Resolution of Severe Hepatosteatosis in a Cystic Fibrosis Patient with Multifactorial Choline Deficiency: A Case Report

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Short title: Treatment of Multifactorial Choline Deficiency in Cystic Fibrosis

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Key words: bile; choline; CF; enterohepatic cycle; lipoproteins; phosphatidylcholine; rs12325817; steatosis

Abstract

In Cystic fibrosis (CF), 85-90% of patients develop exocrine pancreatic insufficiency. In spite of enzyme substitution, low pancreatic phospholipase A2 (sPLaseA2-IB) activity causes fecal loss of bile phosphatidylcholine and choline deficiency. We report on a female CF patient with progressive hepatosteatosis from 4.5y onwards. At 22.3y, the liver comprised 27% fat (2385mL volume) and transaminases were strongly increased. Plasma choline was 1.9µmol/L (normal: 8-12µmol/L). Supplementation with 3x1g/d choline chloride decreased liver fat and volume (3 months: 8.2%;1912mL) and normalized transaminases. Plasma choline increased to only 5.6µmol/L upon supplementation, with high trimethylamine oxide levels (12-35µmol/L; normal:3±1mol/L) proving intestinal microbial choline degradation. The patient was homozygous for rs12325817, a frequent single nucleotide polymorphism in the PEMT gene, associated with severe hepatosteatosis in response to choline deficiency. Resolution of steatosis required 2 years (4.5% fat). Discontinuation/resumption of choline supplementation resulted in rapid relapse/resolution of steatosis, increased transaminases and abdominal pain.

Introduction

Cystic Fibrosis (CF) is an autosomal recessive disease due to Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene mutations, with an incidence of 1:3.300-1:4.800 in Caucasians [1]. Frequent (85-90%) exocrine pancreas insufficiency impacts on nutrient assimilation, growth and organ functions [2,3,4]. The clinical course is dominated by progressive lung disease (99%) and CF-associated liver disease (CFALD; 20-60%), ranging from mild steatosis to cirrhosis, with liver failure being the 2nd-3rd cause of death [1,5,6,7]. In spite of pancreatic enzyme substitution and adjusted nutrient supply, the pathognomonic choline deficiency in CF patients was hardly acknowledged. It is due to sPLaseA2-IB deficiency, fecal loss of bile phosphatidylcholine (PC), and impacts on choline requirement for hepatic fat secretion via very low density lipoproteins (VLDL, 20% PC) [8,9,10]. Moreover, small intestinal dysbiosis [11], and single nucleotide polymorphisms (SNP) of the phosphatidylethanolamine-N-methyltransferase (PEMT) gene [12], aggravating choline deficiency, were not described in CF patients before.

Small intestinal hyperacidity and low activity of sPLaseA2-IB (pH optimum ~8) [13,14] prevent the cleavage and re-uptake of bile PC, causing fecal choline loss in CF patients [8]. This is critical to choline homeostasis, as ~50% of hepatic PC (~23g/1500g liver≈3150mg choline) daily passes through the enterohepatic cycle. This cycle has priority over VLDL, and therefore hepatic fat secretion [13, 15]. PEMT, converting phosphatidylethanolamine to PC, is required for VLDL assembly and reduces choline requirement. Its SNP rs12325817 (25-44% homozygous in Caucasians), however, increases choline requirement and choline deficiency impairs hepatic VLDL secretion [16,17]. In this context, we report on a female CF patient with exocrine pancreas insufficiency, small intestinal dysbiosis and homozygosity of rs12325817, who was cured from severe hepatosteatosis by choline supplementation.

Case Report

The patient (35 weeks+5 days gestational age; 2610g) with intrauterine small intestine perforation (see Table 1) underwent abdominal surgery at postnatal d1, and anus praeter revision at 6 months. Cystic fibrosis and exocrine pancreas insufficiency were confirmed by pilocarpine iontophoresis and fecal pancreas elastase determination. Genetic analysis confirmed compound heterozygoty for F508del/del exons19+20. After initial growth retardation (0-13 months), intensified feeding and adjustment of pancreatic enzyme substitution resulted in catch up growth, although steatorrhea persisted (15±1%). Body weight and size were at the 25th and 50th percentile at 4 ½ and from 15y onwards, respectively. Antibiotic therapy was continuously adjusted to respiratory bacterial status. Forced expiratory volume percent predicted [FEV1pp] was ~90-105%, and forced expiratory flow at 75% vital capacity (FEF75) continuously increased (40%→55%) at 18-24 years.

