FEBS Letters 584 (2010) 2689–2698

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

An allostatic control of membrane lipid composition by SREBP1

Rachel M. Hagen *, Sergio Rodriguez-Cuenca, Antonio Vidal-Puig **

University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Level 4, Box 289, Addenbrooke's Treatment Centre, Addenbrooke's Hospital, Hills Rd., Cambridge CB2 OQQ, UK

ARTICLE INFO

Article history:
Received 16 February 2010
Accepted 3 April 2010
Available online 10 April 2010

Edited by Stefan Hohmann

Keywords:
Unsaturated fatty acid biosynthesis
Membrane lipid composition
Sterol receptor element-binding protein

ABSTRACT

The maintenance of membrane lipid composition within strict limits is critical to maintain optimum cellular function. The biophysical properties of the membrane can be influenced among other factors by the saturation/unsaturation of the phospholipid fatty acyl chain. The rate-limiting enzyme in unsaturated fatty acid biosynthesis is the desaturase enzyme which in turn is regulated by the lipid transcription factor sterol regulatory element binding protein (SREBP1). In this review, we collect some evidence suggesting SREBP1 network as an important allostatic regulator necessary to maintain the pool of unsaturated fatty acid lipid species that can be incorporated into biological membranes.

1. Introduction of the concept of SREBP/Insigs as allostatic mediators

In this review we explore the concept that the sterol regulatory element binding protein 1 (SREBP1) network may be an allostatic node contributing to lipid membrane composition quality control mechanisms. As SREBP1 is central in the control of a variety of lipid biosynthetic pathways (in particular lipid desaturation and phospholipid biosynthesis), our view is that SREBP1 may be an important allostatic mediator that induces adaptive responses to dietary/environmental stresses potentially resulting in lipid perturbations to maintain optimal membrane lipid composition and ensure cell survival and function (Fig. 1). The concept of allostasis was introduced by Sterling and Eye in 1993 to capture the idea of "maintenance of stability through change". From this concept it can be implied that biological systems are flexible, with set points, internal controls and adaptive responses that allow them to maintain key fundamental biological parameters (e.g. glucose, blood pressure, biological membrane lipid composition and biophysical properties) within strict physiological homeostatic limits [1]. These allostatic responses can require energy and can themselves be a cause of stress. Maintenance of these allostatic adaptations imposes different degrees of stress to the components of a system that on their own may have different degrees of vulnerability. Following this, allostatic adaptations can occasionally cause inappropriate activation of alternative paths that on their own can become toxic causing collateral damage and disease. We think that the concept of allostasis can be applied to the mechanisms controlling membrane lipid composition quality and suggest the possibility that the SREBP1/Insig could be an important node of this allostatic responses.

Lipids are as fundamental to life as are DNA and proteins. Unlike other classes of biomolecules such as proteins and DNA, the structure of lipids varies tremendously. This is in our opinion probably the reason why studies focused in lipid biochemistry are well behind compared to studies involving nucleic acids or proteins. In fact the eukaryotic cell uses approximately 5% of its genes to generate thousands of different lipid species [2] suggesting there has been evolutionary pressure to be able to synthesise a large number of diverse lipid species. Lipids are involved in a wide array of processes from compartmentalisation of the cell with membranes to provide efficient energy storage and specific substrates for cell signalling. The importance of lipids is outlined by the large number of human pathologies that in one way or another have been linked to altered lipid and membrane properties, including cardiovascular diseases, diabetes, obesity, inflammation, autoimmune diseases, neurological diseases and cancer [3 and references within]. Thus

Abbreviations: bHLHLZ, basic helix loop helix leucine zipper; CCT, CTP:phosphocholine cytidylyltransferase; DAG, diacylglycerol; ER, endoplasmic reticulum; FAS, fatty acid synthase; HFD, high fat diet; HUFA, highly unsaturated fatty acids; Insig, insulin induced gene; LPA, lysophosphatidic acid; LPLAT, lysophospholipid acyltransferases; LXR, liver X receptor; mGPAT, mitochondrial glycerol-3-phosphate acyltransferase; MUFA, monounsaturated fatty acids; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids; SCAP, SREBP cleavage activating protein; SCD1, stearoyl-CoA desaturase 1; SRE, sterol regulatory element; SREBP, sterol receptor element-binding protein; SSD, sterol-sensing domain; TAG, triacylglycerol

