Original Article

MOLECULAR METABOLISM

Dietary PUFAs drive diverse system-level changes in lipid metabolism

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ABSTRACT

Objective: Polyunsaturated fatty acid (PUFA) supplements have been trialled as a treatment for a number of conditions and produced a variety of results. This variety is ascribed to the supplements, that often comprise a mixture of fatty acids, and to different effects in different organs. In this study, we tested the hypothesis that the supplementation of individual PUFAs has system-level effects that are dependent on the molecular structure of the PUFA.

Methods: We undertook a network analysis using Lipid Traffic Analysis to identify both local and system-level changes in lipid metabolism using publicly available lipidomics data from a mouse model of supplementation with FA(20:4n-6), FA(20:5n-3), and FA(22:6n-3); arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively. Lipid Traffic Analysis is a new computational/bioinformatics tool that uses the spatial distribution of lipids to pinpoint changes or differences in control of metabolism, thereby suggesting mechanistic reasons for differences in observed lipid metabolism.

Results: There was strong evidence for changes to lipid metabolism driven by and dependent on the structure of the supplemented PUFA. Phosphatidylcholine and triglycerides showed a change in the variety more than the total number of variables, whereas phosphatidylethanolamine and phosphatidylinositol showed considerable change in both which variables and the number of them, in a highly PUFA-dependent manner. There was also evidence for changes to the endogenous biosynthesis of fatty acids and to both the elongation and desaturation of fatty acids.

Conclusions: These results show that the full biological impact of PUFA supplementation is far wider than any single-organ effect and implies that supplementation and dosing with PUFAs require a system-level assessment.

Keywords Lipid metabolism; PUFA supplementation; Traffic analysis; Metabolic network

1. INTRODUCTION

Dietary interventions are an attractive means for treating metabolic disease. They are drug-free, pain-free, and are easy to personalise. Foods are typically a mixture of a variety of molecular species across several nutrient groups, meaning that with a judicious use of different foods, several nutrients can be administered in one intervention. This can be helpful in studying and treating deficiencies such as those of polyunsaturated fatty acids (PUFAs). Foods such as fish or plant oils comprise a mixture of PUFAs, which may be used to partially or fully meet the dietary needs of humans. Several PUFAs are considered essential for humans as they cannot be made either *de novo* or from other FAs endogenously. These PUFAs can be administered in excess and together, and the effects are studied at once. Such mixtures have been used to study effects in several different organs in randomised controlled trials (RCTs) (Table 1).

These studies (Table 1) show that the administration of PUFA mixtures on a population is typically aimed one organ in particular. Specifically, the evidence from these trials suggests that FA(22:6n-3) has an important role in a number of conditions, with FA(20:5n-3) and FA(18:3n-3) important in others. The evidence from RCTs also shows that there are differences between organs. For example, there is no effect of FA(20:5n-3) in reducing the heart rate, but it does improve mood disorders and reduces the risk of stroke. This shows that several organs are affected simultaneously. However, to date, there are no studies that measure the effects of PUFAs simultaneously across all these different outcomes in the same cohort and at a system-wide level. Evidence from lambs shows that PUFA

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Table 1 — Summary of meta-analyses and original research investigating the relationship between FA supplementation or concentration and physiological or clinical effects. Fish oil typically comprises FA(22:6n-3) and FA(20:5n-3) but no FA(20:4n-6). *Meta-analysis; **α-Linolenic acid. ADHD, attention-deficit hyperactivity disorder; CHD, coronary heart disease; CNS, central nervous system; CVS, cardiovascular system; EFA, essential fatty acid; NAFLD, non-alcoholic fatty liver disease.

Target organ	FA supplemented	Species	Result	Ref
Liver	FA(22:6n-3)	Human (obese, NAFLD diag.)	Decreased liver fat	[4]*
Liver	Mixed	Human (obese, NAFLD diag.)	Improved NAFLD	[5]*
Liver	Deficient in PUFAs	Piglets	Protection of EFA-containing PEs	[15]
Lungs	n-3 and fish oil	Human (smokers)	Reduced emphysema and chronic bronchitis, and low spirometry values	[16]*
Heart	FA(22:6n-3) and FA(20:5n-3)	Human	Reduced heart rate with FA(22:6n-3) but not FA(20:5n-3)	[17]*
CVS	Fish oil	Human	Effects unclear, may be some benefit	[18]*
CVS	FA(22:6n-3)	Human	Can improve risk of factors for CVD after menopause	[19]
CVS	FA(20:5n-3, 22:6n-3)	Human	Decreased hospitalisations with CHD	[20]*
Circulation	FA(18:3n-3)**	Human (infant)	Some, but not much, FA(22:6n-3) is made from FA(18:3n-3)	[21]
Circulation	Fish oil	Human (infant, malnourished)	Increase in circulating PUFAs but no short-term effects on the development	[22]
Brain, liver, heart, and lung	Mixed/fish oils	Rats	No increase in oxidation	[11]
Skin	PUFA intake	Human (transplant recip.)	Reduced risk of squamous cell carcinoma	[23]
Skin	FA(18:3n-3)** intake	Human (transplant recip.)	Reduced risk of basal cell carcinoma	[23]
CNS	FA(20:5n-3, 22:6n-3)	Human	Lower risk of stroke	[20]*
CNS	FA(20:5n-3)	Human	Improvement in mood disorders	[24]*
CNS	FA(22:6n-3)	Human	Reduced cognitive decline	[24]*
CNS	LPC(22:6n-3)	Mice	Improves memory	[25]
CNS	Fish oil	Human (healthy adults)	Little effect on mood or cognition	[26]
CNS (ADHD)	N/A	Human	High ratio of FA(20:4) to FA(22:6) assoc. with ADHD DHA assoc. with behaviour not cognition	[27]
CNS (Alzheimer's)	N/A	Human	Loss of FA(20:4, 22:4, and 22:6) in PE, increase in FA(14:0, 16:0, and 18:0) in PE. PC stable	[28]

