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Multiple 'Genetic Bottleneck Theory' of Humans and the House Mouse via Chilling Enzyme Δ12-Desaturase

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Review Article

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Abstract

The super Toba volcano eruption in Indonesia around 70,000-75,000 years ago might have reduced the human's world population to around 10,000 individuals. Such a dramatic scenario is indicated by the term 'genetic bottleneck theory' because the whole human species was on a global scale of nearly extinct. Here we show for modern humans and the common house mouse (strain C57bl6) they both possess a 'chilling' enzyme Δ12-desaturase in order to avoid that the body core temperature drops below 35.0 °C (95.0 °F). We postulate the hypothesis that the survivors of the Toba volcano eruption had been selected on their ability to maintain a relatively constant brain- and body core temperature in the face of a notoriously inconstant environmental temperature characterized by extreme adverse cold environmental conditions due to a following 'Toba glacial winter'. Surviving organisms originating from this event must have been in possession of the chilling enzyme Δ12- desaturase.

Keywords: Cold Protection; 'Chilling Enzyme' Δ12 Desaturase; Lc-Ms; Global Dispersal; Modern Humans; House Mouse; Toba Volcano Eruption; Toba Volcano Glacial Winter

Introduction

Homo sapiens, is known for its desire for expansion that results in a worldwide spread over our planet [1]. In the history book of humanity, the chapters on hunter-gatherers cover by far the most pages. At least, that should have been the time when humanity mainly populated the earth as a nomad and a hunter-gatherer. Possibly 1.5 to 1 million years ago, the first humanoids learned to use fire that was important to prepare food, but also to temporarily protect the body and brain against harsh cold climate conditions [2]. It was important to have the ability to maintain a relatively constant brain temperature in the face or a notoriously unstable ambient temperature. The evolution of hominoid ancestors that lived a few million years ago involved modern people with different types of transitions, characterized by important biological changes, such as the evolution of bipedalism and large brains [3]. The problem of these changes can be associated with searching for food for example by the bounty of the hunt [4], and perhaps a consequence of, extreme population constrictions. From the 150,000 to 200,000 years that modern people live on this planet, they have lived the longest time as hunter-gatherers with their characteristic 'expansion behaviour'. This *Homo sapiens* spread from Africa over the rest of the world between 120,000 and 60,000 years ago [1-4]. A life as a hunter-gatherer had everything to do with survival. By collecting edible wild plants and hunting animals, they provided enough food for their families. The hunter-

gatherers usually did not have a fixed place to stay. They traveled in small groups of usually 20-50 people, always on their way to areas with sufficient food. Hunting, collecting and expanding their foraging area was thus the first and most successful adaptation of humanity to at least 90 percent of human history [5].

Using a Systems Biology, *lipidomics* based approach with LC-MS techniques at *post-mortem* human brains - because our evolution is to our awareness literally engraved in our human brain- we demonstrated that the human brain contains a 'chilling enzyme' Δ12 desaturase until presently only observed in unicellular organisms and insects [6-11]. This is the enzymatic step from Oleic Acid (C18:1) towards Linoleic Acid (C18:2, ω-6; LA) Ridgway N, et al. [12], in order to protect the brain against hypothermia under environmental extreme cold exposure conditions. Here we postulate the hypothesis that this chilling enzyme may have played a central role in the negative selection pressure on humanity after the Toba volcano eruption about 75,000 years ago [13], when a very small population of between 1,000 and 10,000 breeding pairs - which survived the short intensive glacial winter period which lasted around 1,000 years became the ancestors of modern humanity. This phenomenon that a species is almost extinct due to, for example [14], extreme harsh environmental conditions such as a rapid glacial period is called a 'genetic bottleneck theory'. Members of this group went back to Africa, and spread over Australia [15], Europe and Asia [1,16]. The 'chilling enzyme' Δ12 desaturase comes back into the 'evolutionary dispersal picture of modern man' about 30,000 years ago when a small group of around 4,500 individuals migrated to the North American continent by a land bridge in the Bering Straits, created by the lowered sea levels due to the large ice sheets [17,18]. This is our second hypothesis how this 'chilling enzyme' Δ12 desaturase protected the human brain against extreme cold and plays a central role in the human evolution and dispersal of *Homo sapiens* all over the globe, even to the Americas [19]. But this group was probably according to Darwinian thought preselected during the Toba volcano eruption and possessed already the 'chilling enzyme' Δ12 desaturase to protect the human brain. In addition, our observations in this study of the occurrence of Δ12 desaturase in the whole brain and blood plasma of the common house / laboratory mouse (*Mus musculus*) gave supportive evidence for a multiple 'genetic bottleneck theory' which also affected small mammals (Figure 1).

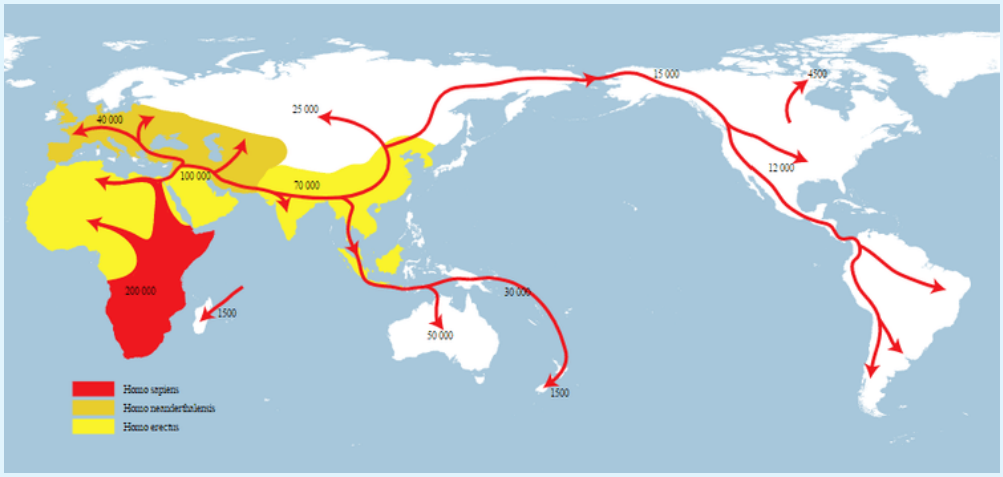


Figure 1: Dispersal of early Hominids across our planet. Numbers indicate number of persons involved in such a 'wave' of nomadic migration. The 'chilling enzyme' in human brain was an important protection mechanism when a small group of estimated around 4,500 individuals migrated to the North American continent by a land bridge in the Bering Straits created by the lowered sea levels due to the large ice sheets (Courtesy: Wikimedia Commons).

Results

With the described LC-MS techniques we could separate based on mass and polarity around 94 individual lipids compounds of the seven major lipid classes: lyso-phosphatidylcholines (LPC); phosphatidylcholines (PC); sphingomyelins (SPM); phosphatidylethanolamines (PE); triacylglycerols (TG) and cholesteryl-esters (ChE) in human *post-mortem* neocortex material (Figure 1). Because no standards for the plasmalogens exist, mass lists determined by using atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) mass

spectrometry (MS) techniques were used [20]. These mass lists were published on internet www.byrdwell.com/plasmogens and www.byrdwell.com/-PhosphatidylEthanolamine. In addition, from the ChE group based on product/precursor ratio's we calculated enzymatic activity. We earlier mentioned we used biomarkers of human *post-mortem* neocortex material and whole mouse brain of a C57bl6 mouse model in order to signify which were the major ChE enzymatic activities.

In Figure 2 are for red and white tissue of *post-mortem* neocortex human brain homogenate the factor spectrum images depicted for a healthy male Control (Co) group (aged > 65 y) versus an age matched Type 2 diabetic (T2DM) group. As can be seen from these factor spectra (Figure 2), the most important components that make up the human brain (measured by our LC-MS method) are in particular the triacylglycerols as the most important lipid class and to a lesser extent the phosphatidylcholines. At first glance, there are no major visual differences between Co patients and type T2DM patients.

