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Medium-chain fatty acids accelerate the incorporation of eicosapentaenoic acid into lung tissue

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Short report

Keywords: asthma, fat metabolic rate, immune response, long-chain polyunsaturated fatty acids, medium-chain fatty acids, resolving inflammation, resorption kinetics

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Medium-chain fatty acids accelerate the incorporation of eicosapentaenoic acid into lung tissue

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Abstract

Long-chain polyunsaturated fatty acids (LCPUFAs) are reported to resolve chronic inflammation in asthma and other lung diseases. This study aimed to accelerate the incorporation of eicosapentaenoic acid (EPA) into lung tissue through the coapplication of medium-chain fatty acids (MCFAs) which enhance the fat-metabolic rate.

Female C57BL/6 mice were supplemented with either 1363,6 mg EPA or 1363,6 mg EPA and MCFAs at 30% of the total fat per kg body weight per day for 28 days (each group size: n=21). The resorption of EPA into the peripheral blood and lung tissue was monitored over 63 days including the wash-out phase.

In the peripheral blood plasma and clots the supplementation with EPA always led to higher EPA concentrations than the administration of EPA with MCFAs pointing to a preferred EPA incorporation into tissues induced by MCFAs (EPA in plasma at day 26: EPA 12.33 wt% ± 1.41; EPA and MCFAs 3.91 wt% ± 0.32; Δ 8.42; p< 0.001; EPA in clots at day 26: EPA 16.44 wt% ± 1.82; EPA and MCFAs 4.47 wt% ± 1.26; Δ 11.97; p< 0.001). In the lung tissue the EPA-incorporation at day 26 was increased by MCFAs compared to the EPA-administration alone (EPA in lung tissue at day 26: EPA 1.28 wt% ± 0.18; EPA and MCFAs 1.83 wt% ± 0.17; Δ 0.55; p< 0.01).

The present study recommends the use of dietary LCPUFA supplementation with MCFAs to support their incorporation into lung tissues.

Keywords

asthma, fat metabolic rate, immune response, long-chain polyunsaturated fatty acids, medium-chain fatty acids, resolving inflammation, resorption kinetics

Abbreviations

EPA: eicosapentaenoic acid

DHA: docosahexaenoic acid

MCFAs: medium-chain fatty acids

LCPUFAs: polyunsaturated fatty acids

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Ethics Statements File

All animal procedures were performed according to protocols approved by the German Animal Subjects Committee (Gen.Nr.FK/1036).

Competing interests

The authors declare that they have no competing interests.

Introduction

Polyunsaturated fatty acids (LCPUFAs) are essential components of cellular membranes that are important for maintaining the structure and function of cells, tissues and organs. In addition to treating heart diseases or mental illnesses, dietary LCPUFA supplementation has been discussed as a treatment that is used to reverse fatty acid profile alterations and to resolve chronic inflammation in asthma and other lung diseases in different experimental models (Levi & Serhan, 2014). Bronchial allergic asthma, for instance, is characterized by dysregulated immune reactions, with the hyperinflammation of the lung bronchi and, in the late phase, lung tissue infiltration by eosinophilic granulocytes, provoking typical symptoms (Schubert et al., 2009). LCPUFAs are metabolic precursors of several lipid-mediators regulating the initiation, the containment and resolution of inflammation (Beermann, Neumann, Schubert & Zielen, 2016). Due to ongoing inflammation, a lack of immune-regulative LCPUFAs can occur in the cellular membranes of lung tissue and immune cells which should be retreated (Fussbroich et al., 2019). The absorption and incorporation process into tissues of dietary LCPUFAs is metabolically complex and time consuming. To establish effective LCPUFA-supplementation strategies against immunological hyperreactions in asthma and other lung diseases, this study aimed to accelerate the incorporation of orally applied LCPUFAs into lung tissue by coapplying medium-chain fatty acids (MCFAs, C6:0 - C12:0). This study is part of a mouse trial focusing on the immunological and pathophysiological aspects of LCPUFA supplementation in asthma (Fussbroich et al., 2020).