supplementation using flax seeds leads to different accumulation of FA(18:3n-3) in the muscle, liver and heart [1]. Furthermore, FA(20:5n-3) were and FA(22:6n-3) were accumulated in the liver and kidney [1]. These results suggest that the traffic of FAs in mammalian systems may be controlled more carefully than previously thought. Following the evidence from an ovine model, it is expected that the traffic and accumulation of PUFAs would also vary in humans. This may be important in understanding the positive effects assoicated with PUFA supplementataion, but also the effects of PUFA intake differing in humans by geographical region [2]. One negative effect has been associated with PUFA supplementation. PUFA deficiency can reverse the effects of alcohol on mitochondrial energy metabolism [3], which complicates the use of PUFAs for treating liver-related disease [4,5].

Organs such as the liver, spleen and heart exchange different FAs to one another with the circulation and thus all contribute differently to the supply of FAs in it [6—9]. Studies show that for FA metabolism, there is interdependency between liver-intestine-heart [6], liver-adipose-muscle [7], liver-adipose-testes [8] and across the CNS [9]. This hints at the presence of a metabolic network whose activity is shaped by factors such as dietary intake. Thus, the effects of supplementing PUFAs not only imply that several organs can be affected simultaneously but also that there are general, systemic effects dependent on the inter-organ traffic. However, a full systemic analysis of PUFA supplementation has not yet been performed. Furthermore, the questions of which PUFAs have what effect(s) and where, whether unintended or undesired effects can be avoided, and how rapidly and specifically the desired effect can be achieved on the target organ remain unresolved. The evidence for several possible effects of PUFAs on several organs and throughout a system motivated us to investigate the relationship between the supplementation of individual PUFAs and changes to system-wide lipid metabolism.

We therefore tested the hypothesis that the supplementation of individual PUFAs affects several organs simultaneously and has system-level effects that are dependent on the molecular structure of the PUFA. Publicly available lipidomics data collected from a mouse model of dietary supplementations of FA(20:4n-6), FA(20:5n-3) and FA(22:6n-3) (arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively) [10] were used to investigate the particular effects of each PUFA throughout the organism. Fortunately, because there is no evidence for increased FA oxidation on supplementation with PUFAs [11], supplementation reflects changes in control of metabolism rather than oxidation.

To identify and characterise systemic effects, we used a Lipid Traffic Analysis (LTA) [12—14]. The network of tissues used in the LTA of the mouse model of the PUFA supplementation network used in this study is shown in Figure 1. This network shows several aspects of lipid metabolism, including storage, biosynthesis, structure, and oxidation. LTA is a relatively new tool for analysing metabolomics data, which uses the distribution of metabolites to determine how the control of metabolism differs between groups. Several studies have used LTA for determining the effects of the dietary intake, including finding that paternal nutritional programming is associated with changes in the control of lipid traffic [12] and obese-gestational diabetes (GDM) is associated with the altered timing of changes in lipid metabolism in pregnancy [13] and changes in lipid metabolism that outlast pregnancy [14].

For example, LTA has shown that lipids found only in the liver of post-weaning dams who had had GDM were also found in the heart of post-weaning dams who did not develop GDM [14]. This difference in metabolite distribution must mean the two systems were being controlled differently. Although the difference could be affected through several possible mechanisms (absorption, oxidation, transport, etc.), only by using LTA was it possible to show that the accumulation of lipid molecules in the livers of obese-GDM post-weaning dams was