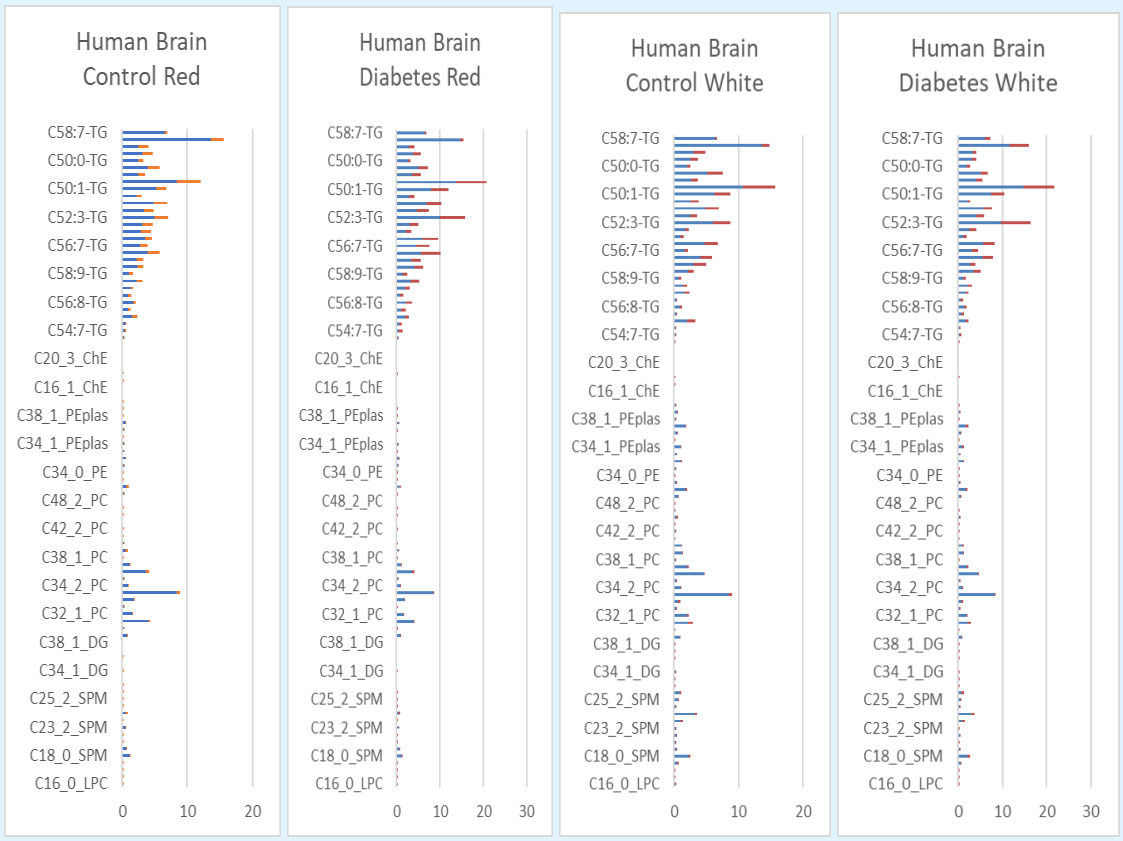


Figure 2: Factor-spectrum mean ± std of *post-mortem* Human Brain of a Control group (n=8 Red, n=8 White) and a matching Type 2 Diabetes group (n=8 Red, n=8 White); material kindly provided by the Netherlands Brain Bank (Amsterdam, Netherlands).

The strength of a Systems Biology approach Kitano H [21] in order to get an impression of enzymatic activity- is based on product/precursor ratios (Tables 1 & 2) [12].

From Table 1 we can clearly see 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. Like other dietary saturated fatty acids (palmitic acid, lauric acid), this fatty acid is usually associated with negative consequences for human health [22]. In contrast, the saturated Fatty Acid (FA) Palmitic Acid (C16:0) (from dietary sources like palm oil, meat & 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 (BMI) 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 3 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 [23]. The two main families of PUFAs are the Ω-3 (starting at α-Linolenic Acid (C18:3, Ω-3)) and Ω-6 (starting at Linoleic acid (C18:2, Ω-6)) families (Figure 2). The Ω-3 and Ω-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) [12].

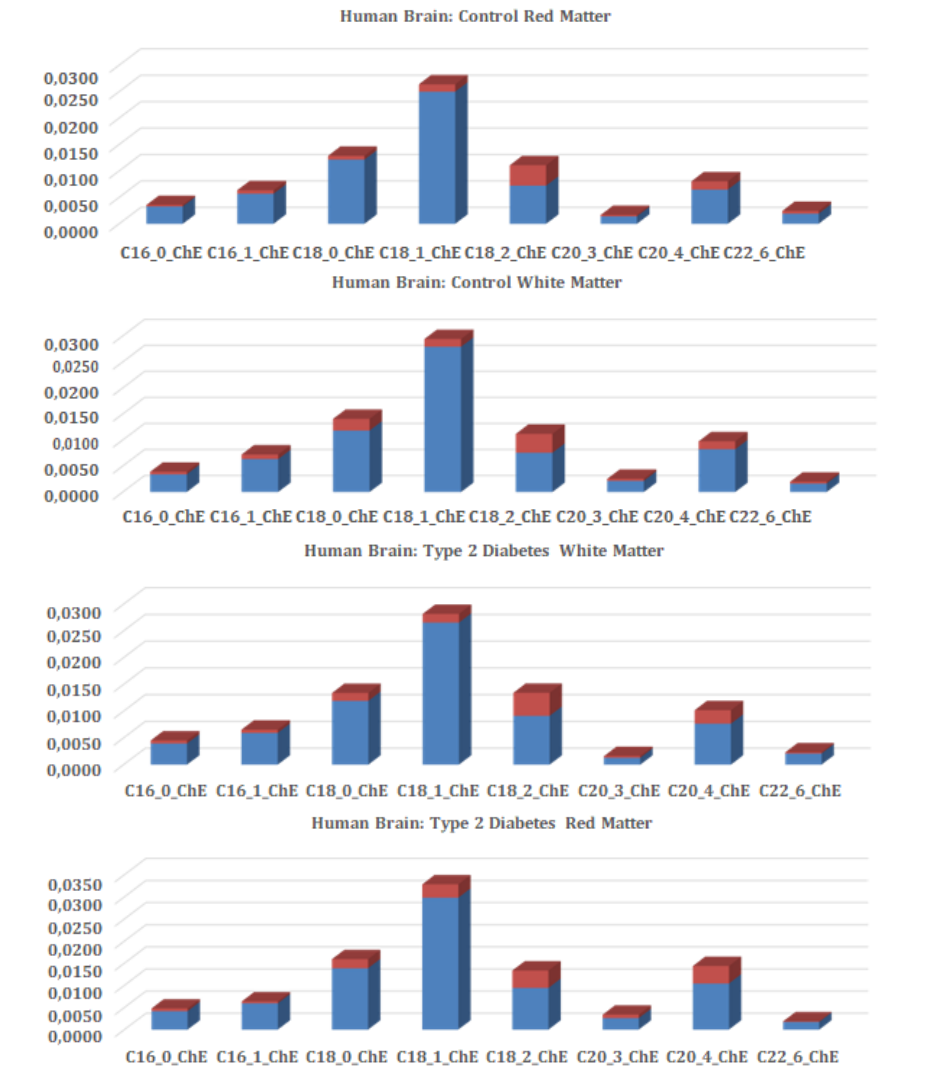


Figure 3: Biomarkers from the Cholesteryl-ester (ChE) fraction based on LC-MS- measurements at *post-mortem* human brain for four different groups: a). Control Red matter; Control White Matter, Type 2 Diabetes White Matter, Type 2 Diabetes Red matter. Oleic Acid (C18:1) is the major Mono Unsaturated 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. Blue colour is 'Mean' while red colour is 'Standard Deviation'

From Table 1 we can observe that -with exception of Myristic acid (C14:0)- all MUFAs are present in *post-mortem* human brain neocortex brain homogenate: Palmitic acid (C16:0), Palmitoleate (C16:1), Stearic acid

(C18:0) and Oleic acid (C18:10). A significant increase was observed for Palmitic acid (C16:0) for white *post-mortem* human neocortical brain homogenate with (P≤ 0.017*), and for red post-mortem human neocortical brain homogenate with (P≤0.014*). Interestingly, for the PUFAs only lipid compounds of the Ω-6 array were measured: Linoleic Acid (C18:2, Ω-6; LA), Dihomo-γ-Linoleic Acid (C20:3, Ω-6; DGLA), Arachidonic Acid (C20:4, Ω-6; ARA) and the important 'fish oil' Docosahexaenoic Acid (C22:6, Ω-3; DHA). No significant differences were observed between these PUFA lipid compounds between Control- and T2DM-group of *post-mortem* human brain homogenate for both red and white matter. In Figure 3 the lipid compounds of Table 1 are visualized and stipulated with the phrase biomarkers.

Lipid Compound	Co-White (n=8) Mean ± std	T2DM-White (n=8) Mean ± std	Co-T2DM White P-value	Co-Red (n=8) Mean ± std	T2DM-Red (n=8) Mean ± std	Co-T2DM Red P-value
Palmitic acid [C16:0]	0.003 ± 0.00048	0.004 ± 0.00061	P≤0.017*↑	0.003 ± 0.000027	0.004 ± 0.00058	P≤0.014*↑
Palmitoleate [C16:1]	0.006 ± 0.00090	0.006 ± 0.00044	P≤0.297	0.006 ± 0.00066	0.006 ± 0.00060	P≤0.471
Stearic acid [C18:0]	0.012 ± 0.00225	0.014 ± 0.00206	P≤0.078	0.012 ± 0.00073	0.012 ± 0.00150	P≤0.713
Oleic acid [C18:1]	0.028 ± 0.00149	0.030 ± 0.00303	P≤0.145	0.025 ± 0.00141	0.027 ± 0.00163	P≤0.059
Linoleic Acid [C18:2, Ω6] (LA)	0.008 ± 0.00363	0.009 ± 0.00394	P≤0.338	0.007 ± 0.00386	0.009 ± 0.00431	P≤0.369
Dihomo-γ- Linolenic Acid [C20:3; Ω6] DGLA	0.002 ± 0.00038	0.003 ± 0.00079	P≤0.197	0.001 ± 0.00026	0.001 ± 0.00032	P≤0.386
Arachidonic Acid [C20:4; Ω6] ARA	0.008 ± 0.00154	0.010 ± 0.00396	P≤0.1772	0.006 ± 0.00157	0.008 ± 0.00252	P≤0.2841
Docosahexaenoic Acid (DHA) [C22:6; Ω3]	0.002 ± 0.0034	0.002 ± 0.00014	P≤0.5172	0.002 ± 0.00052	0.002 ± 0.00024	P≤0.4978

Table 1: 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 3, [12].

