0.06	1.22 ± 0.34
C 22:0	0.37 ± 0.11	0.38 ± 0.15	0.87 ± 0.15	1.39 ± 0.27
C 24:0	0.19 ± 0.12	0.11 ± 0.08	0.43 ± 0.20	0.51 ± 0.45
Total SFAs	24.18 ± 6.18	17.98 ± 6.36	56.78 ± 2.27	65.33 ± 5.82
C 12:1	0.29 ± 0.21	0.04 ± 0.03	0.67 ± 0.52	0.15 ± 0.06
C 14:1	0.06 ± 0.05	0.14 ± 0.21	0.13 ± 0.09	0.43 ± 0.57
C 16:1	1.47 ± 1.24	0.46 ± 0.20	3.24 ± 2.20	1.73 ± 0.64
C 18:1n-7	0.77 ± 0.31	0.27 ± 0.12*	1.81 ± 0.62	1.01 ± 0.31
C 18:1n-9	10.84 ± 1.74	4.24 ± 1.27*	26.01 ± 4.64	16.09 ± 4.14*
C 20:1n-9	0.20 ± 0.11	0.11 ± 0.03	0.49 ± 0.35	0.43 ± 0.16
C 22:1n-9	0.22 ± 0.04	0.24 ± 0.08	0.51 ± 0.02	0.89 ± 0.32
C 24:1n-9	0.15 ± 0.08	0.33 ± 0.12	0.39 ± 0.28	1.24 ± 0.46*
Total MUFAs	13.98 ± 2.35	5.82 ± 1.82*	33.24 ± 2.17	21.97 ± 5.11*
C 18:2n-6	1.81 ± 0.63	0.68 ± 0.23*	4.53 ± 2.31	2.84 ± 1.50
C 18:3n-6	0.11 ± 0.03	0.09 ± 0.09	0.27 ± 0.06	0.32 ± 0.27
C 20:2n-6	0.16 ± 0.18	0.21 ± 0.15	0.33 ± 0.32	0.83 ± 0.59
C 20:3n-6	0.12 ± 0.08	0.15 ± 0.11	0.26 ± 0.13	0.58 ± 0.36
C 20:4n-6	0.20 ± 0.09	0.11 ± 0.03	0.46 ± 0.10	0.45 ± 0.18
C 22:2n-6	0.30 ± 0.22	0.31 ± 0.10	0.67 ± 0.33	1.19 ± 0.37
C 22:4n-6	0.09 ± 0.11	0.16 ± 0.10	0.23 ± 0.33	0.58 ± 0.23
C 22:5n-6	n.d.	n.d.	n.d.	n.d.
Total n-6	2.80 ± 0.73	1.73 ± 0.56	6.76 ± 2.24	6.81 ± 2.68
C 18:3n-3	0.17 ± 0.15	0.13 ± 0.06	0.43 ± 0.39	0.49 ± 0.27
C 20:3n-3	0.21 ± 0.13	0.40 ± 0.35	0.47 ± 0.19	1.34 ± 0.83
C 20:5n-3	0.13 ± 0.10	0.18 ± 0.08	0.29 ± 0.21	0.66 ± 0.33
C 22:5n-3	0.11 ± 0.09	0.19 ± 0.17	0.24 ± 0.15	0.76 ± 0.58
C 22:6n-3	0.38 ± 0.32	0.22 ± 0.14	0.82 ± 0.52	0.78 ± 0.27
Total n-3	1.00 ± 0.55	1.12 ± 0.46	2.26 ± 0.80	4.03 ± 0.56*
n-6/n-3	3.54 ± 2.56	1.78 ± 0.88	3.54 ± 2.56	1.78 ± 0.88
Total	41.96 ± 9.16	26.65 ± 7.83		

Data are mean ± SD ($n = 3$–4)

