Fatty acid profile in mice is modified by *Silybum marianum* oil supplementation in butter

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Butter, a dairy product, is rich in saturated fatty acids (FA). Its consumption was thought to be associated with cardiovascular disease (CVD). FA profile is associated to CVD and is influenced by *Silybum marianum* (SM). This study aims to investigate the effect of butter and SM oil supplementation in butter on the FA profile in mice red blood cells (RBC) and liver. Three groups are included: group feeding on a normal diet, group feeding on a normal diet plus 10 % butter and group feeding on a normal diet plus butter supplemented with SM oil (1%). In red blood cells, butter consumption is associated with increased total saturated FA (SFA) and decreased total unsaturated FA (UFA) mainly monounsaturated FA (MUFA). The supplementation of SM oil decreased further trans FA. However, for the liver, butter consumption did not affect the total SFA and UFA but SM oil supplementation is associated with a significant decrease in SFA and increase in UFA. The conclusion was that SM oil supplementation is in favour of a protective FA profile in mice.
Keywords: Butter, desaturase, fatty acids, mice, silybum marianum oil

INTRODUCTION

Numerous studies have examined the association between dietary fatty acids (FA) and the development of diseases, particularly cardiovascular diseases [1]. It is widely recognised that total saturated fatty acids (SFA) are linked to cardiovascular disease. Numerous investigations, however, contradicted this theory by demonstrating a neutral relationship between the total SFA and coronary heart disease [2]. According to other studies that focused on particular saturated fatty acids, increased long-chain SFA (LCSFA: C12 to 18) in the diet has been linked to an increased risk of cardiovascular disease [3]. Butter has a significant amount of these saturated fatty acids, yet there has been controversy over its link to cardiovascular disease and all-cause mortality. Butter was associated with myocardial infarction [4] and inversely associated with the risk of major adverse coronary events (cardiovascular disease, stroke…) [5]. However, it was inversely linked to diabetes [6].
Moreover, a blood FA profile has been linked to the risk of developing multiple diseases including diabetes [7], a cardiovascular disease [8], and breast cancer [9]. In fact, FA in blood and enzymes implicated in FA metabolism could be considered as a disease predictor [10].
The investigations available are not many, though, examining the connection between blood and dietary FAs. The majority of research focuses on the relationship between dietary FA and blood lipids specifically triglycerides, cholesterol (HDL and LDL) rather than blood FA [11]. It will be of great interest to investigate if dietary FA's impact on blood FA could explain the link between disease and diet. However, the blood concentration and nature of fatty acids depend on FA food intake, intestinal FA absorption, and FA metabolism. Any disturbance in those three pathways impact the blood fatty acid profile. As an example of factors that may influence those pathways, we

can include i/ FA supplemented diet [12] ii/ Alcohol extract of nutmeg downregulated the expression of fatty acid synthase [13] iii/ Sake lees extract ameliorates the hepatic lipid accumulation via suppressing fatty acid-induced intracellular lipid accumulation[14] iiii/ Water extract of Curcuma longa L. suppressed the expression levels of fatty acid transport proteins[15] iiiii/ Pomegranate seed oil and bitter melon extract affect the fatty acid composition and metabolism in the hepatic tissue in rats [16] iiiiii/ Grape skin extracts affect the Stearoyl-CoA Desaturase-1 expression in Caco 2 cells [17]. In fact, the fatty acid profile is influenced by oil and extract added to food.

Silybum marianum (SM) or milk thistle is common in the central region of Tunisia. It has been known to be used as a medicinal plant because it contains silymarin [18]. Silymarin is composed of a group of polyphenolic flavonoids with excellent hepatoprotective activities and hypocholesterolemic, neuroprotective, skin-protective and chemoprotective activities [19]. These biological activities are attributed to the antioxidant properties of silymarin [20]. Additionally, SM oil has been shown to attenuate hepatic steatosis and oxidative stress in high fat diet-fed mice [21]. Silybum marianum has a strong potential for usage in a variety of different disciplines besides medicine, including: human and animal nutrition, the cosmetic industry, phytoremediation, and bioenergy products [22].

The objective of our study is to investigate the relationship between butter consumption and the fatty acid profile in mice red blood cells (RBC) and liver, and then to see if the S.M oil supplementation to butter affect this relationship.

MATERIALS AND METHODS

BIOLOGIC MATERIALS

Silybum marianum (SM) oil (provided by huilerie Ben Selma) was extracted by cold pressure from seeds. The plant was collected from the central area of Tunisia.