* Corresponding author. Fax: +44 1223 330598.
** Corresponding author. Fax: +44 1223 330598.
E-mail addresses: rmh51@cam.ac.uk, rhagen19@hotmail.com (R.M. Hagen), ajv22@medschl.cam.ac.uk (A. Vidal-Puig).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.04.004

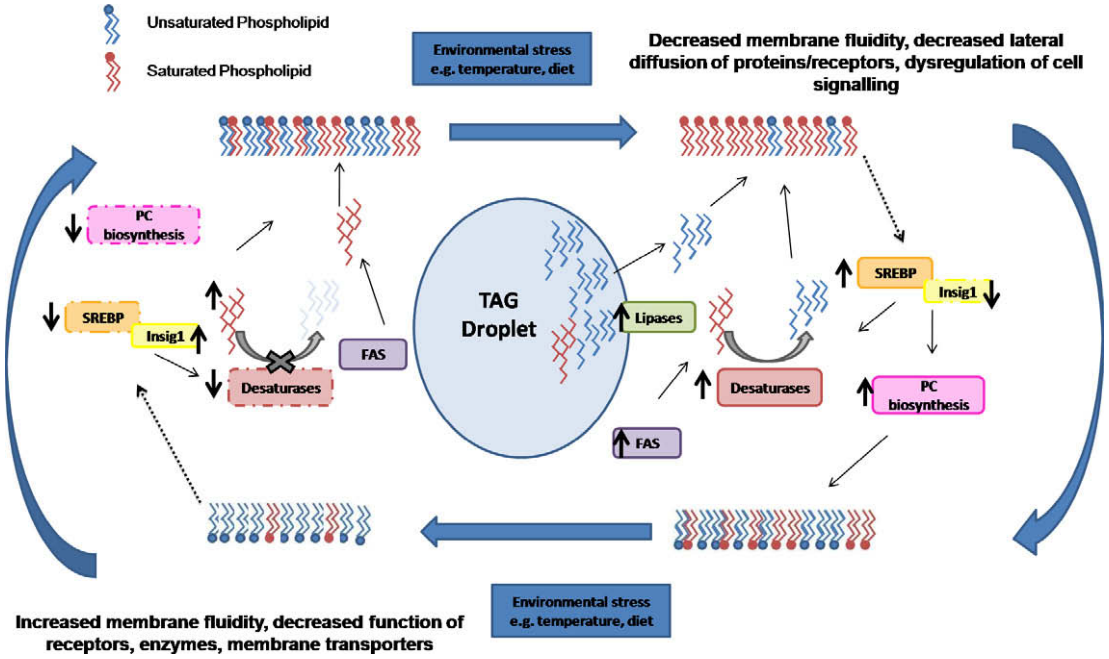

Fig. 1. A cartoon interpretation of how SREBP1 can maintain membrane composition through control of fatty acid desaturation and phosphatidylcholine (PC) biosynthesis. A decrease in membrane fluidity may be sensed by SREBP/Insig complex. A reduction in Insig-1 facilitates SREBP cleavage and increases SREBP activation. SREBP upregulates SCD1, FAS, lipase action and PC biosynthesis resulting in increased biosynthesis of monounsaturated fatty acids and PC which can increase membrane fluidity. If the membrane becomes too fluid, SREBP is no longer activated (possibly through an increase of the SREBP anchor Insig-1 and desaturase and PC biosynthesis) and membrane fluidity is reduced.

the understanding of how specific components of the membrane lipid composition affects the physiological function of the cell is important if we are going to determine how lipid alterations influence cell signalling in pathologies where lipid changes have been extensively reported, such as type 2 diabetes or atherosclerosis.