Significant differences between ad libitum fed and fasted mice are denoted by * $p < 0.05$

differences in the proportions of NEFA in serum, further supporting that adipose tissue does not preferentially liberate one fatty acid species over another during a short-term fast. This finding differs from prior studies where lipolysis was maximally stimulated (Raclot et al. 1997; Conner et al. 1996), but is not incongruous with that work. Differences in the selective mobilization of TAG fatty acids between short-term and long-term fasting likely exist, and can be explained, at least in part, by differences in the enzymatic regulation of lipolysis as it occurs under basal and stimulated conditions. The short-term duration of the fasting conditions examined were not expected to significantly elevate serum glucocorticoids (Nowland et al. 2011) or maximally stimulate adipocyte lipolysis (Szkudelski et al. 2004). Without strong hormonal activation of lipolysis, ATGL is the major regulator of basal NEFA release (Miyoshi et al. 2008). In the current study, ATGL was likely the predominant contributor to adipose tissue

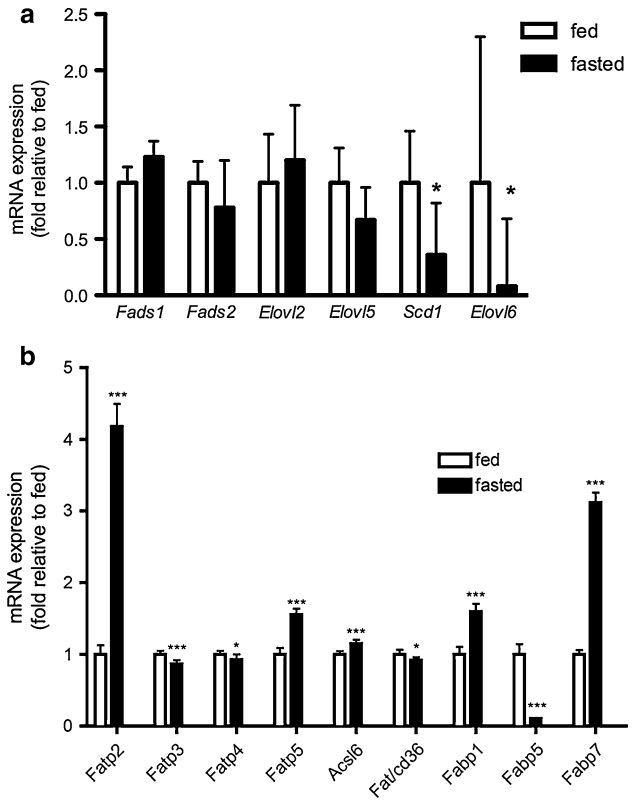

Fig. 2 Short-term fasting alters hepatic expression of enzymes involved in PUFA synthesis and fatty acid uptake. **a** Genes involved in MUFA and PUFA biosynthesis. **b** Genes involved in fatty acid transport. *Fads1* fatty acid desaturase 1, *Fads2* fatty acid desaturase 2, *Elovl2* elongase 2, *Elovl5* elongase 5, *Scd1* stearoyl-CoA desaturase 1, *Elovl6* elongase 6, *Fatp2* fatty acid transport protein 2, *Fatp3* fatty acid transport protein 3, *Fatp4* fatty acid transport protein 4, *Fatp5* fatty acid transport protein 5, *Acsl6* acyl-CoA synthetase long-chain 6, *Fat/cd36* fatty acid translocase/cluster of differentiation 36, *Fabp1* fatty acid binding protein 1, *Fabp5* fatty acid binding protein 5, *Fabp7* fatty acid binding protein 7. Data are mean ± SEM; $n = 6$–7; $^{*}p < 0.05$

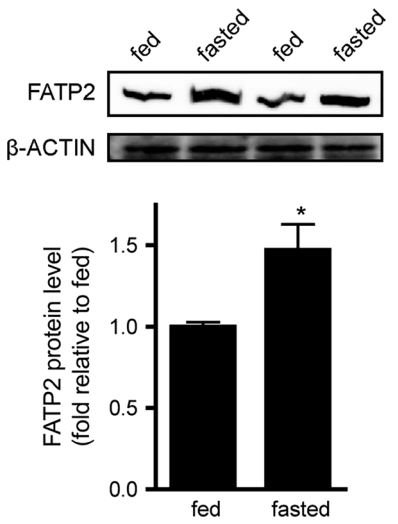

Fig. 3 FATP2 protein levels in fasted versus fed mice. Data are mean ± SEM; $n = 4$; $^{*}p < 0.05$

