Journal of Steroid Biochemistry & Molecular Biology 121 (2010) 619–625

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Adaptation of cholesterol synthesis to fasting and TNF-α: Profiling cholesterol intermediates in the liver, brain, and testis☆

Klementina Fon Tacer[a], Denis Pompon[b], Damjana Rozman[a,*]

[a] Center for Functional Genomic and Biochips, Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Zaloška 4, SI-1000 Ljubljana, Slovenia
[b] LIPM, Centre de Génétique Moléculaire du CNRS, Avenue de la Terrasse-Bât. 23B, 91198 Gif-sur-Yvette, France

ARTICLE INFO

Article history:
Received 30 November 2009
Received in revised form 16 February 2010
Accepted 24 February 2010

Keywords:
Cholesterol biosynthesis
Sterol intermediates
Inflammation
Fasting

ABSTRACT

Key players in pathogenesis of metabolic disorders are disturbed cholesterol balance and inflammation. In addition to cholesterol also sterol intermediates are biologically active, however, surprisingly little is known about their synthesis and roles. The aim of our study was to assess the interplay between the inflammatory cytokine TNF-α and cholesterol synthesis by measuring cholesterol and its intermediates in the liver, brain, and testis. Liquid chromatography–mass spectrometry has been applied to profile sterols of normally fed mice, during fasting and after TNF-α administration. In mice on normal chow diet, sterols other than cholesterol represent 0.5% in the liver, 1% in brain and 5% in testis. In the liver only 7-dehydrocholesterol, lanosterol and desmosterol were detected. Major sterol intermediates of the brain are desmosterol, testis meiosis activating sterol (T-MAS), and 7-dehydrocholesterol while in testis T-MAS predominates (4%), followed by desmosterol, lanosterol, 7-dehydrocholesterol and others. In 20 h fasting there is no significant change in cholesterol of the three tissues, and no significant change in intermediates of the liver. In the brain sterol intermediates are lowered (significant for zymosterol) while in the testis the trend is opposite. TNF-α provokes a significant raise of some intermediates whereas the level of cholesterol is again unchanged. The proportion of sterols in the liver rises from 0.5% in controls to 1.2% in TNF-α-treated mice, which is in accordance with published expression profiling data. In conclusion, our data provide novel insights into the interaction between the inflammatory cytokine TNF-α and the tissue-specific cholesterol biosynthesis of the liver, brain and testis.

1. Introduction

The current epidemics of obesity together with sedentary lifestyle underlie the increasing prevalence of metabolic disorders in modern world. Metabolic syndrome is associated with an increased risk of diabetes and cardiovascular diseases [1]. Furthermore, recent studies have linked obesity to insulin resistance in the brain with subsequent cognitive impairment and neurodegeneration [2]. Metabolic syndrome, in particular obesity, also affects testicular function by reducing total testosterone, as well as having a detrimental effect on spermatogenesis [3]. The key players in the pathogenesis of metabolic disorders are disturbed cholesterol balance [4] and inflammation [5].

Cholesterol is an important building block of mammalian membranes. In brain, it is a crucial constituent of myelin sheath, essential for proper functioning of the nervous system. In testis, it plays a significant role during spermatogenesis and is essential for germ cell development [6]. It is also a precursor of several physiologically active molecules, such as bile acids in the liver and steroid hormones in endocrine tissues. Cholesterol synthesis starts from two-carbon acetyl-CoA. Squalene synthase, the first enzyme committed to cholesterol, produces lanosterol [7], that is converted to the final product by a series of reactions (Fig. 1) [8,9]. Intermediates of the pre-squalene part of the pathway supply cells with a variety of molecules, such as coenzyme Q10, heme A, etc. [10]. In 1995 it was discovered that also the post-squalene intermediates have roles not dedicated solely to cholesterol. Two sterols, 4,4-dimethylcholesta-8(9),14,24-trien-3β-ol (FF-MAS – follicular fluid meiosis activating sterol) and 4,4-dimethylcholesta-8(9),24-dien-3β-ol (T-MAS – testis meiosis activating sterol) were identified as potential meiosis activating molecules in gonads [11]. MAS accumulate in ovary and testis during sexual maturation, have the capacity to trigger oocyte meiosis *in vitro* [11], contribute to oocyte maturation in humans [12] and are synthesized in sperm *in situ* [13]. Furthermore, Engelking et al. [14] implicated accumulation of sterol precursors as a key factor in the genesis of inborn errors of cholesterol synthesis. Cholesterol precursor accumulation interferes with midline

☆ Article from special issue on "Steroid profiling and analytics: going towards Sterome".

* Corresponding author. Tel.: +386 1 543 7591; fax: +386 1 543 7588.
E-mail address: damjana.rozman@mf.uni-lj.si (D. Rozman).