Cow butter was purchased from the local market.

EXPERIMENTAL PLAN

Male Swiss mice, weighing between thirty and thirty-five grams were provided by the Society of Pharmaceutical Industries of Tunisia (SIPHAT). To discard the effect of sex hormones on fatty acid metabolism, male mice were used. Before starting the experiment, the mice were acclimatised for two weeks. Polyethylene home cages were used with sawdust covering the floor. They were maintained under controlled conditions: 12/12 h light/dark cycle, Temperature close to 22°C (21-23) with water and food (standard commercial pellet chow) offered ad libitum. The mice were housed in accordance with the EEC 609/86 Directives regulating the welfare of experimental animals. The experimental protocol was approved by the Ethics Committee for Research in Life Science and Health of the Higher Institute of Biotechnology of Monastir (CERSVS/ISBM011/2024) in compliance with the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) [23]. .

Three groups of five mice each were created: Group1 (control group): following a normal diet, Group 2 (Butter group): feeding a normal diet enriched with 10% commercial butter, Group 3 (SMB group): feeding a normal diet enriched with 10% commercial butter supplemented with SM oil (1% and stored at 4°C).

The experience lasted one month. The animals were ultimately sacrificed. The red blood cells were obtained after manipulating blood and liver samples were collected and stored at -80°C for analysis.

ANALYTICAL METHODS

According to the analytical techniques outlined in the European Union Regulations EEC 2568/91 and EEC 1429/92, the peroxide value (PV), free fatty acid content (% of oleic acid), and UV absorption characteristics at 232 nm (K232) and 270 nm (K270) were measured [24].

To determine the fatty acid composition of SM oil, the Fatty acids were converted into fatty acid methyl esters (FAMEs) prepared according to the Regulation EEC/2568/91, EEC/1429/92 of the European Union Commission and the International Olive Council [24,25]. However for butter, red blood cells and liver, fatty acids were extracted according to the Folch method, and then methylated by BF3 in methanol [26]. The chromatographic separation was provided by a gas chromatography using model 5890 series II instrument (Hewlett-Packard Ca Palo Alto, Calif. USA) equipped with a flame ionisation detector and a fused silica capillary column HP - INNOWAX (30 m length × 0.25 mm and 0.25 µm of film thickness). The temperature was programmed to increase from 180 to 240°C. Nitrogen (1 ml/min) was used as gas carrier. The results were expressed as a relative area percent of the total FAMES.

Estimated fatty acid desaturase and elongase activities are determined by the product/precursor ratio: D9D = C16:1w7/C16:0; D6D = C18:3w6/C18:2w6; D5D = C20:5w3/C20:4w3; D4D = C22:6w3/C22:5w3; Elongase = C18:0/C16:0.

The phenolic compounds were estimated colorimetrically at 765 nm [27]. The result was expressed as mg of gallic acid equivalents/ g of oil (mg GAE/g). 5 g of oil are mixed with 5 ml of the methanol/tween solution (80%/20%), the mixture is stirred for one minute using an ultra turrax before being centrifuged for 12 min at 2850 rpm, and finally, we recover the upper phase. The extraction is repeated 3 times with the residue and the methanolic fraction is left at -20°C overnight. To 200 µl of extract, 800 µl of distilled water and 5 ml of folin reagent are added. The mixture is incubated for one minute in the dark and then 4 ml of sodium carbonate (7.5%) is added. After vortexing, the mixture is left in the dark for 2 hours and then the absor-

bance is read at 765 nm. Under the same conditions, a standard range is prepared with gallic acid.
Total flavonoid content was evaluated by colorometric method at 510 nm [28]. 1 ml of the methanolic extract is mixed with 4 ml distilled water and 0.3 ml $NaNO_2$ (5%). After 5 min, 0.3 ml of $AlCl_3$ (10%) is added and following a 6 min rest, 2 ml NaOH (1M) is added. The volume is adjusted to 10 ml by 2.4 ml of distilled water. After using a vortex to homogenise the mixture, the absorbance at 500 nm is measured. The result was expressed as mg of catechin equivalent / g of oil).
Oxidative stability measures the resistance of the oil against oxidation. Measurement of the induction period was determined by using a Rancimat apparatus, model 734 (Metrohm, Herisau, Switzerland). The temperature was 120°C and the air flow was 20 l/h [29].
Antioxidant Activity (DPPH Radical Scavenging Assay) was evaluated according to the method described by Blois et al.[30].
Anti-pancreatic lipase activity was determined as described by Gooda Sahib et al. [31].
Calcium and zinc concentrations were measured by atomic absorption spectroscopy "Spectrum SP-AA 4000" (NF V 05-113, 1972).