Enzymatic Activity	Co-White (n=8) Mean ± std	T2DM-White (n=8) Mean ± std	Co-T2DM White P-value	Co-Red (n=8) Mean ± std	T2DM-Red (n=8) Mean ± std	Co-T2DM Red P-value
C16:1/C16:0 SCD1, Δ9 desaturase	1.870 ± 0.22825	1.448 ± 0.18324	P≤0.471	1.739 ± 0.18705	1.520 ± 0.20724	P≤0.044*↓
C18:0/C16:0 Elongase ELOVL1,3,6	3.578 ± 1.1008	3.370 ± 0.52550	P≤0.234	3.729 ± 0.47050	3.054 ± 0.49003	P≤0.014*↓
C18:1/C18:0 Stearoyl-CoA desaturase, SCD5, Δ9 desaturase	2.493 ± 0.47625	2.179 ± 0.29431	P≤0.599	2.064 ± 0.15383	2.253 ± 0.24877	P≤0.091
C18:2/C18:1 'Chilling enzyme' Δ12-desaturase	0.269 ± 0.12919	0.312 ± 0.11551	P≤0.674	0.288 ± 0.14791	0.340 ± 0.14463	P≤0.494
C20:4/C20:3 Δ5-desaturase D5D or FADS1	3.879 ± 0.80163	3.986 ± 0.69749	P≤0.0004**↑	4.559 ± 0.67073	5.915 ± 0.9207	P≤0.0054**↑

Table 2: 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 [12].

From *post-mortem* human brain we can clearly see in Figure 3 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 major culprit of this research manuscript is the calculated enzymatic activities based on product/precursor ratios. In the next session we will split it up in enzymatic activities of Mono-unsaturated Fatty Acids (MUFAs) and Polyunsaturated Fatty Acids (PUFAs). For the MUFAs a significant decline with P≤0.044 of Δ9 desaturase (SCD1; C16:1/C16:0) was observed in the T2DM Red Neocortex Human Brain homogenate group together with a strong significant decline (P≤ 0.014) in the similar Neocortex Human Brain group. Most important observation is that the 'chilling enzyme'' Δ12-desaturase (C18:2/C18:1) is present in both Co and T2DM group. In addition, for the PUFAs, 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 2). 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 2) and the "important" fish oil-like PUFA Eicosapentaenoic Acid (C20:5, ω3) EPA.

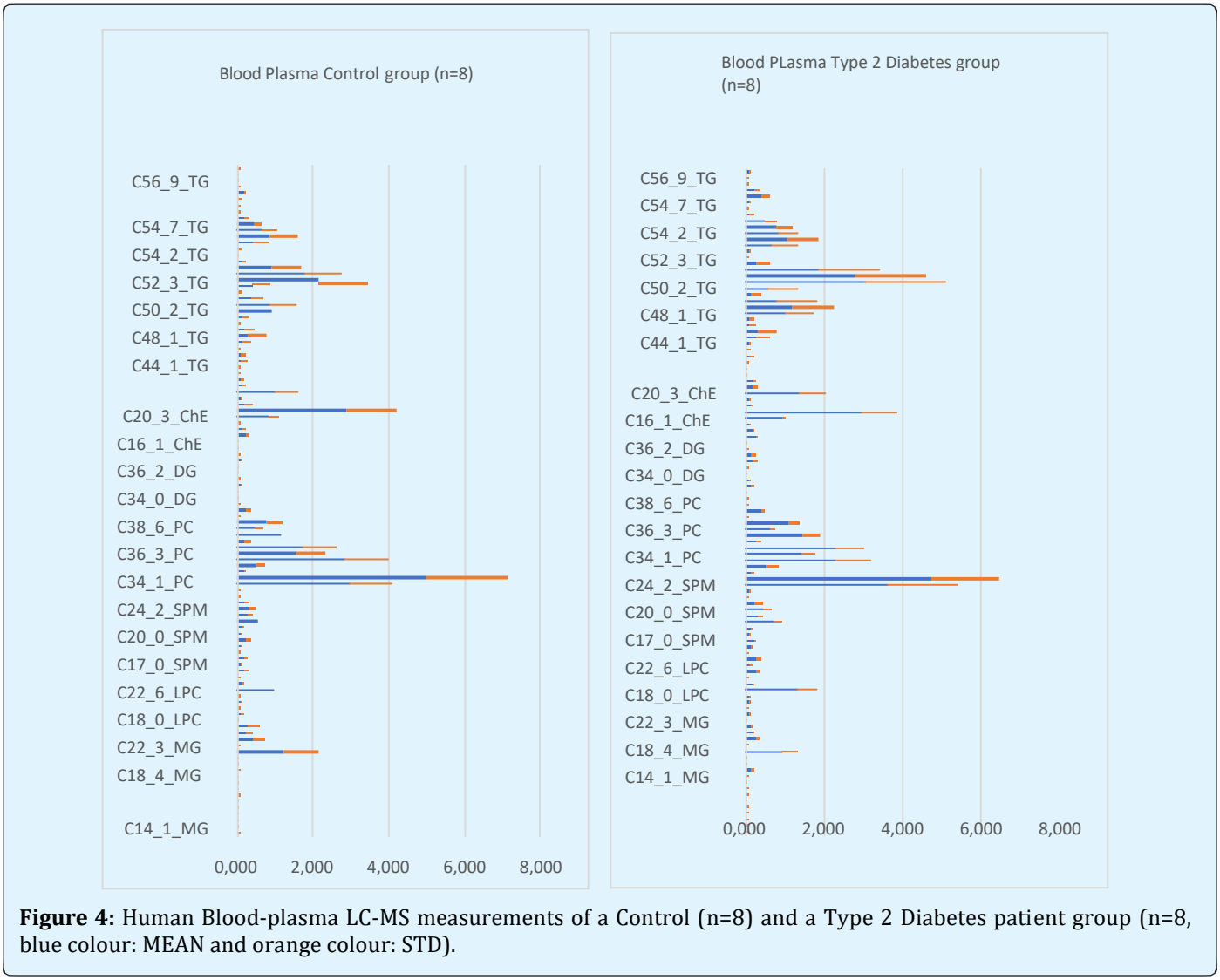


Figure 4: Human Blood-plasma LC-MS measurements of a Control (n=8) and a Type 2 Diabetes patient group (n=8, blue colour: MEAN and orange colour: STD).

In (Figure 4) are the factor spectrum images depicted for human blood plasma of a healthy male Control group (aged > 65 y) versus an age matched Type 2 diabetic group. As can be seen from these factor spectra (Figure 1), the most important components that make up the human brain (measured by our LC-MS method) are in particular the Triacylglycerols (TGs), Cholesteryl-esters (ChE), while the most abundant are the Phosphatidylcholines (PC) as major lipid group. At first glance, there are no major visual differences between control- healthy patients and type 2 diabetes patients.

From Table 3 we can see that all MUFAs -including Myristic Acid C14:0 (which was absent in human neocortex brain homogenate) are present. For the PUFAs the major lipid fractions are present Linoleic Acid (LA), α-Linolenic Acid (ALA), Dihomo-γ-Linolenic Acid (DGLA), Arachidonic Acid (ARA), and remarkable in comparison with human brain neocortex brain homogenate both 'fish oils' Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) are present in human blood plasma. In addition, no significant differences were observed for MUFAs and PUFAs for measured lipid compounds between Control and T2DM group.

Human blood plasma using LC-MS	CO (n=8) Mean (std)	T2DM (n=8) Mean (std)	T-Test CO-T2DM
Myristic Acid	0.013	0.012	P≤0.723
C14:0	(0.009)	(0.005)	
Palmitic Acid	0.220	0.254	P≤0.380
C16:0	(0.089)	(0.060)	
Palmitoleate	0.154	0.161	P≤0.809
C16:1	(0.075)	(0.035)	
Stearic Acid	0.050	0.060	P≤0.406
C18:0	(0.797)	(0.010)	
Oleic Acid	0.797	0.905	P≤0.361
C18:1	(0.302)	(0.118)	
Linoleic Acid	2.895	2.981	P≤0.879
[C18:2, Ω-6] (LA)	(1.314)	(0.850)	
α-Linolenic Acid	0.196	0.130	P≤0.368
[C18:3, Ω-3] (ALA)	(0.197)	(0.046)	
Dihomo-γ-Linolenic Acid	0.070	0.078	P≤0.754
[C20:3, Ω-6] (DGLA)	(0.054)	(0.043)	
Arachidonic Acid	0.983	1.354	P≤0.270
[C20:4, Ω-6] (ARA)	(0.620)	(0.671)	
Eicosapentaenoic Acid	0.114	0.186	P≤0.251
[C20:5, Ω-3] (EPA)	(0.131)	(0.111)	
Docosahexaenoic Acid	0.106	0.170	P≤0.134
[C22:6, Ω-3] (DHA)	(0.080)	(0.083)	

Table 3: Comparison between a Control (CO) and a Type 2 diabetic group (T2DM) (age & gender matched: >65 y all male) of the 'Netherlands Brain Bank' for human blood plasma of the Cholesteryl-ester (ChE) lipid fractions of the elongase /desaturase array of post-mortem brain samples of the same donor. Both groups 8 individuals [12].

In Figure 5 are the Cholesteryl-esters depicted of human blood-plasma for the Control versus the Type-2 Diabetes group. Clearly visible is that Linoleic Acid (C18:2, Ω-6, LA) is the major Poly Unsaturated Fatty Acid (PUFA) and can based on measured arbitrary amounts [dimensionless] (Table 1), be indicated as a biomarker for this ChE major lipid class. This biomarker is followed by Arachidonic Acid (C20:4, Ω-6, ARA) while the third biomarker in human blood plasma is a MUFA Oleic Acid (C18:1).

As mentioned earlier, the main objective of this manuscript is to indicate enzyme presence and possibly even activity in the brains of a human *post-mortem* control (healthy) and type 2 diabetes (T2DM) patient group. Present enzymes are shown in Table 4. What is striking is that there are no significant differences between the control and T2DM group for human plasma enzymes. What is immediately noticeable is that a whole series of desaturases is present. Within the MUFA group: Δ9-desaturase and Δ12-desaturase and within the PUFA group: Δ6-desaturase, Δ5-desaturase, Δ17-desaturase and Δ4-desaturase. Furthermore, the following elongase

enzymes are still present, within the MUFA group C14/16 elongase and C18:0/C16:1 elongase and within the PUFA group the C18/20 elongase and C20/22 elongase The most important observation to support our hypothesis for a 'genetic bottleneck theory' is that the 'chilling' enzyme Δ12-desaturase is present in human blood plasma. So, in this way not only the human brain but the whole body was protected against harsh cold environmental exposure and undercooling.