Material and methods

Mice keeping

Female C57BL/6 mice (Charles River Laboratories, Wilmington, Delaware) at the age of 6–8 weeks were maintained in stainless steel cages under sterile conditions with an alternating 12 h light/dark cycle. Mice were fed water and laboratory chow (Ssniff, Soest, Germany) with 6% fat content (C 14:0: 0.01%; C 16:0: 0.68%; C 16:1:0.04%; C 18:0: 0.22%; C 18:1: 1.44%; C 18:2: 3.21%; C 18:3: 0.37%; C20:0: 0.03%; C 20:1: 0.01%) ad libitum. The body weight of the mice was documented twice per week. All animal procedures were performed according to protocols approved by the German Animal Subjects Committee (Gen.Nr.FK/1036).

Study design and dietary supplementation

Mice were randomized into two dietary treatment groups, eicosapentaenoic acid, (EPA C20:5 n3) (n=21) and EPA + MCFAs (n=21) groups, as well as a control group (n=3). Each treatment group was fed the experimental diet for 28 days (wash-in period); afterwards, the wash-out period without any supplementation was observed until day 63.

92 An experimental diet containing 1363,6 mg EPA or 1363,6 mg EPA plus MCFAs at 30% of the
93 total fat per kg body weight per day was applied orally to each mouse daily from day 1 up to
94 day 28. The experimental diet was applied orally through a feeding needle (canula, 0.70 x 30
95 mm, LL, curved, Knopf C, Robert Helwig GmbH, Berlin, Germany) connected to a 1 mL syringe
96 (Becton Dickinson, Frankfurt/Main, Germany). During supplementation, the experimental diet
97 was freshly mixed on a daily basis from commercially available dietary EPA and MCFA oil and
98 diluted in 0.5% (w:v) gum arabic solution (gum arabic powder, Carl Roth, Karlsruhe, Germany;
99 sterile water) to a final volume of 200 µL per dose to ensure that a defined and precise
100 application volume was given. Just before administration, emulsions were homogenized using
101 an ultrasonic homogenizer (Sonopuls, Bandelin, Berlin, Germany).

102 Blood and tissue collection

103 Blood was collected on day 2 and then every second day until day 26 by venipuncture of the
104 vena facialis. On days 0, 28, 31, 35, 42, 49, 56 and 63 mice were sacrificed by cardiac
105 withdrawal under anesthetic. All blood samples were collected in EDTA-microvettes (Sarstedt,
106 Nümbrecht, Germany). Whole blood was centrifuged by using a Biofuge-Fresco (Heraeus,
107 Hanau, Germany) at 10 000 rpm for 10 minutes, and the remaining cruor was stored in
108 collection tubes at -80 °C until further measurement. Lungs were flushed with PBS through the
109 pulmonary artery to wash out erythrocytes. After this, the lungs were removed and stored at -
110 80 °C until further measurement.

111 Analysis of the fatty acid profile

112 Total lipids of lung tissue, blood clots and plasma were extracted according to the method of
113 Bligh & Dyer (Bligh & Dyer, 1959). For the lipid extraction, the lung tissue and blood clots were
114 lysed and homogenized with an ammonium chloride potassium lysis buffer (Sigma-Aldrich,
115 Taufkirchen, Germany). The total lipid fraction from the extractions was completely dried using
116 a CentriVap (Jouan RC1010, Thermo Scientific, Milan, Italy), overlaid with nitrogen, and then
117 derivatized to fatty acid methyl esters according to the method of Kohn (Kohn, van der Ploeg,
118 Möbius, & Sawatzki, 1996). After derivatization, the organic solvent was completely dried and
119 the fatty acid methyl esters were resolved in hexane (Sigma-Aldrich, Taufkirchen, Germany)
120 for measurement by capillary gas chromatography flame-ionization detection (Fussbroich et
121 al., 2020).