STATISTICAL ANALYSIS

All experiments were carried out in triplicate. Results were expressed as means ± standard deviations. The comparison was performed using the student-t test and ANOVA. Data were analysed with SPSS (version 12.00 for Window, SPSS Inc., Chicago, IL 2003). Differences were deemed significant at P<0.05.

RESULTS

SM OIL CHARACTERISATION

Table I provides a summary of the extracted SM oil's characteristics. Total phenol and total flavonoid concentrations are important in SM oil. In Parallel, the antioxidant capacity (DPPH, reducing power) is high. The SM oil has a high content of UFA (81.93%), with PUFA being the most common (53.61%).

BUTTER CHARACTERISATION

Butter has low water content and is very rich in lipids. Butter has a dry matter content of 86.15 ± 1.05 percent (Table II). The percentage of lipid content are 76.25 ± 3.18%. Almost 69% of fatty acids are saturated (Table II). Palmitic acid is the most common SFA (29.71%). Unsaturated fatty acids (29.96%) are represented mainly by monounsaturated fatty acids (oleic acid (25.12%). The concentrations of calcium and zinc are respectively 10.9 and 0.782 mg/100 g of butter.

FATTY ACIDS IN MICE

RBC Fatty acid variation between all groups is summarised in Table III. Saturated fatty acids increased in butter and SMB groups compared to the control group ($p < 0.001$). Inversely, UFA is lower in the SMB and butter groups than in the control group ($p < 0.001$). From UFA, MUFA decreased significantly in the butter group. Compared to the control group, trans FA dropped in the butter group and dropped more in the SMB group ($p < 0.001$). The difference between butter and the SMB groups is significant (p

Table I - Characteristics and fatty acid composition of *Silybum marianum* seed oil

Characteristics			
Acidity [%]	0.733 ± 0.028	Total phenol (mg GAE/g)	5.1 ± 0.85
PV [MeqO2/Kg]	5.33 ± 0.001	Total flavonoid (mg CE/g)	3.92 ± 0,03
K232	1.908 ± 0.004	DPPH (%)	64
K270	0.169 ± 0.005	Reducing power	0.306
OSI: oxidative stability Index [hour]	4.14	Lipase inhibition (%)	75.45
Fatty acid (%)			
Palmitic acid C16:0		13.37	
Palmitoleic acid C16:1		0.157	
Margaric acid C17:0		0.093	
Margaroleic acid C17:1		0.139	
Stearic acid C18:0		1.492	
Oleic acid C18:1		28.029	
Linoleic acid C18:2		52.954	
Linolenic acid C18:3		0.656	
Arachidic acid C20:0		2.203	
SFA		17.164	
UFA		81.935	
MUFA		28.324	
PUFA		53.611	

Total phenol (mg GAE/g)
Total flavonoid (mg of catechin equivalent /g of oil)
DPPH free radical scavenging activity
SFA: saturated fatty acids, UFA: unsaturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table II - Physicochemical properties, composition and fatty acids of commercial butter

Physicochemical properties		**Composition**	
Peroxide value	4.66 ± 0.11	Lipid content [%]	76.25 ± 3.18
TBARS	0.76 ± 0.08	Calcium [mg/100g]	10.9 ± 1.36
Dry matter [%]	86.15 ±1.05	Zinc [mg/100g]	0.782 ± 0.58
Fatty acids [% of total FA]			
C4 :0	4.59 ± 0.83	C18:1 n11 (trans)	0.034 ± 0.02
C6 :0	4.31 ± 0.53	C18:1 n-9 (cis)	23.88 ± 2.47
C8 :0	3.58 ± 1.11	C18:1 n-7(cis)	1.046 ± 0.26
C10 :0	2.11 ± 0.16	C18:2 (t9,t12)	0.113 ± 0.14
C12 :0	2.78 ± 0.29	C18:2 (c9,t12)	0.017 ± 0.01
C13 :0	0.232 ± 0.06	C18:2 (t9,c12)	0.072 ± 0.05
C14 :0	9.737 ± 0.59	C18:2 n-6 (c9,c12)	0.123 ± 0.10
C14 :1 n-9	0.058 ± 0.02	C18:2 (c9,t11)	0.019 ± 0.02
C15 :0	0.142 ± 0.07	C18:2 (t10,c12)	0.011 ± 0.01
C16 :0	29.71 ± 0.77	C18:3 n-3 (cis)	0.944 ± 0.26
C16 :1 n-9	0.017 ± 0.02	C18:3 n-6	0.028 ± 0.01
C16:1 n-7 (cis)	2.235 ± 0.15	C20 :0	0.157 ± 0.13
C17:0	0.838 ± 0.13	C20 :1	0.671 ± 0.09
C17 :1	0.592 ± 0.13	C22 :0	0.090 ± 0.03
C18 :0	11.08 ± 0.88	C21 :0	0.526 ± 0.07
C18:1 n-9 (trans)	0.013 ± 0.001	C24 :0	0.119 ± 0.04