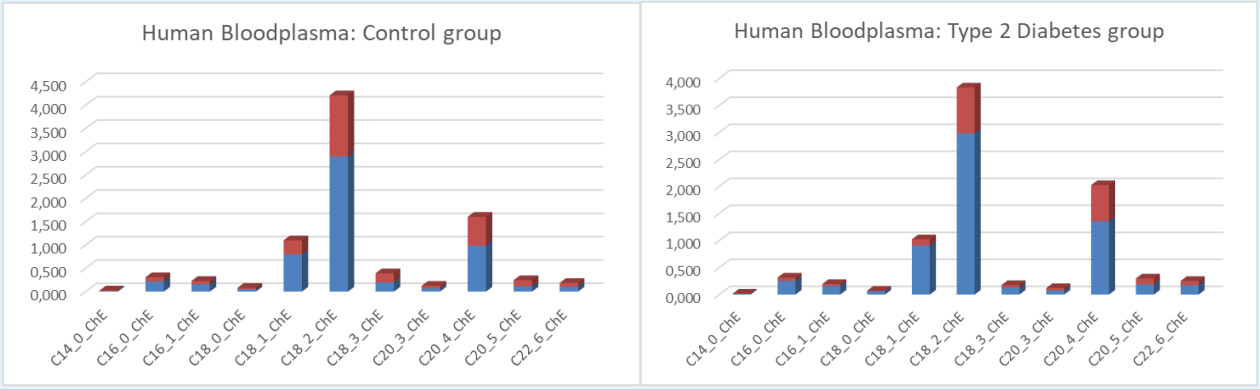


Figure 5: Biomarkers from the Cholesteryl-ester (ChE) fraction based on LC-MS- measurements at *post-mortem* human blood plasma for two different groups: a). Control group and a Type 2 Diabetes group. Per group depicted the Mean ± Std of 8 individuals. Blue colour is 'Mean' while red colour is 'Standard Deviation'.

Enzymatic Activity	Control (n=8) Mean ± std	Type 2 Diabetes (n=8) Mean ± std	Control *vs.*Type 2 Diabetes P-value
C14/16 elongase	19.609 ± 7.831	23.201 ± 8.695	P≤0.400
C16:1/C16:0 SCD1, Δ9 desaturase	0.742 ± 0.314	0.668 ± 0.218	P≤0.594
C18:0/C16:1 Elongase ELOVL1,3,6	0.318 ± 0.066	0.395 ± 0.133	P≤0.168
C18:1/C18:0 Stearoyl-CoA desaturase, SCD5, Δ9 desaturase	17.517 ± 4.262	15.436 ± 3.184	P≤0.287
C18:2/C18:1 'Chilling enzyme' Δ12-desaturase	3.506 ± 0.745	3.283 ± 0.903	P≤0.599
C18:3/C18:2 Δ6 desaturase	0.058 ± 0.050	0.045 ± 0.013	P≤0.485
C20:3/C18:3 C18/20 elongase	0.503 ± 0.302	0.596 ± 0.303	P≤0.551
C20:4/C20:3 Δ5-desaturase D5D or FADS1	15.858 ± 4.796	20.350 ± 9.236	P≤0.242
C20:5/C20:4 Δ17-desaturase	0.101 ± 0.074	0.147 ± 0.100	P≤0.308
C22:6/C20:5 C20/22 elongase & Δ4-desaturase	1.223 ± 0.433	1.085 ± 0.448	P≤0.541

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 human blood plasma material from the 'Netherlands Brain Bank' [12].

Figure 6 shows factor spectra of whole mouse brain (strain C57bl6), a control group, high-fat diet group and 24-hour starvation group. What is immediately noticeable is that the most important lipid fraction is the Phosphatidylcholines and the Plasmamalogens lipid fraction. Furthermore, it is noticeable -in comparison with *post-mortem* human neocortex- that in the Control group and 24 h starvation group of whole mouse brain no Triacylglycerols are detected. An important result - which goes beyond the central theme of this manuscript namely 'genetic bottleneck theory' - is the observation that Triacylglycerols are observed in the mouse whole brain in the 40-day High Fat diet-induced Insulin Resistant obese C57bl6 mouse model. This is our first observation of diet induced brain growth or 'encephalization' in a mouse model. This observation has been discussed in another document [24].

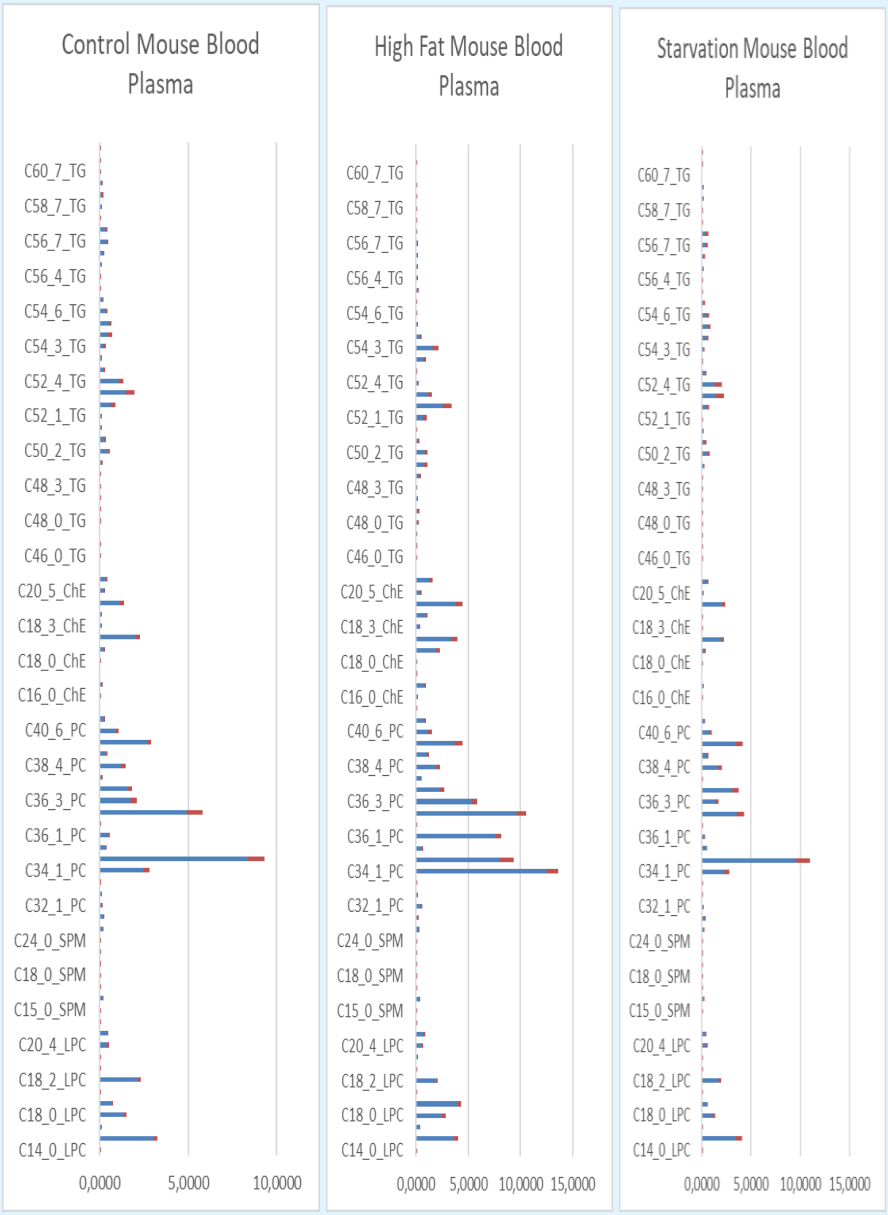
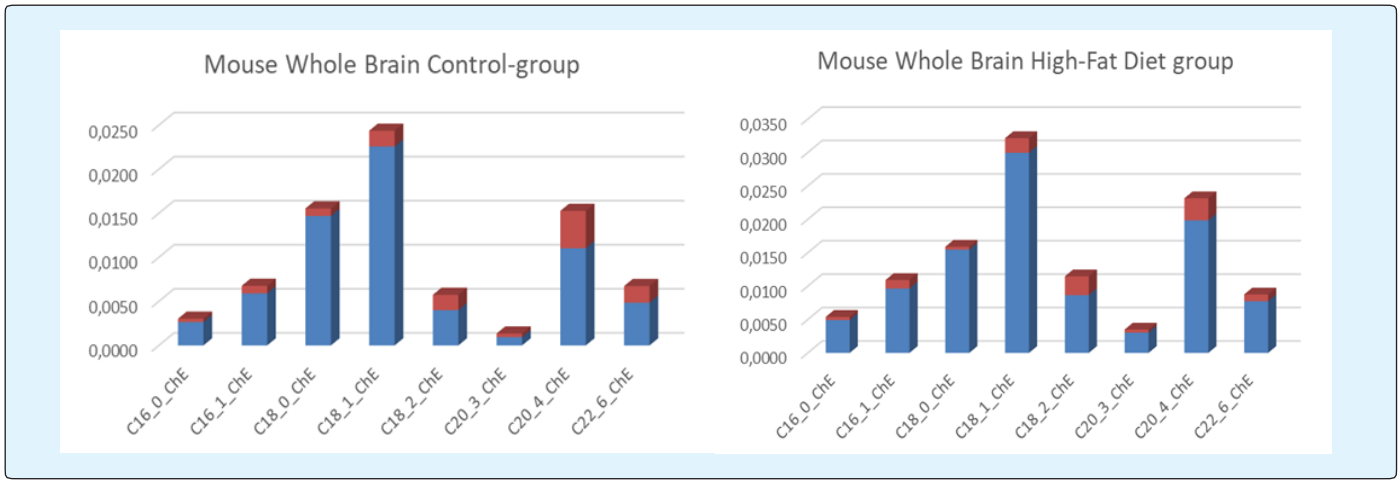


Figure 6: Factor-spectrum mean ± std of a C57bl6 whole mouse brain of a Control-chow group (n=5), a 40 days High-Fat diet group (n=5) and a 24 h starvation (n=5) C57bl6 rodent group.