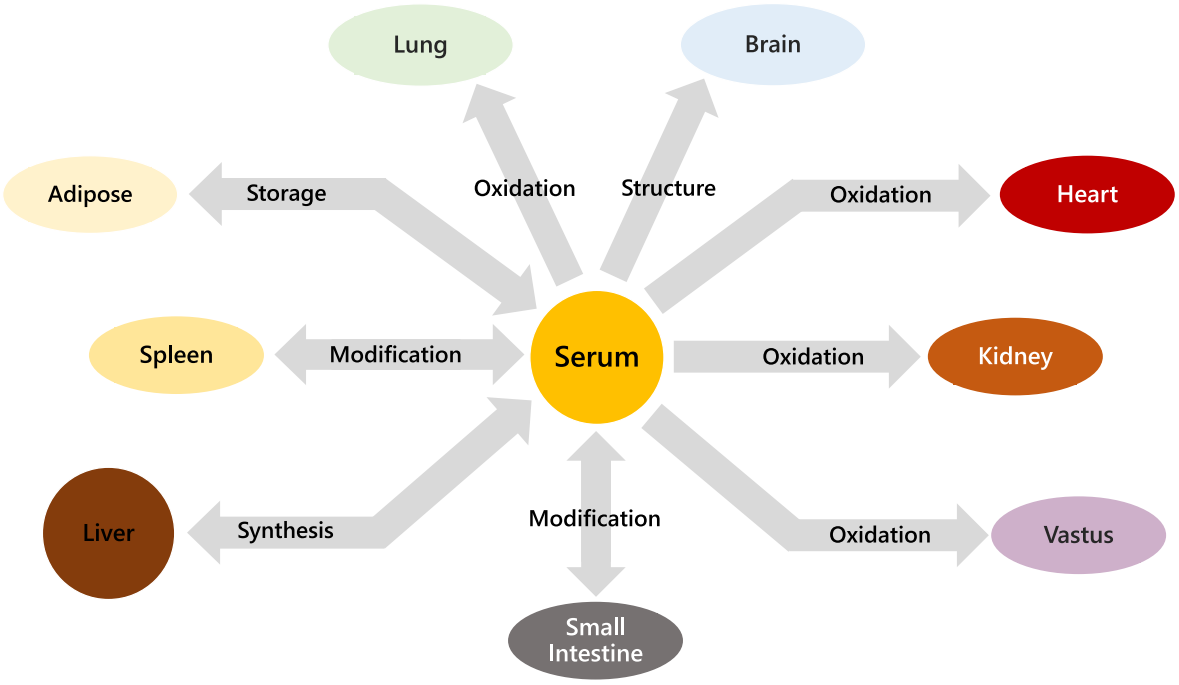

Figure 1: The mouse model of PUFA supplementation used in this study. This shows the tissue network of the mouse model of PUFA supplementation. The arrows show the metabolic connections between compartments.

matched by an appearance in other compartments in the control group, representing transport. These changes in the control of lipid metabolism offer an entirely different type of analysis to other approaches such as biomarker discovery or tissue—tissue comparisons. Indeed, the results from LTAs described above are not obtainable through tissue—tissue comparisons. However, tissue—tissue comparisons are useful for assessing local effects, and these have been performed for PUFA supplementation [10], providing a valuable insight into the effects or PUFA supplementations within each organ/compartment. However, the use of LTA as a system-level analysis plots the metabolism through a system, pinpointing where metabolic changes occur (*e.g.,* transport from the liver into the circulation), providing mechanistic explanations for changes in the behaviour of the system, something not achieved in the original study. LTA therefore goes beyond biomarker discovery results because it is capable of contextualising and even identifying roles for metabolites both within organs and through the system. This makes LTA an ideal tool to identify and characterise both systemic and local effects of nutrients known to modulate metabolism in several organs.

It is important to test this hypothesis using a system-level analysis because effects of nutrient supplementation over the whole organism are inevitable but poorly understood and useful to know, so that deficiencies can be treated accurately. The current limited understanding makes judging the dose and timing of a given supplement difficult.

2. MATERIALS AND METHODS

2.1. Animal model and lipidomics data

The animal model used to generate the lipidomics data was C57BL/6J. Male mice were fed the modified diet for 14 d from around 10 w of age [10]. Lipidomics data for this study were collected using LCMS and made publicly available through the Open Access publication of the original study [10]. Up to 1200 lipid variables were identified in the liver, brain, heart, lung, adipose, spleen, kidney, small intestine, vastus muscle, and plasma. Lipidomics data were reformatted for this study but not reprocessed.

2.2. Lipid Traffic Analysis

The analysis in this study was based on a known map of the tissues as a biological/metabolic network (Figure 1). Categories for the switch analysis were ***A***, ***B***, and ***U***-type lipids [12]. ***A***-type lipids were found throughout the system, ***U***-type lipids were found in only one compartment, and ***B***-type lipids were found in pairs of adjacent compartments, such as liver-serum. These lipid types show that, for example, a lipid may be found throughout the system in one group (an ***A***-type lipid) but may only found in part of the network in another (*e.g.,* ***B***-type for liver-serum, serum-heart, and serum-brain). ***U***-type lipids are isolated, implying they are synthesised locally and not transported. The categorisation of lipids in this way shows how transport, accumulation, and endogenous biosynthesis differ between phenotypes, and importantly, where differences occur.

Jaccard-Tanimoto coefficients (JTCs, J) and associated p values were used as a non-parametric measure of the distinctions between lipid variables associated with phenotype(s). These were used to calculate the overlap between the identities of the variables and the probability that this occurred by random chance, respectively. Where the probability is 1.0, the variables in one group all appear in the other group. The p value associated with each J represents the probability that the difference between the lists of variables for the two phenotypes occurred by random chance. It represents both the number of variables only found in either of the two groups and the order of the binary list. When the p is below 0.5, there are some shared variables, but at least one variable that only appears in one each of the two groups. When the probability is 0, there is no overlap between the lists of variables at all. Variables were regarded as present if they had a signal strength >0 in $\geq$66% of samples per group. The original data were reformatted for LTA and can

be found in Supplementary Information (SI2—Original data formatted for LTA). The switch analysis outputs from the LTA were combined into one document and also included in the Supplementary Information (SI3—Switch analysis (PE, PC, TG, and PI)).

2.3. Statistical methods

Univariate and bivariate statistical calculations were performed in Microsoft Excel 2016. Graphs were prepared in Excel 2016 or OriginLab2018. The activity of enzymes that modify the structure of FAs can be inferred from their activity index, calculated from the ratio of the abundance of the product and substrate of that reaction [29,30]. Lipid Traffic Analysis v2.3 was used for this study [13]. The code was executed in RStudio (v1.2.5x) using R v3.9. The full code for Lipid Traffic Analysis v2.3 used in this study can be found in the Supplementary Information file 1 and *via* Github (https://doi.org/10.5281/zenodo.5499760).

