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Research Article

"Foetal Origins of Mental Diseases" Hypothesis Explained by a MUFAs towards PUFAs Obstruction via the Enzyme Stearoyl CoA Desaturase (SCD) in *Post-Mortem* Human Brain

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Abstract

Background: The molecular bases of "The Foetal Origin Hypothesis of Mental Disorders" FOHOMD-hypothesis remains elusive. We aimed to give comprehensively an explanation of the molecular mechanisms based on LC-MS studies at *post-mortem* Type 2 diabetes (T2DM) human brain for red and white matter supported by whole brain matter of a juvenile C57bl6 mouse model.

Methods: We used a Systems Biology *lipidomics* approach to identify lipid abnormalities in *post-mortem* human brains of subjects with Type 2 diabetes (N = 8) compared to age-matched controls (N = 8) (all elderly > 65 y male). Based on product/precursor ratio's we calculated enzymatic activities via elongase-desaturase array for the different lipid compounds of the Cholesteryl-lipid fraction.

Findings: Our observations suggest that the conversion from Palmitic acid (C16:0) towards palmitoleic acid (C16:1) by the action of Stearoyl CoA Desaturase (SCD) is obstructed.

Major outcome of this study is that conversion of saturated fatty acids to monounsaturated fatty acids (MUFAs) by the enzyme stearoyl-Co-A-desaturase (SCD) is obstructed by a significant decline (P≤0.044*) of Δ9 desaturase (SCD1; C16:1/C16:0), which was observed in the T2DM Red Neocortex Human Brain homogenate group together with a strong significant decline of C18:0/C16:0 elongase (ELOV1,3,6) (P≤ 0.014*) in the similar red T2DM Neocortex Human Brain group.

Also, of extremely importance for the development of a healthy brain during foetal development is the important polyunsaturated fatty acids (PUFAs) ω-6 lipid dihomo-γ-linoleic acid (DHGL) (C20:3) and the conversion of this Fatty Acid (FA) to Arachidonic Acid (C20:4ω-6) which is based on enzymatic activity of the Δ-5-desaturase enzyme and was significantly increased (P≤0.005*) in red matter of these *post-mortem* brains. The juvenile C57bl6 mouse model gave similar results.

Interpretation: Although there is a compensation mechanism of DHGL for PUFAs via Δ-5 desaturase enzymatic activity of (C20:3ω-6/C20:4ω-6) (DGLA/ARA) also known as the triene-to-tetraene ratio. This parameter is used as a biochemical marker of Essential Fatty Acid (EFAs) deficiency. The major conclusion of this study is that mental disorders due to T2DM and foetal programming already starts at the early initial part of the lipid elongase-desaturase array (MUFAs) before the dietary as Essential Fatty Acids (EFA) stipulated C:18 inflammatory Linoleic acid (C18:2, ω-6) and the C:18 non-inflammatory α-Linolenic acid (C18:3, ω-3). So, we hypothesize not only these two EFAs must be supplied by the diet during pregnancy but also the MUFAs: Palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0) and oleic acid (C18:1) in order to elevate an obstruction of a combined Stearoyl CoA Desaturase (SCD-1) and C18:0/C16:0 elongase (ELOV1,3,6) limitation.

Keywords: *Mental Diseases; MUFAs; PUFAs; Stearoyl CoA Desaturase*

Introduction

The concept of "Foetal Origin of Adult Disease" popularized by Barker [1] arose from a robust association between small size at birth and the risk of chronic adult diseases for cardiovascular diseases (CVDs) in adult life. The concept of foetal programming states that changes in the foetal environment during sensitive periods of organ development may cause long-lasting changes in the structure and functioning of these organs later in life and influence the risk for chronic diseases such as coronary heart disease, type 2 diabetes (T2DM), hypertension and osteoporosis which is also called the "Foetal Origin of Adults Disease Hypothesis" [2,3]. The culmination of all these epidemiologic associations is referred to as the "Barker hypothesis". These observations form the basis of the "developmental origins of health and disease" hypothesis, which suggests that the intrauterine signals that compromise foetal growth also act to "program" tissue differentiation in a manner that predisposes to later illness including mental diseases: "Foetal Origins of Mental Diseases" FOHOMD-hypothesis [4,5]. In a recent editorial we extended "the Foetal Origins Hypothesis of Barker" towards the "Foetal Origins Hypothesis of Mental Diseases" FOHOMD-hypothesis [6]. Based on the following physiological observations it is presently acknowledged that:

1. Foetus and the placenta are completely dependent on Essential Fatty Acids (EFAs) the mother can offer for their growth and development;
2. While the third trimester of pregnancy is a crucial period for fat deposition in the human foetus, important phospholipids in placenta blood vessels and uterine vessels require maternal EFA provision for the formation of eicosanoids from the moment of conception;
3. There is a progressive enrichment of the concentration of Arachidonic Acid (C20:4, ω-6) AA and Docosahexaenoic Acid (C22:6, ω-3) DHA in circulating lipids in the foetus during the third trimester;
4. Furthermore, a significant increase in Arachidonic Acid is retained from the mother while for the DHA content of foetal brain it has been observed during the last trimester of pregnancy. During the first postnatal period months, a total amount of 600g EFAs are transferred from mother to foetus during one term pregnancy.
5. Most of these ω-3 fatty acids entering the foetal circulation are obtained by the mother even if the maternal ω-3 concentrations are low [7].

Based on these observations we hypothesized that the nutritional status of the future mother determines the "quality" of the brain of the foetus and the morbidity of mental illness in later life like early dementia. Finally, while the observed relation between foetal growth and adult health has garnered considerable attention, the molecular mechanisms of these associations remains to be determined. There are both considerable promise and important challenges for future studies of the foetal origins of mental health [8]. PUFAs are of particular importance for the nervous system for two major reasons because these fatty acids participate actively both in the development of the nervous system during embryonic and early postnatal life [9]. Along with these actions, currently incurable pathological conditions of the nervous system, including neurodegenerative diseases, mental disorders, stroke, and trauma, involve deregulated contents of fatty acids (FAs). It is therefore believed that these changes contribute in their own right by as yet incompletely understood mechanisms to those pathological processes. Here, we hypothesize that based on our small epidemiological cohort of T2DM patients and our earlier LC-MS studies at T2DM brains that mental diseases are caused by a disordered lipid metabolism [10]. In the present research we will search for biomarkers using LC-MS techniques following a *lipidomics* approach at the elongase-desaturase series in order to elucidate molecular mechanism behind the "Foetal Origins Hypothesis of Mental Diseases" FOHOMD-hypothesis [6].

Research in context

Evidence before this study

Concerns have been raised about the tremendously increase of psychiatric disorders on a global scale among the adult population but also expressing themselves at an earlier age ("childhood mental disorders"). We reviewed the literature related to psychological-, psychiatric- disorders in humans and brain disorders in mainly rodent-model studies, and less in human peripheral blood mononuclear cells or *post-mortem* human brain, in combination with supplied food respectively feed data published in English or on PubMed. These studies described the influences in variation of the dietary intake of linoleic acid and α-linoleic acid -both stipulated as Essential Fatty Acids (EFAs)- and related polyunsaturated fatty acids (PUFAs) on the membrane lipids of cells. Alteration of the PUFA content of cell membranes

has a large impact on membrane fluidity and the functioning of a variety of membrane-associated proteins, lipid signalling, membrane trafficking and nutrient digestion which resulted in altered cell functions. Furthermore, there was a substantial body of evidence linking Ω-3-PUFA deficiency to hyperactivity disorders, coronary heart disease, retarded foetal growth, dementia, reduced visual and cognitive function, clinical depression, bipolar disorder, schizophrenia, and other problems of a dual psychological and physiological nature. Nearly no publications nor *post-mortem* human brain publications focussed on the Monounsaturated Fatty Acid (MUFA) trajectory from Myristic Acid (C14:0) via Palmitic Acid(C16:0), Stearic Acid (C18:0), Oleic Acid (C18:1) towards both EFAs so this research area remains obscure.