In Table 5 are in whole mouse brain -with exception of Myristic Acid (C14:0)- all MUFAs present: Palmitic Acid (C16:0), Palmitoleate (C16:1), Stearic Acid (C18:0) and Oleic Acid (C18:1). And for the PUFAs the Ω-6 members such as Linoleic Acid (C18:2, Ω-6) LA, Dihomo-γ-Linolenic Acid (C20:3, Ω-6) DGLA, Arachidonic Acid (C20:4, Ω-6) ARA and the important Ω-3 'fish oil' Docosahexaenoic Acid (C22:6, Ω-3) DHA. In the comparison Control-High Fat Diet are all earlier mentioned lipids -with exception of the MUFA Stearic Acid (C18:0) (P≤0.176)- significantly different. In addition, in the comparison Control-Starvation all earlier mentioned lipids -with exception of the MUFA Palmitic Acid (C16:0) Acid (P≤0.016*)- are not significantly different. Furthermore, in the comparison for the MUFAs in the comparison High-Fat Diet - Starvation are -with exception of Palmitoleate (C16:1) which is significantly different (P≤0.036*)- all lipid comparisons not significantly different. For the earlier mentioned two PUFAs in the comparison High Fat Diet *vs*. Starvation the following observations are significantly different Dihomo-γ-Linolenic Acid (C20:3, Ω-6) DGLA and Arachidonic Acid (C20:4, Ω-6) ARA with respectively (P≤0.023*) and (P≤0.030*). The other measured PUFAs Linoleic Acid (C18:2, Ω-6; LA) and Docosahexaenoic Acid (C22:6, Ω-3; DHA) are not significantly different.

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 5: 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 [12].



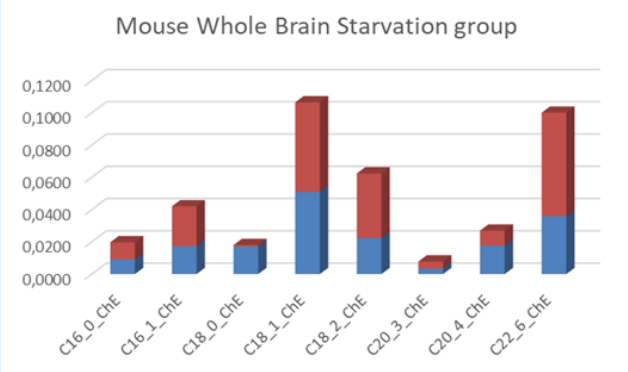


Figure 7: Biomarkers from the Cholesteryl-ester (ChE) fraction based on LC-MS- measurements at whole moue brain for three different groups: a). Control Chow; b). High- Fat Diet; 24 h Starvation. Oleic Acid [C18:1] is the major Mono Unsaturated Fatty Acid (MUFA) and can based on measured arbitrary amounts [dimensionless] (Table 1), be indicated as a biomarker for this ChE major lipid class. Per group depicted the Mean ± Std of 5 individuals. Blue colour is 'Mean' while red colour is 'Standard Deviation'.

From LC-MS measurements of whole mouse brain, we can clearly see in (Figure 7) that the MUFAs have a slow ascending order from Palmitic Acid (C16: 0) towards Oleic Acid (C18: 1) for the Control and High-Fat diet group. For the starvation group solely Oleic acid (C18:1) is with a large standard deviation the largest compound. For the PUFAs for Control and High- Fat diet group Arachidonic Acid (C20:4, Ω-6; ARA) is the largest compound while again for the starvation group with large standard deviation the Ω-3 PUFA Eicosapentaenoic Acid (C20:5, Ω-3; EPA) has the highest value.

Based on product/precursor ratio's for whole mouse brain we could calculate enzymatic activities. The most important observation in order to prove the 'genetic bottleneck theory', also for small mammals, is the demonstration of the Δ 12 desaturase 'chilling enzyme' for the whole brain of the common house / laboratory mouse (strain: C57bl6) which is indeed the case for all three groups Control, High-Fat diet and 24 h starvation. Other desaturase enzymes present in all three groups are Δ9- (SCD1), Δ9- (SCD5) and Δ5- (D5D)-desaturase. One C18:0/C16:0 elongase enzyme is present in all three groups. No clear pattern is visible in significance in the pairwise comparison of all three groups (Tabel 6).

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 'Chilling enzyme' 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 6: 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 [12].

In Figure 8 are the factor spectrum images depicted for blood plasma of juvenile male C57bl6 mouse model split up to three groups Control-chow, 40 days High-Fat diet and 24 h starvation. As can be seen from these factor spectra (Figure 6), the most important components that make up the blood plasma (measured by our LC-MS method) are in to some extent the Triacylglycerols (TGs), Cholesteryl-esters (ChE), Lyso-phosphatidylcholines (LPC) while the most abundant are the Phosphatidylcholines (PC) as major lipid group. At first glance, lipid components within the groups seems to be affected by the treatment exposed to.

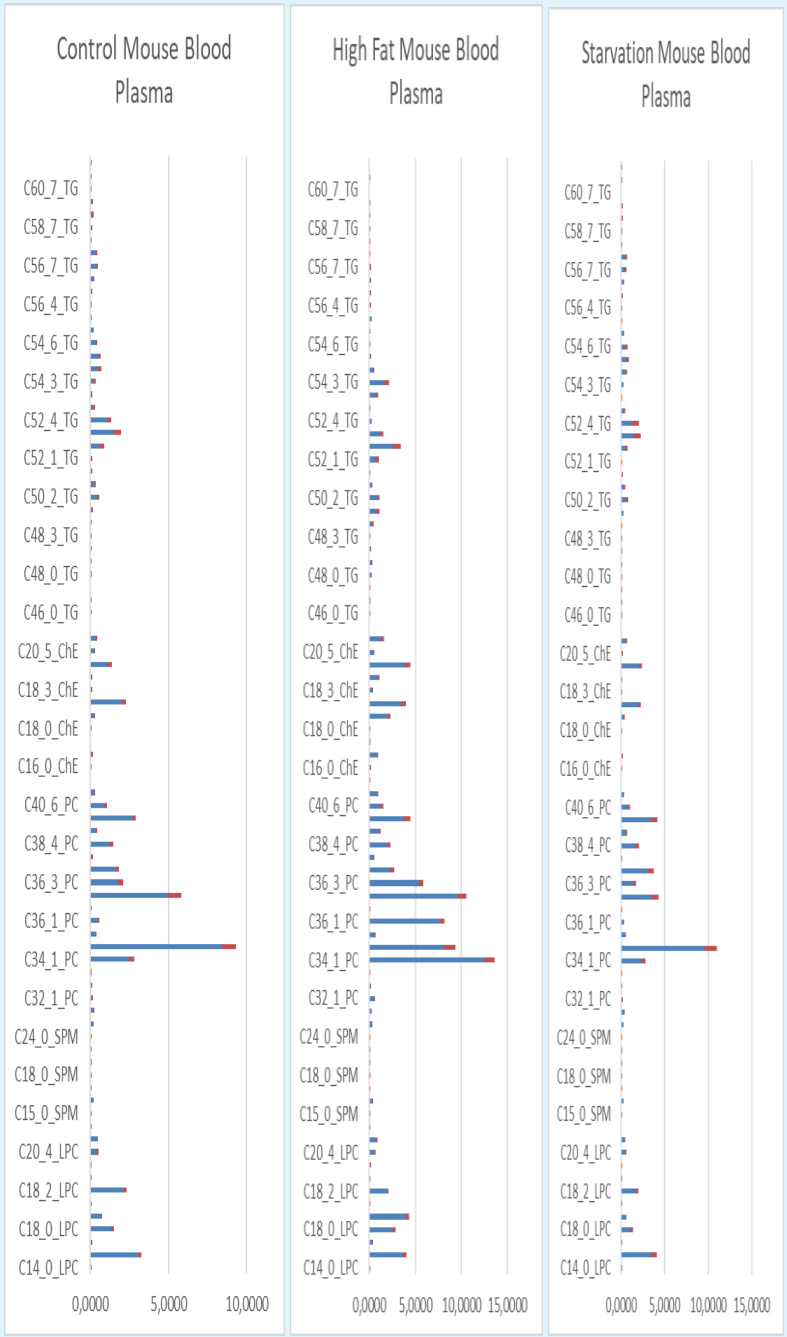


Figure 8: Factor-spectrum mean ± std of the blood plasma of a C57bl6 mouse model of a Control-chow group (n=5), a 40 days High-Fat diet group (n=5) and a 24 h starvation (n=5) C57bl6 rodent group.

In Table 7 are in the mouse blood-plasma of this C57bl6 strain the following lipid components found for MUFAs and PUFAs. The MUFAs consist of Myristic Acid (C14:0), Palmitic Acid (C16:0), Palmitoleate (C16:1), Stearic Acid (C18:0) and Oleic Acid (C18:1). It is important to remark that we found another lipid compound in whole mouse plasma not reflected in the central scheme of Figure 9 (Material & Methods). This 'new' lipid compound is the MUFA: Hexadecadienoic acid (C16:2).

Mouse-model C57bl6 Blood plasma using LC-MS techniques	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-3] (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 7: 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 [12].