122 Statistics

123 Data are displayed as the mean ± standard error of the mean, and were analyzed using a two-
124 way ANOVA with Bonferroni post hoc analysis using GraphPad Prism 5 (GraphPad Software,
125 La Jolla, CA). Differences were considered statistically significant at $p < 0.05$.

126 **Results and Discussion**

127 Fatty acids undergo different metabolic fates depending on their chain length and degree of
128 saturation. Ingested LCPUFAs are absorbed in the small intestine, and re-esterified in
129 enterocytes to newly formed triglycerides or phospholipids to be subsequently packaged into
130 chylomicrons within hours in the postprandial phase (Green & Riley, 1981; Mu et al., 2006).
131 These LCPUFA-loaded lipoproteins are secreted to the lymph and afterwards to the
132 bloodstream through the thoracic duct. At least in the peripheral tissues and organs, LCPUFAs
133 are released by lipoprotein lipases and are transported into the cells to undergo different
134 metabolic fates, including membrane and lipid mediator synthesis, as well as oxidation in
135 mitochondria or esterification and storage in lipid droplets (Roche & Gibney, 1999). Depending
136 on the applied amount, concise LCPUFA incorporation into cellular membranes of erythrocytes
137 for up to 28 days is needed in humans, and into organ tissues for even more time (Lopez-
138 Miranda, Williams & Lairon, 2007). In this study with mice, an EPA supplementation period of
139 28 days was established followed by a 35-day wash-out phase without any supplementation.

140 To improve the effectiveness of dietary LCPUFA supplementation, MCFAs might be helpful to
141 accelerate tissue incorporation. Generally, short carboxylic acids with 6- to 12-carbon-long
142 aliphatic chains are derived from palm kernel or coconut oil, as well as from milk fat in the form
143 of triglycerides. They are used in low-carbohydrate, high-fat ketogenic diets for the treatment
144 of epilepsy, for fat tissue reduction against obesity, or in athletic preparations (Levy, Cooper,
145 Giri & Weston, 2012). In contrast to LCPUFAs, the resorption of consumed MCFAs begins by
146 the oral and gastric mucosa and is ultimately ingested by the small intestine. Then MCFAs are
147 transported in the portal blood system, associated with serum albumin, directly to the liver.
148 Independent of the carnitine acyltransferase transport system, MCFAs immediately undergo
149 mitochondrial oxidation. In different animal models and in obese subjects, MCFA
150 supplementation increases postprandial energy expenditure, thermogenesis and attenuated
151 weight accretion or weight loss (Rego Costa, Rosado & Soares-Mota, 2012). In another clinical
152 trial, dietary supplementation of healthy volunteers with MCFAs and LCPUFAs led to increased
153 blood fat clearance and fat oxidation (Beermann et al., 2003). Fat metabolism stimulated by
154 MCFAs with increased fatty acid resorption together with preferred mitochondrial MCFA
155 oxidation competitive with LCPUFAs might metabolically direct ingested LCPUFAs away from
156 catabolic digestion and towards tissue incorporation.

157 The incorporation kinetics of LCPUFAs into cellular membranes diverge depending on the
158 LCPUFA species, the amount of application, and the type of supplemented cellular membrane
159 system. In particular, the incorporation of EPA and docosahexaenoic acid (DHA, 22:6 n3) into
160 cellular membranes is different depending on the type of cell. Moreover, studies with rats have
161 demonstrated that the incorporation of EPA into peripheral and organ tissues is lower than that

