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Odd-Numbered Fatty Acids in Phosphatidyl Choline Versus Phosphatidyl Ethanolamine of Vitamin B_{12}-Deprived Rats[1] (41151)

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Abstract. In response to a 20-week dietary deficiency of vitamin B_{12} rats accumulated two to four times more heptadecanoic acid (17:0) and pentadecanoic acid (15:0) in phosphatidyl choline (PC) of their cerebrum. Considerably smaller amounts of these odd-numbered fatty acids (ONFA) were present in phosphatidyl ethanolamine (PE) of either the cerebrum or liver of rats deprived of, or supplemented with, vitamin B_{12}. Vitamin B_{12}-deficient rats had twice as much ONFA in PC of their cerebrum than in PC of their liver, and the vitamin deficiency had little or no effect on the amounts of ONFA in PE of the cerebrum or liver. The greater incorporation of ONFA into cerebral PC appears to be correlated with a greater abundance of palmitic acid (16:0) and related chain length–even-numbered fatty acids (14:0) and 16:1) in this phospholipid of the rat. Similar relationships between the abundance of ONFA and 16:0 in neural PC were previously found in an infant with genetically defective B_{12}–coenzyme systems.

Neuropathies characteristically develop in individuals with (a) genetically defective B_{12}–coenzyme-dependent systems (1); (b) pernicious anemia (2); and (c) severe deficiencies of vitamin B_{12} (3–5). It has been speculated (1, 6, 7) that such neurological defects may be partially related to accumulations of small amounts of odd-numbered (ONFA) and branched chain (BCFA) fatty acids in membrane lipids of neural tissues. When the B_{12}–coenzyme-dependent mutase reaction is blocked, the resulting elevated tissue levels of propionyl CoA and methylmalonyl CoA stimulates some increased *de novo* synthesis of, respectively, ONFA and BCFA. Small amounts of unbranched (ONFA), but not branched (BCFA), isomers of 15 and 17 carbon chain length fatty acids have been detected in peripheral nerve lipids of pernicious anemic subjects (7). Increased amounts of ONFA were also found in serum and liver lipids of an infant with a defective propionyl CoA carboxylase system (11). Detectable amounts of ONFA and BCFA can be found in liver lipids of vitamin B_{12}-deficient baboons (12), and ONFA is increased in liver and brain of rats as their tissues are depleted of vitamin B_{12} (13–15). Severe neurological damage was apparent in the infant having ONFA and BCFA in its neural tissues (1), and more subtle neuropathies were evident when only ONFA was detected in neural tissues of pernicious anemic subjects (7) and in aneural tissues of an infant with a defective propionyl-CoA carboxylase system (11). Recent studies suggest that vitamin B_{12}-deficient rats can experience blood and bone marrow changes characteristic of pernicious anemia (16), and these deficient rats may also exhibit some symptoms of neurological dysfunction (17).

Kishimoto *et al.* (1) reported that disproportionately greater amounts of available ONFA and BCFA were incorporated into phosphatidyl choline (PC) of neural tissues of the infant with the defective B_{12}–coenzyme systems. Comparatively smaller to negligible amounts of ONFA and BCFA were present in phosphatidyl ethanolamine (PE) of other phospholipids of the neural tissues, and these odd-numbered fatty acid isomers appeared to be more abundant in neural than in most aneural tissues. We previously reported (14) that altered patterns of polyunsaturated fatty acids

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(PUFA) were more apparent in PC than PE of liver and brain lipids of vitamin B_{12}-deficient rats fed soy protein diets. Our vitamin B_{12}-deficient rats also had increased amounts of ONFA in PC of both their cerebrum and liver, and cerebral PC contained approximately twice as much ONFA as that in liver PC (14). No attempt was made to measure the effects of vitamin B_{12} deficiency on the distribution of ONFA into PE or other phospholipids of neural or liver tissues of these rats. There is an obvious need to determine whether the various symptoms of vitamin B_{12} deficiency in rats can also be correlated with an apparent preferential incorporation of more ONFA into PC of their neural tissues similar to that reported by Kishimoto et al. (1). Some limited data have been reported on the distribution of 17:0, but not 15:0, in brain and liver lipids of 18-day-old rats depleted of vitamin B_{12} (15).