For the PUFAs the Ω-6 members such as Linoleic Acid (C18:2, Ω-6) LA, Dihomo-γ-Linolenic Acid (C20:3, Ω-6) DGLA, Arachidonic Acid (C20:4, Ω-6) ARA and for the Ω-3 'wing' the essential Fatty Acid (FA) α-Linoleic Acid (C18:3, Ω-3) ALA and in the end of the schedule (Figure 9 Material & Methods) the important Ω-3 'fish oils' Eicosapentaenoic Acid (C20:5, Ω-3) EPA & Docosahexaenoic Acid (C22:6, Ω-3) DHA. In the comparison Control *vs.* High Fat Diet all earlier mentioned lipids are with exception of the PUFAs Linoleic Acid (C18:2, Ω-6) (P≤0.1432) and Eicosapentaenoic Acid (C20:5, Ω-3) (P≤0.1710) significantly different. In addition, in the comparison Control-Starvation the following lipids were significantly different: Myristic Acid (C14:0) (P≤0.0115*) & Hexadecadienoic acid (C16:2) (P≤0.0065**) for the MUFAs while Linoleic Acid (C18:2, Ω-6) LA, (P≤0.0145*); α-Linoleic Acid (C18:3, Ω-3) ALA (P≤0.0105*) & Eicosapentaenoic Acid (C20:5, Ω-3) EPA, (P≤0.002**) for the PUFAs. In the comparison of the High-Fat diet group *vs.* 24 h starvation all lipid compounds were significantly different with one exception: Eicosapentaenoic Acid (C20:5, Ω-3) (P≤0.1529), which was not significantly different.

In (Figure 9) are the lipid compounds of this C57bl6 mouse model depicted and clearly visible is that Linoleic Acid (C18:2, Ω-6, LA) and Arachidonic Acid (C20:4, Ω6, ARA) are the major Poly Unsaturated Fatty Acid (PUFA) and can based on measured arbitrary amounts [dimensionless], be indicated as a biomarker for this ChE

major lipid class. Remarkable in the High-Fat diet C57bl6 mouse group the MUFAs Palmitoleate (C16:1) and Oleic Acid (C18:1) as well as the PUFAs Dihomo-γ-Linolenic Acid (C20:3, Ω-6) (DGLA) & Docosahexaenoic Acid (C22:6, Ω-3) DHA rise tremendously, probably as a consequence of the nutritional intervention with exposure for 40 days to the High-fat diet.

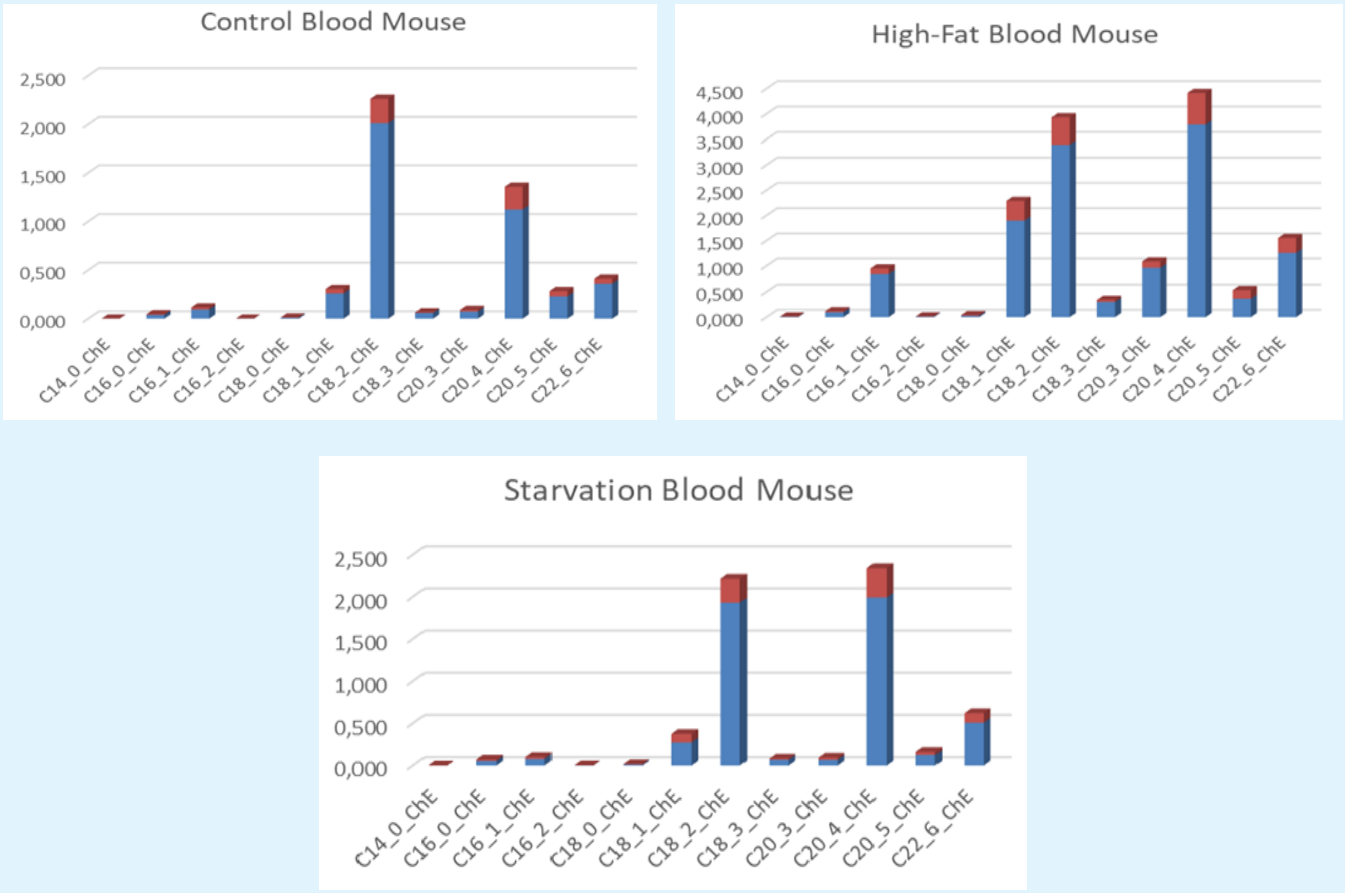


Figure 9: Biomarkers from the Cholesteryl-ester (ChE) fraction based on LC-MS- measurements at mouse blood plasma for three different groups: a). Control group; b). High-Fat diet group; c). 24 h starvation group. Per group depicted the Mean ± Std of 5 individuals. Blue colour is 'Mean' while red colour is 'Standard Deviation'.

The main theme of this publication is additional physiological / biochemical evidence for a 'genetic bottleneck' theory some 75,000 years ago as a result of a rapid violent glacial time- period lasting some 1,000 years that, according to our initial hypothesis, could only be survived if organisms had a chilling enzyme Δ12 desaturase at their disposal to keep the body core temperature and also that of the brain in the physiological range. So, our initial hypothesis states that only the survivors of this recent rapid ice time possessed this chilling enzyme and that this must now be demonstrated in the offspring. We have done this before in Table 2 for modern humans (*post-mortem* neocortical brain homogenate) & Table 4 (blood-plasma) and now in Table 6 (whole brain) & Table 8 (blood-plasma) for the normal house / laboratory mouse (strain: C57bl6). This enzyme has so far only been demonstrated in single-celled organisms and an insect (see introduction and discussion) and now in this manuscript also in humans and a smaller mammalian species the mouse. In addition, the following desaturases were observed in blood plasma of this small rodent mammal (C57bl6): the desaturases Δ9 (SCD1), Δ9 (SCD5), Δ12, ('Chilling enzyme'), Δ6 (C18:3/C18:2), Δ5 (D5D), Δ17 (C20:5/C20:4) and Δ4-desaturase. The significant differences in enzymatic activities between the

experimental groups in this C57bl6 mouse are given in Table 8.

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 'Chilling enzyme' 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 Δ6 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/C20:3 Δ5-desaturase D5D or FADS1	12.751 ± 0.629	3.988 ± 0.833	15.377 ± 1.262	P≤0.00001***	P≤0.0062**	P≤0.00001***
C20:5/C20:4 Δ17-desaturase	0.425 ± 0.016	0.094 ± 0.031	0.209 ± 0.051	P≤0.0.00001***	≤0.0.0004**	P≤0.0039**
C22:6/C20:5 C20/22 elongase & Δ4-desaturase	0.903 ± 0.043	3.759 ± 0.925	1.612 ± 0.312	P≤0.0013**	P≤0.0066**.	P≤0.0047**

Table 8: 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 [12].