At 11m, abdominal ultrasound showed normal size of liver and spleen (97th percentile), but no CF-associated liver disease (CFALD). Progression of steatosis and hepatomegaly, but no enlargement of the spleen, was seen from 4½ years onwards by ultrasound imaging. At 22.3y the patient reported on right upper abdominal pain since 6 months. Magnetic resonance imaging showed 27% liver fat and 2385mL liver volume (Fig 1A). Liver enzymes were increased (Fig. 2A-D). Plasma choline was 1.9µmol/L (Controls: 10.85±0.96 µmol/L) and PC was 0.80µmol/L (Controls: 2.18±0.16mmol/L), indicative for severe choline deficiency and impaired hepatic VLDL secretion [8,14,18].

We supplemented the patient with 3x1g/d choline chloride (2200mg/d choline, 4-5.5fold adequate intake) [18,19,20]. Pain relief occurred within 2 weeks, and liver fat was decreased to 8% at 3 months (Fig 1C). Liver enzymes normalized (Fig. 2), and HDL cholesterol increased (Table 2). However, plasma choline did not rise to expected 10-15µmol/L [18]. Homozygosity for rs12325817 of the PEMT gene suggested impaired hepatic PC metabolism, and trimethylamine oxide (TMAO) indicated bacterial choline degradation within the small intestine

[14,15] (Fig. 3A+C). Nevertheless, plasma PC was doubled at 3m, and continuously increased (Fig. 3B) in parallel to decreasing liver fat and volume (Fig. 1C+D). Discontinuation of choline treatment resulted, within 3 months, in a relapse of abdominal pain, hepatosteatosis (17% fat), decreased plasma PC and increased enzymes. Treatment resumption completely normalized liver parameters (Fig. 1B-D, Fig2-3).

Discussion

This case shows the resolution of a severe hepatosteatosis in a female compound heterozygous CF patient, diagnosed for CFRLD at 4½ years. If there had been further deterioration of liver function, organ transplantation would have been among the options to discuss [21]. Severity of steatosis, extent of choline deficiency, and delayed resolution, however, forced us to regard this case in more detail.

The extrahepatic origin of steatosis in CF is suggested by its rapid development in liver allografts [22]. Choline deficiency in CF patients, due to exocrine pancreas insufficiency and fecal loss, was first described in 2005 [8], impairing the secretion of liver fat via VLDL [15]. Here, choline deficiency as primary cause of hepatosteatosis, was confirmed by effective resolution following supplementation, relapse after cessation and resolution after resumption of choline treatment.

However, resolution of hepatosteatosis lasted 2y, increase in plasma choline was low, and TMAO was far above normal values, suggesting intestinal choline degradation. CF patients frequently show dysbiosis, and the intestinal microbiota can degrade choline to trimethylamine, followed by its hepatic oxidation to trimethylamine oxide (TMAO) [14]. Moreover, this patient was homozygous for rs12325817, causing severe hepatosteatosis following choline deprivation [12]. Hence, this CF patient developed severe hepatosteatosis, apparently caused by exocrine pancreas insufficiency, small intestinal dysbiosis and rs12325817, which was resolved by choline supplementation.

5. Conclusions

Whereas choline deficiency is frequent in CF patients, severe hepatosteatosis can be caused by additional factors, particularly small intestinal dysbiosis and SNPs of the PEMT gene. Severe hepatosteatosis may require the determination of choline and PC, TMAO as an indicator of reduced bioavailability and PEMT gene sequencing. High choline supplementation may resolve steatosis under such conditions.

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Table 1. Patient data

Sex	female
CFTR Genotype	F508 del + deletion exon 19 & 20
Relevant SNP of *PEMT* gene	rs12325817, homozygous
Delivery Mode	Cesarean Section
Gestational Age at Birth	35w + 5d
Birth Weight	2610g
Surgery	Abdominal surgery (anus praeter) postnatal day 1 due to small intestinal volvulus and perforation; change to Bishop-Coop fistula at day 6, resection after 6 months.
Clinical Situation at Start of Choline Treatment	Abdominal Pain, Hepatosteatosis, hepatomegaly, beginning liver cirrhosis, increased AST, ALT, gGT, AP

Abbreviations: CFTR, Cystic Fibrosis Transmembrane Regulator; SNP, single nucleotide polymorphism; PEMT, phosphatidylethanolamine-N-methyltransferase; AST, aspartate transaminase; ALT, alanine transaminase; gGT, γ-glutamyltransferase; AP, alkaline phosphatase; w, week; d, day

Table 2. Plasma lipids from start of choline treatment onwards.