lipolysis in fasted mice, explaining the lack of preferential depletion of any particular class or individual species of fatty acid within adipose TAG. Thus, although results from our work differ from prior reports, they do not disagree with the notion of a predominant role for HSL in mediating the selective hydrolysis of PUFA in adipose tissue when lipolysis is maximally stimulated and HSL is fully activated (Miyoshi et al. 2006), such as during a prolonged fast. Our results, however, extend this work by characterizing effects occurring during a short-term fast.

Despite the lack of relative change in adipose TAG and serum NEFA with short-term fasting, differences in liver TAG fatty acyl species were evident. Hepatic TAG had lower levels of MUFA, but were enriched with both n-3 and n-6 PUFA during fasting, although a greater preference for n-3 PUFA enrichment was demonstrated by the significant reduction in n-6 to n-3 ratio. Because dietary sources of fatty acids were unavailable with fasting, these n-3 and n-6 PUFA must have come from either the elongation and desaturation of precursor lipids or the enhanced selective incorporation of blood NEFA mobilized from adipose. We first investigated hepatic synthesis as a possible mechanism for increased endogenous generation of liver n-3 and n-6 PUFA, and for decreased MUFA. Hepatic expression of *Elovl6* and *Scd1* was significantly reduced by short-term fasting, which may have contributed to the reduction in 18:0 and 18:1n-9 that were observed. However, hepatic expression of the major genes involved in n-3 and n-6 PUFA biosynthesis, *Fads1*, *Fads2*, *Elovl2*, and *Elovl5,* was not different between the fasted group and the fed group. Furthermore, there was an increase in the relative and absolute concentrations in hepatic TAG of 18:2n-6 and 18:3n-3, which are the major substrates for synthesis of the longer-chain n-6 and n-3 PUFA, respectively. If these were being used in elongation and desaturation reactions, a relative decrease in their abundance would be expected. These data, therefore, suggest it is unlikely that the enrichment of hepatic TAG PUFA was a result of increased biosynthesis.

Thus, we next examined the hepatic expression of several fatty acid transporters for insight into the shift in composition of stored liver fatty acids. Interestingly, there was a highly specific regulation of different fatty acid transporters with fasting, such that transporters with a greater affinity for n-3 and n-6 PUFA tended to be increased, while transporters with a greater affinity for SFA and MUFA tended to be down-regulated. Specifically, expression of *Fabp7*, *Fatp2*, *Fabp1*, and *Acsl6* increased by 3.7-fold, fourfold, 1.5-fold, and approximately 1.1-fold, in the fasted group compared with the fed group. These

transporters preferentially mediate uptake of n-3 and n-6 PUFA into cells and show high affinity for 22:6n-3 (Norris and Spector 2002; Marszalek et al. 2005; Xu et al. 1996; Mita et al. 2010; Nemecz et al. 1991; Melton et al. 2011, 2013). Expression of the *Fatp5* transporter was also 1.5-fold higher in fasted animals compared with ad libitum fed animals. *Fatp5* transports long-chain (14–20 carbon) and very long chain (>22 carbon) fatty acids, but it is unknown whether it has a preference for unsaturated fatty acids that may help to explain effects observed in fasted mice (Falcon et al. 2010; Nie et al. 2012; Krammer et al. 2011). Conversely, transporters specific for longer-chain saturated fatty acids and 18-carbon fatty acids, such as *Fatp3*, *Fatp4*, *Fabp5*, and *Fat/cd36* (Hardwick et al. 2013; Krammer et al. 2011; Zimmerman et al. 2001), showed a decrease of 10–90 % that likely contributed to the reduced proportions of TAG 18:0 and 18:1n-9 that were evident in livers with fasting. Taken together with our findings on adipose TAG fatty acid and serum NEFA composition, these data suggest that selective uptake by the liver plays an important role in mediating the observed changes in hepatic fatty acid composition that occurred with fasting.