Discussion

According to the 'genetic bottleneck theory', between 50,000 and 100,000 years ago, human populations have fallen sharply to 3,000-10,000 surviving individuals [25]. It is supported by a number of genetic observations suggesting that the current human is descended from a very small population of between 1,000 and 10,000 breeding pairs that existed around 70,000 years ago [13]. Proponents of the controversial 'genetic bottleneck theory' suggest that the eruption of the Toba resulted in a global ecological disaster, including destruction of vegetation along with severe drought in the tropical rain forest belt and in monsoon regions. For example, a 10-year volcano winter caused by the eruption may have largely destroyed human food sources and has greatly reduced the population size [26]. These environmental changes may have caused 'population bottlenecks' in many species, including hominids [27]. This, in turn, may have accelerated differentiation from within the smaller human population. Therefore, the genetic differences between modern humans reflect changes over the past 70,000 years, rather than gradual differentiation over hundreds of thousands of years [25]. The point that humanity has survived from a population of 10,000 to the present about 7 to 8 billion might be the case. It is supported by some genetic evidence that suggests that today's humans are descended from a very small

population of between 1,000 and 10,000 breeding pairs that existed about 70,000 years ago. According to the National Human Genome Research Institute (NHGRI) about 99.9 percent of the DNA sequence is identical in all people [28], which give supportive evidence for a small group of common ancestors and a 'genetic bottleneck theory' for *Homo sapiens*. So, we assume that the current world population of mankind is descendants of this small population that survived the outbreak of the global climate 'glacial winter' lasting around 1,000 year some 75,000 years ago [29]. The Toba volcano eruption has been associated with a 'genetic bottleneck' in human evolution about 70,000 years ago [14,30], which was possibly the result of a serious decrease in the total human population as a result of the Toba event which is the most super volcano burst ever studied [26,31]. Some evidence points to 'genetic bottlenecks' in other animals in the aftermath of the Tobaeruption. The populations of the Bornean orangutan [32], central Indian macaque [33], cheetahand tiger [34], all recovered from very low numbers around 70,000-55,000 years ago. In addition, the separation of the nuclear gene pools from eastern and western lowland gorillas in Africa has been estimated to have happened around 77,700 years ago Thalmann O, et al. [35] which could be indicative for a 'multiple bottleneck theory' and consequently a global disaster. Our observations at the common house mouse (*Mus musculus*) -an experimental animal presently multiple used in scientific laboratory- support this theory because as we demonstrated in the Results paragraph the 'chilling enzyme' Δ12 desaturase was also found in whole brain and blood plasma of a C57bl6 mouse strain. The laboratory mouse originates from ancestors in the Middle East in the area that is now Pakistan. Genome analysis has confirmed that the laboratory mouse is a blend of these four different species or subspecies of the genus *Mus*: *Mus musculus musculus* (eastern Europe), *Mus musculus domesticus* (western Europe), *Mus musculus castaneus* (south-east Asia), and *Mus musculus molossinus* (Japan). The laboratory mouse genome is mostly from *M. m. domesticus*, ranging from estimates of 68% to 92% on average, while the C57bl6 mouse strain was developed as inbred strain. So, the origin of the region of the genes encoding the genome of this C57bl6 was probably to most extent determined by *Mus musculus domesticus* (western Europe) [36]. From this 'history map' of the genes of the C57bl6 mouse strain we can cautiously conclude that if ≈75,000 years ago the Toba volcano eruption took place, their ancestors of *M. m. domesticus* has been Darwinian selected in their area of origin: western Europe [36]. So, based on this information we can make two conclusions: a). Other small (prey) mammals were also affected by the 'Toba glacial winter'; b). This glacial winter also affected the region western Europe and was probably global.

Modern humans are a vivid account of the most recent complexity theory to explain the emergence of *Homo sapiens* in Africa some hundred thousand years ago - 'Out of Africa theory' [1,4] and the rapid and unprecedented spreading of our species in a variety of environments in Australia and Eurasia, including the Arctic (Siberia) [17], and the Beringia land bridge between Eastern Siberia and present-day Alaska. Man lived in the Americas at the end of the last ice age, or more specifically known as the late glacial maximum, about 16,000 - 13,000 years before present [18], starting between 75,000 and 60,000 years ago. Archaeological discoveries since 2000, however, show that *Homo sapiens* occupied the high- lying geographical area between Northeast Asia and Northwest North America (that is Beringia [19], before 30,000 years ago and the last glacial maximum (LGM) [37]. Generally, the brain and body are protected from exposure to cold and hypothermia from dry clothing [38].

Following on from Darwinian thought, we have earlier postulated the hypothesis that the way modern man lived at that time as hunter-gatherer and travelled like nomads exploring new areas in search for new food resources, they were exposed to new cold, harsh environments such as Arctic and Siberia [17,18]. Consequently, this way of living required physiological and biochemical adjustments to body and brain. Here, we have demonstrated on the basis of a Systems Biology approach Kitano H [21] following *lipidomics* performed studies with LC-MS techniques Van Ginneken V, et al. [24, 39-41] at human *post-mortem* neocortex material [41] and the common whole mouse brain (strain C57bl6) Van Ginneken V [24] contain a 'chilling enzyme' Δ12 desaturase to protect the brain from hypothermia under extreme conditions of exposure to extreme cold. We hypothesize that these survivors were (pre)selected due to the Toba volcano eruption Robock A, et al. [26], Rose WI, et al. [31] followed by a rapid glacial period of around 1,000 year [29]. Because the survivors of this disaster had this Δ12-desaturase 'chilling enzyme' in their brains they could cope with the Siberian cold and other harsh cold environmental conditions in their search for food and new ecological habitats.

So far, this 'chilling' enzyme Δ12 desaturase has only been found in single-celled organisms such as cyanobacteria the gut bacterium *Escherischa coli*, yeast but also in multicellular organisms like the house cricket *Acheta domesticus* [6-11]. It occurs in these organisms after cold exposure in order to prevent that the 'fluid

mosaic membrane' - consisting of Polyunsaturated Fatty Acids (PUFAs) Batcabe JP, et al. [11] was biochemical damaged by adaptations of the Cholesteryl esters, the building blocks of these PUFAs. Poikilotherms (prokaryotic and eukaryotic) must quickly adapt to temperature stress by cooling to survive. In an early study at these species an Δ12 fatty acid bifunctional desaturase enzyme has been identified as the key to low temperature adaptation [42]. The activity of this enzyme is mainly increased by gene expression and new protein synthesis. In addition, this enzyme is independently induced by oxygen and temperature, while cooling alone is not sufficient to increase enzyme activity. Cooling and reduced temperature cause an increase in desaturase activity, increased membrane unsaturation and subsequently fluidity [43]. 'Membrane regulation' for stress responses such as exposure to cold in higher 'warm-blooded' vertebrates - such as the common house mouse and other rodents or humans - has hardly been studied. In poikilotherms [36-44], membrane rigidification may be the first response to cold perception: reducing the fluidity of membranes from membranes at physiological temperatures is associated with increased cold-inducibility of a number of genes, including desaturases. Stress-induced remodeling of membrane lipids can influence generation, transduction and deactivation of stress signals, either by global effects on the fluidity of the membrane matrix, or by specific interactions of boundary (or fluent) lipids with receptor proteins, lipases, ion channels, etc. The data from point to membranes not only as targets of stress, but also as sensors in triggering a stress response [45].

In order to test our two hypotheses, enzymatic activity in the *post-mortem* human neocortical brain and in the whole mouse brain, Δ12 desaturase enzymatic activity was calculated as earlier performed based on product-precursor ratios [24,40,41]. The expression of genes for desaturase -with emphasis on the 'chilling' enzyme Δ12-desaturase- is very important since it provides the molecular basis for the acclimation of organisms to changing environmental temperatures. In particular -until presently studied- in the case of plants, cyanobacteria yeast, gut bacteria and insects [5-7,8-10,46].

Finally, in the biosynthesis of essential fatty acids (EFA), the two 18 C-atoms are Linoleic acid (C18: 2; Ω6) (LA) and α-linolenic acid (C18: 3, Ω3) (ALA) - both polyunsaturated fatty acids (PUFAs): an elongation is alternated with different desaturases by repeatedly inserting an ethyl group and then forming a double bond (Figure 9). Maintenance of the structure and function of membranes in cells is important when the temperature changes and the membrane is under pressure. Fatty acid desaturases are enzymes that introduce double bonds in fatty acyl chains [11,47].

The premise of this manuscript is cold exposure (hypothermia) can be tolerated by numerous species: and even man's tolerance to cold exposure may be enhanced if adaptive mechanisms of these organisms can be identified and made applicable to human biology. Much progress is being made towards understanding mechanisms that allow numerous species to tolerate cold exposure or 'chilling' via a mechanism of torpor or 'suspended animation'. In case of the eruption of the Toba volcano Rampino MR, et al. [13] followed by a rapid short global glacial period of about 1,000 years [14] like we earlier mentioned, this might have had a tremendous impact on a global scale for unicellular and multicellular organisms including (small) mammals like the common mouse *(Mus musculus*) but even nearly wiped out modern man (*Homo sapiens*). Our Systems Biology [21] *lipidomics* based observations at brains of mouse and modern man give supportive evidence for the 'genetic bottleneck theory' - which has been already been demonstrated for a number of species earlier mentioned in this manuscript- so we cautiously conclude the Toba volcano glacial winter of around 1,000 years resulted in a multiple nearly wipe out of many species.

The exact geographical distribution of anatomically modern human populations at the same time of eruption is not known, and surviving populations may have lived in Africa and then migrated to other parts of the world. Analysis of mitochondrial DNA 60,000-70,000 years ago, corresponds to dating from the eruption of the Toba until about 75,000 years ago [48]. The most significant "recent" wave occurred about 70,000 years ago via the so-called "southern route", which spread rapidly along the coast of Asia and reached Australia about 65,000-50,000 years ago [15], while Europe was populated by an early spur which established the Near East and Europe less than 55,000 years ago [16]. Subsequently, people lived in the Americas at the end of the last ice age, or more specifically what is known as the late glacial maximum, about 16,000 - 13,000 years before the current beginning between 75,000 and 60,000 years ago. Archaeological discoveries since 2000, however, reveal that *Homo sapiens* occupied the latitude between Northeast Asia and Northwest North America, that is Beringia before 30,000 years ago and the Last Glacial Maximum (LGM). So, this small group of about 15,000 people crossing the Beringia land bridge between East Siberia and present- day Alaska [17,18], were the descendants and survivors of the eruption of the Toba volcano≈75,000 years ago Rampino MR, et al. [13]

followed by a brief, dramatic cooling or 'volcanic winter' or ice age of around 1,000 y, which resulted in a drop of the global mean surface temperature by 3- 5°C [14]. So, this group that populated America already had the advantage that they were pre- selected - by the cold period after the eruption of the Toba volcano - on the Δ-12 desaturase 'chilling' enzyme in the human brain. The eruption of the Toba volcano - followed by a short intensive cold period Rampino MR, et al. [13] was the first evolutionary pressure of selection by cold exposure for humanity. While the second was during the crossing of around 5,000 breeding pairs via the frozen Beringia land bridge to both Americas [19]. This group was also selected evolutionarily by exposure to cold, but had the advantage that their ancestors had survived the eruption of the Toba volcano and consequently were exposed to an intensive rapid cold period. In other words, were preselected via the brain enzyme Δ-12 desaturase 'chilling' enzyme, on cold exposure.