Age	Time from Start of Choline treatment	Choline treatment	HDL-Cholesterol	LDL-Cholesterol	Trigly-cerides
y	y		mg/dL (>44)	mg/dL (<161)	mg/dL (<201)
22.3	0.00	no	**24**	59	84
22.6-24.3	0.25-1.94	yes	41-43	43-69	79-132
24.5	2.16	no	**29**	45	84
24.8-25.1	2.45-2.72	yes	41-42	57	132-172

*: Data in parentheses define reference values. Blood was taken after over night fasting. Abbreviations: y, years; HDL, high density lipoprotein; LDL, low density lipoprotein,

Figure legends

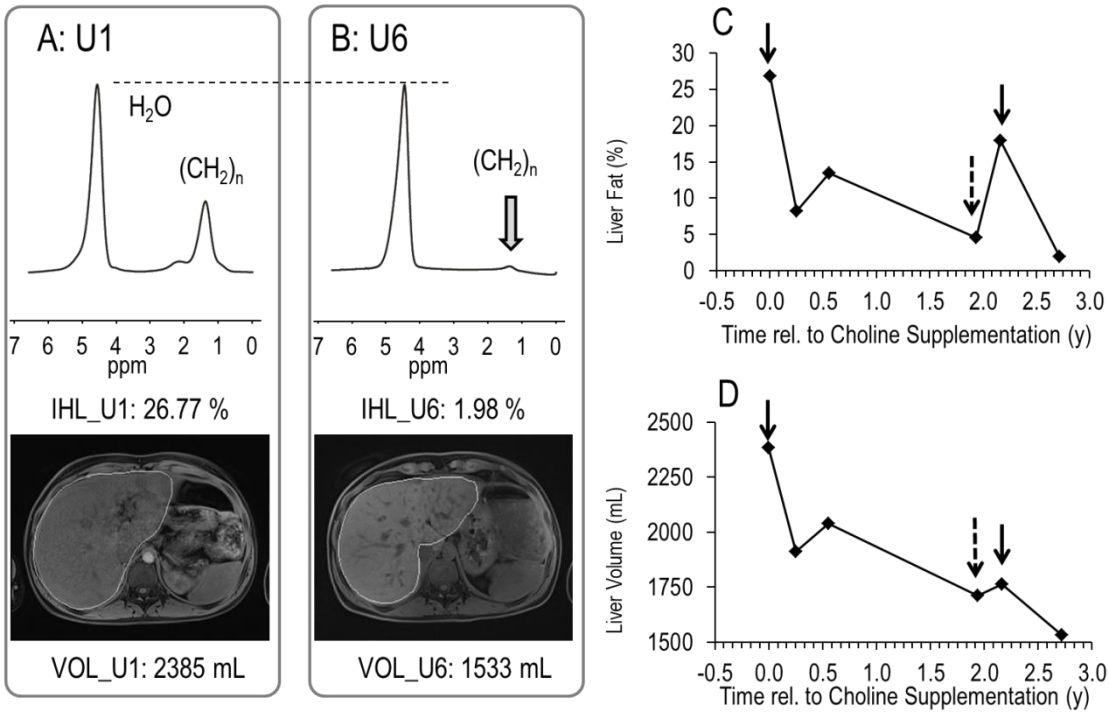


Figure 1: *Hepatosteatosis/-megaly prior to and after choline treatment.* **A** and **B** show liver fat and volume prior to choline supplementation and after 2.5y, **B** and **D** demonstrate their concentrations relative to the starting point of supplementation (3x1g choline chloride/d). Solid arrows: starting point and resumption of treatment; dashed arrows: intermittent cessation of treatment.