Added value of this study

This biochemical LC-MS study at *post-mortem* human brain of Type 2 Diabetic (T2DM) patients and a matching Control group -including our extensive literature research studies- supports the perception that Fatty Acids (FA) are important for healthy brain development from the foetus towards the 6-years old child. Functional Food Programmes might focus on FA from the PUFAs like the Essential Fatty Acids (EFAs) from C:18 origin such as the inflammatory Linoleic acid (C18:2, ω-6) and the non-inflammatory α-Linolenic acid (C18:3, ω-3). However, the major outcome of this study is that the MUFAs like Palmitic acid (C16:0) (palm oil, meat and diary), Palmitoleic acid (C16:1) (plants and marine resources), Stearic acid (C18:0) (meat, cocoa butter) and Oleic acid (C18:1) (avocado and olive oil), might not be neglected. So, we give a new definition for Essential Fatty Acids (EFAs) including the MUFAs which are mainly essential during early mental health development.

Implications of all the available evidence

Presently on a global scale, one billion people are acute starving (0 kcal/caput/day), two billion people are malnourished and lack essential nutritional elements in their food in order to perform labour. Our predictions and possibly implications of the FOHOMD-hypothesis, are that it might have a tremendous impact in countries were the mothers are exposed to starvation or malnutrition. In addition, the brain of a new born child develops until an age of 6-years old. As a consequence, childhood growth stunting -including brain development under these conditions- is a major nutritional challenge that affects over ≈165 million children globally. Psychiatric disorders at adult age can be prevented by acknowledging the FOHOMD-hypothesis with both the importance of MUFAs and PUFAs and its conversion by Stearoyl CoA Desaturase (SCD). Food Assistance Programs can assist in these WHO, FAO and UN tasks.

Materials and Methods

Experimental design: First of all, human *post mortem* brain material and blood plasma from the Dutch Brain Bank - in the comparison Control (Co) *versus* Type 2 diabetes (T2DM) - was used to trace the molecular mechanisms by means of a System Biological *lipidomics*-based approach using LC-MS techniques via biomarkers in order to confirm the FOHOMD-hypothesis. Firstly, in the patient material the morbidity of brain diseases in the Control (Co) group was compared with that of the type 2 diabetes (T2DM) group. Co and T2DM group which were comparable in age (> 65 years, elderly) and gender (all male). T2DM was confirmed in the blood plasma with a fructosamine test (11.van Ginneken et al 2017b). Furthermore, in a C57B16 mouse model (both High-fat induced Insulin Resistant (IR) and 24 h starvation), relevant biomarkers were searched by using the same LC-MS techniques for whole brain homogenate and blood plasma to provide evidence to confirm the FOHOMD-hypothesis. A Systems Biology *lipidomics* based approach was used and these biomarkers were searched in the elongase-desaturase series (Figure 1) based on product-precursor ratios expressing the enzymatic activity.

Brain-tissue: Human Brain tissue was obtained from the "Netherlands Brain Bank". All anonymous brain donors were of the male gender, elderly (aged > 65 y). With statically no difference in age, the autopsy time, the pH of the cerebrospinal fluid and the brain weight between Control (n = 8) and Type 2 diabetes (N = 8) group. With respect to early Alzheimer disease all patients were in stages varying between 0 - 2 with exception of patient Co-7 which was at stage 3. The origin of the piece of cortex brain material in most of these brain donors was the gyryus area. The "cause of death" was also given and varied but gave heart failure and respiratory problems as the main causes of death. Visually, a clear separation could be made between red and white matter in the cortex. A tissue homogenate of the brain tissue (red or white; ~10% wet weight/vol) in phosphate-buffered saline (PBS) was made with the Mini-beat Beater [11].

In order to test for Type 2 diabetes (T2DM) a fructosamine test was performed on blood plasma of all individuals Control (n = 8) and T2DM (n = 8) which gave a significantly increase ($P \leq 0.04^*$) in the T2DM group. This fructosamine test is a reflection of the glycated

plasma proteins, the most important of which is albumin. Because the half-life albumin is 19 days, fructosamine gives an estimation of average glycemic control over the previous 2 - 3 weeks. In the T2DM group four donors had no mental diseases, one cancer, two Parkinson and one vascular dementia [11].

Mass spectrometry (LC-MS): As described earlier [12-15], fifty µl of the well mixed tissue homogenate was mixed with 1000 µl IPA containing four internal standards. In addition, blood plasma samples of 10 µl plasma were extracted with 300 µl of isopropanol (IPA) containing several internal standards (IS: C17:0 lyso-phosphatidylcholine, di-C12:0 phosphatidylcholine, tri-C17:0 glycerol ester, C17:0 cholesteryl ester and heptadecanoic acid (C17:0)). Samples were placed in an ultrasonic bath for 5 minutes. After mixing and centrifugation (10000 rpm for 3 minutes) the supernatant was transferred to an autosampler vial. Thereaftere10 µl of the sample was injected on the LC-MS Instrument (Thermo Electron, San Jose, USA). A Thermo LTQ is a linear ion-trap LC-MS instrument (Thermo Electron, San Jose, USA). Lipids were separated on a 150 x 32 mm id C4 Prosphere column (Alltech, USA) using a methanol gradient in 5 mM ammonium acetate and 0.1% formic acid (mobile phase A: 5% methanol, mobile phase B: 90% methanol). The flowrate was 0.4 ml/min and the gradient was as follows: 0 - 2 minutes - 20%B, 2 - 3 minutes - 20% to 80%B, 3 - 15 minutes - 80% to 100%B, 15 - 25 minutes - hold 100%B, 25 - 32 minutes - condition at 20% B. The instrument used was a Thermo LTQ equipped with a Thermo Surveyor HPLC pump Data were acquired by scanning the instrument from m/z 300 to 1200 at a scan rate of approximately 2 scans/s in positive ion ESI mode.

Estimation of enzyme activity of desaturases and elongases in brain homogenate or blood plasma

In addition, elongase-desaturase series, from which enzym activities could be calculated based on product-to-precursor ratios of individual measurement of fatty acids (FA) as earlier performed and as depicted in figure 1 [14].

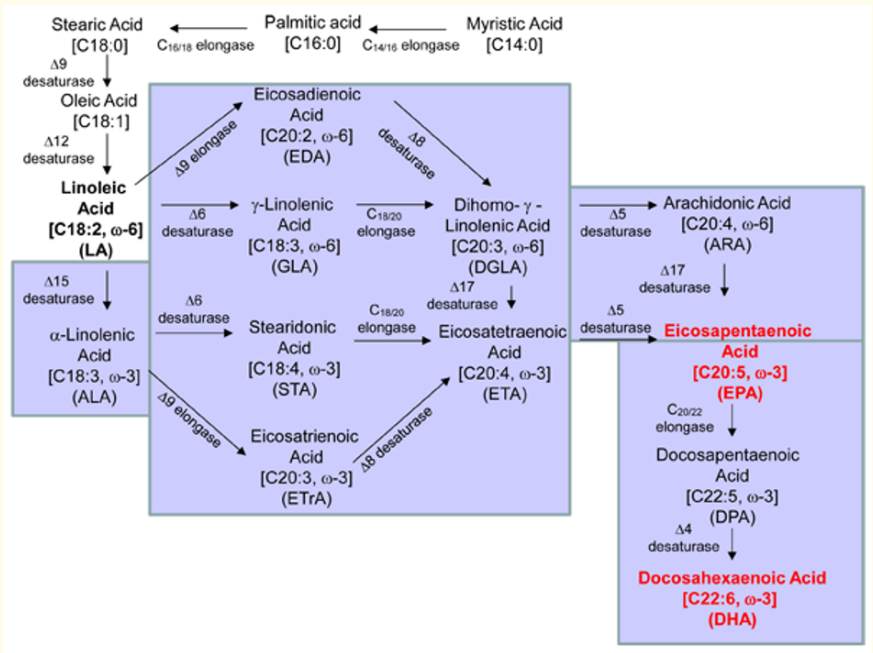

Figure 1: *Biosynthesis of long-chain omega-6 and omega-3 polyunsaturated fatty acids from dietary commodities following an elongase-desaturase series in mammals. Enzym activities can be calculated based on product-to-precursor ratios of individual measurement of fatty acids (Modified: 15. Ridgway and McLeod 2016).*