0960-0760/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2010.02.026

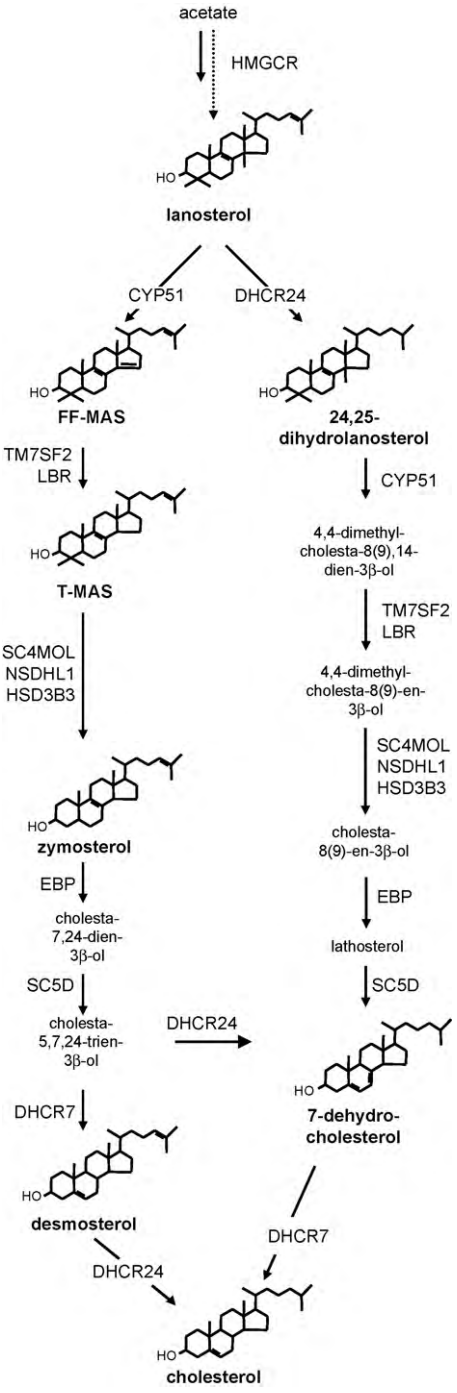

Fig. 1. Schematic representation of post-squalene part of cholesterol biosynthesis. Abbreviations of enzyme names correspond to UniGene Symbols. HMGCR: HMG–CoA reductase; LBR: lamin B receptor; TM7SF2: 14-dehydrocholesterol reductase; SC4MOL: sterol C4-methyl oxidase, NSDHL: 3-β-hydroxy-Δ5-steroid dehydrogenase, HSD3B3: 3β-keto-steroid reductase, EBP-sterol 8,7 isomerase; SC5D: sterol-C5 desaturase; DHCR7: 7-dehydrocholesterol reductase. FF-MAS: follicular fluid meiosis activating sterol, 4,4-dimethyl-5α-cholesta-8,14,24-triene-3β-ol; T-MAS: testis meiosis activating sterol, 4,4-dimethyl-5α-cholesta-8,24-diene-3β-ol.

fusion of facial structures [14] and impairs hair development [15].

Several lines of evidence suggest that in addition to cholesterol also sterol intermediates are biologically active and may play considerable part in physiology and pathology.

Pathogenesis of many metabolic disorders result from interactions between the immune system and lipid metabolism where caloric restriction may offer new therapeutic approaches [16,17]. Herein we show that fasting and TNFα-induced inflammation provoke tissue-specific changes in the sterol composition of liver, testis and brain. We report that the major changes are not in cholesterol but in the post-lanosterol intermediates of the synthesis pathway.

2. Materials and methods

2.1. Materials

Recombinant human TNF-α with specific activity of 3.3×10^7 U/mg was generously provided by V. Menart († Lek, d.d.). TNF-α was freshly diluted in sterile 0.9% saline to 0.15 mg/ml. Cholesterol, desmosterol, 7-dehydrocholesterol, 24,25-dihydrolanosterol and lanosterol were purchased from Steraloids. FF-MAS and T-MAS are laboratory standards from A.G. Byskov (Laboratory of Reproductive Biology, University Hospital of Copenhagen).

2.2. Animals and treatment

All *in vivo* procedures were in accordance with the Amsterdam Protocol on Animal Protection and Welfare and were approved by Veterinary Administration of the Republic of Slovenia. Animals were housed in the Medical Experimental Center of Faculty of Medicine, University in Ljubljana. Experiments were performed on C57BL/6 mice (Harlan), 10–12 weeks of age of both genders. Mice were assigned into three groups; control, fasted, and TNF-α-treated (6 animals per group, 3 males and 3 females). Animals from control group had free access to food throughout the study. In fasted and TNF-α-treated group, food was removed at the time of TNF-α administration, because TNF-α induces anorexia [18]. Mice were *i.v.* injected with a human recombinant TNF-α (30 μg/animal in 200 μl), dose known to have a significant effect without leading to mortality, or a corresponding volume of saline. Mice were sacrificed 20 h after injection at the beginning of the light phase. Mice from the three groups were sacrificed within 1 h, one animal at the time, from alternating groups. Liver, brain, and testes were harvested, snap frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

2.3. Sterol extraction and LC–MS analysis

Total sterols from liver, brain and testes were extracted as previously described [19,20]. Briefly, frozen tissues were freeze-dried and lipids were extracted in 75% n-heptane–25% isopropanol (v/v). The organic phase was saponified in 0.5 M potassium hydroxide in 80% ethanol at 60 °C for 60 min. After cooling to room temperature, pH was neutralized with 1 M KH_2PO_4 and the sterols extracted with 75% n-heptane–25% isopropanol (v/v). The organic phase was dried, redissolved in acetonitrile and subjected to LC–MS analysis [21,22]. 50 μl of sample was loaded on XTerra RP18 3.5 μm 4.6 × 100 mm column (Waters). The column temperature was maintained at 60 °C with flow rate 1.3 ml/min. All elution steps were performed with a water–acetonitrile gradient including a constant concentration of 0.01% (v/v) formic acid. Column was equilibrated in 20% acetonitrile (v/v) and eluted with a multistep linear acetonitrile gradient from 50 to 75% (v/v) for the first 10 min followed by 75–87.5% gradient for the next 10 min and finally 5 min from 87.5% to 100% acetonitrile. In such conditions, lanosterol eluted at 16.07 min, 24,25-dihydrolanosterol at 16.9 min, FF-MAS at 14.24 min, T-MAS at 15.74 min, zymosterol at 13.09 min, 7-dehydrocholesterol at 13.82 min, desmosterol at 13.26 min, and cholesterol at 15.05 min (Table 1). The effluents were delivered to the quadrupole mass detector (Micromass ZQ) set in the positive electrospray ionization mode using the following settings:

Table 1
The measured cholesterol intermediates in LC–MS analysis.