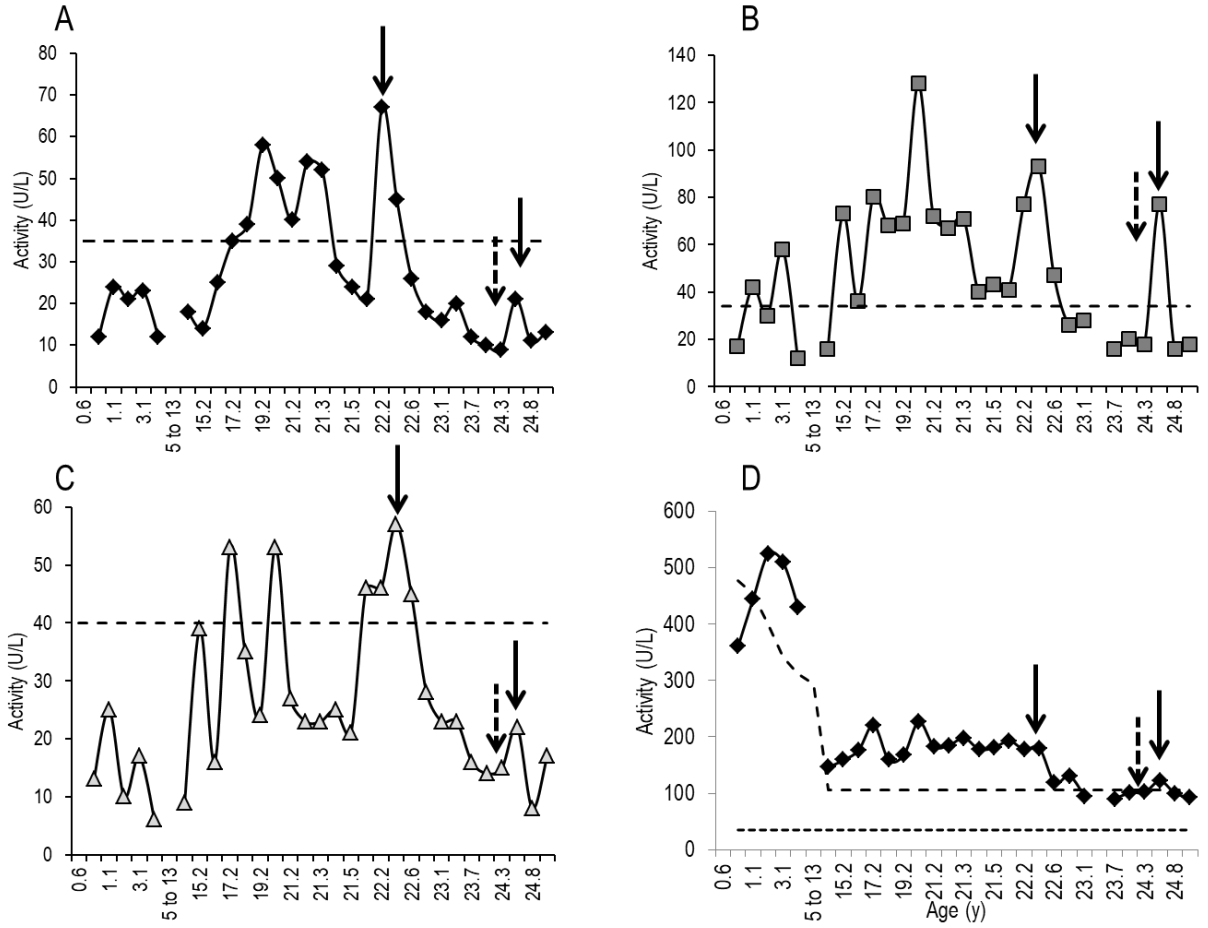


Figure 2: Time course of liver enzymes. A: Aspartate aminotransferase; B: Alanine aminotransferase); C: gGT=gamma glutamyl transferase; D: Alkaline phosphatase. See legend to Fig. 1C+D.