Calculations and statistics

We applied the techniques of biomarkers in this *lipidomics* based Systems Biology LC-MS study because they allowed us to make easier and faster decisions about the importance of fatty acids (MUFAs and PUFAs), including enzymatic activity. Following a Systems Biology approach [16] based on product-precursor ratios we calculated enzymatic activities in whole brain of mice together with plasma and also

in neocortex *post-mortem* human brain, red- and white matter. From figure 1 we see -applicable in animal and human tissues- there are four families of fatty acids that are derived from the precursors Palmitoleic-, Oleic-, Linoleic-, and Linolenic acids. Of these, Linoleic- and Linolenic-acids are essential dietary elements for humans and all higher animals. The four precursor fatty acids are metabolized (through desaturation and chain elongation) to form the Long-Chain Polyunsaturated Fatty Acids (LC-PUFAs). The principal fatty acids derived from linoleic acid are arachidonic acid, which contains four double bonds (tetraene) and Dihomo-γ-Linolenic Acid (DGLA), whereas products formed from Linolenic Acid (LA) are Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). The major products from Palmitoleic- and Oleic-acids are the 20-carbon Fatty Acids with three double bonds (triene). In the presence of adequate dietary amounts of essential fatty acids, tetraene products predominate in plasma. In addition, Δ6-desaturase which transforms Linolenic Acid (LA) into γ-Linolenic Acid (by making an additional double bond) is the slowest step of the Fatty Acid (FA) metabolism. Its activity is impaired by many physiological and pathologic factors and leads to γ-Linolenic Acid (GLA) deficiency [17]. The γ-linolenic acid supplementation in diet allows to omit the inefficient delta6-desaturase system which has an effect in rising of Dihomo-γ-Linolenic Acid (DGLA), Arachidonic Acid (AA) and their derivatives [18].

When the intakes of both linoleic and linolenic acids are low, triene formation is high and hence the triene/tetraene ratio in plasma is used to assess the index of Essential Fatty Acid (EFA) deficiency [18]. The ratio of [Dihomo-γ-Linolenic Acid]/[Arachidonic Acid] known as the triene-to-tetraene ratio, is used as a biochemical marker of essential fatty acid deficiency, with a value greater than 0.2 considered indicative of deficiency [19]. For all parameters, the mean value of the control- and T2DM-group was compared to the mean value of the fatty-diet group. Statistics were performed via SPSS [20], using a two-tailed T-Test for differences between the human *post mortem* neocortex brain homogenate provided by the "Netherlands Brain Bank" for Control- and T2DM- groups (red- and white matter) and corresponding human blood plasma. Similar statistics were also performed on the whole brain homogenate and blood plasma of the high fat diet induced obese IR C57bl6 mouse model including the 24h starvation group. In addition, P ≤ 005 was considered as statistically significant. Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov and F_{max} tests, respectively.

Results

With the described LC-MS techniques we could separate based on mass and polarity around 150 individual lipids of the five major lipid classes: Lyso-phosphatidylcholines (LPC); Phosphatidyl-cholines (PC); Sphingomyelins (SPM); Phosphatidylethanolamines (PE); Triacyl-glycerols (TG) and Cholesteryl-esters (ChE). From the latter group based on product/precursor ratio's we calculated enzymatic activity. We earlier mentioned we used biomarkers in order to signify which were the major Che compounds.

Our C57bl6 mouse model was juvenile and therefore we can assume the impact of the nutritional interventions (40 days HF-diet and 24h starvation) has a large impact on the developing brain of rodents. In this C57bl6 High Fat diet induced IR obese mouse model we found a strongly significant difference (P<0.005**, decrease) between the Control and 24 h Starvation mouse group for Δ9-Desaturase (C16:1/C16:0) and a significant difference (P<0.028*) for elongase (C18:0/C16:0). While between the Control and the High-Fat diet group a strong significant difference (P<007**) was observed for elongase *C18:0/C16:0) and a strong significant difference (P<001**) for Δ9 desaturase (C18:1/C18:0, stearoyl-CoA desaturase) (Table 2).

We will only discuss the observed significant differences between the Control-Starvation and Control-High Fat group because significant differences between the High Fat diet and Starvation group are irrelevant as studying material. We will discuss the elongase-desaturase array here in detail to start from the initial Fatty Acid (FA) Myristic Acid [C14:0] in the conversion to Palmitic acid [C16:0]. The [C14/C16] elongase activity is important in the conversion of Myristic acid [C14: 0] to Palmitic acid [C16: 0]. For the C57bl6 juvenile mouse model this enzyme was not found which is also confirmed in our human *post-mortem* brains data. The other elongase [C16/C18] that is important in the conversion of Palmitic acid [C16:0] to Stearic acid [C18:0] decreases significantly (P≤0.007**) in the High-Fat diet group while in the 24 h starvation group it also decreases significantly (P≤0.028*). So, from our observations for whole mouse brain (Table 2) we found a strongly significant difference between the Control and 24 h Starvation mouse group for Δ9-Desaturase (C16:1/C16:0) (P<0.005***) and a strong significant difference for the elongase (C18:0/C16:0) (P<0.028*) (both decrease). While between the Control and the High Fat diet a strongly significant difference was observed for elongase (C18:0/C16:0) (P<0.007**) and Δ9-desaturase (C18:1/C18:0) ratio. (P<0.001**).

These results of this juvenile C57bl6 mouse model gave on major important topics - namely confirmation of an obstruction via the enzyme Stearoyl CoA Desaturase (SCD) from MUFAS towards PUFAs – supportive evidence to the data of the post-mortem Type 2 diabetes elderly (> 65 y, all male) cohort described in this study. So, we can conclude that our juvenile C57bl6 mouse model is complementary towards our elderly human neocortex elderly (> 65 y) brain model from the Netherlands brain bank. So from our observations, here we give final prove for the 'The Fetal Origin Hypothesis or Mental Disorders' or FOHOMD hypothesis.

Mouse-model C57bl6 Whole mouse brain using LC-MS	CO Mean (std)	HF Mean (std)	STARV Mean (std)	T-Test CO-HF	T-Test CO-STARV	T-Test HF-STARV
Palmitic Acid C16:0	0.003 (0.0004)	0.005 (0.0013)	0.004 (0.0009)	P ≤ 0.03*	P ≤ .0.016*	P ≤ 0.251
Palmitoleate C16:1	0.006 (0.0009)	0.010 (0.0030)	0.006 (0.0003)	P ≤ 0.029*	P ≤ 0.0618	P ≤ 0.036*
Stearic Acid C18:0	0.015 (0.0009)	0.016 (0.0031)	0.016 (0.0012)	P ≤ 0.176	P ≤ 0.059	P ≤ 0.957
Oleic Acid C18:1	0.023 (0.0018)	0.031 (0.0054)	0.025 (0.0026)	P ≤ 0.019*	P ≤ 0.087	P ≤ 0.066
Linoleic Acid [C18:2, Ω-6] (LA)	0.004 (0.0018)	0.009 (0.0035)	0.005 (0.0014)	P ≤ 0.040*	P ≤ 0.371	P ≤ 0.080
Dihomo-γ-Linolenic Acid [C20:3, Ω-6] (DGLA)	0.001 (0.0004)	0.003 (0.0012)	0.001 (0.0003)	P ≤ 0.010*	P ≤ 0.096	P ≤ 0.023*
Arachidonic Acid [C20:4, Ω-6] (ARA)	0.011 (0.0042)	0.020 (0.0044)	0.014 (0.0024)	P ≤ 0.011*	P ≤ 0.268	P ≤ 0.030*
Docosahexaenoic Acid [C22:6, Ω-3] (DHA)	0.005 (0.0019)	0.007 (0.0012)	0.007 (0.0013)	P ≤ 0.039*	P ≤ 0.036*	P ≤ 0.923