Name	Chemical nomenclature	Chemical formula	Mw	$[M+H^+-H_2O]$	RT (min)	LOD (μg/g)
Lanosterol	4,4,14α-trimethyl cholesta-8(9),24-dien-3β-ol lanosta-8,24-dien-3β-ol	$C_{30}H_{50}O$	426.7	409	16.07	0.05
24,25-Dihydrolanosterol	4,4,14α-trimethyl cholesta-8(9)en-3β-ol	$C_{30}H_{52}O$	428.7	411	16.9	0.05
FF-MAS	4,4-dimethylcholesta-8(9),14,24-trien-3β-ol	$C_{29}H_{46}O$	410.7	393	14.24	0.05
T-MAS	4,4-dimethylcholesta-8(9),24-dien-3β-ol	$C_{29}H_{48}O$	412.7	395	15.74	0.05
Zymosterol	cholesta-8(9),24-dien-3β-ol 8, 24(5α)-cholestadien-3β-ol	$C_{27}H_{44}O$	384.6	367	13.09	0.01
7-Dehydrocholesterol	cholesta-5,7-dien-3β-ol 5,7-cholestadien-3β-ol provitamin D3	$C_{27}H_{44}O$	384.6	367	13.82	0.01
Desmosterol	24-dehydrocholesterol 5,24-cholestadien-3β-ol 3β-hydroxi-5,24-cholestadiene	$C_{27}H_{44}O$	384.6	367	13.26	0.01
Cholesterol	3β-cholesta-5-en-3β-ol	$C_{27}H_{46}O$	386.6	369	15.05	0.05

desolvation and cone flow 400 l/h and 100 l/h nitrogen respectively, capillary potential 3.5 kV, cone potential 33 V, extraction potential 3 V. Others fine quadrupole tunings were set according to the manufacturer's recommendations for analysis of ions in the 200–500 *m/z* range. The mass spectrometer was simultaneously operated in scan (*m/z* range = 295–450) and multiple channel single ion (SIR) modes using as settings the sterol molecular mass −17 to monitor the $[+H^+-H_2O]$ ions (Table 1). Limits of detection (LOD) for each sterol were between 0.05 and 0.01 μg/g (Table 1).

2.4. Statistics

Kruskal–Wallis test with post-hoc pair-wise multiple comparisons was used to compare sterols among treatments within each tissue. Statistical analysis was performed using SPSS software and $p < 0.05$ was considered to be significant.

3. Results

3.1. Sterol composition in the liver, brain, and testis

The tissue profile of 7 analyzed sterols (lanosterol, FF-MAS, T-MAS, zymosterol, 7-dehydrocholesterol, desmosterol, and cholesterol) is depicted in Fig. 2 . Sample chromatograms of standards and tissue extracts are shown in supplementary data (Figs. 1 and 2). Cholesterol represents 99.5% of total sterols in the liver, 99% in the brain, and 94.3% in the testis (Fig. 2B). As expected, brain contains at least 3 times more cholesterol (4.9 mg/g) compared to liver and testis (~1.5 mg/g in both tissues) (Fig. 2A) [23]. Liver has a limited spectrum of sterol intermediates. With exception of 7-dehydrocholesterol (4 μg/g, representing 0.24% of total sterols), lanosterol (1.6 μg/g, 0.15%), and desmosterol (0.8 μg/g, 0.08%), other sterols were below limit of detection (Fig. 2). In contrast to the liver, sterols other than cholesterol represent 1% of total sterols in the brain and even 5.7% in the testis.

In the brain, we found all measured intermediates. The major intermediates are desmosterol (23 μg/g, 0.5%), T-MAS (15 μg/g 0.2%), and 7-dehydrocholesterol (8 μg/g, 0.16%) (Fig. 2). The major sterol of the testis is T-MAS (71 μg/g, 4.06%), followed by desmosterol (15 μg/g, 0.89%), lanosterol (5 μg/g, 0.43%), 7-dehydroholesterol (2.7 μg/g, 0.21%), FF-MAS (1.2 μg/g, 0.08%), and zymosterol (0.27 μg/g, 0.02%) (Fig. 2). 24,25-Dihydrolanosterol was below limit of detection in all tissues (data not shown). It seems that lanosterol represents the preferential substrate for CYP51 not only in the liver [24] but also in brain and testis. Since mostly sterols with unsaturated Δ24 bond were detected, it seems that this branch of the synthesis pathway predominates (Figs. 1 and 2). Our results also show that desmosterol is the major precursor of cholesterol in testis and brain (Fig. 2). Only in the liver there are comparable amounts of desmosterol and 7-dehydrocholesterol, suggesting that

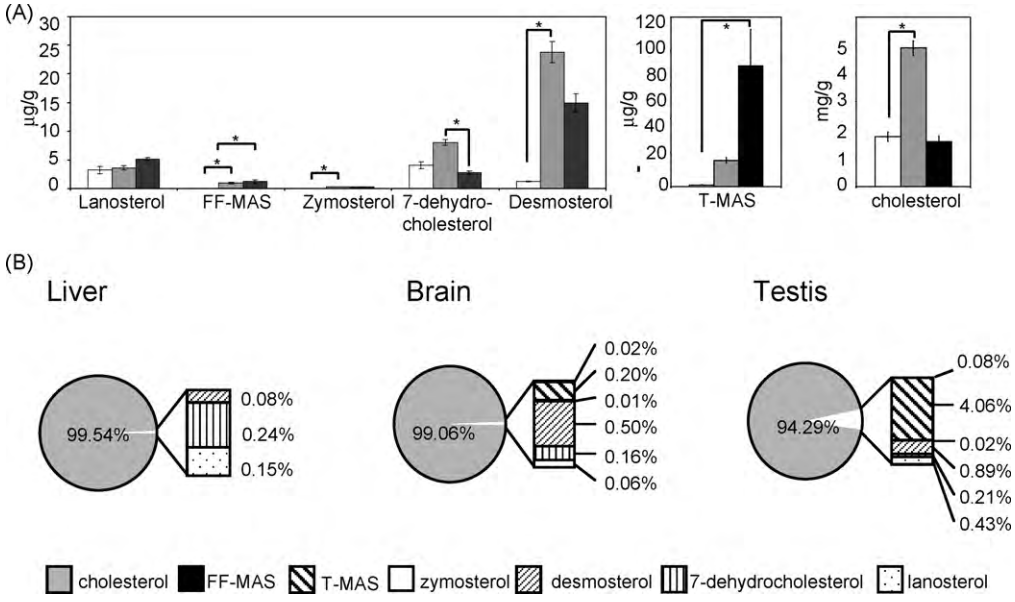

Fig. 2. Sterol intermediates of cholesterol synthesis in the liver, brain, and testis of normally fed mice. (A) Data represent the amount of sterol intermediates (μg/g) and cholesterol (mg/g) in liver (white bars), brain (gray bars), and testis (black bars). Mean ± S.E.M. of six animals of both genders (except for testis, 3 animals) is presented. *$p < 0.05$. (B) Pie-charts representing sterol composition of the liver, brain, and testis.