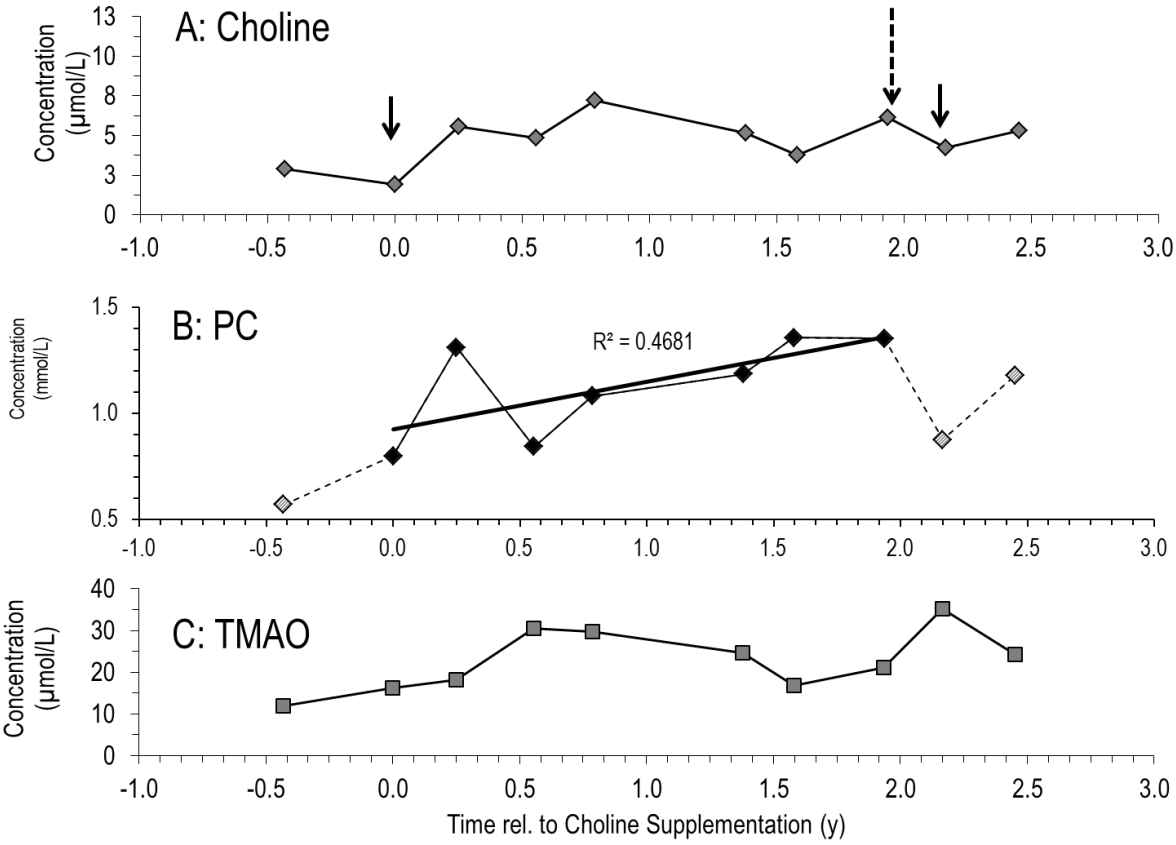
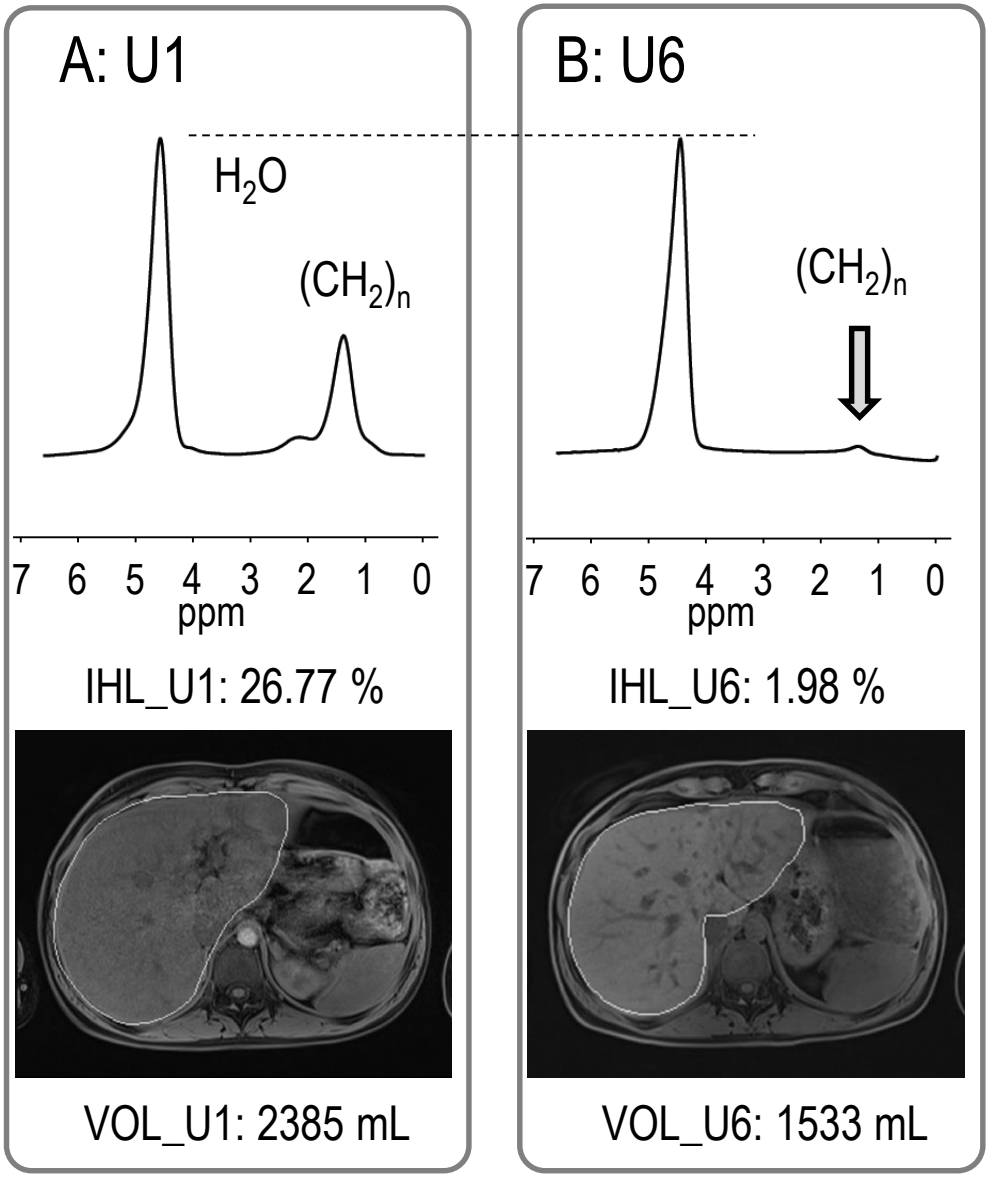