Table 1: *Comparison between a Control (CO), High-Fat (HF) diet and 24 h Starvation (STARV) C57bl6 mouse model whole mouse brain for Cholesteryl-ester (ChE) lipid fractions of the elongase/desaturase array. All three groups 5 individuals (Source modified: 15. Ridgway and McLeod 2016).*

Enzymatic Activity	Control (n = 5) Mean ± std	High-Fat (n = 5) Mean ± std	24h-starvation (n = 5) Mean ± std	CO-HF P-value	CO-STARV P-value	HF-STARV P-value
C16:1/C16:0 SCD1, Δ9 desaturase	2.22 ± 0.0950	2.095 ± 0.4501	1.540 ± 0.29503	P ≤ 0.573	P ≤ 0.005**	P ≤ 0.055
C18:0/C16:0 Elongase ELOVL1,3,6	5.646 ± 1.0915	3.410 ± 0.7898	4.047 ± 0.6409	P ≤ 0.007**	P ≤ 0.028*	P ≤ 0.201
C18:1/C18:0 Stearoyl-CoA desaturase, SCD5, Δ9 desaturase	1.541 ± 0.1286	1.928 ± 0.1039	1.568 ± 0.1159	P ≤ 0.001**	P ≤ 0.734	P ≤ 0.001**
C18:2/C18:1 Δ12-desaturase	0.176 ± 0.0752	0.273 ± 0.0986	0.200 ± 0.070205	P ≤ 0.123	P ≤ 0.618	P ≤ 0.219
C20:4/C20:3 Δ5-desaturase D5D or FADS1	12.697 ± 6.7899	6.433 ± 1.1628	10.052 ± 0.9849	P ≤ 0.108	P ≤ 0.435	P ≤ 0.001**

Table 2: *Enzymatic activity calculated based on product-precursor ratio following the elongase-desaturase array as depicted in the previous figure for the Cholesteryl fraction measured by LC-MS-techniques of C57bl6 mouse whole brain material (Source modified: 15. Ridgway and McLeod 2016).*

Mouse-model C57bl6 Blood plasma using LC-MS	CO Mean (std)	HF Mean (std)	STARV Mean (std)	T-Test CO-HF	T-Test CO-STARV	T-Test HF-STARV
Myristic Acid C14:0	0.001 (0.001)	0.010 (0.002)	0.001 (0.0001)	P ≤ 0.0006***	P ≤ 0.0115*	P ≤ 0.0005***
Palmitic Acid C16:0	0.047 (0.010)	0.098 (0.020)	0.040 (0.006)	P ≤ 0.0025**	P ≤ .0.2347	P ≤ 0.0022**
Palmitoleate C16:1	0.121 (0.019)	0.838 (0.112)	0.094 (0.020)	P ≤ 0.0001***	P ≤ 0.0668	P ≤ 0.0001***
Hexadecadienoic acid C16:2	0.002 (0.001)	0.013 (0.002)	0.001 (0.0001)	P ≤ 0.0003***	P ≤ 0.0065**	P ≤ 0.0003***
Stearic Acid C18:0	0.0111 (0.001)	0.027 (0.012)	0.011 (0.001)	P ≤ 0.0366**	P ≤ 0.5251	P ≤ 0.0397*
Oleic Acid C18:1	0.271 (0.062)	1.898 (0.391)	0.259 (0.046)	P ≤ 0.0006***	P ≤ 0.7406	P ≤ 0.0006***
Linoleic Acid [C18:2, Ω-6] (LA)	2.868 (0.500)	3.399 (0.533)	2.011 (0.250)	P ≤ 0.1432	P ≤ 0.0145*	P ≤ 0.0022**
α-Linolenic Acid [C18:3, Ω-6] (ALA)	0.089 (0.015)	0.303 (0.038)	0.059 (0.005)	P ≤ 0.0001***	P ≤ 0.0105*	P ≤ 0.0001***
Dihomo-γ-Linolenic Acid [C20:3, Ω-6] (DGLA)	0.093 (0.020)	0.962 (0.126)	0.073 (0.016)	P ≤ 0.0001***	P ≤ 0.1223	P ≤ 0.0001***
Arachidonic Acid [C20:4, Ω-6] (ARA)	1.182 (0.238)	3.790 (0.624)	1.123 (0.234)	P ≤ 0.0003***	P ≤ 0.7029	P ≤ 0.0003***
Eicosapentaenoic Acid [C20:5, Ω-3] (EPA)	0.503 (0.104)	0.365 (0.171)	0.229 (0.054)	P ≤ 0.1710	P ≤ 0.0020**	P ≤ 0.1529
Docosahexaenoic Acid [C22:6, Ω-3] (DHA)	0.451 (0.081)	1.259 (0.294)	0.359 (0.052)	P ≤ 0.0026**	P ≤ 0.0725	P ≤ 0.0020**

Table 3: *Comparison between a Control (CO), High-Fat (HF) diet and 24 h Starvation (STARV) C57bl6 mouse model in blood plasma for Cholesteryl-ester (ChE) lipid fractions of the elongase/desaturase array. All three groups 5 individuals (Source modified: 15. Ridgway and McLeod 2016).*

Enzymatic Activity	Control (n = 5) Mean ± std	High-Fat (n = 5) Mean ± std	24h-starvation (n = 5) Mean ± std	CO-HF P-value	CO-STARV P-value	HF-STARV P-value
C14/16 elongase	51.21 ± 12.711	10.76 ± 4.443	65.86 ± 12.216	P≤0.0011**	P≤0.090	P≤0.0001***
C16:1/C16:0 SCD1, Δ9 desaturase	2.66 ± 0.532	8.71 ± 1.233	2.37 ± 0.412	P≤0.0.0001***	P≤0.363	P≤0.0001***
Hexadecadienoic acid/ palmitoleate C16:2/C16:1	0.020 ± 0.002	0.015 ± 0.001	0.015 ± 0.004	P≤0.0042**	P≤0.0475*	P≤0.865
C18:0/C16:0 Elongase ELOVL1,3,6	4.444 ± 0.789	2.136 ± 0.7777	8.655 ± 2.189	P≤0.0016**	P≤0.010*	P≤0.0015**
C18:1/C18:0 Stearoyl-CoA desaturase, SCD5, Δ9 desaturase	25.237 ± 4.495	76.354 ± 23.246	23.234 ± 2.658	P≤0.0071**	P≤0.422	P≤0.007**
C18:2/C18:1 Δ12-desaturase	10.752 ± 0.889	1.803 ± 0.104	7.892 ± 1.186	P≤0.0001***	P≤0.0031**.	P≤0.0003***
C18:3/C18:2 Δ15 desaturase	0.031 ± 0.001	0.089 ± 0.004	0.030 ± 0.003	P≤0.0.00001***	P≤0.3841	P≤0.00001***
C20:3/C18:3 C18/20 elongase	1.046 ± 0.092	3.234 ± 0.674	1.234 ± 0.256	P≤0.0017**	P≤0.1830	P≤0.0014**
C20:4/C18:2 Lineoyl-CoA desaturase	0.50616 ± 0.01876	1.281 ± 0.150	0.03849 ±0.0257	P≤0.00001***	P≤0.00001***	P≤0.00001***
C20:4/C20:3 Δ5-desaturase D5D or FADS1	12.751 ± 0.629	3.988 ± 0.833	15.377 ± 1.262	P≤0.0.00001***	P≤0.0062**	P≤0.00001***
C22:6/C20:5 C20/22 elongase and Δ4-desaturase	0.903 ± 0.043	3.759 ± 0.925	1.612 ± 0.312	P≤0.0013**	P≤0.0066**	P≤0.0047**

Table 4: *Enzymatic activity calculated based on product-precursor ratio following the elongase-desaturase array as depicted in the previous figure for the Cholesteryl fraction measured by LC-MS-techniques on collected C57bl6 mouse blood plasma material (Source modified: 15. Ridgway and McLeod 2016).*

