INCREASING DIETARY SATURATED FAT IS NEGATIVELY ASSOCIATED WITH PLASMA CONCENTRATIONS OF NON-HIGH-DENSITY LIPOPROTEIN CHOLESTEROL AND PERFLUOROOCTANOATE (PFOA) IN APOE*3-LEIDEN.CETP MICE AT CONSTANT LOW-LEVEL DIETARY PFOA

Doc 2 Annex 3

Abstract 1876

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Overview

Cross-sectional (C-S) studies have observed positive associations of serum perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) with non-high-density-lipoprotein cholesterol (non-HDL-C). These associations weaken substantially at serum PFOA and PFOS concentrations >50 ng/mL. Laboratory investigation has established that PFOA and PFOS would be expected to reduce serum cholesterol, and a recent longitudinal occupational study has found no association of increasing serum PFOA and PFOS with non-HDL-C over the concentration range where previously observed in C-S studies. In an attempt to explain this paradox, we hypothesized that differences in dietary composition may lead to similar non-causal associations between non-HDL-C and PFOA/PFOS from low levels of exposure in diet. We tested this hypothesis in the male APOE*3Leiden.CETP transgenic mouse model by varying the relative proportions of sugar (sucrose) and fat (cocoa butter) as caloric sources in diets to which were added approximately 30 ug/kg PFOA (and PFOS). This transgenic mouse model has attenuated clearance of apoB-containing lipoproteins and exhibits a human-like lipoprotein metabolism on a Western-type diet. We found that changes in relative proportions of sugar and fat in diets affected plasma non-HDL-C and PFOA in the same direction and conclude that the associations in the C-S studies are likely non-causal and potentially related to dietary differences.

Methods

Test substances: Perfluorooctanoic acid, ammonium salt (NH_4 PFOA, lot 15822) and perfluorooctanesulfonate, potassium salt (K PFOS FC-95, lot 217) were supplied by the 3M.

Animal & husbandry: Forty male APOE*3Leiden.CETP transgenic mice (10-11 weeks old) were obtained from SPF breeding stock at TNO Biosciences (Leiden, The Netherlands). Mice were housed in macrolon cages (1-4 mice per cage) in clean-conventional animal rooms with environmental controls (relative humidity 50-60%, ~21 C, light cycle 7 am to 7 pm). Food and acidified tap water were provided *ad libitum*. Experiments were performed in conformance with the rules and regulations set forward by the Netherlands Law on Animal Experiments. Experiments had been approved by the Animal Experiment Committee of TNO under registration number 2956.

Experiment: The overall study design is depicted in Figure 1. Five weeks prior to the dietary exposure to PFOA (and PFOS), mice (n 10/group) were fed various modified diets (Figure 2A) containing different amounts of cacao butter and sucrose and equal amounts of corn oil, cholesterol and casein. Diets were formulated to achieve an isocaloric intake by adjusting the ratio of calories from cocoa butter to calories from sucrose (Figure 2B). The % fat in diets varied to yield 5%, 15%, 25 % and 35% in Dietary Group 1, 2, 3, and 4, respectively. After initial 5-week run-in period, modified diets fortified with PFOA (and PFOS) to target concentrations approximating 30 ug/kg diet (adjusted for expected differences in food consumption) were given to the mice for additional 4 weeks.

Results

No adverse clinical signs were observed during the study. At sacrifice, no abnormal macroscopic aberrations were observed. The different diet preparations had little influence on food intake and body weight (data not shown). PFOA concentrations in diets were 28.4, 27.4, 25.3, and 26.2 ug/kg and, on average, were 81 4% of targeted concentrations. PFOS concentrations averaged 57 27% of targeted concentrations. In part, the lack of homogeneity in diet PFOS concentrations increased variability in the data associated with PFOS and resulted in no statistically significant associations. During the "run-in" period without PFOA (and PFOS), distinct cholesterol and lipoprotein (latter not shown) profiles were established between Dietary Groups (compare Figure 3A and 3B). As proportion of fat to sugar increased, non-HDL-C decreased and HDL-C increased. After 2 and 4 weeks on diets containing PFOA (and PFOS), the established cholesterol (and lipoprotein) profiles persisted and were not changed by the addition of fluorochemicals (see Figure 3C and 3D as well as Figure 4A). In addition to reducing non-HDL-C, increasing proportion of fat vs. sugar in diets had the effect of reducing plasma [PFOA] (see insets, Figure 3C and 3D as well as Table 1, and Figure 4B). The reductions in plasma [PFOA] with increasing dietary fat appeared to result from decreased uptake of dietary PFOA (see Table 1). Because the effects of diet composition on plasma non-HDL-C and [PFOA] were in the same "direction", the association between plasma non-HDL-C and [PFOA] at Week 2 and 4 was positive even though fluorochemical in diet at low concentration had no direct effect on plasma non-HDL-C (Figure 5).