3. RESULTS AND DISCUSSION

3.1. Phospholipid traffic is modulated by supplementation with PUFAs

LTA was used in this study to investigate the consequences of PUFA supplementation across all lipid classes throughout the organism. We began with the two most abundant phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC). LTA begins by categorising lipids into ***A***-, ***B***-, *or* ***U***-types, ones that were found in all compartments, in adjacent compartments, and only in one compartment, respectively (see *Methods*). 74 PE species (configurations) were detected across the 10 tissues, with only 6 found as ***B***-type lipids (Figure 2A). This means that fewer than 10% of PE variables were trafficked throughout the system. Moreover, nearly half the PE variables were ***U***-type. This suggests that PE is configured locally from only a small number of PEs (***B***-type PEs). Around a quarter of PCs were trafficked through the system; 97 PCs were detected across the 10 tissues, with 23 ***B***-type and 44 ***U***-type variables (Figure 2B). This is considerably more than for PE but is consistent with PC's role as a major structural lipid and also with the delivery of PUFAs such as FA(20:4) [31]. Despite there being 4-5 ***B***-type configurations of PE in the control group, only PE(16:0/22:6) was found throughout the network on supplementation with FA(20:5n-3) (Figure 2A). Therefore, FA(22:6n-3)-containing PE is maintained and protected across all tissues, consistent with low-PUFA feeding studies in which it was also protected [15]. PE(16:0/22:6) is also found in spermatozoa from a range of mammals, including herbivores whose diet is FA(22:6)-poor [32]. The evidence for the wide distribution of PE(16:0/22:6) in this analysis, alongside the existing evidence of its importance and presence irrespective of the dietary intake, shows that the supply this lipid must be maintained under all circumstances across the whole system. Like PE, PC is known to have a key structural role but is also known to be important in the storage and transport of FA(20:4) [31]. LTA showed that the supplementation of PUFAs drove several changes in the molecular profile of the PC fraction, Figure 2B. PC(16:0/20:4) and PC(18:0/20:4) were maintained throughout all compartments with all treatments, consistent with the long-established concept that PCs were crucial for the storage and transport of FA(20:4) [31]. PC(18:2/20:4) was detected almost throughout the entire control, FA(20:4n-6)- and FA(20:5n-3)-supplemented systems, but not found at all in FA(22:6n-3)-supplemented mice. LTA therefore shows that the PC's role as a store/transport vehicle for FA(20:4) is system-wide and can be modulated by supplementation with FA(22:6n-3). The prominence of FA(20:4) distribution in PC may explain some of the results of PUFA feeding trials in humans.

The feeding of fish oils (that are high in FA(20:5n-3) and FA(22:6n-3) but typically low in FA(20:4n-6)) has shown neutral or mixed effects on cognition in children [33], been associated with poor prosocial behaviour and language skills [34], and shown to have a little effect on mood or cognition in healthy adults [26]. A study of attention-deficit hyperactivity disorder found that the plasma concentration of FA(20:4) and FA(22:6) was positively correlated with cognition, and a high ratio of FA(20:4) to FA(22:6) was the most important for behaviour [27]. A study of neurodegeneration found the loss of both FA(22:6) and FA(20:4) in the CNS in Alzheimer's disease [28]. This suggests that a good supply of both FA(20:4) to FA(22:6) is associated with the optimum CNS activity, which is consistent with around 12% of the dry mass of the human brain being FA(20:4) and FA(22:6) together, and evidence for specific transporters of both FA(20:4n-6) and FA(22:6n-3) into the CNS [35]. Therefore, supplementation with FA(22:6n-3) and FA(20:5n-3) alone will result in a proportional reduction of FA(20:4n-6) and thus potentially a limiting of the positive effect on CNS-related outcomes. The traffic analysis performed in this study shows that there are at least two protected phospholipid isoforms comprising FA(20:4) that are found throughout the system, *viz.* (PC(16:0/20:4) and PC(18:0/20:4). This is expanded when the dietary supply of FA(20:4) is higher, with PC(18:2/20:4) being found throughout the system, except for adipose, in the FA(20:4n-6) group, appearing to replace other variables, *e.g.,* PC(16:0/20:5). This explains how FA(20:4n-6) is trafficked to reach all parts of the body and even to the CNS where it likely has a under-appreciated developmental role.

Other lipid pathways were also affected. There were 32 configurations of PI, of which 13 were ***B***-type and 15 ***U***-type, Figure 3A. Thus 28 of the 32 variables were ***U***- or ***B***-type variables. The number of configurations of PI increased on supplementation with FA(20:5n-3) and more so with FA(22:6n-3), and was generally lower with FA(20:4n-6). This contrasts with PE in which the control group showed the greatest variety of ***B***-type variables, with the FA(20:5n-3) supplement group showing the narrowest variety. PE and PI provide contrasting but complimentary routes for the distribution of PUFAs. There was also commonality between PI and PE. PI(18:1/18:2) was found in the small intestine, spleen, liver and heart of control mice but was not found in supplemented mice at all. PE(16:0/18:2) was not found on supplementation, and P(18:0/18:2) was lost from all but two of the compartments in the supplemented groups. These changes across phospholipid pathways demonstrate the long reach of PUFA supplementation. There are routes for all supplemented PUFAs to all compartments that differ as a result of supplementation, at least one unique derivative associated with it and also changes in the supply of FA(16:0) and FA(18:2).