For blood plasma in this C57bl6 mouse model strongly significantly differences (P ≤0.001**) & (P ≤0.0001***) were observed between the Control and the High-Fat diet mouse group for all in the Table 4 mentioned enzymes: C14/16 elongase, Δ9 desaturase, palmitoleate C16:2/C16:1, C18:0/C16:0 Elongase, C18:1/C18:0 Stearoyl-CoA desaturase, C18:2/C18:1 Δ12-desaturase, C18:3/C18:2 Δ6 desaturase, C18/20 elongase, C20:4/C20:3 Δ5-desaturase; C20:5/C20:4 Δ17-desaturase and C20/22 elongase & Δ4-desaturase. In the comparison of Co vs. HF the enzymatic activity of Lineoyl CoA-desaturase (C20:4/C18:2) increased significantly (P≤0.00001***) which is indicative for an ≈250% increase of the enzymatic activity following a HF-diet. Lineoyl CoA-desaturase plays a key role in the turnover of 18:2 (ω-6) towards 20:4(ω-6) and consequently determines the total amount of PUFAs. Furthermore, in the comparison Control-Starvation for blood plasma of this C57bl6 mouse model a strongly significantly differences (P≤0.001***) was observed for C18:2/C18:1 Δ12-desaturase, C20:4/C18:2 Lineoyl-CoA desaturase, C20:4/C20:3 Δ5-desaturase, C20:5/C20:4 Δ17-desaturase which all gave -except for C20:4/C20:3 Δ5-desaturase- a decrease in enzymatic activity. Notify that C20:4/C20:3 Δ5-desaturase is the important conversion ('shunt') from Dihomo-γ-Linoleic Acid (C20:3, Ω-6; DGLA) towards Arachidonic Acid (C20:4, Ω-6; ARA) in order to compensate for the PUFA rate limiting 'slow' enzyme Δ6 desaturase from Linoleic Acid (C18:2, Ω-6; LA) towards γ-Linoleic Acid (C18:3, Ω-6; GLA).

We used T2DM *post mortem* neocortex human brain homogenate (grey and white) versus a Control group and the whole brain of a High fat diet Insulin Resistant (IR)/Type 2 diabetes (T2DM) C57bl6 mouse model in comparison with a starvation group and their control. The principle of the method to search for biomarkers in terms of product-precursor ratios has earlier been performed in our LC-MS studies with a C57bl6 mouse model. We hope that this approach, using enzymatic biomarkers, will be a valuable tool to confirm the existence of this "Foetal Origins of Mental Diseases" hypothesis and its mechanisms.

Figure 2: *Biomarkers from the Cholesteryl-ester (ChE) fraction based on LCMS-measurements at post-mortem human brain for four different groups: a). Control Red matter; Control White Matter, Type 2 Diabetes White Matter, Type 2 Diabetes Red matter. Oleic Acid [C18:1] is the major Mono Unsaturated Fatty Acid (MUFA) and can based on measured arbitrary amounts [dimensionless] (Table 1), be indicated as a biomarker for this ChE major lipid class. Per group depicted the Mean ± Std of 8 individuals.*

Lipid Compound	Control-Red (n = 8) Mean ± std	Diabetes-2 Red (n = 8) Mean ± std	P ≤ 0.05	Control-White (n = 8) Mean ± std	Diabetes-2 White (n = 8) Mean ± std	P ≤ 0.05
Palmitic acid [C16:0]	0.0033 ± 0.00027	0.0039 ± 0.00058	P ≤ 0.014*↑	0.0034 ± 0.00048	0.0042 ± 0.00061	P ≤ 0.017*↑
Palmitoyl-CoA [C16:1]	0.0057 ± 0.00066	0.0059 ± 0.00060	P ≤ 0.471	0.0063 ± 0.00090	0.0059 ± 0.00044	P ≤ 0.297
Stearic acid [C18:0]	0.0121 ± 0.00073	0.0119 ± 0.00150	P ≤ 0.713	0.0118 ± 0.00225	0.0139 ± 0.00206	P ≤ 0.078
Oleic acid [C18:1]	0.0250 ± 0.00141	0.0266 ± 0.00163	P ≤ 0.059	0.0279 ± 0.00149	0.0298 ± 0.00303	P ≤ 0.145
Linoleic Acid [C18:2, Ω6] (LA)	0.0072 ± 0.00386	0.0091 ± 0.00431	P ≤ 0.369	0.0075 ± 0.00363	0.0094 ± 0.00394	P ≤ 0.338
Dihomo-γ-Linolenic Acid [C20:3; Ω6] DGLA	0.0014 ± 0.00026	0.0013 ± 0.00032	P ≤ 0.386	0.0022 ± 0.00038	0.0026 ± 0.00079	P ≤ 0.197
Arachidonic Acid [C20:4; Ω6] ARA	0.0065 ± 0.00157	0.0077 ± 0.00252	P ≤ 0.284	0.0082 ± 0.00154	0.0104 ± 0.00396	P ≤ 0.177
Docosahexaenoic Acid (DHA) [C22:6; Ω3]	0.0019 ± 0.00052	0.0021 ± 0.00024	P ≤ 0.498	0.0016 ± 0.00034	0.0017 ± 0.00014	P ≤ 0.517

Table 5: *Lipid compounds detected by LC-MS-techniques and given following the elongase-desaturase array for the Cholesteryl fraction of human post-mortem neocortex material as depicted in Figure 2 (Source modified: 15. Ridgway and McLeod 2016).*

From *post-mortem* human brain we can clearly see in figure 2 that Oleic Acid (C18:1) comprises around 40% of the total human ChE fraction followed by Stearic Acid (C18:0) ≈ 18-19%, Linoleic Acid (C18:2, ω6) ≈ 11 - 13%, Arachidonic Acid (C20:4, ω6) ≈ 10-13%, Palmitoyl CoA (C:16-1) ≈ 8-9%, Palmitic Acid (C16:0) ≈ 5 - 6%; Dihomo-γ-Linolenic Acid (C20:3, ω6) ≈ 2-3% while the important Docosahexaenoic Acid (C22:6, ω3) was also ≈ 2-3%.

The strength of a Systems Biology approach is to get an impression of enzymatic activity is based on product/precursor ratios (See table 2). From table 3 we can clearly see for the C57bl6 mouse model that Myristic acid, -the 14-carbon saturated fatty acid (C14:0)- usually accounting for small amounts (0.5%-1% weight of total fatty acids) in animal tissues, is not found in *post-mortem* human brain (See table 5). Like other dietary saturated fatty acids (palmitic acid, lauric acid), this fatty acid is usually associated with negative consequences for human health. In contrast, the saturated Fatty Acid (FA) Palmitic Acid (C16:0) (from dietary sources like palm oil, meat and diary), increases significantly in the T2DM group, both for Red- and White human brain tissue. This might indicate Palmitic Acid is synthesized *de novo* or obtained in increased extent from the diet in the T2DM group. The latter cannot be excluded due to an increased appetite and Body Mass Index in T2DM patients.

Palmitic acid (C16:0) is followed in this elongase-desaturase array by the MUFA Oleic Acid (C18:1) which is indicated in figure 2 as most important biomarker of all detected Cholesteryl-esters (ChE).