In Medical Sciences, cold exposure of the human brain could also have the potential in practical implications like hypothermic neurosurgery [49]. Presently, with an overcrowded planet reaching around ten billion people at the midst of the 21st century, induced hibernations in combination with brain hypothermia -also called synthetic torpor- could be useful for applications such as long-duration space-flight Griko Y [50] in a further expansion of humanity towards *example gratia* Mars planet.

Material and Methods

Brain-Tissue

Human Brain tissue (neocortex) was obtained from the "Netherlands Brain Bank". Causes of death causes were earlier presented (Table 1) [41]. Time of *post-mortem* dissection varied between 4.4 and 24.5 hours (Table 1) [41]. The region of *post-mortem* collection of the tissue material was in all cases the (neo) cortex (Table 1) [41]. Visually, a clear separation could be made between grey and white matter in the cortex. A tissue homogenate of the brain tissue (grey or white; ~10% wet weight/ volume) in phosphate-buffered saline (PBS) was made with the Mini-beat Beater.

Blood Plasma

Blood was centrifuged at 8000 x *g* for 5 min. The plasma was aliquoted and stored at -80ºC at the "Netherlands Brain Bank" pending analysis.

LC-MS of Lipids and Fatty Acids in Blood Plasma of Mouse and Men

Lipids and free fatty acids (FFA) were analysed with electrospray LC-MS as earlier performed [24,39,40,41]. Samples, 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 cholesterol ester and heptadecanoic acid (C17:0)). After mixing and centrifugation the supernatant was transferred to an autosampler vial. Lipids were separated on a 150 x 3.2 mm i.d. 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 gradients were as follows: 0-2 min - 20%B, 2-3 min - 20% to 80%B, 3-15 min - 80% to 100%B, 15-25 min - hold 100%B, 25-32 min -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.

The FFA LC-MS platform employs the same sample and similar HPLC conditions as the lipid method. The ammonium acetate concentration is 2 mM instead of 5 mM and no formic acid was added. The gradient: 0-2 min - 30%B, 2-3 min - 30% to 70%B, 3-10 min - 70% to 100%B, 10-15 min - hold 100%B, 15-20 min -condition at 30% B. Detection of FFA is performed in negative ion ESI mode. Combined the two methods provide (semi) quantitative data for approximately 200 different identified lipids and FFA.

Each extract was injected three times (10 µl), once for the LC-MS FFA platform and two times for the LC-MS lipid platform. Furthermore, a quality control (QC) sample was prepared by pooling the samples. The pool was divided into 10 µl aliquots that were extracted the same as the study samples. The QC samples were placed at regular intervals in the analysis sequence (one QC after every 10 samples). The QC samples served two purposes. The first is a regular quality control sample to monitor the LC-MS response in time. After the response has been characterized, the QC samples were used as standards of unknown composition to calibrate the data.

In plasma samples, the 6 dominant lipid classes observed with these two methods are the lyso-phosphatidylcholines (IS used: C17:0 lyso-phosphatidylcholine), phosphatidylcholines (IS used: di-

C12:0 phosphatidylcholine), sphingomyelines (IS used: di-C12:0 phosphatidylcholine), cholesterylesters (IS used: C17:0 cholesteryl- ester), triacylglycerols (IS used: tri-C17:0 glycerol ester), and free fatty acids (IS used: C17:0 FFA) (Figure 1, Annex 1). In addition to these lipids, the extracts also contain minor lipids, but these were either not detected (concentration too low relative to very abundant lipids like phosphatidylcholines and diacylglycerols, DG) or they were not included in data processing with exception of the DG. The LC-MS lipid and LC-MS FFA data were processed using the LC- Quan software (Thermo).

LC-MS of Lipids and Fatty Acids in Brain Homogenate of Mouse and Men

Fifty µL of the brain homogenate was mixed with 1000 µL IPA containing 4 internal standards. Samples were placed in an ultrasonic bath for 5 minutes. Thereafter, samples were centrifuged at 10,000 rpm for 3 minutes followed by injection of 10 µL 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). Internal standard C24:0 PC was used to correct the data for small variations in analysis, such as sensitivity and injection volume.

In brain homogenate, the 8 dominant lipid classes observed with these two methods are the lyso-phosphatidylcholines (IS used: C17:0 lyso-phosphatidylcholine), plasmalogens, phosphatidylcholines (IS used: di-C12:0 phosphatidylcholine), sphingomyelines (IS used: di- C12:0 phosphatidylcholine), ethanolamines, cholesterylesters (IS used: C17:0 cholesteryl ester), triacylglycerols (IS used: tri-C17:0 glycerol ester), and free fatty acids (IS used: C17:0 FFA) [24,39,40,41].

Analysis of Plasmalogens

Analysis of cholesterylesters (ChE), lysophosphatidyl-cholines (LPC), phosphatidylcholine (PC), sphingomyelin (SPM) and triacylglycerols (TG) were based on molecular mass and retention time using internal standards. Because no standards for the plasmalogens exist, mass lists determined by using atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) mass spectrometry (MS) techniques were used [20]. These mass lists were published on internet www.byrdwell.com/plasmogens and www.byrdwell.com/- PhosphatidylEthanolamine.

Calculating Enzymatic Activity

The elongase- and desaturase- enzymes are alternately active in the synthesis of long chain unsaturated fatty acids. The elongase-enzyme extends the carbon chain with two carbon atoms.

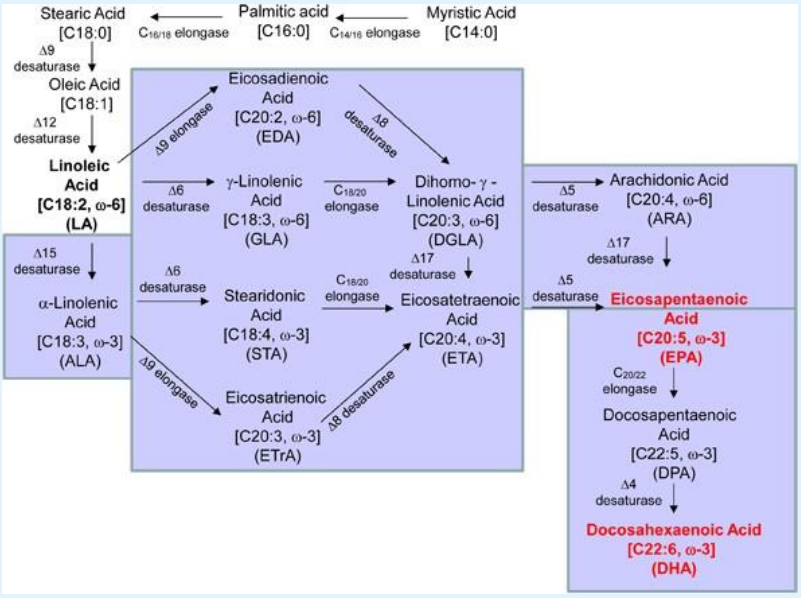


Figure 10: Biosynthesis of long-chain ω-6 and ω-3 polyunsaturated fatty acids from dietary commodities following an elongase-desaturase series in vertebrates and mammals. Enzym activities can be calculated based on product-to-precursor ratios of individual measurement of fatty acids (FAs) [12].

The desaturase enzyme makes a carbon-carbon double bond in the extended fatty acid chain. Both types of enzymes are specifically active for carbon atoms at a certain distance from the carboxyl group. Two series of unsaturated compounds arise. These are referred to in the literature as ω-3 fatty acid and ω-6 fatty acid. To date, higher mammals are believed to have three desaturases delta5, delta6 and delta9 fatty acyl CoA desaturases. These enzymes have a broad chain length specificity and occur mainly in liver [51,52]. However, they are incapable of desaturation beyond the C9 carbon atom. Nevertheless, certain polyunsaturated fatty acids are vital for maintaining health, in particular the ω-6 family members, dihomo-γ-linoleic acid and arachidonic acid. These are 20 carbon chain fatty acids that are precursors of the eicosanoid hormones, i.e. the prostagladins, thromboxanes and leukotrienes, which contain 20 carbon atoms [51,52]. In this evolutionary related hypothesis-manuscript we will limit ourselves to the 'chilling enzyme' Δ12 desaturase, the conversion step from Oleic Acid [C18:1] towards Linoleic Acid [C18:2, Ω-6, LA] (Figure 10).

Statistics

For all measured parameters the mean value of the control *post-mortem* human group was compared to the mean value of the *post-mortem* T2DM human group. Statistics were performed via SPSS using one-way ANOVA for differences between control and T2DM groups. $P \leq 0.05$ was considered as statistically significant. Normality of the data and homogeneity of variances were checked by Kolmogorov-Smirnov and F_{max} tests, respectively [53]. In *lipidomics* the number of objects/subjects is often small and therefore a statistical method often used is classification: the assignment of subjects to discrete categories [12]. In our case healthy (n=8) or Diabetic 2 (T2DM, n=8) for human brain & human blood plasma and a Control-chow C57bl6 mouse group (n=5), an Insulin Resistant (IR) High Fat diet induced obese C57bl6 mouse group (n=5) and a 24 h starvation C57bl6 mouse group (n=5) for whole brain homogenate and blood plasma.

Data Availability

All relevant data are available upon request.

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