162 of DHA (Owen et al., 2004). Therefore, in the present study, EPA was used as a low-
163 incorporation resorption marker for LCPUFAs. Applying EPA alone resulted in an increased
164 concentration of this fatty acid in the peripheral blood plasma, reaching a plateau at day 10 up
165 to day 28 of supplementation (Fig. 1 A). In contrast, a combined administration of EPA with
166 MCFAs never reached this concentration level (EPA in plasma at day 26: EPA 12.33 wt% ±
167 1.41; EPA and MCFAs 3.91 wt% ± 0.32; Δ 8.42; $p < 0.001$). After day 28, during the wash-out
168 phase, the EPA concentration rapidly decreased in plasma. A comparable outcome was
169 evident for peripheral blood clots (Fig. 1 B). Generally, erythrocytes are a good indicator of
170 LCPUFA incorporation into cellular membranes in stable diets. However, they vary more in the
171 pattern of incorporation compared to organ tissues during the LCPUFA-supplementation
172 period (Owen et al., 2004). One factor for this might be the shorter life span of erythrocytes, at
173 40 days in mice, than of lung tissue cells (Horký, Vácha, & Znojil, 1978). In this study,
174 supplementing EPA alone led to an increased concentration of this fatty acid in the peripheral
175 blood clots, reaching an unsteady concentration plateau at day 10 up to day 26 of
176 supplementation, which was always higher than the combined administration of EPA with
177 MCFAs pointing to a preferred internal incorporation of EPA into cellular membranes of organ
178 tissues induced by MCFAs (EPA in clots at day 26: EPA 16.44 wt% ± 1.82; EPA and MCFAs
179 4.47 wt% ± 1.26; Δ 11.97; $p < 0.001$; Fig. 1 B). Indeed, MCFAs significantly increased the EPA
180 incorporation into the bronchial tissue of the lung up to day 26 by more than a one-third
181 compared to EPA administration alone (EPA in lung tissue at day 26: EPA 1.28 wt% ± 0.18;
182 EPA and MCFAs 1.83 wt% ± 0.17; Δ 0.55; $p < 0.01$; Fig. 2). Due to the preferred mitochondrial
183 MCFA oxidation, the supplemented EPA seem to be spared from catabolic processes and
184 preferentially shifted towards incorporation into cellular membranes of the lung tissue. The
185 body weights of both experimental groups never altered in this setting. In contrast to our
186 findings, in rats faltering LCPUFA-contents have already been observed after 2 days of
187 supplementation in different organs, such as in the myocardium and skeletal muscle.
188 Furthermore, stable maximal incorporation occurred after 28 days of supplementation (Owen
189 et al., 2004). In this study, a stable saturated level of EPA could never be reached in the lung
190 tissue within 28 days of supplementation, which may be due to the different basal metabolic
191 rates of mice compared to rats. Interestingly, the removal of cellular-associated EPA was also
192 enhanced by MCFAs beyond the supplementation phase compared to EPA alone, indicating
193 that the stimulated rate of fat metabolism continues beyond supplementation (EPA in lung
194 tissue at day 31: EPA 0.81 wt% ± 0.08; EPA and MCFAs 0.58 wt% ± 0.06; Δ 0.23; $p<0.01$; Fig.
195 2). This finding has to be considered in the supplementation strategy for immune-regulative
196 LCPUFA supplementation against lung inflammation.

197 **Conclusions**

198 Based on the illustrated accelerating effects of MCFAs on the incorporation and elimination
199 kinetics of dietary LCPUFAs into and from lung tissue, the present study suggests to
200 accompany dietary supplementation with both LCPUFAs and MCFA to support LCPUFA
201 incorporation into lung tissue. Upon the termination of supplementation, MCFAs should be
202 discontinued to stabilize the established LCPUFA status in the body.

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256

257 **Legend**

258 **Fig. 1. Concentration of EPA in the peripheral blood plasma (A) and clots (B) during and**
259 **after supplementation.** Female C57BL/6 mice were supplemented with 1363,6 mg EPA
260 (group size: n=21) or 1363,6 EPA and MCFAs at 30 % of the total fat per kg body weight per
261 day (group size: n=16) for 28 days. **$p<0.01$, ***$p<0.001$, ###$P<0.01$ compared to baseline.