TBARS (Thiobarbituric acid reactive substances)

= 0.024). n-3 FA decreased in the SMB group compared to the butter group ($p < 0.001$). However, there is no noticeable difference from the control group. Liver fatty acid variation between all groups is summarised in Table IV. Total SFA and UFA did not differ between the control and butter groups.

In the SMB group SFA decreased and UFA with PUFA increased significantly compared to the control and butter groups. Also, n-3 FA increased in the SMB group compared to the control group (mainly DHA). Whereas trans FA did not differ between all groups.

There are no significant variations in D6D, D5D, and elongase across the three groups regarding desaturase and elongase indexes in RBC (figure 1). In contrast to the butter group, the SMB group has a lower D9D index. The D4D index was higher in the butter group compared to the control group with no difference in the SMB group.

In the liver, D4D and D5D indexes increased in the butter and SMB groups compared to the control group. There was no difference in the elongase index between the butter and SMB groups; instead, it declined in the butter group and then increased in the SMB group. There was no significant difference in the D9D and D6D indexes between all groups (figure 2).

DISCUSSION

The SM oil has a significant antiradical activity and oxidative stability. As far as we know, few studies focused on phenolic compounds and antiradical activity of SM seed oil produced under cold pressure [32] [33]. Our findings confirmed the high total phenolic content of the Tunisian variety reported by Meddeb et al. [32]. In fact, the method used in our and in the last-mentioned study to measure polyphenols is not the optimal one. Other investigations, using HPLC technique, have found lower concentrations of phenolic compounds [33]. In contrast to numerous previous researches, ours has a better oxidative stability index [32].

Results on fatty acid composition from multiple studies are nearly identical [32] [34] [35]. The major fatty acid was Linoleic acid C18:2 (59.95%), followed by oleic acid (20.029%). The amount of those fatty acids varies throughout the research. Environmental variables or the use of various extraction techniques could account for this discrepancy [35]. Because of its fatty acid composition, SM oil has a significant impact on human health, particularly in terms of preventing cancer and cardiovascular diseases [36]. Our results, on FA in butter, are consistent with those of numerous earlier studies in which butter is high in saturated fat (almost 60%) [37].

In this work, we investigated how mice's fatty acid profiles, as well as desaturase and elongase indices, were affected by butter and butter supplemented with SM oil. We demonstrated that the butter group had higher SFA in RBC. The rise in butyric acid, capric acid, lauric acid, myristic acid, and palmitic acid is the primary cause of the increased SFA. Butter has those FAs in it. Thus, diet intake may account for their increase. Moreover, the butter and SMB groups showed an increase in butyric acid, one of the short-chain FAs (SCFA). Butyric acid, or SCFA, can be synthesised in the intestine by the "gut microbiota" or

consumed by food [38]. We can propose that adding SM oil to butter stimulates the gut microbiota to produce SCFA. Because SM oil contains a high concentration of phenolic compounds, a previous study showed that phenolic compounds have a beneficial effect on gut microbiota [39].

FA with more than 12 molecules of carbon increased in the butter group but decreased in the SMB group. Moreover, FA which decreased in the butter group, decreased more in the SMB group. This could be explained by the fact that the SM oil inhibits pancreatic lipase (shown in this study) and therefore some of the

Table III - Fatty acid composition of red blood cells in different groups of rats; control group (normal diet), butter group (diet supplemented with butter), SMB group (diet supplemented with butter and SM oil)