Reported here are the effects of a 20-week dietary deficiency of vitamin B_{12} on the comparative distribution of ONFA, both 15:0 and 17:0, into PC and PE of cerebral and liver tissues of rats. Included are data on the concentrations of related carbon chain length–even-numbered fatty acids, myristic (14:0), palmitic (16:0), and palmitoleic (16:1) acids,[4] in PC and PE of the brain and liver.

Materials and Methods. Female Spraque–Dawley rats (Charles River Breeding Lab, Wilmington, Mass.) weighing 35–50 g were fed a vitamin B_{12}-supplemented diet (5 μg vitamin B_{12}/100 g diet) for 1 week and then divided into two groups having equivalent body weights. These groups were fed the same basic diet, with or without supplements of vitamin B_{12}, for the next 20 weeks. The basal diet, described previously (14), included 24% soy protein, 43.2% sucrose, 3.5% linoleate and 20% total fat, 3% sodium propionate, 0.25% L-cystine, 0.01% thyroid powder, and optimal amounts of mineral and other vitamins. With these experimental conditions the 20 weeks of vitamin B_{12} deprivation induces a significant metabolic block of the B_{12}–coenzyme mutase system as evidenced by a 6 to 7-fold increase of total methylmalonic acid in the liver and a doubling of ONFA in PC of the brain and liver (14). Unique biochemical markers of a blocked B_{12}--coenzyme system include elevated tissue levels of methylmalonyl-CoA and increasing urinary excretion of methylmalonic acid (16, 18, 19) and increased amounts of ONFA in neural and other tissue lipids (1, 7, 12–15). A 20 to 21-week deprivation of vitamin B_{12} and dietary conditions similar to ours will cause a 30-fold increase of urinary excretion of methylmalonic acid and 80%, or more, decreases of vitamin B_{12} from the brain, liver, and plasma of rats (16). Sodium propionate and thyroid powder were included in our experimental diets to promote a more rapid depletion of vitamin B_{12} from tissues of the rats (20, 21).

At the termination of the study, the rats were starved overnight and then exsanguinated while being anesthetized with ethyl ether. The liver and cerebrum of each rat were immediately removed, freed of extraneous tissues, weighed, sealed in plastic bags, and frozen until they could be subjected to lipid analyses. The cerebrum refers to the forebrain obtained by severing the brain at the cerebellum–forebrain junction and removal of the olfactory tracts.

Total lipids were quantitatively isolated by the method of Folch *et al.* (22). Thin-layer chromatography (tlc) techniques used to isolate PC and PE and the gas–liquid chromatography (glc) methods used for quantitative analyses of fatty acid components in these phospholipids have been described by Peifer and Lewis (14). Silica gel-coated tlc mitochromatoplates (23) were used to fractionate total lipids into bands of PC and PE using a solvent system of chloroform–methanol–water (80:25:3).[5] The bands of PC and PE were scraped from the tlc microchromatoplates and the respective phospholipids were eluted from the silica gel with chloroform–methanol (1:1).[5] Methyl ester derivatives of the phospholipid fatty acids were prepared by a

[4] Fatty acids are designated by carbon chain length:number of double bonds.

[5] Volumetric ratios.

