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Short-term energy deficit causes net accumulation of linoleoyl-enriched triacylglycerols in rat liver

Zhen-Yu Chen and Stephen C. Cunnane

Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada

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Energy depletion by reduced food intake over 4 days resulted in a 73% reduction in total rat liver triacylglycerols (TG). In liver TG of energy-depleted rats, dilinoleoyl oleoyl glycerol (OLL) and trilinoleoyl glycerol (LLL)) were quantitatively increased by 85% and 147%, respectively. The net increase in linoleoyl-enriched species could be quantitatively accounted for by the release of linoleate from monolinoleoyl species and its subsequent reacylation into dilinoleoyl species and trilinolein during energy depletion. Hence while palmitate, oleate and some linoleate are being hydrolyzed, presumably for oxidation, some linoleate is retained and contributes to the remodelling of hepatic triacylglycerols during energy deficit.

Triacylglycerol; Fasting; Liver; Oxidation

1. INTRODUCTION

Fasting causes accumulation of arachidonic (20:4n-6) and docosahexaenoic acids (22:6n-3), but depletion of palmitic (16:0), palmitoleic (16:1n-7) and oleic acids (18:1n-9) from the liver and plasma triacylglycerols (TG) [1–4]. The mechanism by which some long-chain fatty acids are selectively utilized while others are retained during fasting is not well understood but is likely to be a protective mechanism preventing essential fatty acid deficiency under high requirements for oxidizable fatty acid substrates. It is not clear whether individual TG species are preferentially retained or, indeed, whether some TG species are also remodelled during fasting-induced TG depletion. Quantitative changes in individual liver TG species during short-term energy deficit were therefore determined in the present study.

2. MATERIAL AND METHODS

Male Wistar rats (200–250 g; Charles River Canada, St. Constant, Quebec) were given a semi-purified diet with sunflower oil providing 39 g/kg linoleic acid [4]. The rats were fed the diet for a total of 28 days with actual food intake determined during week 3. For 4 days (day 23–day 27), half of the rats were given 25% of their normal ad libitum intake (short-term energy deficit). On day 28, all of the rats were sacrificed under diethyl ether anesthesia. The abdomens were opened and livers removed, washed in saline and then frozen.

The total liver lipids were extracted, and TG separated by thin-layer chromatography and quantitated as described previously [3]. The TG band was recovered and eluted from the silica with 60 ml hexane/diethyl ether (90:10, v/v). Individual TG species were analyzed using a Varian GLC 3700 equipped with TG column coated with 65% phenylmethyl silicone film (Quadrex, New Haven, CT, USA) and a movable cold on-column (J & W Scientific, Folsom, CA, USA).

An aliquot of the total TG was hydrogenated at 70°C in a small conical vial. The TG sample was dissolved in 300 μl of hexane/methanol (2:1, v/v) and transferred to the hydrogenation vial, followed by addition of 1 mg rhodium on alumina powder (5%, Aldrich Chemical Co., Milwaulkee, WI, USA) as a hydrogenation catalyst. The TG sample was bubbled with hydrogen gas for 5 min. Hexane (200 μl/min) was added to compensate for loss of solvent by evaporation. After the hydrogenation was completed (verified by GLC), the sample was centrifuged, and the hexane layer was saved for analysis of hydrogenated TG species.

Correspondence address: S.C. Cunnane, Department of Nutritional Sciences, University of Toronto, Toronto, Ontario M5S 1A8, Canada

3. RESULTS

Energy deficit over 4 days caused a 73% reduction in total hepatic TG (16.5–4.4 mg/g wet liver weight) as well as a change in the fatty acid composition of liver TG as previously reported [3]: decreased proportions of 16:0, 16:1n-7 and 18:1n-9, but increased proportions of 18:0, 18:2n-6 and 20:4n-6. Fed compared to energy-depleted rats had distinctly different liver TG species profiles (Fig. 1A). Individual TG species have been identified by reference to standards, from the known fatty acid composition of the sample and by reference to Kuksis et al. [6]. With the column used, resolution of TG species is on the basis of total carbon number (C) and number of double bonds. Without stabilization by hydrogenation, TG species containing 20:4n-6 and 22:6n-3 were unrecoverable due to their high thermal sensitivity. Hydrogenation increased the thermal stability of the 20:4n-6 and 22:6n-3 TG species by converting them to saturated 20 and 22 carbon fatty acids, respectively. Following hydrogenation, C56 (probably containing 20:4n-6) and C58 (probably containing 22:6n-3) were resolved as saturated TG