Fatty acids	control	Butter group	SMB group	pANOVA
C4:0	0.058 ± 0.012	0.741 ± 0.324♙	♦♦♦2.315 ± 0.115***	0.00
C6:0	0.020 ± 0.001	0.400 ± 0.541	♦1.439 ± 0.428*	0.005
C8:0	0 .024 ± 0.003	0.594 ± 0.719	1.211 ± 1.104	0.215
C10:0	0.022 ± 0.001	0.149 ± 0.077♙	♦♦1.659 ± 0.486**	0.000
C12:0	0.022 ± 0.001	1.073 ± 0.284♙♙	♦♦♦1.491 ± 0.249	0.000
C13:0	0.064 ± 0.057	0.318 ± 0.052♙♙	♦♦0.239 ± 0.040	0.000
C14:0	0.696 ± 0.109	2.248 ± 0.459♙♙	0.594 ± 0.100***	0.000
C14:1n-9	1.369 ± 0.012	1.097 ± 0.138♙	1.141 ± 0.227	0.133
C15:0	1.356 ± 0.167	0.153 ± 0.060♙♙♙	1.083 ± 0.333**	0.000
C16:0	26.180 ± 2.214	33.345 ± 2.262♙	25.135 ± 1.735**	0.001
C16 :1 n-9	3.402 ± 0.294	0.527 ± 0. 225♙♙♙	♦1.785 ± 0.897*	0.001
C16 :1 n-7 cis	1.401 ± 0.136	2.238 ± 0.278♙♙	1.242 ± 0.117**	0.000
C17:0	5.885 ± 1.052	0.875 ± 0.149♙♙♙	5.132 ±1.158***	0.000
C18:0 acidestéarique	10.729 ±1.086	11.397 ± 0.312	10.394 ± 0.931	0.335
C18 :1 n-9 cis	27.118 ± 0.657	23.916 ± 0.737♙♙	27.181 ± 5.155	0.325
C18:1n-7cis	1.079 ± 0.160	0.718 ± 0.279	0.956 ± 0.124	0.112
C18:1 n-9 trans	0.624 ± 0.160	0.261 ± 0.060♙♙	0.521 ± 0.081**	0.004
C18:2 t9c12	1.193 ±1.621	1.754 ± 0.507	0.079 ± 0.038**	0.068
C18:2 c9t12	1.802 ± 1.791	0.097 ± 0 .031	0.437 ± 0.183*	0.090
C18 :2 c9c12	12.561 ± 1.410	13.762 ±0.786	13.007±3 .742	0.808
C18 :3 n-3 cis	0.502 ± 0.082	0.934 ± 0.255♙	0.356 ± 0.113**	0.004
C18 :2 t10c12	0.351 ± 0.091	0.511 ± 0.068♙	0.297 ± 0.040**	0.005
C18 :2 t9 t11	0.150 ± 0.090	0.173 ± 0.016	0.073 ± 0.057*	0.096
C18 :2 c11t13	0.495 ± 0.108	0.614 ± 0.118	0.353 ± 0.88*	0.024
C18:3 n-6	0.493 ± 0.107	0.488 ± 0.231	0.389 ± 0.127	0.653
C20:0	0.112 ± 0.049	0.080 ± 0.021	0.063 ± 0.019	0.173
C20:1 n-9	0.141 ± 0.066	0.076 ± 0.047	0.080 ± 0.023	0.191
C21:0	0.149 ± 0.064	0.204 ± 0.044	0.081 ± 0.017**	0.013
C22:0	0.527 ± 0.062	0.590 ± 0.082	♦0 .363 ± 0.097*	0.014
C20:3 n-3	0.102 ± 0.097	0 .035 ± 0.033	0.035 ± 0.030	0.275
C20:4 n-6	0.112 ± 0.050	0.058 ± 0.013	0.072 ± 0.024	0.127
C20:4 n-3	0.239 ± 0.237	0.073 ± 0.035	0.111 ± 0.076	0.279
C23:0	0.131 ± 0.053	0.078 ± 0.040	0.127 ± 0.046	0.272
C20:5	0.147 ± 0.023	0.094 ± 0.046	♦0 .093 ± 0.029	0.140
C24:0	0.216 ± 0.065	0.136 ± 0.045	0.141 ± 0.022	0.096
C22:5 n-3	0.152 ± 0.096	0.021 ± 0.014♙	0.146 ±0.101*	0.092
C22:6 n-3	0.177 ± 0.129	0.137 ± 0.086	0.081 ± 0.065	0.424
C24:1	0.223 ± 0.087	0.022 ± 0.008♙♙	♦0 .082 ± 0.045*	0.003
SFA	46.193±1.855	52.388±0.641♙♙	♦♦51.473±0.901	0.000
UFA	53.804±1.854	47.613±0.643♙♙	♦♦48.525±0.901	0.000
MUFA	35.360±0.333	28.858±0.549♙♙♙	32.991±4.423	0.036
PUFA	18.444±2.185	18.755±0.785	15.534±3.668	0.213
n-6 FA	16.020±2.338	16.183±0.586	13.893±3.736	0.434
n-3 FA	1.667±0.497	1.689±0.112	1.120±0.123***	0.031
Trans FA	4.587±0.365	3.411±0.772	♦1.761±0.203**	0.013

