Uptake, elimination and toxicokinetic modeling of 13C4-8:2 diPAPs given with food to twelve males

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References

Aim

The aim of the study was to assess 1) if humans uptake an FTOH-derived diPAP with food, 2) to measure uptake profiles, 3) to obtain kinetic parameters by modeling the experiment 4) if diPAPs metabolise and hence are sources of perfluorocarboxylic acids (PFCAs), in humans.

Conclusions

The data confirms that up to 7% (preliminary result) of 8:2 diPAP is uptaken by humans with food, and metabolises to PFCAs, which confirms that FTOH based PFAS are sources of human PFCA exposure. The elimination kinetics is non-linear, and consequently the half-life is not constant and increases with a decreasing blood concentration. Half-lives of other PFAS determined in highly exposed humans might therefore be underestimated. The study suggests that diPAP might distribute to another compartment than serum.

Results and Discussion

Figure 1 shows that the 13C4-8:2 diPAP is uptaken by humans in 0.9-6% from food as in rats (D’eon et al., 2011), but that the serum concentrations decrease linearly with time in a log-log plot, which has not previously been observed. This rules out regular linear clearance kinetics of the type C=Ae-t and suggests an empirical power-law C=A-t. Non-linear pharmacokinetics has previously been observed in animals such as the rat (Tuszynski et al., 2008), where the power-law signifies that two competing and saturable transport/metabolic mechanisms exist (Fig. 2). The expected metabolites 13C4-8:2 FTOA, PFCA and 13C4 PFNA were found, and their concentrations increased with time. This confirms that PFCAs can stem from diPAP, and likely also other FTOH based PFAS. 8:2 monopAP was not found, similarly to what has been found in rats (D’eon et al., 2011). The MS/MS method allowed us to search for non-target metabolites.

Numerical fitting of the model shown in Fig. 2 shows that the elimination, i.e. the half-life of 8:2 diPAP is not constant. Initially, at high 8:2 diPAP serum concentrations, the half-life is short (0.9-1.4 days) and similar to the half-life in rats. With decreasing serum concentrations, the elimination slows down, and half-lives become 6-11 times longer (5.7-15.4 days). If other PFAS behave similarly, the elimination of PFAS in averagely exposed humans might therefore be slower than estimated, based on half-lives determined from highly exposed humans. The identity of the second compartment remains unknown, but possibly PFCAs containing a hydrophobic polyfluorinated part of diPAPs can distribute to both red blood cells and to fatty tissue as FTOH does (Bull et al., 2014).

Materials and Methods

The study included only healthy males (21-50 years), who were given one dose of 700 µg 13C4-8:2 diPAP (prepared as 0.7 mg/ml in 96% ethanol) spiked to a breakfast meal and left to absorb for 1 h. Clean food was therefore supplied for two weeks, and instructions were given on how to otherwise minimise PFAS exposure. The study received ethical approval by the Danish Ethics Committee, since the 8:2 diPAP dose corresponded to a daily intake of 67% of a provisional limit value of 90 µg/kg body weight (unit C, 2008 ESFA TDI value of 1.5 µg PDFA/kg body weight), over the two weeks - 20 mL of blood (and urine) were collected from the volunteers on Day 0 before eating the dose(s) and on days 1-2:3-6:10:14-27. Blood was immediately prepared as serum and frozen until analysis. Triplicate analyses were made on 0, Duplicate on Days 1,3,4,27 and Single on Days 2, 6, 10. An in-house method was developed and validated using 350 µL serum, ultrasonic bath for 30 min/10000 G for 2 min, transferred to a frozen aluminum block, and the liquid decanted from the fat pellet into 1.5 mL EPP tubes. 2 nm internal standard, then 1500 µL cold AcN was added, mixed, shaken for 10 min, centrifuged 20 min/15000 G/C. The supernatant was transferred to a 350 µL tip vial, and the samples were blown down to N2 to 0.12 mL, and 80 µL AqN was added (40% organic strength). The sample was filtered through 0.2 µm PTFE filter and 50 µL injected into the ISPE-EEQ (OT/OP-FMS) (Aldrich 1290-1390-0500), using C18 columns for SPE 3.0 mm id *1.5µm/20mm) and for the analytical column (Agqacy C18 0.1 mm id *1.5µm/100 mm) and 10 mm guard column. Analytical column eluents were water/AcN (A1: 95:5, B1: 95:5), buffer formic acid/ammonia (pH 9) and 2 ml ammonia fluoride. Online SPE eluents were A1: 100% water (same strength of additives as above) and B1. The PFAS were analysed using an in house validated MS-Sim method, with acceptance criteria of 5-10 ppm accuracy. Calibration curves were made in bovine serum treated as the samples at levels 0.125 – 0.25 – 0.5 – 1.0 – 1.5 – 2.5 – 5.0 – 10 ng/mL. LODs of 9 diPAPs ranged from 0.02 ng/mL to 0.5 ng/mL (8:2 diPAP: 0.08 ng/mL), and RSDs were 2.3 – 7%.

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Fig. 2: Proposed kinetics and metabolic pathway for 8:2 diPAP in humans. Pipes symbolise linear kinetic pathways. Transport belts with limited speed symbolise saturable transport/metabolism. Note that two such saturable mechanisms compete for the same substrate (8:2 diPAP) in this model, as suggested by the apparent power-law behavior.

Fig. 1: Log-Log time-series of concentration C versus time t on a scale. The data suggests a power-law type of behavior C=A-t with fitting constants A and k.