3.2. PUFA supplementation drives changes to the distribution and supply of energy stored in TGs

The pattern of alterations in the triglycerides (TGs) was similar to those of PC, with little change to the number of variables detected but some change to the profile (Figure 3B). Changes in the TG composition were noted in all compartments, with both ***B***- and ***U***-type TG variables differing in virtually all compartments on supplementation with any of the three PUFAs. This raises the question of which PUFAs were affecting what mechanisms and through which routes, *e.g.* the biosynthesis of TG from PC transferred to the liver is a known phenomenon [36], suggesting the crossover of FAs through this route.

One important contributor to the profile of TGs is endogenous synthesis (*de novo* lipogenesis, DNL) and thus, possible changes to the

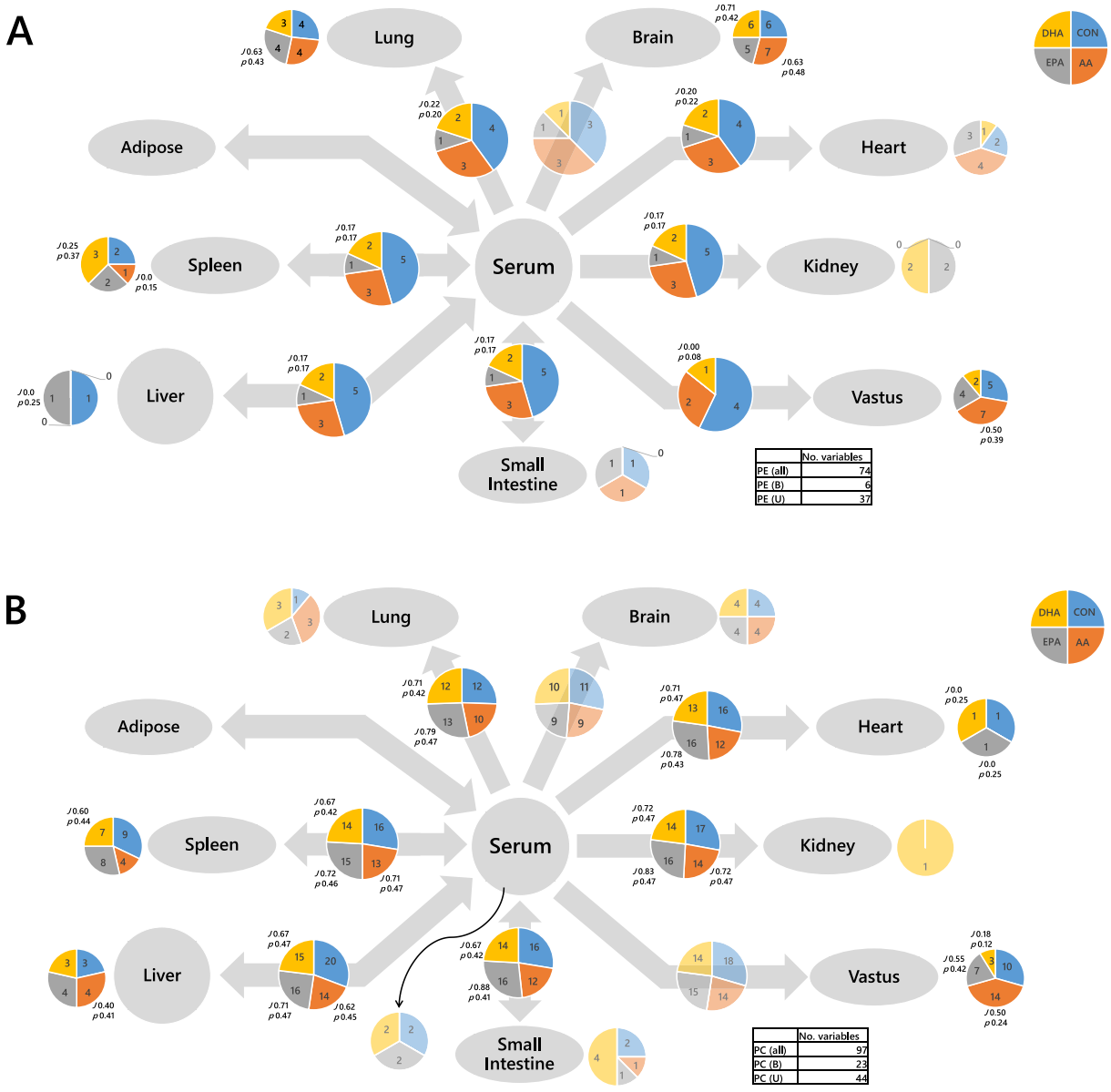

Figure 2: Traffic Analysis of phospholipids in a mouse model of PUFA supplementation. Panel **A**, Switch Analysis of phosphatidylethanolamine (PE) variables. Panel **B**, Switch Analysis of phosphatidylcholine (PC) variables. Pie charts show the number of variables of the appropriate head group in the relevant tissue(s). Large inset pie charts show the ***B***-type species (lipids found in two neighbouring compartments), whereas ***U***-type lipids (lipids found only in one compartment) are depicted with smaller pie charts. The Jaccard-Tanimoto coefficients (*J*) and probability (*p*) values that describe the similarity between sets of variables. Translucent pie charts indicate those in which only the number of variables differs between groups.

biosynthesis of FA(16:0) were tested for. TG markers for DNL, TG(46:0, 46:1, 48:0, 48:1, 48:2, and 50:1) [37], were largely unchanged in several tissues of the supplementation groups (vastus, adipose, lung, brain, and small intestine) or less abundant (spleen, liver, kidney, heart, plasma) in them. Perhaps the clearest example of reduced abundance is the plasma, providing evidence that the supply of DNL variables through the circulation is weaker in all supplemented groups (Figure 4A).