262 In the peripheral blood plasma and clots the supplementation with EPA increased its
263 concentration always higher compared to the combined administration of EPA with MCFAs
264 pointing to a preferred EPA incorporation into tissues induced by MCFAs (EPA in plasma at
265 day 26: EPA 12.33 wt% ± 1.41; EPA and MCFAs 3.91 wt% ± 0.32; Δ 8.42; $p< 0.001$; EPA in
266 clots at day 26: EPA 16.44 wt% ± 1.82; EPA and MCFAs 4.47 wt% ± 1.26; Δ 11.97; $p< 0.001$).

267

268 **Fig. 2. Concentration of EPA in lung tissue along and after supplementation.** Female
269 C57BL/6 mice were supplemented with 1000 mg EPA (group size: n=21) or 1363,6 mg EPA
270 (group size: n=21) or 1363,6 EPA and MCFAs at 30 % of the total fat per kg body weight per
271 day (group size: n=21) for 28 days. ***$p<0.01$ between the groups, ###$p<0.01$ compared to
272 baseline.

273 The combined administration of EPA and MCFAs increased EPA incorporation into the lung
274 tissue up to day 26 (EPA in lung tissue at day 26: EPA 1.28 wt% ± 0.18; EPA and MCFAs 1.83
275 wt% ± 0.17; Δ 0.55; $p<0.01$) and disappeared earlier thereon compared to EPA
276 supplementation alone (EPA in lung tissue at day 31: EPA 0.81 wt% ± 0.08; EPA and MCFA
277 0.58 wt% ± 0.06; Δ 0.23; $p<0.01$).