Significant difference ♙butter/control ♦SBM/control *butter/SMB groups

Table IV - Fatty acid composition of liver in different groups of rats; group1: control (normal diet), group 2: (diet supplemented with butter), group 3: (diet supplemented with butter and SM oil)

Fatty acids	Control	Butter group	SMB group	pANOVA
C4:0	0.058 ± 0.029	1.627 ± 0.336⁋⁋	♦♦0.205 ± 0.041***	0.00
C6:0	0.061 ± 0.028	0.806 ± 0.351⁋	0.067 ± 0.025**	0.001
C8:0	0.021 ± 0.009	1.211 ± 1.528	0.073 ± 0.121	0.158
C10:0	0.100 ± 0.074	1.263 ± 1.138	0.137 ± 0.043*	0.057
C12:0	0.207 ± 0.328	1.953 ± 0.628⁋⁋	♦0.804 ± 0.210**	0.001
C13:0	0.618 ± 0.427	0.111 ± 0.024	♦0.121 ± 0.063	0.017
C14:0	0.728 ± 0.192	0.787 ± 0.152	0.633 ± 0.174	0.432
C14:1 n-9	0.469 ± 0.189	0.376 ± 0.326	0.721 ± 0.366	0.299
C15:0	0.155 ± 0.121	0.067 ± 0.095	0.168 ± 0.087	0.325
C16:0	20.087 ± 0.434	20.042 ± 0.722	18.863 ± 1.398	0.201
C16 :1 n-9 trans	0.439 ± 0.036	0.429 ± 0.042	0.422 ± 0.033	0.819
C16 :1 n-7 cis	1.107 ± 0.010	1 .059 ± 0.060	1.146 ± 0.191	0.633
C17:0	0.581 ± 0.040	0.634 ± 0 .603	0.898 ± 0.771	0.737
C18:0	25.691 ± 0.262	23.775 ± 1.313	24.104 ± 1.867	0.248
C18 :1 n-9 cis	0.637 ±0.172	0.809 ± 0.297	0.706 ± 0.285	0.703
C18:1 n-7 cis	7.624 ± 0.911	9.027 ± 2.856	7.715 ± 0.9634	0.508
C18:1 n-9 trans	1.229 ± 0.790	0.644 ± 0.246	0.860 ± 0.170	0.234
C18:2 t9c12	0.073 ± 0.003	0.076 ± 0.035	1.785 ± 2.456	0.256
C18:2 cis 9t12	0 .127 ± 0. 015	0.117 ± 0.021	2.193 ± 3.746	0.404
C18 :2 n-6 c9c12	1.208 ± 0.078	1.092 ± 0.175	1.505 ± 0.502	0.245
C18 :3 n-3 cis	6.596 ± 0.308	7.344 ± 2.627	7.390 ± 2.297	0.867
C18 :2 t10c12	0.108 ± 0.008	0.082 ± 0.028	0.112 ± 0. 021	0.163
C18 :2 t9 t11	0.297 ± 0.014	0.224 ± 0.152	0.321 ± 0.084	0.405
C18 :2c11t13	0.007 ± 0.003	0.005 ± 0.003	0.016 ± 0.022	0.506
C18:3 n-6	1.723 ± 0.024	1.344 ± 0.552	1.497 ± 0.368	0.496
C20:0	0.596 ± 0.036	0.490 ± 0.366	0.533 ± 0.103	0.828
C20:1 n-9	0.083 ± 0.011	0 .094 ± 0.066	0.091 ± 0.031	0.940
C21:0	0.242 ± 0.006	0. 285 ± 0.112	0 .240 ± 0.069	0.674
C22:0	0.336 ± 0.056	0 .663 ± 0.025⁋⁋⁋	♦♦0.652 ± 0.085	0.000
C20:3 n-3	0.593 ± 0.060	0. 634 ± 0.036⁋	0.629 ± 0.143	0.856
C20:4 n-6	10.775 ± 0.486	9.583 ± 0.538⁋⁋	10.158 ± 0.688	0.079
C20:4 n-3	0.580 ± 0.018	0.207 ± 0.146	♦♦0.178 ± 0.129	0.003
C23:0	0.715 ± 0.036	0.236 ± 0.142⁋⁋	0.268 ± 0.117	0.001
C20:5	2.388 ± 0.050	1.684 ± 0.981	1.903 ± 0.861	0.537
C24:0	4.788 ± 0.173	0.563 ± 0.089⁋⁋⁋	0.726 ± 0.313	0.000
C22:5 n-3	8.253 ± 0.133	4.583 ± 1.893⁋	♦5.207 ± 1.403	0.020
C22:6 n-3	0.576 ± 0.331	6.008 ± 3.179⁋	♦♦6.854 ± 2.397	0.016
C24:1	0.109 ± 0.111	0.051 ± 0.056	0.084 ± 0.033	0.531 BioMed
SFA	54.990 ± 0.562	54.519 ± 1.540	♦48.497 ± 2.888**	0.003
UFA	45.008 ± 0.561	45.479 ± 1.540	♦51.503 ± 2.890**	0.003
MUFA	11.700 ± 0.624	12.492 ± 3.155	11.749 ± 1.900	0.859
PUFA	33.307 ± 0.455	32.987 ± 4.441	♦39.754 ± 3.606*	0.032
n-6 FA	14.014 ± 0.365	12.295 ± 1.002⁋	17.252 ± 3.60	0.148
n-3 FA	16.600 ± 0.061	18.778 ± 2.614	♦20.259 ± 2.041	0.098
Trans FA	1.847 ± 0.784	1.158 ± 0.183	5.292 ± 5.481	0.248