The considerable change in the abundance of biomarkers for DNL across much of the system shows that the endogenous production of FA(16:0) is suppressed by supplementation with PUFAs within 14 d of supplementation commencing and thus shows how the PUFA-driven suppression of DNL in the liver affects the supply of lipids to other tissues. However, as seen in sheep [1], the magnitude of changes in lipid species reflected the half-life of FAs in different organs. In mice, FAs have a half-life of around 12–24 h in the liver, whereas in adipose, it is closer to 14 d [38] and in brain is 36–40 d [39]. This suggests that although most of the FAs in the system will have been turned over during the supplementation period of this model, this occurs only unevenly across tissues. The half-lives of FAs were consistent with the magnitude of the changes observed on supplementation but also with the concept that there is evidence that DNL is modulated observable in every tissue. However, DNL is a complicated process involving a number of enzymes in different cell compartments. To deepen the effects of PUFA supplementation on endogenous FA metabolism, we also looked at local FA metabolism across the network.

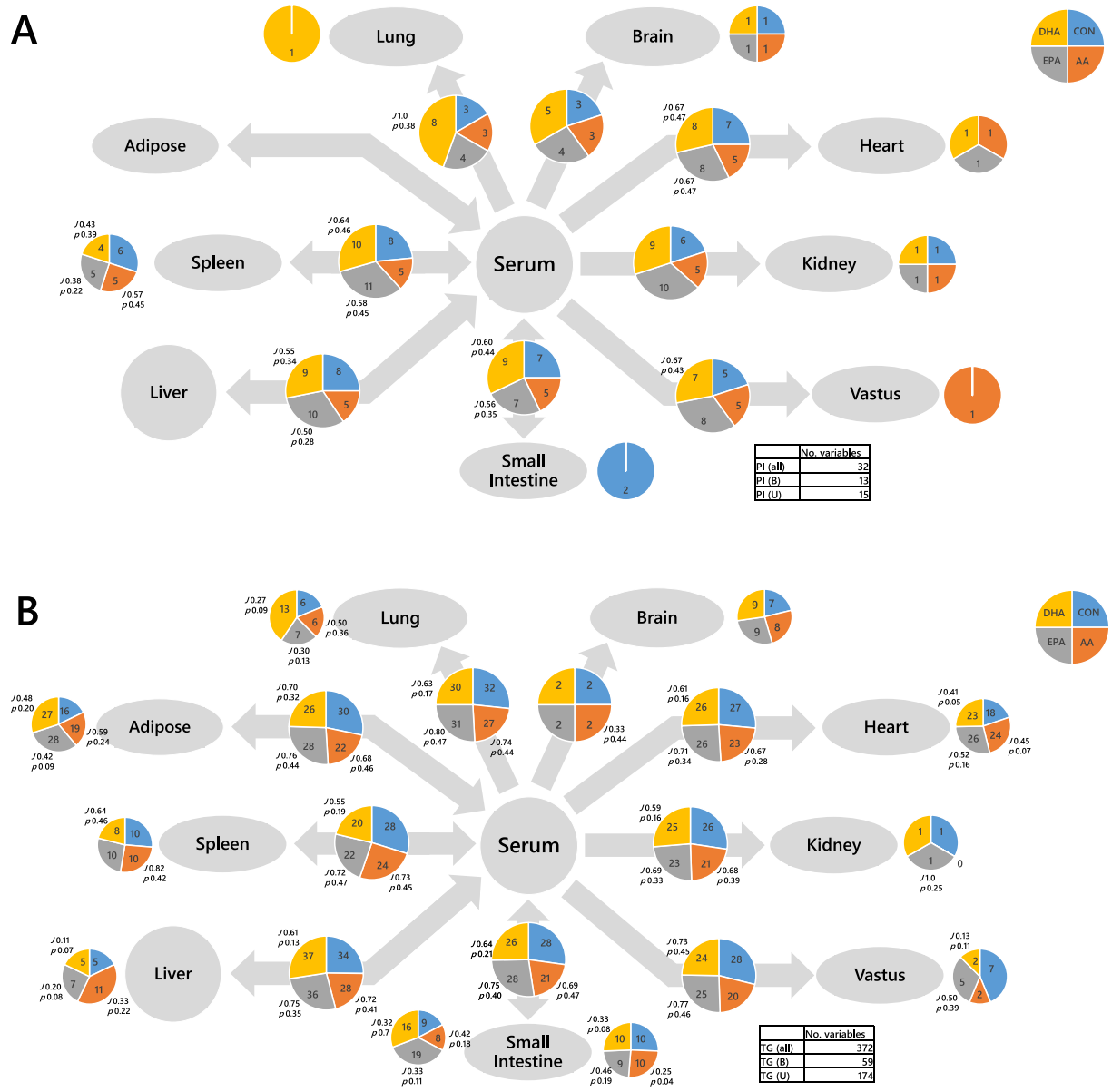

Figure 3: Traffic Analysis of lipids in a mouse model of PUFA supplementation. Panel **A**, Switch Analysis of phosphatidylinositol (PI) variables; Panel **B**, Switch Analysis of triglyceride (TG) variables. Pie charts show the number of variables of the appropriate head group in the relevant tissue(s). Large inset pie charts show the ***B***-type species (lipids found in two neighbouring compartments), whereas ***U***-type lipids (lipids found only in one compartment) are depicted with smaller pie charts. The Jaccard-Tanimoto coefficients (*J*) and probability (*p*) values that describe the similarity between sets of variables. Translucent pie charts indicate those in which only the number of variables differs between groups.