Figure 1. EPA concentration in blood

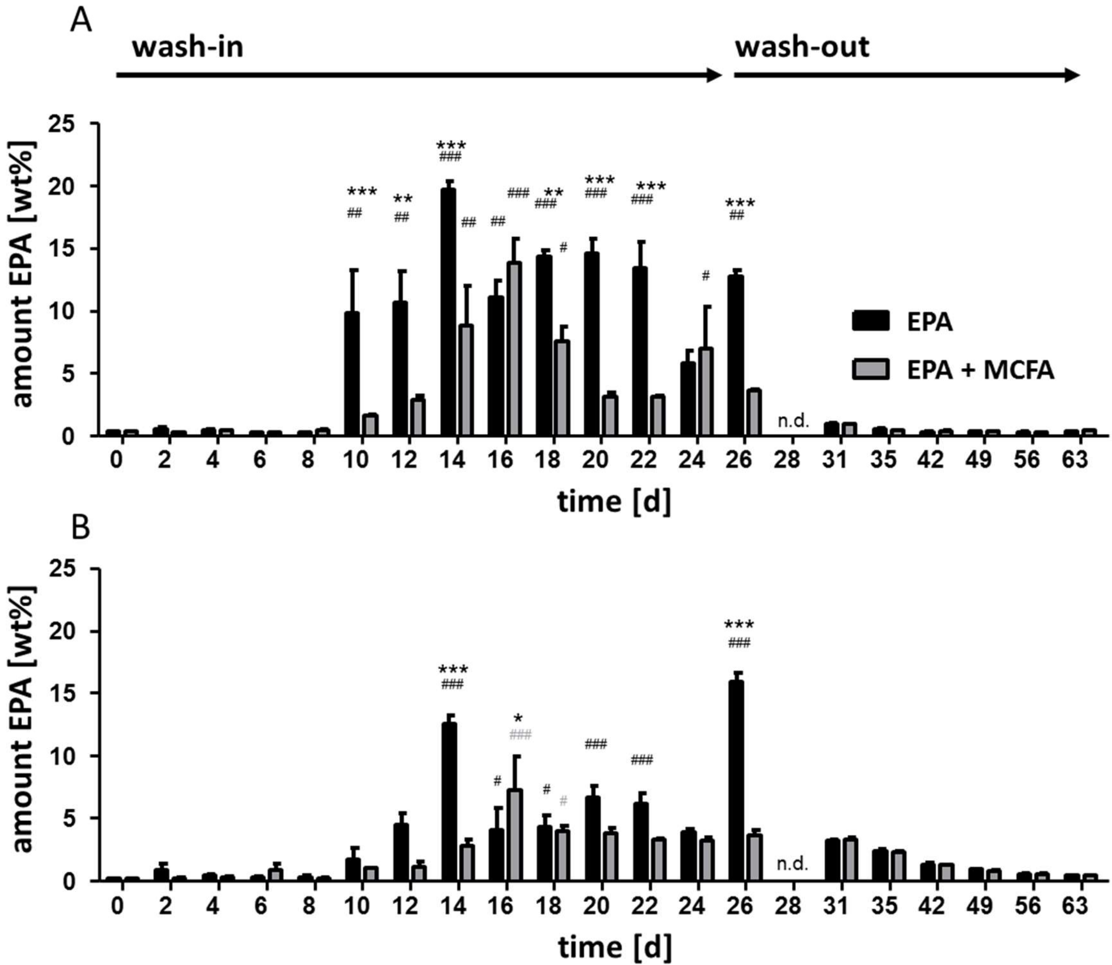

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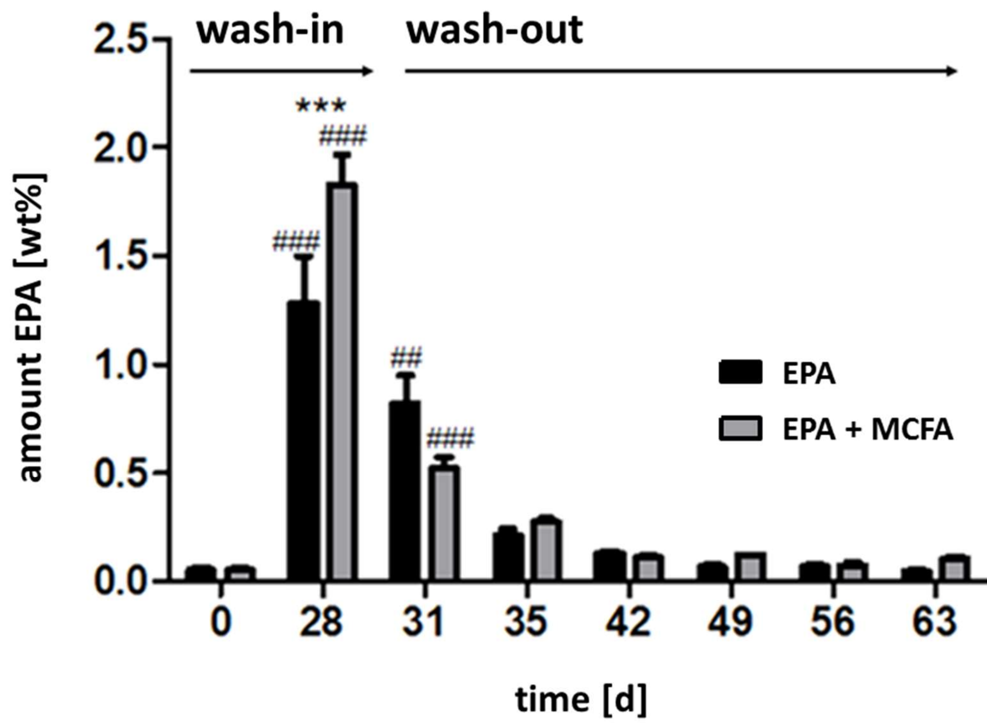
Figure 2. EPA incorporated into lung cells