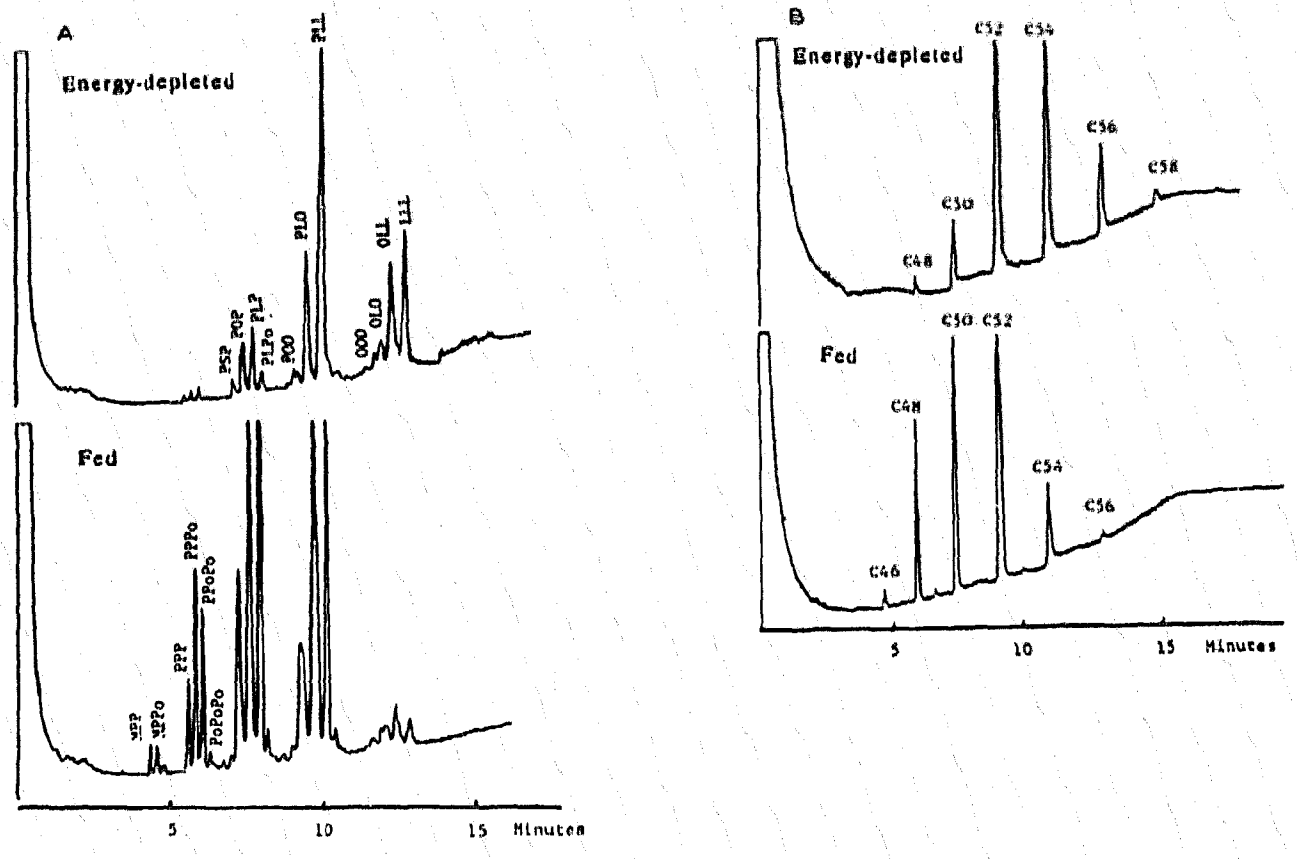

Fig. 1.(A) Gas liquid chromatograms of liver triacylglycerol species from a fed (bottom) or energy-depleted (top) rat. (B) Gas liquid chromatograms of hydrogenated liver triacylglycerol species from a fed (bottom) or energy-depleted (top) rat. Column, fused silica capillary (25 m × 0.25, i.d.) coated with 65% phenylmethyl silicone. Temperature, 320°C–355°C at 2°C/min. Carrier Gas hydrogen (25 psi). Abbreviations: MPP, dipalmitoyl myristoyl glycerol; MPPo, myristoyl palmitoyl palmitoleoyl glycerols; PPP, tripalmitoyl glycerol; PPPo, dipalmitoyl palmitoleoyl glycerol; PPoPo, dipalmitoleyl palmitoyl glycerol; PoPoPo, tripalmitoleoyl glycerols; PSP, dipalmitoyl stearoyl glycerol; POP, dipalmitoyl oleoyl glycerol; PLP, dipalmitoyl linoleoyl glycerol; PLPo, palmitoyl linoleoyl palmitoleoyl glycerol; POO, dioleoyl palmitoyl glycerol; PLO, palmitoyl linoleoyl oleoyl glycerol; PLL, dilinoleoyl palmitoyl glycerol; OOO, trioleoyl glycerol; OLO, dioleoyl linoleoyl glycerol; OLL, dilinoleoyl oleoyl glycerol; LLL, trilinoleoyl glycerol.

(Fig. 1B). In energy-depleted rats, C46 was not detectable; C48 was reduced from 11.3% to 1.4% and C50 from 32.1% to 6.1% of total TG; C52 was proportionally unchanged but C54 increased from 7.8% to 33.0%, C56 from 1.3% to 10.5% and C58 from 0% to 2.2% of total TG (Table I). Within the C52 species, PLL was proportionally increased and quantitatively decreased; the other two C52 TG species (POO and PLO) were proportionally and quantitatively decreased. Within the C54 species, proportions of OLL and LLL were significantly increased. In general, the absolute amount (μg/g liver weight) of individual liver TG species was therefore markedly reduced due to reduction of total liver TG size except for OLL, LLL, C56 and C58, which were quantitatively increased (Table I).

4. DISCUSSION