sodium methoxide-catalyzed transesterification reaction and quantitative analytical techniques described previously (14, 24). The glc analyses of these fatty acid–methyl esters was accomplished using glass columns (2 m × 6 mm i.d.) packed with 10% (w/w) of phosphoric acid treated (2%) diethyleneglycol succinate polyester (DEGS) on 100 to 120-mesh Gas Chrom Q (Applied Science Lab, State College, Pa.). Operating conditions included a column oven temperature of 180°, pure nitrogen as the carrier gas, and quantitative glc techniques described previously (14, 24). Identification and quantitative analyses of specific fatty acids were based on multiple glc data including relative retention times and equivalent chain length values (ECL) obtained for the phospholipid fatty acid–methyl esters and pure methyl ester reference compounds. Reference compounds (Nu Chek Prep, Inc., Elysian, Minn.) included methyl esters of myristic acid (14:0), pentadecanoic acid (15:0), palmitic acid (16:0), palmitoleic acid (16:1), heptadecanoic acid (17:0), stearic acid (18:0), and other longer chain saturated and unsaturated fatty acids. Quantitative analyses of fatty acids ranging from 14:0 to 22:6 by these glc techniques are described elsewhere (14, 24). The glc analyses on 10% SE-30/80 to 100-mesh Gas Chrom Q packed glass columns (1 m × 2.5 mm i.d.) at 175° further established the identity and relative quantity of 15:0 and 17:0 in liver PC of some of the vitamin B_{12}-deficient rats. The effects of vitamin B_{12} deficiency on linoleate (18:2), arachidonate (20:4), eicosapentaenoate (22:5) and other longer chain saturated and unsaturated fatty acids in PC and PE have been reported previously (14). In this report, all of these fatty acids are reported as the sum of the long chain fatty acids (LCFA-18:0+) in Table I.

Statistical treatments. Data are presented as means ± SEM for fatty acid patterns found in PE and PC of cerebral and liver tissues of six individual rats deprived of vitamin B_{12} and six individual controls receiving supplements of the vitamin. Each rat had detectable amounts of ONFA in both PE and PC of its cerebrum and liver. Significant differences between means were calculated by Student's *t* test as described by Steele and Torrie (25).

Results. Terminal body weights of rats deprived of vitamin B_{12} and their controls were, respectively, 248.5 ± 1.3 and 317.1 ± 10.6 g. With our experimental conditions a 20-week dietary deprivation of vitamin B_{12} leads to a six- to sevenfold increase of total methylmalonic acid in the liver, decreased ratios of arachidonic acid to linoleic acid (20:4/18:2) in PC and PE of the liver, and significant increases of ONFA in PC of both the cerebrum and liver of the rat (14). Similar experimental conditions are also reported to promote very significant decreases in the amounts of vitamin B_{12} found in the brain, liver, and plasma of rats (16). Results of the present study show that there are major differences in the relative amounts of ONFA and related even-numbered fatty acids (RENFA) in PC versus PE of the rats' cerebrum (Table I). Fatty acids having 14 to 17 carbon chain lengths (ONFA + RENFA) account for 45.0–47.9% of total fatty acids in cerebral PC but only 5.1–5.2% of those in cerebral PE of the rat. Such differences are a reflection of the significantly greater amounts of both ONFA and RENFA found in cerebral PC. The deficiency of vitamin B_{12} promoted major increases of both 15:0 and 17:0 on cerebral PC, whereas only an increase of 17:0 was apparent in cerebral PE of the deficient rats. Cerebral PC contained approximately 10 times more palmitic acid (16:0) than that in cerebral PE, and it is cerebral PC which obviously incorporates the greater amounts of ONFA in vitamin B_{12}-deficient rats. This tendency for ONFA to be most abundant in that phospholipid, PC of neural tissues, having the greatest abundance of 16:0, was also suggested by data on ONFA in neural phospholipids of an infant with defective B_{12}–coenzyme systems (1).

Major differences are apparent in the concentrations of 14–17 carbon fatty acids present in PC of the liver versus cerebrum of the rats (Table I). These acids (ONFA + RENFA) account for 11.9% of the total acids in liver PC and 45.0% of those in cerebral PC of the controls. The controls have similar patterns of 14–17 carbon fatty acids

TABLE I. EFFECTS OF DEPRIVATION OF VITAMIN B_{12} ON ODD-NUMBERED (ONFA) AND RELATED EVEN-NUMBERED (RENFA) FATTY ACIDS IN PHOSPHATIDYL CHOLINE (PC) AND PHOSPHATIDYL ETHANOLAMINE (PE) OF THE CEREBRUM AND LIVER OF RATS[a]