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Figures

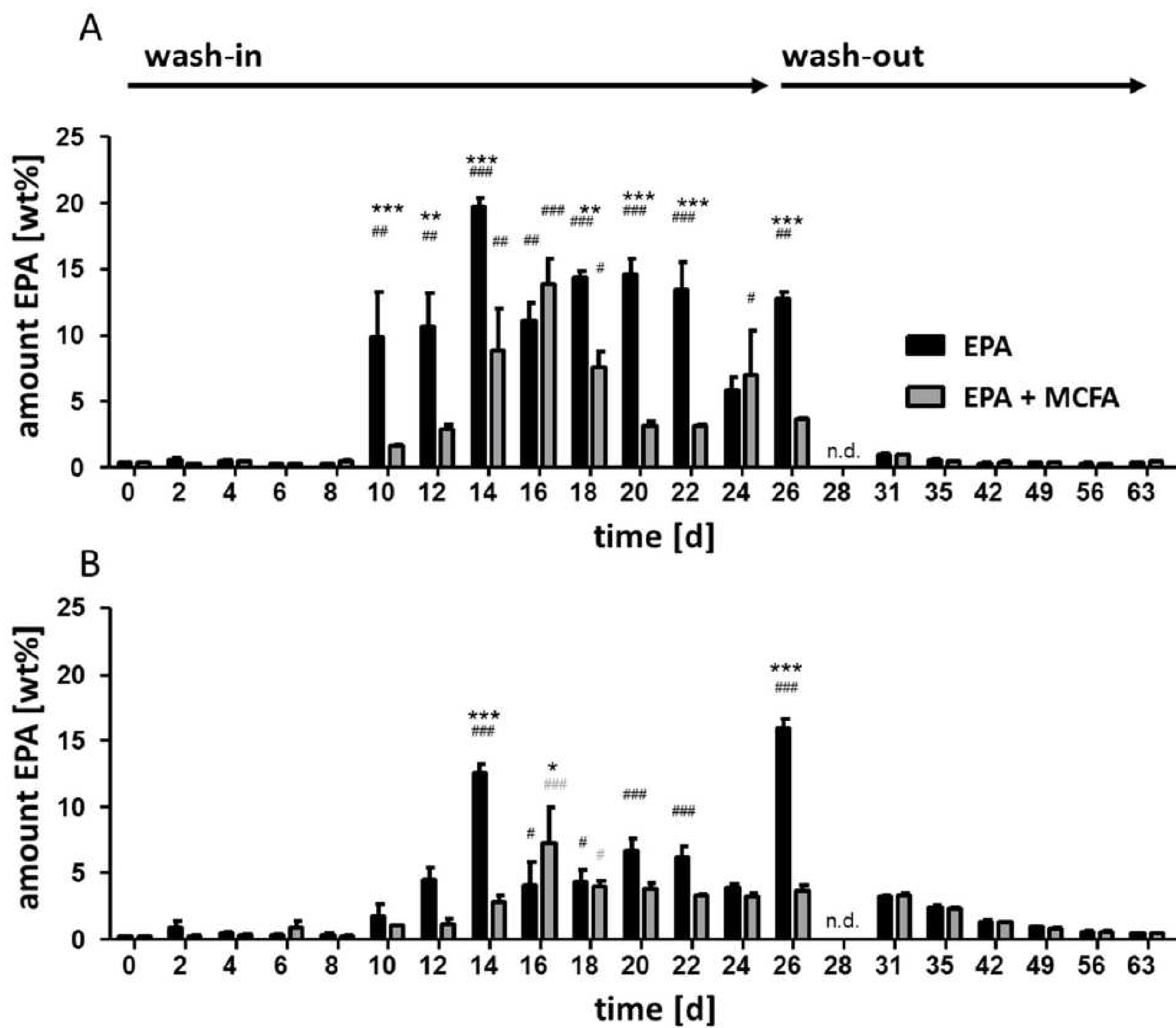

Figure 1