Figure 1:

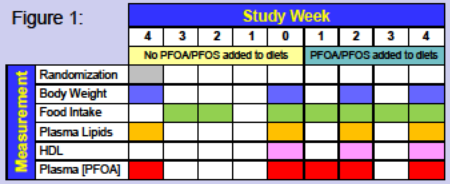


Figure 1 provides a graphic depiction of the overall study design. Measurements made on Week -4 were just prior to placement on modified diets for Dietary Groups 1, 2, 3, and 4. The "run-in" period was 5 weeks prior to adding ~30 ug/kg PFOA (and PFOS) to diets. Week 0 represents a full week to evaluate diet-induced changes in lipoprotein profiles.

Figure 2:

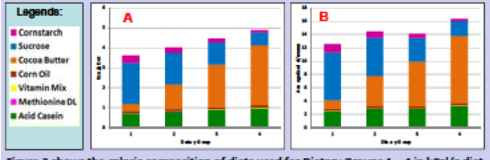


Figure 2 shows the caloric composition of diets used for Dietary Groups 1 – 4 in kCal/g diet (Figure 2A) as well as the average daily caloric consumption by mice in Dietary Groups 1 – 4 in kCal/d/mouse (Figure 2B). Diets were designed to provide, as nearly as possible, an isocaloric balance by varying the proportions of sucrose and cocoa butter.

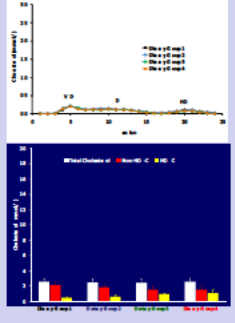
Table 1: The relationship between total ingested dietary PFOA and plasma [PFOA]. As the ratio of dietary kCal from cocoa butter to kCal from sucrose increases from a low of 0.17 in Group 1 to a high of 4.63 in Group 4, the plasma concentration of PFOA per total ingested mass of PFOA decreases.

Dietary Group	Parameter	Week 0	Week 1	Week 2	Week 4	Slope*
1	Accumulated PFOA dose (ug/kg)	0	21.39	2.22	89.83	2.26[a]
	Plasma PFOA (ng/mL)	0	6.01	103.97	19 .68	
2	Accumulated PFOA dose (ug/kg)	0	21.09	2.08	88.16	1.8 [b c]
	Plasma PFOA (ng/mL)	0	35. 6	81.9	157.35	
3	Accumulated PFOA dose (ug/kg)	0	17.62	35.0	7 .32	1.91[b]
	Plasma PFOA (ng/mL)	0	29.95	65.60	1 3.2	
4	Accumulated PFOA dose (ug/kg)	0	18.8	37. 2	79.89	1.65[c]
	Plasma PFOA (ng/mL)	0	28.6	66.95	128.33	

* Mean slope values sho ng the same lette a e not stat st ca ly s gn f cantly d ffe ent

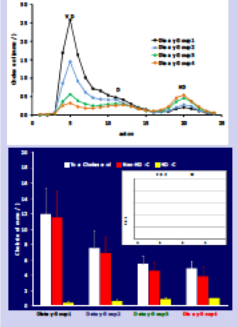
Figure 3:

A (t = week -4)
Cholesterol fraction profile (top) and cholesterol concentrations (bottom) for mice before introduction of modified diets.



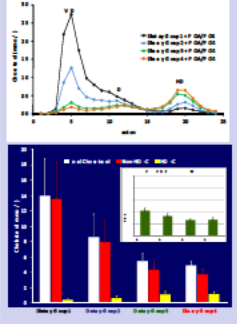
There were no differences in baseline cholesterol profiles and concentrations prior to placement on modified diets.