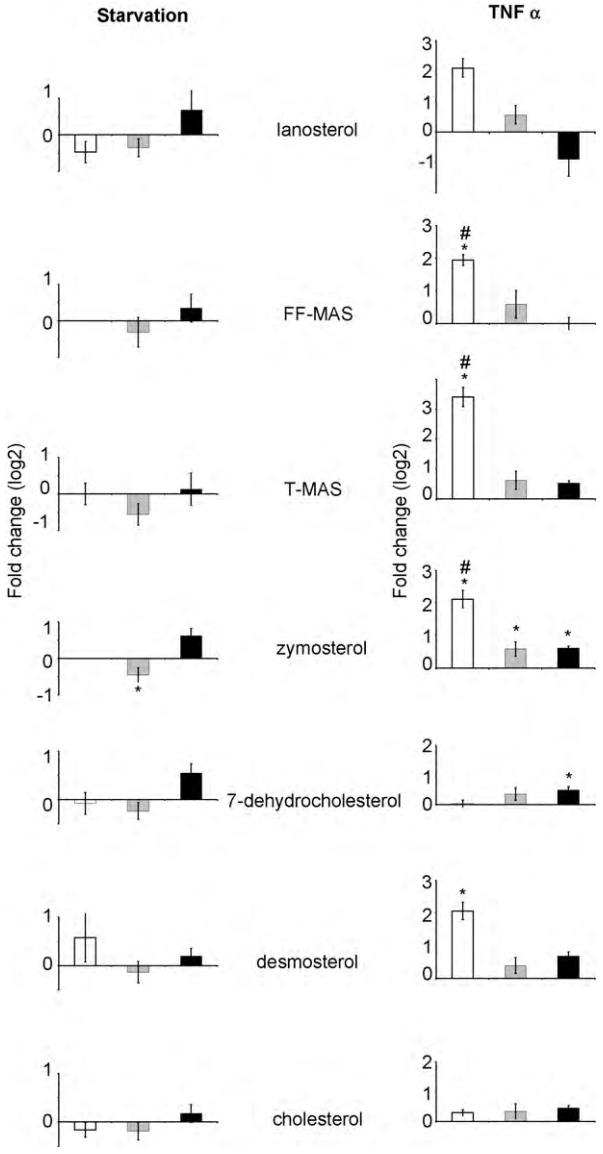

Fig. 3. (A) The impact of fasting on tissue-specific sterol composition. The effect of fasting is expressed as log2 ratio of fasted/normally fed animals in liver (white bars), brain (gray bars), and testis (black bars). (B) The impact of TNF-α on tissue-specific sterol composition. The effect of TNF-α is expressed as log2 ratio of TNF-α/fasted *versus* fasted animals in liver (white bars), brain (gray bars), and testis (black bars). Mean ± S.E.M. of six animals of both genders (except for testis, 3 animals) is presented. $^{*}p < 0.05$, Kruskal–Wallis test with post-hoc pair-wise multiple comparisons. #Sterols were below limit of detection in fasted animals. In these cases the fold change was calculated according to the detection limit for each sterol.

in liver DHCR24 and DHCR7 compete for substrate cholesta-5,7,24-trien-3β-ol (Fig. 1).

3.2. The impact of fasting on sterol composition

Contemporary lifestyle leads to different metabolic disorders as a result of an energy imbalance. In contrast, caloric restriction exerts opposite effects and produces a number of benefits. We assessed the effect of short-term fasting on sterol metabolome of the liver, brain, and testis. Fig. 3A depicts log2 ratios of sterols in 20 h fasted mice *versus* controls that had chow available *ad libitum*. In the three tissues, there is no significant change in cholesterol (Fig. 3A) despite the established inhibition of cholesterogenic genes [21]. Unexpectedly, in the brain the amount of measured intermediates decreased, statistically significant for zymosterol (Fig. 3A, gray bars) while in testis an increase in the amount of almost all intermediates (Fig. 3A, black bars) has been observed (statistically not significant).

3.3. The effect of the inflammatory cytokine TNF-α on sterol composition

Tumor necrosis factor TNF-α is a candidate mediator of insulin resistance in obesity, as it is over-expressed in adipose tissue of rodents and humans and blocks the action of insulin [25]. In is also a mediator of inflammation. Our previous expression profiling analysis demonstrated that 20-h TNF-α treatment provokes up-regulation of several genes encoding acute phase proteins and inflammatory markers in the liver [21]. The reason for food removal during the TNF-α treatment is the fact that TNF-α induces anorexia [18]. This was confirmed also in our pilot experiment where all mice had access to normal chow *ad libitum*. Food intake in the control group was 0.18 g/g of body weight, whereas in TNF-α treated group only 0.04 g/g of body weight. Short-term fasting is thus a classical paradigm when studying the effects of inflammation on metabolism [26].

Fig. 3B depicts log2 ratios of measured sterols in TNF-α treated mice *versus* fasted controls in liver, brain, and testis. After TNF-α administration there is an increase of almost all intermediates in all three tissues (Fig. 3B) whereas the level of cholesterol remains almost unchanged. In the liver the proportion of sterol intermediates rises from 0.5% in controls and fasted mice to over 1.2% after TNF-α treatment. This increase is under-estimated since FF-MAS, T-MAS and zymosterol were below limit of detection in livers of fasted animal. The approximate fold change for these sterols was calculated by using their detection limits (Table 1).