Significant difference ⁋butter/control ♦SBM/control *butter/SMB groups

FA will not cross the intestinal wall.

FA in red blood cells represents an imprint of FA in food [40]. Circulating FA could be considered as biomarkers of disease development [10]. Studies showed that butyrate can decrease the coronary artery disease risk [41]. Another study considers C22:0, C24:0, C26:0, C25 n-3 (EPA), and C20:4 n-6 (AA) as biomarkers of CAD and trans FA as CAD severity predictor [42]. In this study, we showed that butter is linked to higher levels of butyric acid and lower levels of oleic acid. This finding proved the correlation between butter consumption and a preventative FA profile. On the other hand, SM oil supplementation is associated with decreased C22:0, C20:5, and trans FA. Therefore, adding SM oil to a diet helps improve the fatty acid profile, which helps avoid CAD.

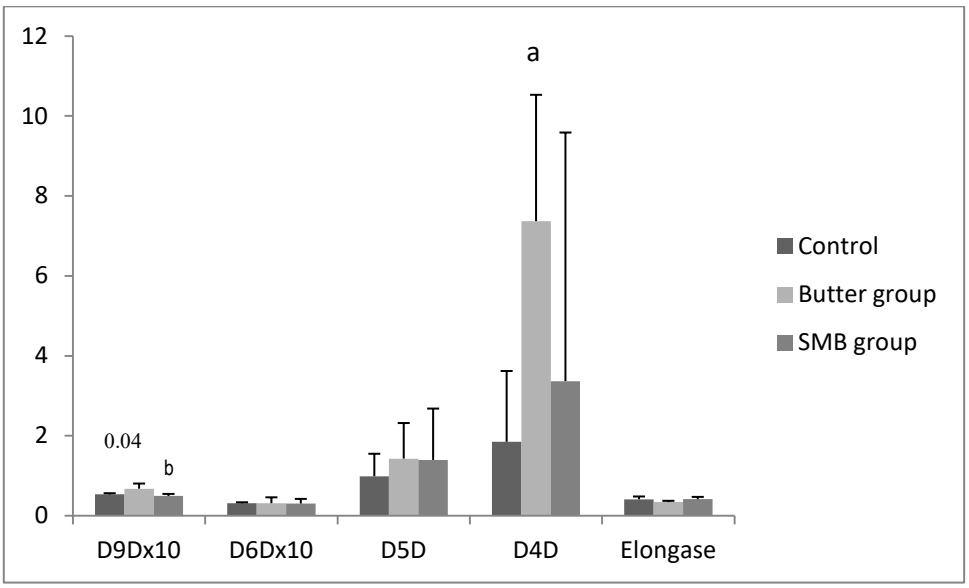

Figure 1 - Estimated desaturase and elongase activity variation in red blood cells of different groups of mice: control (normal diet), Butter (diet supplemented with butter), SMB (diet supplemented with butter and SM oil). D9D = C16:1n-7/C16:0; D6D = C18:3n-6/C18:2n-6; D5D = C20:5n-3/C20:4n-3; D4D = C22:6n-3/C22:5n-3; Elongase = C18:0/C16:0.