The present results support previous observations that 18:2n-6, 20:4n-6 and 22:6n-3 in liver are proportionally increased after short-term fasting [1–4]. We believe this is the first report of differentiation of individual TG species of rat liver. It is clear that as unsaturation of the individual TG species increased to a total of 4 double bonds (PLL), net depletion (or utilization) during energy-deficit was reduced from >95% to <50%. Above a total of 4 double bonds, TG species (OLL and LLL) were actually present in quantitatively higher amounts after energy deficit (Table I; Fig. 2).

These results suggest that during energy deficit, the liver degrades TG species preferentially on the basis of their total carbon chain length and degree of unsaturation, e.g. lower molecular weight and less unsaturated TG species are depleted faster than larger molecular weight more unsaturated TG. Thus, liver TG subclasses C48 and C50 (mainly consisting of 16:0 with 16:1n-7, 18:1n-9, 18:0 and 18:2n-6) serve as an important liver lipid source for energy under these conditions. Exogenous free 18:2n-6 is oxidized more slowly during fasting than other long chain fatty acids [7,8], which is

Table I

Liver triacylglycerol composition (% of total TG and μg/g wet liver weight) in fed and energy-depleted rats

TG	% of Total TG		μg/g liver	
	Fed	Energy deficit	Fed	Energy deficit
MMP	0.8 ± 0.1	ND	134 ± 10	ND
MPPo	0.8 ± 0.1	ND	118 ± 12	ND
Total C46	1.6 ± 0.1		252 ± 10	
PPP	2.2 ± 0.1	0.3 ± 0.1**	345 ± 10	12 ± 2**
PPPo[1]	4.8 ± 0.1	0.4 ± 0.1**	773 ± 12	17 ± 3**
PPoPo[2]	3.7 ± 0.2	0.7 ± 0.1**	598 ± 40	33 ± 2**
PoPoPo[3]	0.6 ± 0.1	ND	61 ± 3	ND
Total C48	11.3 ± 0.1	1.4 ± 0.1**	1776 ± 63	61 ± 4**
PSP	6.1 ± 0.4	0.5 ± 0.1**	999 ± 62	22 ± 2**
POP[4]	12.9 ± 0.4	2.3 ± 0.5**	2135 ± 69	102 ± 4**
PLP[5]	12.5 ± 0.2	2.2 ± 0.1**	2069 ± 191	99 ± 6**
PLPo[6]	0.6 ± 0.1	1.1 ± 0.1	95 ± 17	44 ± 2
Total C50	32.1 ± 0.4	6.1 ± 0.1**	5296 ± 215	268 ± 32**
POO[7]	9.4 ± 0.1	2.8 ± 0.1**	1555 ± 65	125 ± 8**
PLO[8]	20.5 ± 0.3	12.9 ± 1.0**	3376 ± 142	570 ± 44**
PLL	14.1 ± 0.2	30.8 ± 1.4**	2337 ± 32	1335 ± 60**
Total C52	44.0 ± 0.4	46.5 ± 2.5	7267 ± 66	2050 ± 110*
OOO	1.7 ± 0.6	2.6 ± 0.3	282 ± 102	113 ± 18
OLO[9]	1.7 ± 0.4	3.4 ± 1.0	303 ± 92	149 ± 62
OLL	2.0 ± 0.3	13.9 ± 1.0**	330 ± 63	612 ± 62*
LLL	1.7 ± 0.2	13.2 ± 2.3**	235 ± 55	581 ± 101*
Total C54	7.8 ± 0.1	33.0 ± 0.6**	1314 ± 37	1445 ± 53
Total C56	1.3 ± 0.1	10.5 ± 1.0**	219 ± 14	462 ± 66*
Total C58	ND	2.2 ± 0.3	ND	97 ± 18