An increase in the quantity of almost all intermediates was observed after the TNFα stimulus as well in the brain and testis, statistically significant for zymosterol in brain (Fig. 3B, gray bars) and zymosterol and 7-dehydrocholesterol in testis (Fig. 3B, black bars). Raise of cholesterol intermediates is in line with our earlier transcriptome studies showing that TNF-α activates the expression of genes from the hepatic cholesterol biosynthesis [21]. This suggests that TNFα induces cholesterol biosynthesis despite fasting. However, lower utilization or altered distribution of sterols after TNF-α application cannot be excluded since the flux through cholesterol pathway has not been measured.

4. Discussion

While consequences of obesity on metabolic and cardiovascular physiology are well established, epidemiological and experimental data are beginning to define that the central nervous system [27] and reproduction may also be detrimentally affected. Pathogenesis of obesity associated metabolic disorders results from the interplay of disturbed lipid homeostasis and inflammatory stimuli, such as TNF-α. While majority of studies focuses on cholesterol, it is becoming clear that also intermediates of cholesterol synthesis are biologically active, yet poorly characterized molecules. Among the few known roles is their capacity to activate nuclear receptor LXR [28], which influences the regulation of downstream genes of the lipid metabolism and inflammation [29]. Our work focuses on sterol intermediates in the liver, brain, and testis.

After squalene, cholesterol pathway has two possible branches with sterol intermediates containing either the saturated Bloch pathway [30] or Δ24 unsaturated Kandutsch–Russell pathway [31], with desmosterol or 7-dehyrocholesterol as immediate cholesterol precursors (Fig. 1). All enzymatic steps are now defined, however their precise sequence is not yet explained [7]. Using LC–MS analysis of total lipid extracts (Fig. 2) we determined only

7-dehydrocholesterol, lanosterol and desmosterol in the liver. This small spectrum of cholesterol intermediates might results from a coordinate regulation of the biosynthetic pathway and a balanced flux of intermediates. When intracellular cholesterol levels drop, SREBP2 induces cholesterol biosynthesis and uptake [32]. Excess of cholesterol inhibits SREBP2 and activates LXR, which in turn promotes cholesterol export and elimination [29], silences squalene synthase and CYP51 [33], and LDL-receptor mediated uptake [34].

In contrast to liver, brain and testis comprise a wealthier sterol profile in normally fed conditions. This reflects different regulation in organs that are separated from circulation by the organ–blood barrier. Liver has normally a low basic cholesterol synthesis rate with most of cholesterol production taking place in extra-hepatic tissues [35–37]. The blood–brain barrier efficiently protects brain from exchange with lipoprotein cholesterol in the circulation, so almost all cholesterol is a product of local synthesis *de novo* [36,38] and is balanced by excretion of 24S-hydroxycholesterol [36]. Our study shows that the major cholesterol intermediates of the brain are desmosterol, T-MAS, 7-dehydrocholesterol and lanosterol. The excess of desmosterol over 7-dehydrocholesterol suggests desmosterol as a major cholesterol precursor in brain which is in line with previously published findings [39]. As expected [40], T-MAS is the major sterol intermediate in testis. In addition to T-MAS, desmosterol that resides in mature spermatozoa [41] represents 0.89% of total sterols (Fig. 2B). Although MAS are not obligatory for oocyte meiosis [42], there are several indications of their importance in mammalian reproduction. FF-MAS correlates with the oocyte quality [12,43], stimulates oocytes growth derived from ICSI patients *in vitro* [44] and protects oocytes from precocious chromatid segregation [45]. Interestingly, we found substantial amounts of T-MAS also in the brain (Fig. 2), where it has not been described so far. That might suggest a novel role of MAS in brain function and regeneration.

Restricting caloric intake and fasting have beneficial metabolic effects [46]. We measured the level of cholesterol and cholesterol synthesis intermediates after 20 h fasting. This corresponds to phase II of fasting–starvation transition in mammals. Liver glycogen stores are depleted, lipids represent the main source of energy and gluconeogenesis becomes necessary to supply the requirements of the brain and. In humans, this state can be maintained for several weeks and has often been referred to as a period of adapted starvation [47]. In condition of low energy, adenine monophosphate (AMP) activated protein kinase (AMPK) is an energy sensor that restores cellular ATP levels by switching on catabolic and switching off anabolic pathways, such as cholesterol biosynthesis [48].

Liver is the central organ of control system that handles cholesterol within physiological limits [37] and is very responsive to metabolic perturbations, such as feeding cholesterol [49] and fasting [50,51]. In liver, fasting inhibits cholesterol biosynthesis at the transcriptional level [21]. This transcriptional inhibition seems to be synchronous for the entire pathway since intermediates do not accumulate (Fig. 3A). Conversely to expectation, also brain responds to fasting by decreasing the level of sterol intermediates (Fig. 3A) what is in line with decreased sterol synthesis in pineal gland after 24 h fast [52]. However, cholesterol level remains unaffected in all investigated tissues (Fig. 3A), likely due to homeostatic mechanisms. In the liver, cholesterol balance is maintained by concurrent decrease in conversion of cholesterol to bile acids [21] and in the brain by the efficient cholesterol reutilization [23].

A trend of increase of sterol intermediates has been observed in the testis (Fig. 3A) which is consistent with our earlier results [20]. The change does not meet statistical significance possibly due to low number of animals per group. Though starvation does intervene with hypothalamic–pituitary–gonadal axis and represses reproduction [53], the antigonadal effect of short-term starvation can be overcome with high dose of GnRH [54]. Our data imply that testis poses mechanisms to override short-term fluctuation of energy supply in regulation of cholesterol biosynthesis, in particular after sexually maturity [20].