	Odd-numbered fatty acids[b,c]		Related even-numbered fatty acids[b,c]			ONFA + RENFA[b]		LCFA[b,c,d]
	15:0	17:0	14:0	16:0	16:1	14:0	17:0	18:0 +
	(g/100 g TFA)		(g/100 g TFA)			(g/100 g TFA)		(g/100 g TFA)
Cerebral phospholipids								
Phosphatidyl choline								
B_{12} supplemented	0.11 ±0.01	0.43 ±0.04	0.37 ±0.05	43.3 ±0.06	0.98 ±0.09	45.0 ±0.5		55.0
B_{12} deprived	0.46* ±0.12	0.84* ±0.17	0.33 ±0.01	45.5 ±0.8	1.23 ±0.17	47.9 ±0.5		53.1
Phosphatidyl ethanolamine								
B_{12} supplemented	Trace	0.18 ±0.02	Trace	4.7 ±0.1	0.21 ±0.03	5.1 ±0.2		94.9
B_{12} deprived	Trace	0.34* ±0.06	Trace	4.6 ±0.1	0.19 ±0.03	5.2 ±0.1		94.8
Liver phospholipids								
Phosphatidyl choline								
B_{12} supplemented	0.05 ±0.01	0.31 ±0.05	0.19 ±0.05	11.0 ±0.5	0.35 ±0.07	11.9 ±0.6		88.1
B_{12} deprived	0.27** ±0.04	0.43 ±0.03	0.66** ±0.06	15.1** ±0.6	0.43 ±0.03	17.0** ±0.6		83.0
Phosphatidyl ethanolamine								
B_{12} supplemented	Trace	0.31 ±0.04	0.14 ±0.07	10.5 ±0.7	0.21 ±0.04	11.1 ±0.7		88.9
B_{12} deprived	0.05 ±0.02	0.39 ±0.03	Trace	9.4 ±0.6	Trace	9.9 ±0.6		90.1

[a] Means ± SEM for phospholipid fatty acid patterns (PC and PE) found in liver and cerebrum of six rats deprived of vitamin B_{12} and six vitamin B_{12}-supplemented rats.

[b] Percentage of total fatty acids (TFA) in respective phospholipids.

[c] Trace is designated for those fatty acids accounting for less than 0.05% of total fatty acids in PE. Fatty acids are designated according to carbon chain length: degree of unsaturation. Pentadecanoic (15:0), heptadecanoic (17:0), myristic (14:0), palmitic (16:0), and palmitoleic (16:1) acids.

[d] Percentage of phospholipid fatty acids having carbon chain lengths of 18 carbons and longer (LCFA). The relative effects of vitamin B_{12} deficiency on LCFA patterns in PC and PE have been reported previously (14).

* Significantly different from vitamin B_{12}-supplemented control ($P < 0.05$).

** Significantly different from vitamin B_{12}-supplemented control ($P < 0.001$).

in both PC and PE of their liver but widely different patterns of the same fatty acids in PC and PE of their cerebrum. The vitamin B_{12} deficiency promoted significantly greater accumulations of 14:0, 15:0, and 16:0, but not 17:0, in liver PC. Some small increase of 15:0 is the only apparent change in this range of fatty acids of liver PE of vitamin B_{12}-deficient rats. Previous studies (14) demonstrated how soy protein fed–vitamin B_{12}-deficient rats accumulate more 16:0 in liver PC as comparable decreases of polyunsaturated fatty acids occur in this phospholipid.

The comparative effects of vitamin B_{12} deficiency on the distributions of total ONFA into PC and PE of the cerebrum and liver are summarized in Fig. 1. Whereas equivalent small amounts of total ONFA are present in both PC and PE of the liver of control rats, these vitamin B_{12}-supplemented rats incorporate 135% more ONFA into PC than into PE of their cerebrum. When the rats are deficient in vitamin B_{12}, they incorporate 271%more ONFA into PC than into PE of their cerebrum. The deficient rats also incorporate 59% more ONFA into PC than into PE of their liver. Increases of ONFA in cerebral PC of deficient rats is the result of increases of both 15:0 and 17:0, whereas those in liver PC are due to increases of 15:0 alone.