EPA concentration in blood

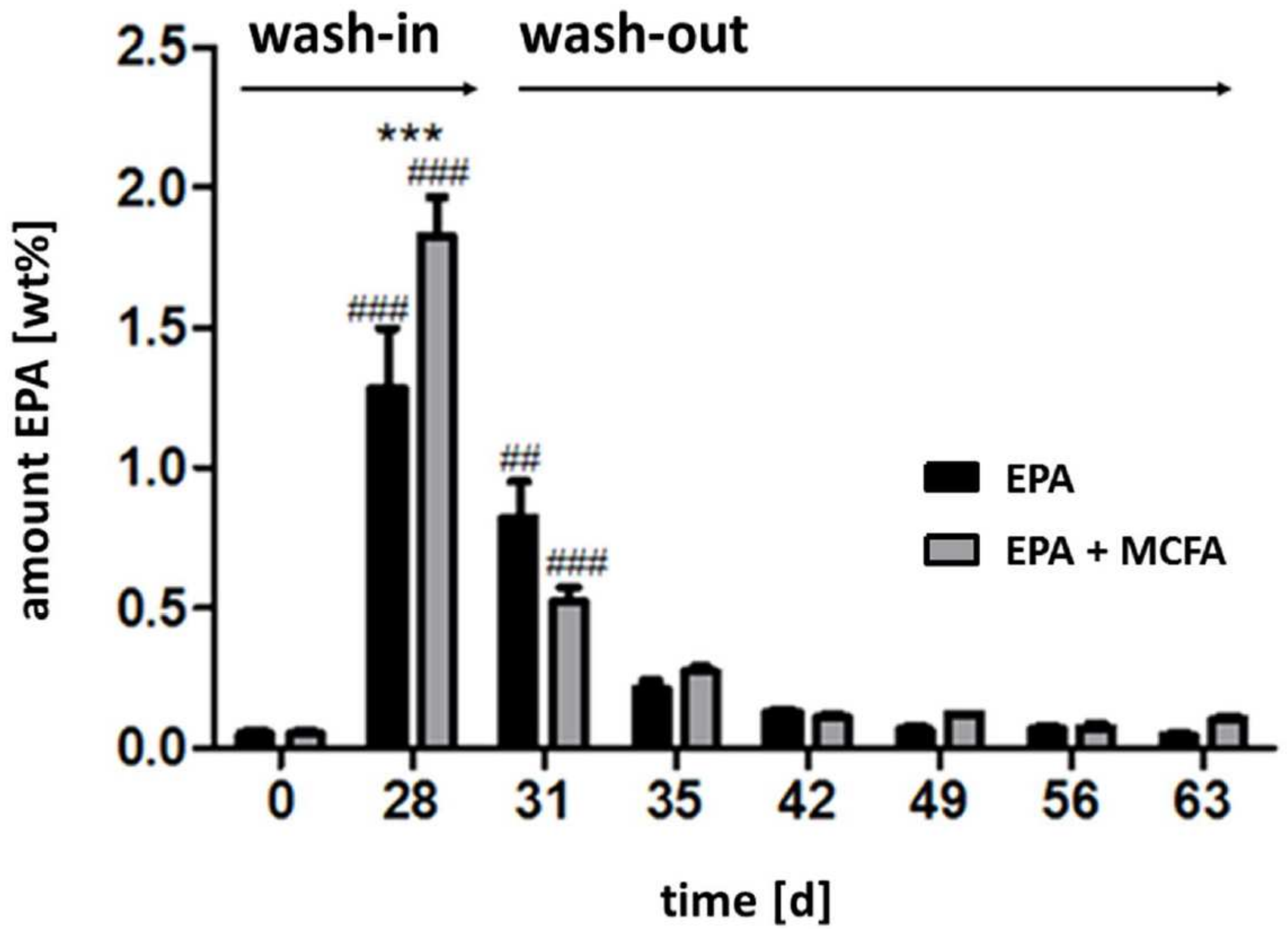

Figure 2

EPA incorporated into lung cells