3.3. Alteration to long-chain FA supply driven by PUFA supplementation

The activity index of elongases ELOVL2 and 5 on FA(20:5n-3) can be calculated using the ratio between both PC(16:0/20:5) and PC(16:0/22:5), and PC(18:0/20:5) and PC(18:0/22:5). The ELOVL2/5 activity based on PC(16:0/22:5)/PC(16:0/20:5) showed that the activity was higher in the lung, heart and vastus muscle on supplementation with FA(20:4n-6) but lower on supplementation with FA(22:6n-3), Figure 4B. Supplementation of FA(20:5n-3) means this measurement of the ELVOL2/5 activity cannot be done for the FA(20:5n-3) group. However, the activity ratio calculated from PC(18:0/20:5) and PC(18:0/22:5) was unchanged in the liver and small intestine with FA(20:4n-6) supplementation, unchanged in the lung after FA(22:6n-3) supplementation, lower in the lung, spleen, serum, and kidney after FA(20:4n-6) supplementation, and lower in the kidney, small intestine, lung, and liver after FA(22:6n-3) supplementation. These results are surprising because they show not only that the biosynthesis of FA(22:5n-3) was much greater in certain compartments but also that its distribution and synthesis were tightly controlled and independent of the liver. Specifically, the supplementation of FA(20:4n-6) and FA(22:6n-3) leads to an increase in PC(16:0/22:5) in the lung, heart, and muscle but a decrease in other tissues, with no change or a reduction in PC(18:0/22:5) throughout the system. This suggests first that the metabolism of PCs is more tightly controlled and more organ specific than expected, and second that when the intake is low, PUFAs are used to substitute one another.

Leg muscle and spleen in pigs have both been found to produce FA(22:5) [40], an observation that is consistent with evidence of the