An important fraction of the ChE are the Polyunsaturated acids (PUFAs) which are Essential Fatty Acids (EFAs), which means that they cannot be synthesised and must come from the diet. The two main families of PUFAs are the omega-3 (ω3) and omega-6 (ω6) families (Figure 1). The omega-3 and omega-6 series are not synthesized in mammals. This metabolism involves insertion of additional double bonds into the aliphatic chain (a process termed desaturation) and addition of further carbon atoms to the acyl chain (a process termed elongation). Thus, Linoleic Acid (18:2n-6) LA is converted sequentially to γ-linolenic acid (18:3, ω-6) GLA, Dihomo-γ-linolenic acid (20:3, ω-6) DGLA and Arachidonic acid (20:4, ω-6) AA. Arachidonic Acid (20:4 ω-6) is the precursor of potent pro-inflammatory eicosanoids. This Ω-6 array takes place in a process that competes with the metabolism of the Ω-3 array where α-Linolenic Acid (C18:3, ω-3) ALA is converted via Stearidonic Acid (18:4, ω-3) STA and Eicosatrienoic Acid (20:4, ω-3) ETrA towards Eicosapentaenoic Acid (20:5, ω-3) (EPA). EPA can be further metabolised to Docosapentaenoic Acid (22:5, ω-3) DPA and finally "the other fish oil" Docosahexaenoic Acid (22:6, ω-3) DHA. Docosahexaenoic Acid (22:6 n-3) (DHA) produces less potent eicosanoids and gives rise to docosanoids with anti-inflammatory properties (i.e., resolvins and protectins). In this research manuscript (and others) EPA (C20:5, ω-3) is not found in the human brain. This remarkable observation will be discussed in another manuscript. The major culprit of this research manuscript are the calculated enzymatic activities. Unfortunately, only Dihomo-γ-Linoleic Acid (C20:3, ω6) DGLA, Arachidonic Acid (C20:4, ω6) ARA and Docosahexaenoic Acid (C22;6, ω3) could be measured from the Very Long Chain Fatty Acids (VLFC) lipid fraction (PUFAs) (Figure 1). This implies that the PUFAs of the omega 6 branch after α-Linolenic Acid (C18: 3, ω3) do not occur in *post-mortem* human brain tissue, or in very low concentrations. Missing PUFA lipid compounds -or below the detection limit- are EDA, GLA, STA, ETrA, ETA, DPA (explanation abbreviations see figure 1) and the "important" fish oil-like PUFA Eicosapentaenoic Acid (C20:5, ω3) EPA (see Discussion).

Enzymatic Activity	Co-Red (n = 8) Mean ± std	T2DM-Red (n = 8) Mean ± std	Co-T2DM Red P-value	Co-White (n = 8) Mean ± std	T2DM-White (n = 8) Mean ± std	Co-T2DM White P-value
Palimitonyl-CoA Desaturase C16:1/C16:0 SCD1, Δ9	1.739 ± 0.18705	1.529 ± 0.20724	P ≤ 0.044*↓	1.870 ± 0.22825	1.448 ± 0.18324	P ≤ 0.471
C18:0/C16:0 Elongase ELOVL1,3,6	3.729 ± 0.47050	3.054 ± 0.49003	P ≤ 0.014*↓	3.578 ± 1.1008	3.370 ± 0.52550	P ≤ 0.234
C18:1/C18:0 Stearoyl-CoA desaturase, SCD5, Δ9 desaturase	2.064 ± 0.15383	2.253 ± 0.24877	P ≤ 0.091	2.493 ± 0.47625	2.179 ± 0.29431	P ≤ 0.599
C18:2/C18:1 Δ12-desaturase	0.288 ± 0.14791	0.340 ± 0.14463	P ≤ 0.494	0.269 ± 0.12919	0.312 ± 0.11551	P ≤ 0.674
C20:4/C20:3 Δ5-desaturase D5D or FADS1	4.559 ± 0.67073	5.915 ± 0.9207	P ≤ 0.0054**↑	3.879 ± 0.80163	3.986 ± 0.69749	P ≤ 0.0004**↑

Table 6: *Enzymatic activity calculated based on product-precursor ratio following the elongase-desaturase array as depicted in Figure 3 for the Cholesteryl fraction measured by LC-MS-techniques of human post-mortem neocortex material (Source modified: 15. Ridgway and McLeod 2016).*

Our observations suggest that the conversion from Palmitic acid (C16:0) towards palmitoleic acid (C16:1) by the action of Stearoyl CoA Desaturase (SCD) is obstructed.

Major outcome of this study is that conversion of saturated fatty acids to monounsaturated fatty acids (MUFAs) by the enzyme stearoyl-Co-A-desaturase in *post-mortem* human brain in red and of a type 2 diabetes group for the ratios of Palmitoleic Acid (C16:1) to Palmitic Acid (C16:0) (SCD-16) and Oleic Acid (C18:1) to Stearic Acid (C18:0) (SCD-18 ≈ Δ-9 desaturase) is obstructed. Also, of extremely importance for the development of a healthy brain during foetal development is the important polyunsaturated fatty acids (PUFAs) ω-6 lipid Dihomo-γ-Linoleic Acid (DHGL) (*C20*:*3*) and the conversion of this Fatty Acid (FA) to Arachidonic Acid (*C20*:*4ω-6*) which is based on enzymatic activity of the Δ-*5-desaturase* enzyme which was significantly increased (P ≤ 0.005*) in red matter of these *post-mortem* brains of the T2DM group.

Control Human Blood	Control Co Mean ± SD (n = 8)	T2DM Mean ± SD (n = 8)	Δ(T2DM/Co)%	T-test Control-T2DM
C16:1/C16:0	0.74 ± 0.314	0.67 ± 0.218	90.05	P ≤ 0.594
C18:0/C16:0	0.23 ± 0.090	0.26 ± 0.101	111.41	P ≤ 0.593
C18:1/C18:0	17.52 ± 4.262	15.44 ± 3.184	88.12	P ≤ 0.287
C18:3/C18:2	0.058 ± 0.050	0.045 ± 0.013	77.55	P ≤ 0.485
C20:3/C18:3	0.503 ± 0.050	0.596 ± 0.303	118.35	P ≤ 0.551
C20:4/C18:0	19.64 ± 8.340	23.296 ± 13.121	118.57	P ≤ 0.518
C20:4/C20:3	15.86 ± 4.796	20.35 ± 9.236	128.32	P ≤ 0.242
C20:4/C18:2	0.329 ± 0.105	0.448 ± 0.161	140.05	P ≤ 0.080
C22:5/C20:5	C22:5 n.d.	C22:5 n.d.	-	-
C22:6/C22:5	C22:5 n.d.	C22:5 n.d.	-	-

Table 7: *Comparison between a Control and Type 2 diabetes (T2DM) human group for blood plasma for Cholesteryl-ester lipid fractions of the elongase-desaturase array derived enzymatic activities based on product/precursor ratios. n.d.=not detected.*

In contrast to brain material, no significant differences were found in blood plasma for the cholesteryl-esters so various calculated enzyme activities that corresponded to those in *post mortem* human brain as shown in table 1 couldn't be calculated.

From the observations given in table 7 we can conclude that for human material from a brain bank blood plasma and brain material for cholesteryl esters do not produce comparable lipid profiles. Possibly because the lipid composition of the human blood plasma in

the preceding weeks of these elderly patients (> 65y) -for which most of them were hospitalized before passing away (see table 1; death cause)- blood lipids might be affected by nutritional protocol, medicines, starvation (cachexia) and other factors.

The observations of a significant increase of Monoacylglycerols (MAG) and Triacylglycerols (TAGs) in blood plasma of T2DM patients are important observations and support the present intumescence that around 90% of short- and median-chain fatty acids are oxidized in the mitochondria, whilst approximately 10% of the very long chain Triacylglycerols are oxidized via β-peroxisomal oxidation. So, there is *a* tight association between *T2DM* and dyslipidemia. Furthermore, it is presently thought that FFAs induce Insulin Resistance (IR) in human muscle at the level of insulin-stimulated glucose transport or phosphorylation by impairing the insulin-signalling pathway as we earlier described. It would be a daunting task to review the interactions of lipids and glucose metabolism in relation to insulin resistance and the development of type 2 diabetes. In addition, it would move us further away from our initial question regarding the Foetal Origin of Mental Disorder FOHOMD-hypothesis.

In table 8 are related to this topic significant differences given of specific lipid compounds in blood plasma of these two "Netherlands Brain Bank" groups, more precisely a T2DM group (n = 8) and an aged matched Control-group (n = 8).