Interplay between inflammation and disturbed cholesterol homeostasis is implicated in the pathogenesis of obesity-related metabolic disorders and neurodegenerative diseases [55] that actually share several common abnormalities. We chose TNF-α to induce inflammatory response as it has a key role in development of insulin resistance in mice [25] and human [56], and plays a major role in neuroinflammation-mediated cell death in Alzheimer's disease [57]. In the liver, TNF-administration results in the appearance of sterol intermediates (Fig. 3B, white bars) not present in normally fed or fasted mice. The increase of intermediates with Δ24 unsaturated double bond and no change in 7-dehydrocholesterol imply that CYP51 is more efficient than DHCR24 in competition for lanosterol after the TNF-α stimulus.

Brain is considered an immunologically privileged site due to the presence of the blood–brain barrier. However, TNF-α was demonstrated to cross the blood–brain barrier [58]. Our data show that TNF-α influences cholesterol synthesis in brain and leads to increase of several intermediates (Fig. 3B, gray bars), statistically significant for zymosterol. Changes in cholesterol metabolism are intimately involved in pathogenic processes of neurodegenerative disorders [59]. The buildup of sterol intermediates might interfere with membrane dynamics and protein/receptor function and might represents a crucial determinant in neurogenesis. It was shown that in cholesterol depleted conditions, desmosterol [60] and 7-dehydrocholesterol [61] are not efficient in restoring ligand binding activity of the serotonin(1A) receptor, in spite of recovery of the overall membrane order. Additionally, DHCR24, the enzyme that catalyzes the conversion of desmosterol into cholesterol, is down-regulated in affected neurons in Alzheimer's disease and its increased expression was found to be protective against Aβ-induced toxicity [62]. APPSLxPS1mut mouse model for Alzheimer's disease display increased levels of the cholesterol precursor desmosterol [63]. Our results provide novel insight into the interaction between inflammation and cholesterol biosynthesis that may contribute to neuropathogenesis through accumulation of sterol intermediates.

In testis (Fig. 3B, black bars), TNF-α-treatment also results in the build up of sterol intermediates. The decrease in lanosterol, no change in FF-MAS and increase in T-MAS and subsequent intermediates might imply that in testis TNF-α accelerates the conversion of FF-MAS to T-MAS. This further implies that after the TNF-α stimulus, CYP51 might represent the rate-limiting step in the post-squalene part of cholesterol synthesis. In testis TNF-α causes statistically significant increase in 7-dehydrocholesterol and desmosterol, suggesting that both branches of cholesterol synthesis are used.

5. Conclusions

We show that liver, brain and testis possess tissue-specific sterol fingerprints that are changed in response to fasting and TNF-α stimulus. This gives novel insights into the regulation of cholesterol homeostasis by inflammatory stimuli. Increase of sterol intermediates triggered by TNF-α might be implicated in the pathogenesis of obesity-related metabolic disorders and in neurodegeneration.

Acknowledgements

We thank Mogens Baltsen and Dr. A.G. Byskov (Laboratory of Reproductive Biology, University Hospital of Copenhagen) for FF-

MAS and T-MAS standards, Helena Klavžar (CFGBC, University of Ljubljana, Slovenia), Martina Perše (Institute of Pathology, Medical Experimental Centre, Faculty of Medicine University of Ljubljana), Dr. Manica Černe (Lek, d.d.) and Dr. Srdjan Novakovič (Institute of Oncology Ljubljana) for help with animals. Dr. K. Košmelj, J. Ačimović and Dr. P. Juvan are also acknowledged for helpful discussions on statistical analysis. The work was supported by the Slovenian Research Agency, Grants J1-6713, P1-0527, Z1-7562-0381, and the funds of Lek Pharmaceuticals, d.d. Klementina Fon Tacer was supported by the fellowship from the Slovenian Research Agency.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsbmb.2010.02.026.