B (t = week 0)
Cholesterol fraction profile (top) and cholesterol concentrations (bottom) for mice after 4 weeks of modified diets without added PFOA/PFOS.



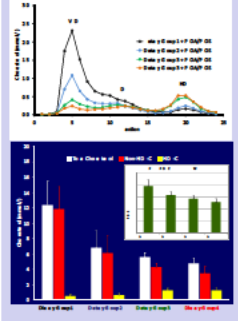
After 5 weeks on modified diets before adding PFOA (and PFOS), notable differences between dietary groups in cholesterol profiles and concentrations became apparent . PFOA was not detectable in plasma samples (inset, see also Table 1). There was a decreasing trend in plasma non-HDL-C and increasing trend in plasma HDL as sugar in diet decreased and fat increased.

C (t = week 2)
Cholesterol fraction profile (top) and cholesterol concentrations (bottom) for mice after 2 weeks of modified diets with added PFOA/PFOS (~ 30 ug/kg diet).



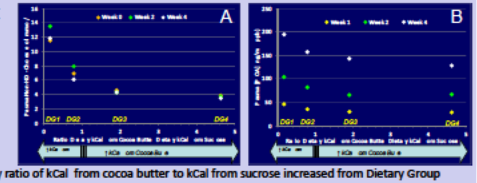
After 2 weeks on modified diets containing PFOA (and PFOS), mean plasma PFOA ranged from 65 – 100 ng/mL (inset, see also Table 1). However, plasma cholesterol levels and lipoprotein profiles did not change significantly compared to Week 0 levels. There was a decreasing trend in both plasma non-HDL-C and PFOA and increasing trend in plasma HDL as sugar in diet decreased and fat increased.

D (t = week 4)
Cholesterol fraction profile (top) and cholesterol concentrations (bottom) for mice after 4 weeks of modified diets with added PFOA/PFOS (~ 30 ug/kg diet).



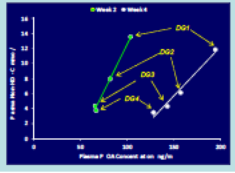
After 4 weeks of modified diets containing PFOA (and PFOS), mean plasma PFOA continued to rise and ranged from 128 – 195 ng/mL (inset, see also Table 1). Plasma cholesterol levels and lipoprotein profiles remained similar to the levels at Week 0 and Week 2. Effect of diets on plasma non-HDL-C, HDL, and PFOA were similar to that seen at Week 0 (non-HDL-C and HDL) and Week 2.

Figure 4:



As the dietary ratio of kCal from cocoa butter to kCal from sucrose increased from Dietary Group (DG) 1 to DG 4, plasma concentration of both non-HDL-C (Figure 4A) and PFOA (Figure 4B) decreased. This effect of dietary composition on plasma non-HDL-C and PFOA concentrations in the same "direction" was observed at all time points for which measurements were obtained and is responsible for the positive, non-causal associations between plasma non-HDL-C and PFOA concentrations observed from data obtained during study Week 2 and Week 4 (see Figure 5, below).

Figure 5:



As shown in Figure 4 above, when the ratio of dietary calories from cocoa butter to dietary calories from sucrose decreases, i.e., as diets change from higher cocoa butter content to higher sucrose content, plasma concentrations of both non-HDL-C and PFOA increase. When the relationship of plasma non-HDL-C and PFOA is examined at a given point in time (cross-sectional), there is a positive correlation that is solely the result of differences in dietary composition, because increasing PFOA exposure does not affect non-HDL-C.

Discussion & Conclusion:

Differences in dietary proportions of sucrose and cocoa butter were shown to produce non-causal, cross-sectional, positive associations between plasma concentrations of non-HDL-C and PFOA in ApoE*3.Leiden.CETP mice, which exhibit human-like lipoprotein metabolism on Western-type diets. Thus, uncontrolled differences in dietary composition may explain similar cross-sectional epidemiological associations in humans at low serum concentrations of PFOA.

Fructose and glucose are known to stimulate lipogenesis and the production of VLDL cholesterol; therefore, increasing the proportion of calories from sucrose may have resulted the up-regulation of lipogenic pathways. In addition, with respect to plasma [PFOA], it is conceivable that PFOA, which resembles fatty acids, may compete with dietary fat for GI uptake. Indeed, the data from Table 1 suggested that this may have been the case. Both factors may have been influential in determining the associations found in this study.