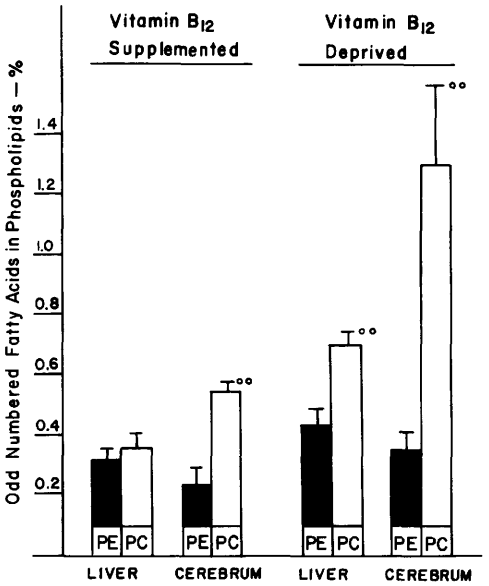

FIG. 1. Odd-numbered fatty acids (ONFA) in phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) of the liver and cerebrum of rats deprived of vitamin B_{12} and their controls. Each bar represents means ± SEM for six rats. Those PC bars with the superscript °° contained significantly more ONFA ($P < 0.01$) than that in PE of the same tissues of the same rats (paired t tests).

Discussion. Myristic (14:0), palmitic (16:0), and palmitoleic (16:1) are the three even-numbered fatty acids which together account for 45–50% of the total fatty acids in PC, but only 5–9% of those in PE, of brain tissues of humans (26) and rats (Table I). Palmitic acid is also the most abundant fatty acid in brain PC of both humans and rats. Significantly greater amounts of 15:0, 17:0, and total ONFA accumulated in PC than PE of neural tissues of rats (Table I and Fig. 1) and in an infant (1) in response to a nutritional deficiency or metabolic block of the B_{12} coenzyme dependent mutase system. In agreement with earlier suggestions of Kishimoto *et al.* (1), proportionately greater amounts of available ONFA are incorporated into neural PC which also incorporates the greater amounts of 16:0 and related chain length–even-numbered fatty acids (14:0 and 16:1). The relative differences between ONFA in PC of the cerebrum and liver also appear to be correlated with relative differences of RENFA in the PC of these two tissues of vitamin B_{12}-deficient rats. Increases of 15:0 were more apparent than increases of 17:0 in cerebral and liver phospholipids of our rats (Table I) and in neural tissue lipids of pernicious anemic subjects (7).

Subtle neuropathies reported for rats deprived of vitamin B_{12} for 7 to 15 months (17) were accompanied by 1–2% increases of ONFA in the total lipids of their brain. It seems likely that these rats had considerably greater amounts of ONFA in PC of their brain lipids (Fig. 1). Further studies are needed to determine whether functional changes in neural tissues of human subjects or rats can be correlated with relative differences of either ONFA or BCFA in PC or other phospholipids of neural tissues.

Except for one contradictory report (27), the available literature suggests that a deficiency of vitamin B_{12} or an equivalent met-

abolic block will stimulate increased accumulations of ONFA into tissues of humans (1, 7), baboons (1) and rats (13–15, 17). Results of this present study (Table I and Fig. 1) and those of Kishimoto *et al.* (1) suggest that proportionately greater amounts of these ONFA are likely to become incorporated into PC of neural tissues of both rats and human subjects.

The combination of PC and PE accounts for 70–76% of the total phospholipids in the rat's brain, and phosphatidyl serine (PS), sphingomyelin (Sph), and small amounts of inositol phosphoglycerides (PI) are the other phospholipids (15, 26). Only 2–6% of the fatty acids in PS and Sph are 16:0 (15, 26, 28), and neither of these phospholipids contained much 16:0, ONFA or BCFA in the infant with defective B_{12}–coenzyme systems (1).

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