References

[1] R.H. Eckel, S.M. Grundy, P.Z. Zimmet, The metabolic syndrome, Lancet 365 (2005) 1415–1428.
[2] S.M. de la Monte, L. Longato, M. Tong, J.R. Wands, Insulin resistance and neurodegeneration: roles of obesity, type 2 diabetes mellitus and non-alcoholic steatohepatitis, Curr. Opin. Investig. Drugs 10 (2009) 1049–1060.
[3] D.G. Goulis, B.C. Tarlatzis, Metabolic syndrome and reproduction. I. Testicular function, Gynecol. Endocrinol. 24 (2008) 33–39.
[4] P. Libby, M. Aikawa, U. Schonbeck, Cholesterol and atherosclerosis, Biochim. Biophys. Acta 1529 (2000) 299–309.
[5] G.E. McKellar, D.W. McCarey, N. Sattar, I.B. McInnes, Role for TNF in atherosclerosis? Lessons from autoimmune disease, Nat. Rev. Cardiol. 6 (2009) 410–417.
[6] L. Hermo, R.-M. Pelletier, D.G. Cyr, C.E. Smith, Surfing the wave, cycle, life history, and genes/proteins expressed by testicular germ cells, Microsc. Res. Tech. 9999 (2009).
[7] H.R. Waterham, R.J. Wanders, Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith–Lemli–Opitz syndrome, Biochim. Biophys. Acta 1529 (2000) 340–356.
[8] L. Liscum, Cholesterol biosynthesis, in: D.E. Vance, J.E. Vance (Eds.), Biochemistry of Lipids, Lipoproteins and Membranes, Elsevier, 2002, pp. 409–431.
[9] J.L. Gaylor, Membrane-bound enzymes of cholesterol synthesis from lanosterol, Biochem. Biophys. Res. Commun. 292 (2002) 1139–1146.
[10] J.L. Goldstein, M.S. Brown, Regulation of the mevalonate pathway, Nature 343 (1990) 425–430.
[11] A.G. Byskov, C.Y. Andersen, L. Nordholm, H. Thogersen, X. Guoliang, O. Wassman, J.V.A.E. Guddal, T. Roed, Chemical structure of sterols that activate oocyte meiosis, Nature 374 (1995) 559–562.
[12] E.V. Bokal, K. Fon Tacer, M. Vrbnjak, S. Leposa, I.V. Klun, I. Verdenik, D. Rozman, Follicular sterol composition in gonadotrophin stimulated women with polycystic ovarian syndrome, Mol. Cell. Endocrinol. 249 (2006) 92–98.
[13] M. Cotman, D. Jezek, K. Fon Tacer, R. Frangez, D. Rozman, A functional cytochrome P450 lanosterol 14 alpha-demethylase CYP51 enzyme in the acrosome: transport through the Golgi and synthesis of meiosis-activating sterols, Endocrinology 145 (2004) 1419–1426.
[14] L.J. Engelking, B.M. Evers, J.A. Richardson, J.L. Goldstein, M.S. Brown, G. Liang, Severe facial clefting in Insig-deficient mouse embryos caused by sterol accumulation and reversed by lovastatin, J. Clin. Invest. 116 (2006) 2356–2365.
[15] B.M. Evers, M.S. Farooqi, J.M. Shelton, J.A. Richardson, J.L. Goldstein, M.S. Brown, G. Liang, Hair growth defects in Insig-deficient mice caused by cholesterol precursor accumulation and reversed by simvastatin, J. Invest Dermatol. (2010).
[16] M. Bauer, A.C. Hamm, M. Bonaus, A. Jacob, J. Jaekel, H. Schorle, M.J. Pankratz, J.D. Katzenberger, Starvation response in mouse liver shows strong correlation with life-span-prolonging processes, Physiol. Genomics 17 (2004) 230–244.
[17] S. Sadruddin, R. Arora, Resveratrol: biologic and therapeutic implications, J. CardioMetab. Syndr. 4 (2009) 102–106.
[18] C. Grunfeld, C. Zhao, J. Fuller, A. Pollack, A. Moser, J. Friedman, K.R. Feingold, Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters, J. Clin. Invest. 97 (1996) 2152–2157.
[19] M. Baltsen, A.G. Byskov, Quantitation of meiosis activating sterols in human follicular fluid using HPLC and photodiode array detection, Biomed. Chromatogr. 13 (1999) 382–388.
[20] K. Fon Tacer, S. Kalanj-Bognar, M.R. Waterman, D. Rozman, Lanosterol metabolism and sterol regulatory element binding protein (SREBP) expression in male germ cell maturation, J. Ster. Biochem. Mol. Biol. 85 (2003) 429–438.
[21] K. Fon Tacer, D. Kuzman, M. Seliskar, D. Pompon, D. Rozman, TNF-alpha interferes with lipid homeostasis and activates acute and proatherogenic processes, Physiol. Genomics 31 (2007) 216–227.
[22] M. Panchal, J. Loeper, J.C. Cossec, C. Perruchini, A. Lazar, D. Pompon, C. Duyckaerts, Enrichment of cholesterol in microdissected Alzheimer disease senile plaques as assessed by mass spectrometry, J. Lipid Res. (2009).
[23] J.M. Dietschy, S.D. Turley, Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal, J. Lipid Res. 45 (2004) 1375–1397.
[24] S.H. Bae, Y.K. Paik, Cholesterol biosynthesis from lanosterol: development of a novel assay method and characterization of rat liver microsomal lanosterol delta 24-reductase, Biochem. J. 326 (1997) 609–616.
[25] K.T. Uysal, S.M. Wiesbrock, M.W. Marino, G.S. Hotamisligil, Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function, Nature 389 (1997) 610–614.
[26] Y. Wang, A.H. Moser, J.K. Shigenaga, C. Grunfeld, K.R. Feingold, Downregulation of liver X receptor-{alpha} in mouse kidney and HK-2 proximal tubular cells by LPS and cytokines, J. Lipid Res. 46 (2005) 2377–2387.
[27] A.J. Bruce-Keller, J.N. Keller, C.D. Morrison, Obesity and vulnerability of the CNS, Biochim. Biophys. Acta 1792 (2009) 395–400.
[28] C. Yang, J.G. McDonald, A. Patel, Y. Zhang, M. Umetani, F. Xu, E.J. Westover, D.F. Covey, D.J. Mangelsdorf, J.C. Cohen, H.H. Hobbs, Sterol intermediates from cholesterol biosynthetic pathway as liver × receptor ligands, J. Biol. Chem. 281 (2006) 27816–27826.
[29] J.J. Repa, D.J. Mangelsdorf, The liver X receptor gene team: potential new players in atherosclerosis, Nat Med 8 (2002) 1243–1248.
[30] K.E. Bloch, Sterol structure and membrane function, CRC Crit. Rev. Biochem. 14 (1983) 47–92.
[31] A.A. Kandutsch, A.E. Russell, Preputial gland tumor sterols, J. Biol. Chem. 235 (1960) 2256–2261.
[32] M.S. Brown, J.L. Goldstein, The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor, Cell 89 (1997) 331–340.
[33] Y. Wang, P.M. Rogers, C. Su, G. Varga, K.R. Stayrook, T.P. Burris, Regulation of cholesterologenesis by the oxysterol receptor, LXRalpha, J. Biol. Chem. 283 (2008) 26332–26339.
[34] N. Zelcer, C. Hong, R. Boyadjian, P. Tontonoz, LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor, Science 325 (2009) 100–104.
[35] J.M. Dietschy, S.D. Turley, D.K. Spady, Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans, J. Lipid Res. 34 (1993) 1637–1654.
[36] J.M. Dietschy, S.D. Turley, Cholesterol metabolism in the central nervous system during early development and in the mature animal, J. Lipid Res. 45 (2004) 1375–1397.
[37] J.M. Dietschy, S.D. Turley, Control of cholesterol turnover in the mouse, J. Biol. Chem. 277 (2002) 3801–3804.
[38] I. Bjorkhem, S. Meaney, Brain cholesterol: long secret life behind a barrier, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 806–815.
[39] D. Lutjohann, A. Brzezinka, E. Barth, D. Abramowski, M. Staufenbiel, K. von Bergmann, K. Beyreuther, G. Multhaup, T.A. Bayer, Profile of cholesterol-related sterols in aged amyloid precursor protein transgenic mouse brain, J. Lipid Res. 43 (2002) 1078–1085.
[40] K. Fon Tacer, T.B. Haugen, M. Baltsen, N. Debeljak, D. Rozman, Tissue-specific transcriptional regulation of the cholesterol biosynthetic pathway leads to accumulation of testis meiosis-activating sterol (T-MAS), J. Lipid Res. 43 (2002) 82–89.
[41] D.S. Lin, W.E. Connor, D.P. Wolf, M. Neuringer, D.L. Hachey, Unique lipids of primate spermatozoa: desmosterol and docosahexaenoic acid, J. Lipid Res. 34 (1993) 491–499.
[42] X. Cao, S.H. Pomerantz, M. Popliker, A. Tsafriri, Meiosis-activating sterol synthesis in rat preovulatory follicle: is it involved in resumption of meiosis? Biol. Reprod. 71 (2004) 1807–1812.
[43] C.L. Marin Bivens, C. Grondahl, A. Murray, T. Blume, Y.-Q. Su, J.J. Eppig, Meiosis-activating sterol promotes the metaphase i to metaphase II transition and preimplantation developmental competence of mouse oocytes maturing in vitro, Biol. Reprod. 70 (2004) 1458–1464.
[44] J.L. Cavilla, C.R. Kennedy, A.G. Byskov, G.M. Hartshorne, Human immature oocytes grow during culture for IVM, Hum. Reprod. 23 (2008) 37–45.
[45] S. Cukurcam, C. Hegele-Hartung, U. Eichenlaub-Ritter, Meiosis-activating sterol protects oocytes from precocious chromosome segregation, Hum. Reprod. 18 (2003) 1908–1917.
[46] V.D. Longo, C.E. Finch, Evolutionary medicine: from dwarf model systems to healthy centenarians? Science 299 (2003) 1342–1346.
[47] T. Wang, C.C. Hung, D.J. Randall, The comparative physiology of food deprivation: from feast to famine, Annu. Rev. Physiol. 68 (2006) 223–251.
[48] L. Hue, M.H. Rider, The AMP-activated protein kinase: more than an energy sensor, Essays Biochem. 43 (2007) 121–137.
[49] K.R. Feingold, M.H. Wiley, G. MacRae, S. Lear, A.H. Moser, G. Zsigmond, M.D. Siperstein, De novo sterologenesis in the intact rat, Metabolism 32 (1983) 75–81.
[50] J.M. Dietschy, M.D. Siperstein, Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat, J. Lipid Res. 8 (1967) 97–104.
[51] S.D. Turley, C.E. West, Effect of cholesterol and cholestyramine feeding and of fasting on sterol synthesis in the liver, Ileum, and lung of the guinea pig, Lipids 11 (1976) 571–577.
[52] T.Y. Chan, P.L. Tang, Factors regulating sterol biosynthesis in the rat pineal gland in vitro, Biochem. Mol. Biol. Int. 34 (1994) 983–992.
[53] L.J. Hoffer, I.Z. Beitins, N.H. Kyung, B.R. Bistrian, Effects of severe dietary restriction on male reproductive hormones, J. Clin. Endocrinol. Metab. 62 (1986) 288–292.