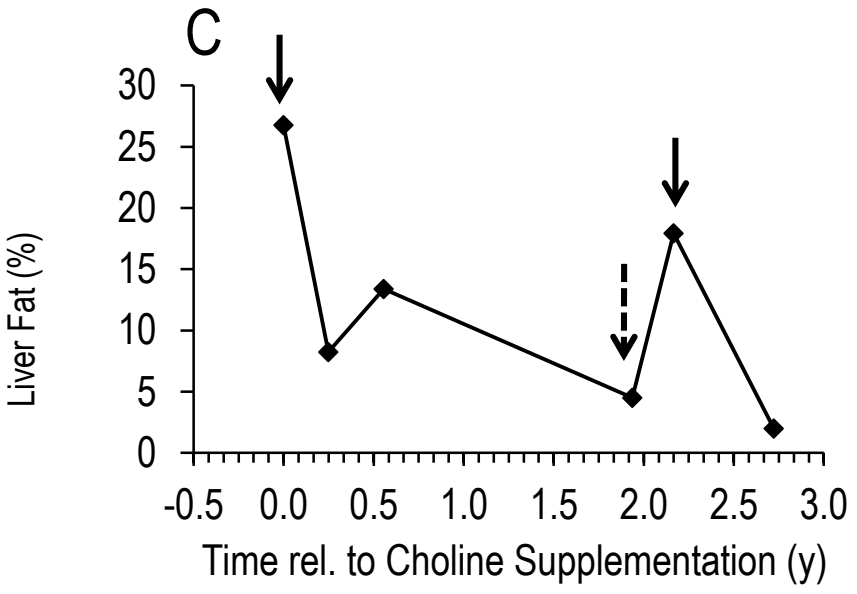