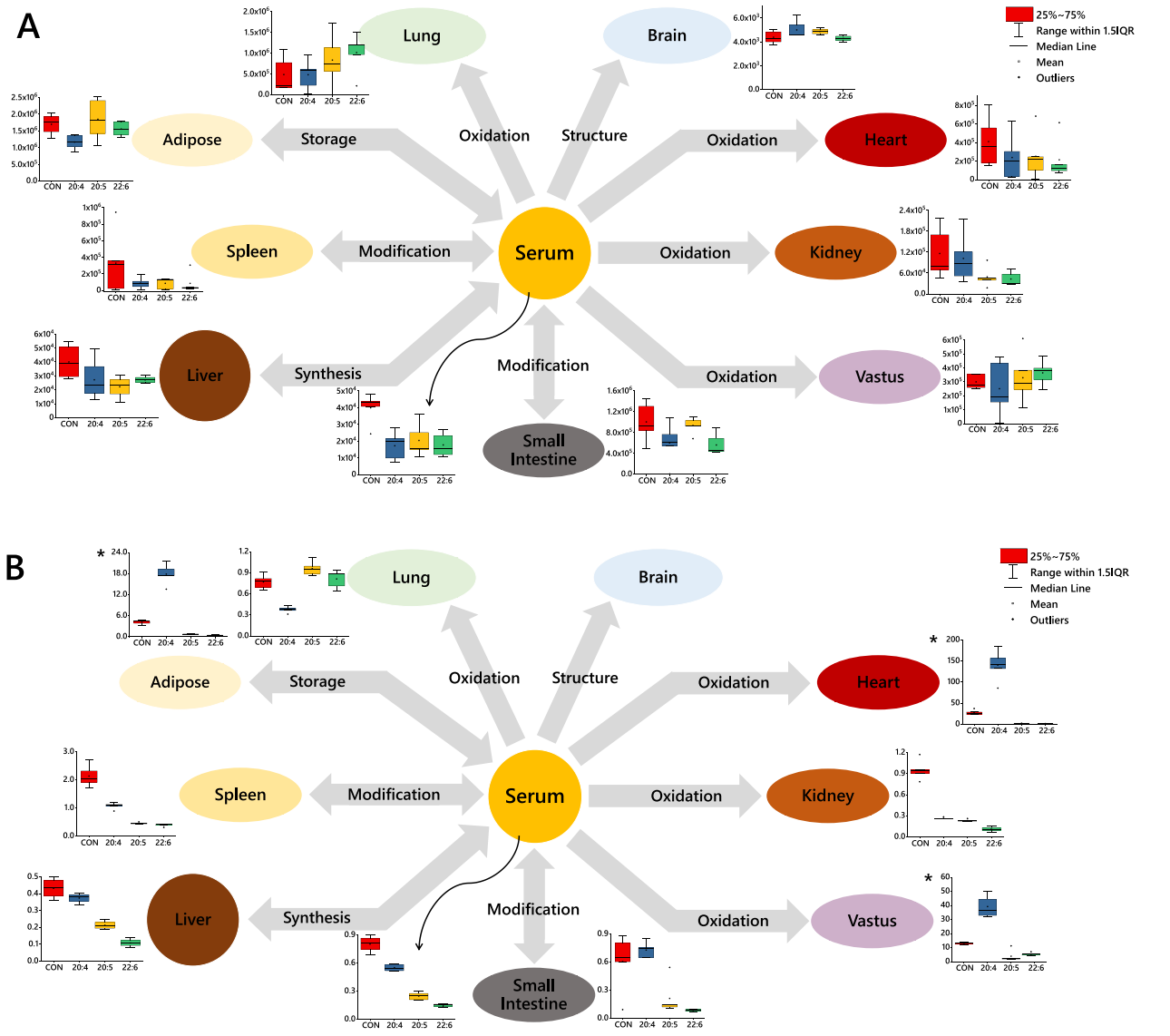

Figure 4: Modifications to FA metabolism associated with supplementation with PUFAs. Panel **A**, Abundance of TGs associated with *de novo* lipogenesis, TG(46:0, 46:1, 48:0, 48:1, 48:2, 50:1) [37], shown as the mean with 1.5 IQR. Panel **B**, the activity of elongases ELOVL2/5 on FA(20:5) expressed as the ratio of the abundance of PC(18:0/22:5) to PC(18:0/20:5) and PC(16:0/22:5) to PC(16:0/20:5), with the latter marked*. The box plots represent the values for mean, standard deviation and spread for $n = 4$ or 5 per group.

presence of this FA in several tissues and lipid classes in mice. Some FA(22:5)-containing lipids were organ-specific in mice, PE(18:2/22:5) was only found in the heart and PE(22:5/22:6) was only found in vastus. However, some compartments were pathway-specific, *e.g.,* almost all of the FA(22:5) in the liver was in triglycerides (TGs). This study therefore suggests that FA(22:5) is produced in at least two separate ways, one resulting in FA(22:5)-containing TGs mainly in the liver and the other in PLs mainly in muscular tissue. These results are important because they show that some tissues are more independent in FA modification than previously known. Furthermore, because FA(22:5) is required for producing protectins and D-series resolvins [41], the evidence for the biosynthesis of this compound in the lung, kidney, vastus, and heart suggests a role for supplementation in the resolution of acute kidney and lung injury by resolvins [42], the resolution of acute inflammation in the heart initiated by myocardial infarction [43], and the biosynthesis of lipokines in the skeletal muscle during and after exercise [44]. These results also show that the conversion of FA(20:5n-3) to FA(22:5n-3) on supplementation with FA(20:4n-6) occurs in localised areas of the organism (Figure 4B), with a possible role in controlling inflammation.

The untargeted nature of the systemic LTA also shows how the metabolism of saturated FAs such as FA(22:0) and FA(24:0) is modified by supplementation with PUFAs. In pigs, there is a significant release of FA(22:0) and FA(24:0) from lung tissue [40], unlike mice in whom FA(22:0)-containing PCs and PEs were found in the CNS and FA(24:0)-containing PCs in both the brain and spleen (as PC(24:00/18:01) and PC(24:00/20:04), respectively) but not the lungs. The clear difference in the profile of FA(22:0)- and FA(24:0)-containing lipids in mice suggests that saturated long-chain FAs were produced independently in at least two places in this model. The biosynthesis of FA(22:0) and FA(24:0) relies upon ELOVL1 [45], suggesting that this enzyme is expressed in the CNS and periphery. Furthermore, there was almost no

change in traffic of FA(22:0) and FA(24:0) associated with PUFA supplementation, suggesting little effect of the PUFA on ELOVL1 supplementation.

4. CONCLUSIONS

It is evident from the applications of LTA to lipidomics data from the model of PUFA supplementation that there are systemic effects in different pathways as well as different effects in different tissues driven by PUFA supplementation. The hypothesis that system-level and local changes in lipid metabolism were associated with PUFA supplementations was correct. Specifically, these affected different lipid pathways differently, with the profile of the PI and PE fractions changing considerably according the PUFA supplement, with more subtle reorganisations in PCs and TGs. Which FAs were made and elaborated was also PUFA-dependent. This provides a mechanistic basis for interpreting results of clinical trials in which PUFAs were administered and shows that all tissues are affected by PUFA supplementation. This study shows that it is not clear what the therapeutic window is or should be for these PUFAs. These results therefore raise important questions about the relevance of PUFA supplementation trials aimed at improving metabolic health because the metabolic response is tissue-dependent and not uniform. The results of this study show that a network analysis is essential for understanding the effects of nutrient supplementation on whole organisms because it is the only type of analysis capable of uncovering effects throughout the system.

AUTHOR CONTRIBUTIONS

SF conceived the research question with AK. SF performed all lipid analyses and wrote the manuscript. SF, SV, SGS, and AK interpreted data. DC conceived improvements to previous code with SF, wrote all code, and contributed to data analysis and figure preparation. AK and DC wrote the original grant proposals. SF and AK revised the manuscript with comments from all authors. All authors commented on the manuscript and approved the final version.

DATA AVAILABILITY STATEMENT

The R code used in this study for Lipid Traffic Analysis v2.3 is publicly available through the original paper [13,14], as is v1.0 [12], and *via* Github (https://doi.org/10.5281/zenodo.5499760) [46], offered with a CC-BY licence. The data used in this study were made publicly available by the authors [10]. The original data were reformatted for LTA and can be found in Supplementary Information (SI2—Original data formatted for LTA). The switch analysis outputs from the LTA were combined into one document and also included in the Supplementary Information (SI3—Switch analysis (PE, PC, TG, and PI)).

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2022.101457.

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