[54] M. Bergendahl, I. Huhtaniemi, Acute fasting is ineffective in suppressing pituitary-gonadal function of pubertal male rats, Am. J. Physiol. 264 (1993) E717–722.
[55] R.M. Adibhatla, J.F. Hatcher, Altered lipid metabolism in brain injury and disorders, Subcell. Biochem. 49 (2008) 241–268.
[56] A. Jellema, J. Plat, R.P. Mensink, Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state, Eur. J. Clin. Invest. 34 (2004) 766–773.
[57] D. Tweedie, K. Sambamurti, N.H. Greig, TNF-alpha inhibition as a treatment strategy for neurodegenerative disorders: new drug candidates and targets, Curr. Alzheimer Res. 4 (2007) 378–385.
[58] W. Pan, A.J. Kastin, TNF-alpha transport across the blood–brain barrier is abolished in receptor knockout mice, Exp. Neurol. 174 (2002) 193–200.
[59] I.J. Martins, T. Berger, M.J. Sharman, G. Verdile, S.J. Fuller, R.N. Martins, Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease, J. Neurochem. 111 (2009) 1275–1308.
[60] P. Singh, R. Saxena, Y.D. Paila, M. Jafurulla, A. Chattopadhyay, Differential effects of cholesterol and desmosterol on the ligand binding function of the hippocampal serotonin(1A) receptor: implications in desmosterolosis, Biochim. Biophys. Acta 1788 (2009) 2169–2173.
[61] P. Singh, Y.D. Paila, A. Chattopadhyay, Differential effects of cholesterol and 7-dehydrocholesterol on the ligand binding activity of the hippocampal serotonin(1A) receptor: implications in SLOS, Biochem. Biophys. Res. Commun. 358 (2007) 495–499.
[62] A. Crameri, E. Biondi, K. Kuehnle, D. Lutjohann, K.M. Thelen, S. Perga, C.G. Dotti, R.M. Nitsch, M.D. Ledesma, M.H. Mohajeri, The role of seladin-1/DHCR24 in cholesterol biosynthesis, APP processing and A[beta] generation in vivo, EMBO J. 25 (2006) 432–443.
[63] T. Vanmierlo, V.W. Bloks, L.C. van Vark-van der Zee, K. Rutten, A. Kerksiek, S. Friedrichs, E. Sijbrands, H.W. Steinbusch, F. Kuipers, D. Lutjohann, M. Mulder, Alterations in brain cholesterol metabolism in the APPSLxPS1mut mouse, a model for Alzheimer's disease, J. Alzheimers Dis. 19 (1) (2009) 117–127.