Figure 3: *Time course of choline (A), phosphatidylcholine (PC) (B) and trimethylamineoxide (TMAO) (C) relative to choline treatment.* See legend to Fig. 1C+D.

Figure 1-3

Fig. 1





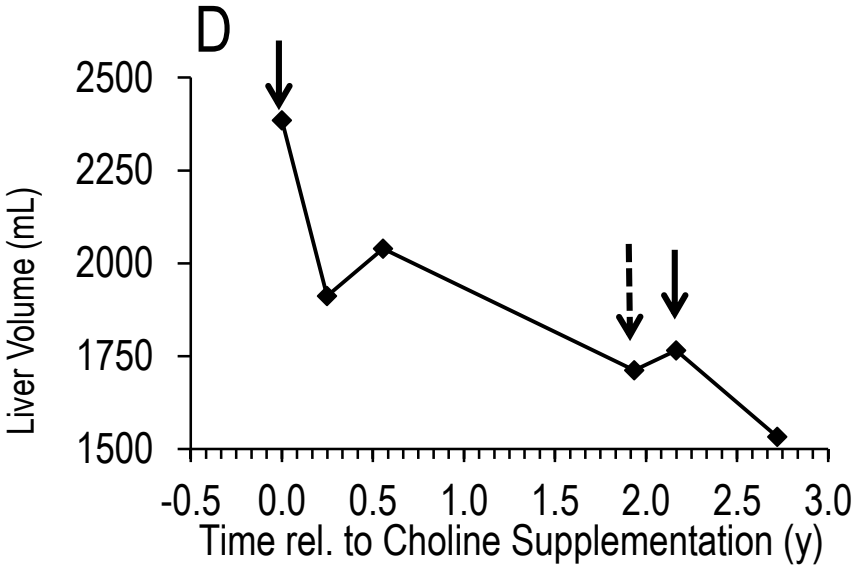


Fig. 2

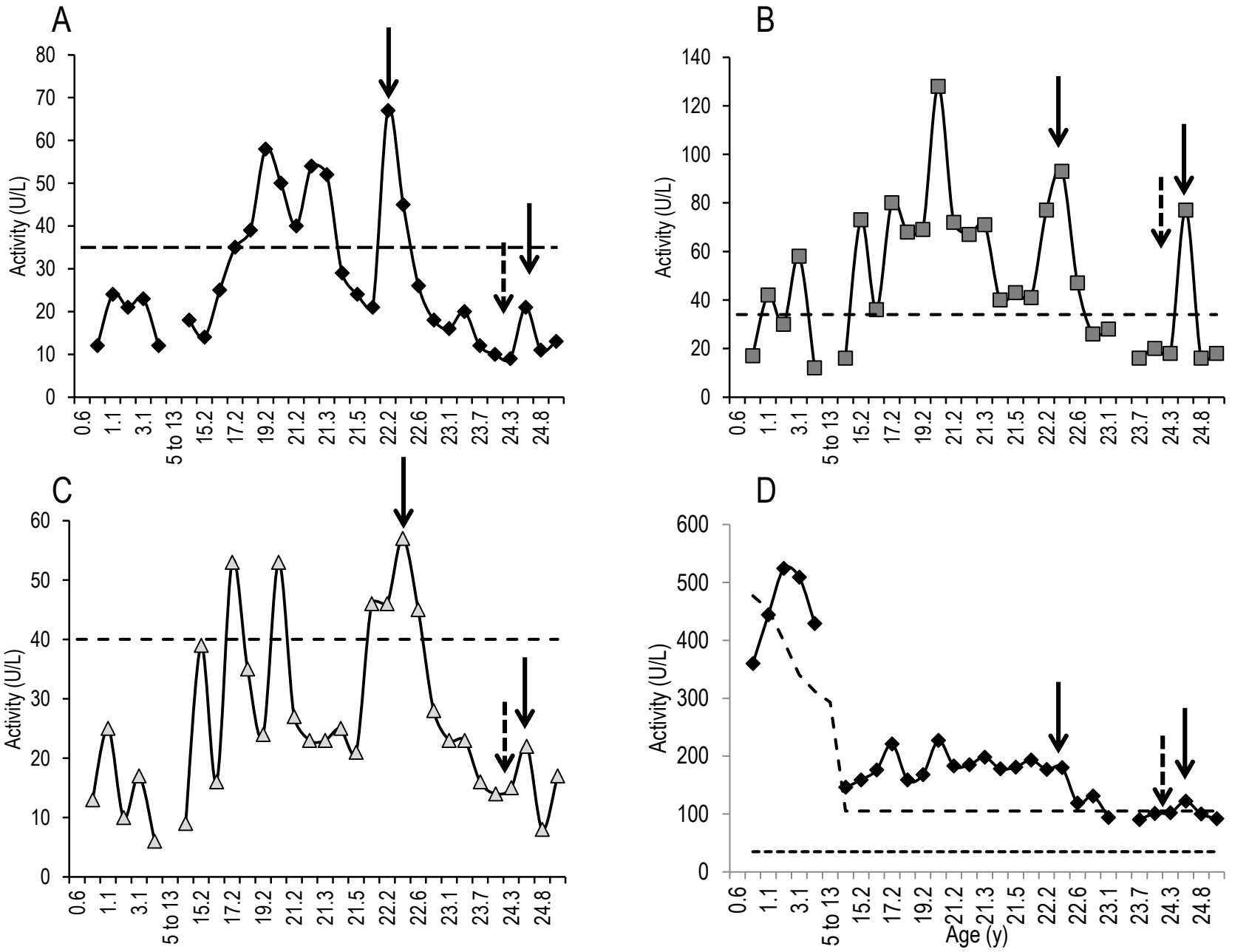
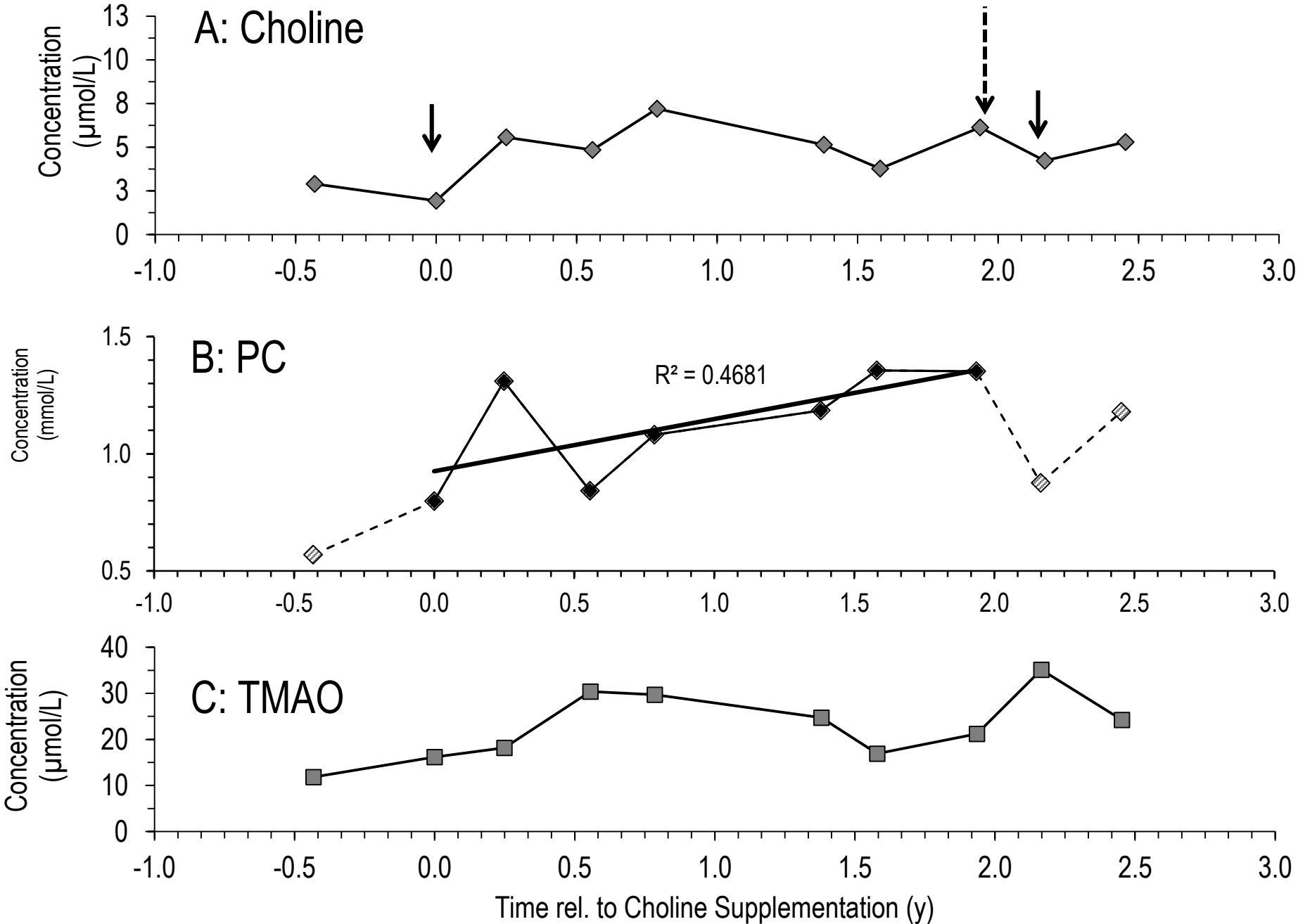

A
B
C
D
Activity (U/L)
Age (y)
0.6
1.1
3.1
5 to 13
15.2
17.2
19.2
21.2
21.3
21.5
22.2
22.6
23.1
23.7
24.3
24.8

Fig. 3


A: Choline
Concentration (µmol/L)
13
10
8
5
3
0
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
B: PC
Concentration (mmol/L)
R² = 0.4681
1.5
1.0
0.5
C: TMAO
Concentration (µmol/L)
40
30
20
10
0
Time rel. to Choline Supplementation (y)

Title:
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Conflict of Interest:
Based on current and previous work of WB, the University of Tuebingen, Medical Faculty, submitted a patent application for the combined administration of choline, ARA and DHA for prevention of developmental disorders associated with very preterm birth. PU has participated in clinical trials sponsored by Vertex Pharmaceuticals, Boehringer Ingelheim, and Chiesi Farmaceutici. He has received payment for lectures from PhysioAssist Germany and Danone Nutricia. The inventor of said patent application (WB) and all other authors indicate that they do not have any conflict of interest to disclose.

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