Redistribution of fatty acids from the liver to other tissues is mediated through the synthesis and secretion of lipoproteins. The liver is the main site of synthesis of circulating phospholipids during fasting (Radding and Steinberg 1960). Circulating phospholipid composition is important since the brain can selectively take up 22:6n-3 as part of a lysophosphatidylcholine (Nguyen et al. 2014). In conditions such as pregnancy, recirculation of fatty acids in blood phospholipids is a major mechanism by which the body transfers n-3 PUFA to the developing fetus (Burdge et al. 2006; Vlaardingerbroek and Hornstra 2004; Postle et al. 1995). This has been demonstrated in both rodents (Chen et al. 1992; Burdge et al. 1994) and humans (Stark et al. 2005; Burdge et al. 2006; Kuipers et al. 2011), suggesting that while rodents have a generally higher metabolic rate than humans (Demetrius 2005), mechanisms governing the metabolism and transport of 22:6n-3 are likely conserved. In the present study, the absolute concentration of 22:6n-3 increased in serum phospholipids during fasting, although the proportion of this species in total phospholipid fatty acids did not change when relative percentages were compared. This was likely a result of the relative dilution of rising DHA by a coordinate significant rise in the concentration of 16:0, as well as smaller non-significant increases in other fatty acids. The specific and tandem increase in both 16:0 and 22:6n-3 in this fraction has biological significance. DHA in plasma and serum phospholipids is typically found at the *sn*-2 position, most often with 16:0 at the *sn*-1 position (Pynn et al. 2011; Farkas et al. 2000; Brand et al. 2010). The coordinate rise in the concentration of both of these fatty acids within the serum phospholipid fraction suggests a specific fasting-mediated increase in the hepatic synthesis of 16:0/22:6n-3 species of phospholipids for recirculation of DHA in lipoproteins. Fasting, even for short periods, is associated with beneficial effects on health (Horne et al. 2012, 2013). Our findings suggest that enhanced redistribution of DHA in circulating phospholipids may constitute a novel mechanism to help explain some of these benefits, since phospholipids are the predominant carrier of n-3 PUFA in serum.

While serum TAG is not typically considered a major pool for the transport of n-3 or n-6 PUFA (Crowe et al. 2008; Balkova et al. 2009), it is still of interest to examine fasting-mediated changes in the composition of this complex lipid in serum. The fatty acid profile of serum TAG is generally expected to mirror that of the hepatic TAG from which it is derived (Hyysalo et al. 2014; Donnelly et al. 2005). In serum TAG from our mice, the major change observed with fasting was a decrease in total MUFA that resulted largely from a reduction in 18:1n-9, similar to the effect that was evident in the liver. The second largest effect of fasting on serum TAG was an increase in the proportion of total n-3 PUFA, which was also apparent in liver. However, unlike effects observed in liver, a specific fasting-mediated increase in 22:6n-3 content was not seen in serum TAG. Additionally, fasting mediated an absolute and relative reduction in the concentration of serum TAG linoleic acid that was opposite to the enrichment in liver TAG 18:2n-6 that was coordinately observed. Overall, our results are in line with prior work indicating that the pools of n-3 and n-6 PUFA in serum TAG are considerably smaller than pools in phospholipid ($\sim$75 to 90 % lower) and that serum TAG 22:6n-3 is a relatively minor transport source in blood (Crowe et al. 2008; Balkova et al. 2009).

In conclusion, this study provides new evidence suggesting that short-term fasting increases the redistribution of adipose DHA, primarily through selective changes in liver uptake and utilization of n-3 NEFA for the synthesis of secreted phospholipid, rather than through selective changes in adipose NEFA release. Future work should examine the specific role of individual hepatic fatty acid transporters in mediating this effect, and the role of hepatic fatty acid transporters in mediating the selective enrichment of plasma phospholipid with DHA that is evident in other conditions, such as pregnancy.

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Compliance with ethical standards

Conflict of interest KA Marks, PM Marvyn, JJA Henao, RM Bradley, KD Start, and RE Duncan declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (University of Waterloo AUPP#13-13). This article does not contain any studies with human participants performed by any of the authors.

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