Lipid Compound	Control vs. T2DM group and value in blood plasma (mean ±std) (Co = 8) and (T2DM = 8)	P-value
C18-2-MG	Control: 0.009 ± 0.0039	P ≤ 0.048*
	T2DM: 0.018 ± 0.00107	
C20-3-MG	Control: 0.011 ± 0.00062	P ≤ 0.048*
	T2DM: 0.030 ± 0.0240	
C24-3-MG	Control: 0.0004 ± 0.0019	P ≤ 0.036*
	T2DM2:0.009 ± 0.00060	
C54-6-TG	Control: 0.200 ± 0.0924	P ≤ 0.042*
	T2DM2: 0.462 ± 0.3190	
C56-7-TG	Control: 0.155 ± 0.0762	P ≤ 0.014**
	T2DM2: 0.397 ± 0.2112	
C56-8-TG	Control: 0.061 ± 0.0364	P ≤ 0.032*
	T2DM2: 0.201 ± 0.1626	
C58-10-TG	Control: 0.011 ± 0.0079	P ≤ 0.013**
	T2DM2: 0.036 ± 0.0236	
C59-9-TG	Control: 0.029 ± 0.0162	P ≤ 0.003***
	T2DM2: 0.076 ± 0.0311	

Table 8: *Following a Systems Biology lipidomics approach we measured 108 individual lipid compounds using LCMS techniques in blood plasma at the five major lipid classes in blood plasma of a Control (n=8) patient group and a Type 2 diabetes group (n=8). It is important to notice that post-mortem brain tissue (red and white) were of the same patient as the sampled LC-MS measured blood plasma.*

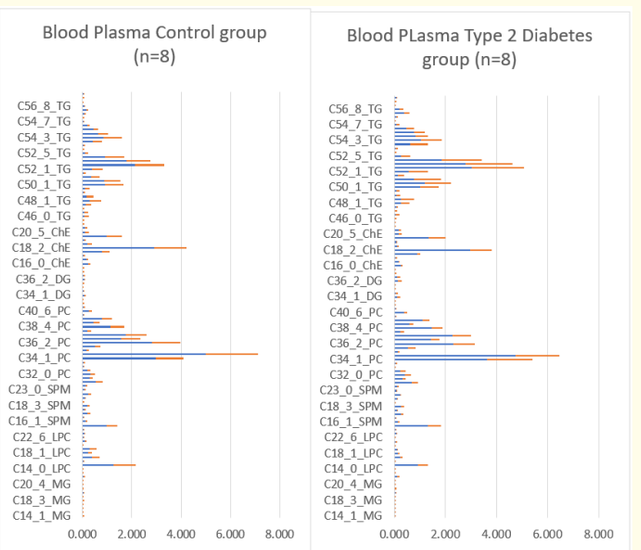

Figure 3: *Human Blood-plasma LCMS measurements of a Control (n = 8) and a T2DM2 patient group (n = 8, blue colour: MEAN and orange colour: STD).*

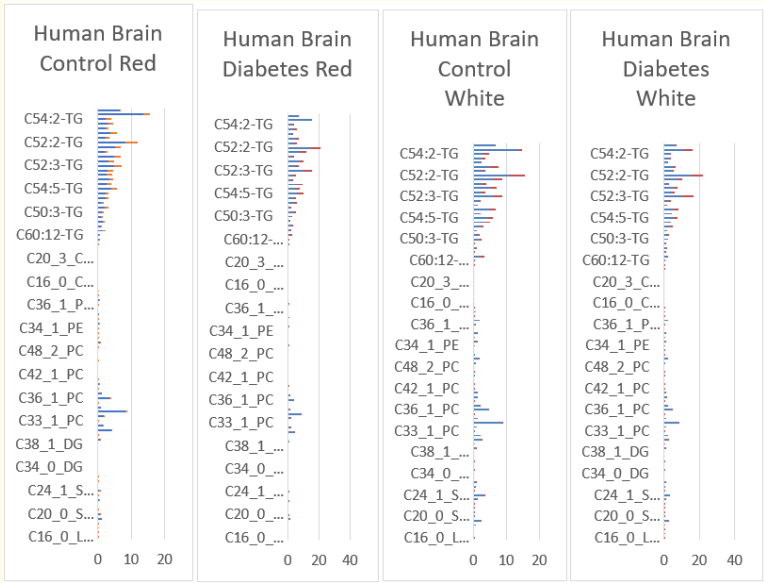

Figure 4: *Factor-spectrum mean ± std of post-mortem Human Brain of a Control group (n = 8 Red, n = 8 White) and a matching Type 2 Diabetes group (n = 8 Red, n = 8 White); material kindly provided by the Netherlands Brain Bank (Amsterdam, Netherlands).*

Discussion

Here, we finally give the ultimate proof of the *lipidomics* related molecular mechanism of the "Foetal Origin Hypothesis of Mental Disorders" FOHOMD-hypothesis through a Systems Biological approach [16]. It is generally acknowledged that the basic structure of the human brain is determined in great extent during the embryonic stage and fetal stage of the third period of the pregnancy [6,8].

An objection to this study to elderly patients might be that the starting material is not suitable for making statements about the "FOHOMD"- hypothesis because the patients were all elderly aged around 65 years [11]. This topic we have tried to make more plausible by supporting our *post-mortem* elderly human brain data by a juvenile High-Fat diet obese induced IR C57bl6 mouse model. Major outcomes were similar and indicated that conversion of saturated fatty acids to monounsaturated fatty acids (MUFAs) by the enzyme stearoyl-Co-A-desaturase (SCD-1) in *post-mortem* human brain in red and white matter of a type 2 diabetes group for the ratios of Palmitoleic Acid (C16:1) to Palmitic Acid (C16:0) (SCD-16) and Oleic Acid (C18:1) to Stearic Acid (C18:0) (SCD-18 ≈ Δ-9 desaturase) is obstructed. While for the C57bl6 mouse model our observations suggest that the conversion from Palmitic acid (C16:0) towards palmitoleic acid (C16:1) by the action of Stearoyl CoA Desaturase (SCD-16) is obstructed in this High-Fat diet IR induced obese whole mouse brain group. Palmitic acid can also be transformed into Stearic Acid (C18:0) by C16/18 elongase activity and subsequently into Oleic Acid (C18:1) by SCD activity. It plays a key role in composition of the fatty acid profile in adipose tissue and animal products such as meat and milk [15]. So, we hypothesize consequently that lactation of pregnant women and breastfeeding of the new-born and the quality of the mother milk for lipids might be in large extent determined by the SCD activity. Consequently, the brain development is dependent on the quality of the mother milk which gave indirectly support for the FOHOMD-hypothesis [21]. mRNA expression of stearoyl-CoA desaturase is dependent on many factors, including diet, hormones, and the activity of other genes [15].

Also, of extremely importance for the development of a healthy brain during foetal development is the important polyunsaturated fatty acids (PUFAs) ω-6 lipid dihomo-γ-linoleic acid (DHGL) (*C20*:*3*) and the conversion of this Fatty Acid (FA) to Arachidonic Acid (*C20*:*4ω-6*) which is based on enzymatic activity of the Δ-*5-desaturase* enzyme which was significantly increased ($P \leq 0.005$*) in red matter of these *post-mortem* brains of the T2DM group. We are convinced the concept of the "developmental programming" and the "FOHOMD"- hypothesis has to become in nearby future well accepted because of the outcomes of this *post-mortem* human brain study are supported by compelling animal studies with a juvenile obese IR C57bl6 mouse model that has precisely defined the outcomes of our T2DM human brain studies.

The outcomes of this study might seem uncontroversial because the traditional intumescence in relation to Polyunsaturated Fatty Acids (PUFAs) was that the human body needs two C:18 essential fatty acids (EFAs), and it can make all but two of them -linoleic acid (the 18-carbon Ω-6 fatty acid) and linolenic acid (the carbon Ω-3 fatty acid). These two fatty acids must be supplied by the diet and are therefore essential fatty acids (EFAs) (Figure 1) [22]. This is a robust elementary fact in clinical nutrition and a "unwritten law" in in any biochemical-, biomedicine-, or clinical nutrition textbook [22,23]. However, based on our observations in this study at post-mortem human brain in comparison to T2DM human brain -supported by LCMS whole brain analysis in a juvenile rodent model- we can conclude our observations give supportive evidence to support FOHOMD-hypothesis.