The values displayed above the histograms are the significant values of pANOVA: difference between three groups. [a]significant difference with control; [b]significant difference with butter group

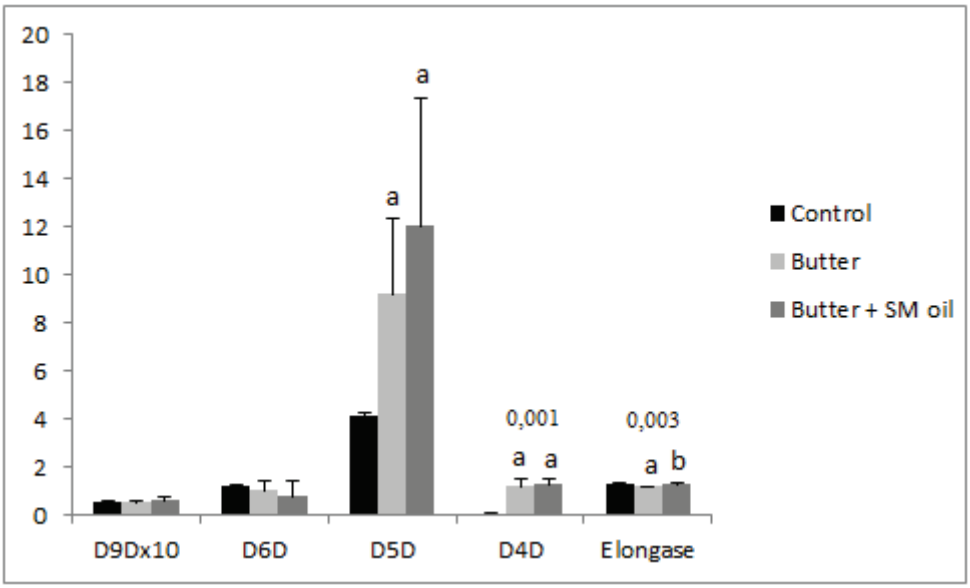

Figure 2 - Desaturase and elongase index variation in liver of different groups of mice: control (normal diet), Butter (diet supplemented with butter), SMB (diet supplemented with butter and SM oil). D9D = C16:1n-7/C16:0; D6D = C18:3n-6/C18:2n-6; D5D = C20:5n-3/C20:4n-3; D4D = C22:6n-3/C22:5n-3; Elongase = C18:0/C16:0.

The values displayed above the histograms are the significant values of pANOVA: difference between three groups. [a]significant difference with control; [b]significant difference with butter group

In the liver, there are no differences in total SFA or UFA between the butter and control groups. However, many SFA increased in the butter group such as C4:0, C6:0, C12:0, and C22:0 and other SFA decreased such as C23:0 and C24:0. The change in fatty acid content in the liver may be the result of changes in dietary FA. The FAs that increased in the butter group, are decreasing in the SMB group compared to the butter one. Thus, two hypotheses are plausible: i- SM oil masks the effect of butter consumption on fat profile. ii- SM oil decreases the content of FA which have raised under the effect of butter consumption.
In summary, the impact of butter and SM oil on fatty acid metabolism may account for the differences in

fatty acid profiles observed in the control, butter, and SMB groups. Butter and SM oil supplementations are associated with increased D5D and D4D enzyme activities. Moreover, butter inhibits the elongase enzyme. However, SM oil activates it. This could be explained by the effect of butter and SM oil compounds (FA, zinc, phenolic compounds, flavonoids...). Previous studies showed that α-linolenic acid and anthocyanins affect desaturase activities [43]. Hydroxytyrosol and high fat diet influence desaturase expression and activity [44]. Silibinin, a phenolic compound of SMO, downregulates many lipogenic genes (FATP5, SREBP-1) and gluconeogenic genes [45]. We can suggest that SMO enhances the lipid (FA) composition of the liver by raising UFA, primarily w3, in addition to its lipogenic effect (ascribed to silibinin).

CONCLUSION

SM oil supplementation influences fatty acid profile in RBC and liver. It enhances the fatty acid profile to help prevent an illness. It would be very interesting to add SM oil to butter for good health. However, to further understand the impact of adding SM oil to the taste and stability of butter, additional research ought to be considered.

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