Each value is the mean ± SD. ND, <0.01% of total TG; * $P<0.05$; ** $P<0.01$, Student t-test. See Fig. 1 for abbreviations. The main TG species have been identified but other species may also be present. [1] +MPO; [2] +MLP; [3] +MLP; [4] +PSPo; [5] +POPo; [6] +MOL; [7] +PLS; [8] +PoOO; [9] +SLL

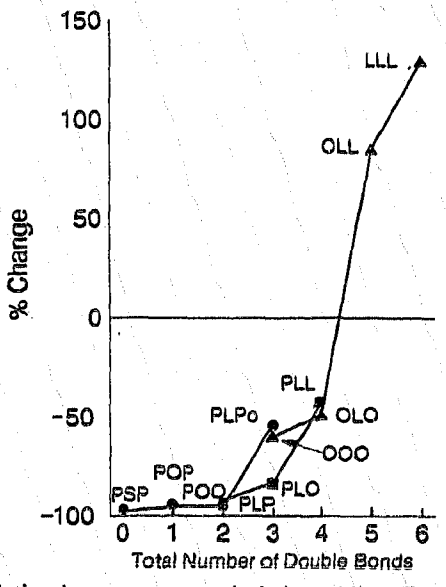

Fig. 2. Relation between percent depletion of liver TG species and total number of double bonds of TG species in energy-depleted rats. (●) = TG species within C50; (■) = TG species within C52; (▲) = TG species within C54.

in agreement with our present data on the net utilization during energy deficit of endogenous 18:2n-6 esterified to liver TG. However, in fed animals, free 18:2n-6 is more easily oxidized than stearic and palmitic acids [8–12]. Hence, anabolic remodelling of hepatic TG species can occur during the catabolic influence of short-term energy deficit.

The net depletion of 16:0 and 18:1n-9 from liver TG during energy deficit appears to have been influenced by the two neighboring fatty acid moieties. Thus, depletion of 16:0 from POO (92%) was greater than from PLO (83%) and PLL (42%). Greater utilization of 18:1n-9 from POO, POP and PLO than from SLO, OLO and OLL was also observed (Table I). This indicates that the net utilization of 16:0 and 18:1n-9 from hepatic TG during energy deficit was dependent on the carbon chain length and degree of unsaturation of the two neighboring fatty acids. From our data, slower utilization of 16:0, 16:1n-7 and 18:1n-9 would also be predicted if the two neighboring fatty acids were 18:2n-6, 20:4n-6 and 22:6n-3. The methods used here have not distinguished individual TG species with

20:4n-6 and 22:6n-3 (present mainly in C56-C60), so this remains speculation.

Although adipose tissue contributes large amounts of 18:2n-6 to the circulation during fasting (Chen and Cunnane, unpublished), the net increase in linoleoyl-enriched species of hepatic TG during energy deficit can be completely accounted for by the depletion of monolinoleoyl species with the subsequent reacylation of 18:2n-6 into di- or trilinoleoyl species. Hence, whilst TG species containing 16:0, 18:1n-9 and 18:2n-6 are undergoing net hydrolysis presumably for oxidation, some 18:2n-6 is retained and contributes to remodelling of hepatic TG during energy deficit.

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