Our observations suggest that the conversion from Palmitic acid (C16:0) towards palmitoleic acid (C16:1) by the action of Stearoyl CoA Desaturase (SCD) is obstructed in *post-mortem* type 2 diabetic brain. Palmitic acid can also be transformed into stearic acid (C18:0) by C16/18 elongase activity and subsequently into oleic acid (C18:1) by SCD activity. Oleic acid can be then converted into longer chain fatty acids by sequential action of elongases and Δ^5 and Δ^6 desaturases. So, the combined actions of desaturases and elongases -which is essential in the transformation of MUFAs towards PUFAs- was obstructed in our *post mortem* human brain model.

Secondly, a biochemical measures of Essential Fatty Acid status and deficiency is the triene-to-tetrane ratio which can be calculated based on the [Dihomo-γ-Linolenic Acid] / [Arachidonic Acid] known as the triene-to-tetraene ratio. This parameter is used as a biochemical marker of Essential Fatty Acid (EFAs) deficiency, with a value greater than 0.2 considered indicative of deficiency [19]. Following the elongase-desaturase array Palmitic acid (C16:0) from the diet or *de novo* synthesis can be elongated via C16/18 elongase to Stearic Acid (C18:0) and desaturated by Δ-9 desaturase to Oleic acid (C18:1) (See figure 1). Further elongation and desaturation of Oleic Acid (18:1) by Δ-6 and Δ-5 desaturase leads to Eicosatrienoic Acid (C20:3 ω3) ETrA. However, Δ-6 desaturase shows strong preference for Linoleic Acid (C18:2 ω-6) LA and α-Linolenic Acid (18:3 ω-3) ALA over Oleic Acid (C18:1). This means that Dihomo-γ-Linolenic Acid (C20 :3 ω-6) DGLA increases only when Linoleic Acid (C18 :2 ω-6) LA and α-Linolenic Acid (18: 3 ω-3) ALA are both limiting, as in a fat-free nutrition or severe fat malabsorption [24]. The ratio of [Dihomo-γ-Linolenic Acid]/[Arachidonic Acid] known as the triene-to-tetraene ratio, is used as a biochemical marker of essential fatty acid deficiency, with a value greater than 0.2 considered indicative of deficiency [19]. An increased triene-to-tetraene ratio is a biochemical marker of failure to inhibit Oleic Acid (C18 :1) desaturation, explained by the promiscuous activity of Δ-6 desaturase with 18-carbon fatty acids and absence of inhibitory effects of Linoleic Acid (C18: 2, ω6) LA and γ-Linolenic Acid (C18: 3, ω3) GLA on Oleic Acid (C18: 1) desaturation. A key point is that the triene-to-tetrane ratio is only useful when both ω-6 and ω-3 fatty acids are limiting; it is not a marker of ω-3 fatty acid deficiency. The triene-to-tetraene ratio is also not useful for monitoring Arachidonic Acid (C20:4, ω-6) ARA in neonates given fish oil or other sources of because Docosahexaenoic Acid (C22:6, ω-3) DHA also inhibits Δ-6 desaturase and hence synthesis of Dihomo-γ-Linolenic Acid (C20 : 3 ω6) DGLA from Oleic Acid (18 : 1) and synthesis of Arachidonic Acid (C20:4, ω-6) ARA from Linoleic Acid (C18 : 2 ω-6) LA. We have to stipulate that detection of this important biochemical triene-to-tetraene ratio in *post-mortem* human brain are rare and are to our awareness solely performed in this research manuscript at elderly (> 65y, all male) patients. Therefore, our juvenile High Fat Diet induced obese C57bl6 mouse model gives supportive The Triene-to-Tetraene biochemical ratio ARA/DGLA was for Control-Red and Control-White *post-mortem* human brain tissue respectively 0,224 ± 0,03577 *vs*. 0,268 ± 0.03795 which was not significantly different (P ≤ 0.0925). While for T2DM-red vs. T2DM-white *post-mortem* human brain tissue, this biochemical parameter was significantly different, respectively 0,1733 ± 0,03110 *vs*. 0,2598 ± 0.05953 (P ≤ 0.00413***). Comparison between the Control- and T2DM-group gave for red *post-mortem* human brain tissue a strong significant difference (P ≤ 0.00935***), while this was for white human brain tissue (comparison Co and T2DM) not the case. Our observations were performed at red brain tissue which was during life of these patients well perfused with blood plasma. So, it would be an interesting extension for this biochemical parameter -valid for essential fatty acid (EFA) deficiency- if it would give a similar pattern in blood plasma, like in red human brain tissue. This was not the case: the DGLA/ARA ratio for the Control blood plasma group was 0,069 ± 0,0226 while for the T2DM group was 0,056 ± 0,00194 (P ≤ 0.256).

It is important to notice, this Triene-to-Tetraene ratio supports our hypothesis because significant differences are observed in T2DM red tissue and not in blood plasma. Brain development in humans -also called synaptic pruning- is influenced by food for up to 6 years and then stops [25].

The High Fat diet induced obesity IR C57bl6 mouse model supported the major observations for this derailed enzymatic activity of the elongase-desaturase array for Type 2.

The major message of this research manuscript is that at an early stage of the elongase-desaturase array from MUFAs towards PUFAs it goes wrong in the *post-mortem* type T2DM brain (red and white matter). These observations were supported by similar results in the whole brain of a juvenile obese IR C57bl6 mouse model exposed to a High Fat diet for 40 days. The results of this study indicate that these additional fatty acids are the monounsaturated fatty acids (MUFA), palmitoleic acid (16:1) and oleic acid (18:1). Fatty acids (FA) synthesised *de novo* and those obtained from the diet are modified through desaturation and elongation on the endoplasmic reticulum membrane. These reactions produce a variety of long-chain and very long-chain saturated, monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs) that have a variety of fates and functions [23]. FA serve as substrates in the synthesis of complex lipid classes, such as triacylglycerols and phospholipids, through which these FA facilitate energy storage and membrane fluidity, respectively [15]. Non-esterified FA also exert unique signalling properties [15].

In a previous manuscript we studied *post mortem* "material" of the Netherlands brain bank. Based on microscopic classification and histological pathophysiological characteristics we quantified in this cohort of elderly Noninsulin-dependent diabetes mellitus (NIDDM≈T2DM) of 204 patients: 39.4% healthy (Co-group); 32.8% Alzheimer (mild form); 13.5% "real" Dementia; 4.2% Multiple Sclerosis; 3.9% several dualistic mix-forms; 3.5% Vascular Dementia and 2.7% Parkinson. So, this gives supportive evidence for that we concluded T2DM patients has an increased morbidity of mental diseases [26]. So, from our observations we can conclude that the major culprit of "Foetal Origins Hypothesis of Mental Disorders" FOHOMD-hypothesis lies in an early derailed transition mechanism from MUFAs towards PUFAs at (δ9-desaturase) Our study is the first one recommending MUFAs as dietary supplement for the mothers during third period pregnancy and afterwards during lactation which is an opinion against the major flow which adds to a growing body of science supporting the benefits of PUFA (EPA and DHA) supplementation on pregnancy and the health and development of the infants.

Conclusion

In conclusion, our biochemical study at the FOHOMD-hypothesis at brain lipid molecular level, including enzymatic activity, opens new research tools to handle already mental disorders -based on the generally acknowledged foetal origin hypothesis of Barker (1995)-at the foetal stage while the brain is still developing and afterwards during lactation while the enzyme SCD obstructs gentle MUFA-PUFA transition- which might affect the lipid quality of the mother milk and consequently the brain development of the new-born. Further studies at rodent models and in an advanced stage clinical trials should bring neuropsychiatry at a higher level in the 21st century. It's a challenge for modern psychiatry - with currently one billion hungry people (often 0 kcal/caput/day) and two billion malnourished – it recognizes that clinical nutrition quality for the mother can prevent mental disorders and psychiatric disorders to children. As a consequence, childhood growth stunting-including brain development under these conditions- is a major nutritional challenge that affects over 165 million stunting growing children globally [27].

Conflict of Interest

None.

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