One aspect of which alterations in lipid composition can have profound effects is on the biophysical properties of the biological membrane. Biological membranes enclose cytoplasmic membranes as well as organelles' membranes acting as a permeability barrier, separating out different compartments and producing optimum environmental conditions for biological processes within the cell. The cell membrane is a highly dynamic structure that needs to be able to grow in size during growth and cell division, and maintain continual turnover and renewal of membrane components under resting conditions [4]. The cell membranes are composed predominantly of a bilayer of phospholipids in which sphingolipids and sterols are also present. However, the membrane bilayer is not homogeneous in nature. Specialised structures such as "lipid rafts" or lipid microdomains characteristically enriched in cholesterol and sphingomyelin serve as communication hubs for signal transduction pathways where receptors cluster to coordinate specific signalling events and cellular functions. The membranes are composed of diverse glycerophospholipids species consisting of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI). They all have a common structure including a polar phosphate containing the head group and two hydrophobic hydrocarbon chains derived from fatty acids. The fatty acid chains are numbered sn-1 and sn-2 and are usually between 14 and 24 carbons in length, whilst one chain is usually unsaturated (usually at the sn-2 position).

Fatty acid chains in phospholipids change in response to varying environmental stresses, such as temperature. The relationship between temperature and membrane fluidity (homeoviscous adaptation) has been extensively studied in bacteria, but many of its characteristics can be applied to plants and animals [5]. As the temperature increases there is a decrease in unsaturated fatty acyl chains and an increase in the average length of fatty acid chains, allowing the membrane to become more rigid. In mammalians, sterols are an additional component that is important in regulating membrane fluidity. In fact increased sterol concentrations within a membrane results in increased rigidity and a reduction in fluidity.

The effect of fatty acid unsaturation on the membrane fluidity is probably one of the best characterised phenomenons, which has been demonstrated in animals, fish, fungi, plants, bacteria and cyanobacteria [6]. In fact the ratio between saturated and monounsaturated fatty acids (MUFA) chains of the phospholipids can directly influence the physical properties of cell membranes. An increase in the saturated fatty acid content results in decreased membrane fluidity, as the phospholipids are able to pack densely together. The importance of this function is revealed by the fact that the enzymes involved in the desaturation of lipids, so called desaturases have been evolutionally conserved [7]. However, it is not only the fatty acyl chain unsaturation characteristics that can affect membrane function. Among other factors, the head group alteration of phospholipids influence membrane behaviour. Choline is a bulky headgroup and thus PC has a cylindrical molecular geometry [8]. In contrast, ethanolamine is a smaller headgroup and thus PE assumes a conical molecular geometry. The smaller headgroup of PE allows tighter packing of the phospholipids and thus, when as the abundance of PE versus PC increases there is a reduction in membrane fluidity.

A combination of the degree and orientation of double bonds within the fatty acid hydrocarbon chain, phospholipid head groups and length of chains has led to the existence of more than 1000 different lipid species in a cell [2]. This diversity in lipid species allows optimising the lipid composition of membranes to fulfill specific functions. Importantly, the lipid composition of membranes

is not homogenous throughout the cell. Each organelle has been found to have a unique lipid composition allowing optimal functionality. For example, the plasma membrane contains a high concentration of sterols and sphingolipids compared with the endoplasmic reticulum (ER) with the consequence that the ER membrane is more fluid [8]. Mitochondria, the Golgi apparatus, peroxisomes, and even the lipid droplets also have distinct lipid composition, which confers the correct biophysical properties to each organelle and contribute to maintain the viability of the cell. Depending on their composition, these biomembranes play an important role in regulating many cellular functions, in addition to the mere compartmentalisation, such as signal transduction, cell adhesion, lipid trafficking, ion channel function, receptor mobility/interaction and viral/bacterial entry [9,10]. Thus, the diverse lipid repertoire provides the advantage of optimising membrane composition to its specialised functions. However, this raises several problems: (a) What are the mechanisms of quality control over membrane lipid composition? (b) How specific are these mechanisms to sense selective lipid defects? (c) How selective are the allostatic responses in fixing selective defects?

2. Where do the unsaturated fatty acids for incorporation into membranes come from?

Hydrocarbon chains of fatty acids can either be classed as saturated, MUFA or polyunsaturated fatty acids (PUFA), depending on the number of double bonds present. Cells have the capacity to synthesise fatty acids de novo via the enzymes acetyl coal carboxylase (ACC) and fatty acid synthase (FAS), resulting in the generation of palmitic fatty acid (16:0) through a series of cyclic reactions (1. condensation, 2. ketoreduction, 3. dehydration and 4. enoyl reduction). Fatty acids derived from the diet and from de novo lipogenesis can be further elongated and double bonds added via elongases and desaturases respectively contribute to generate a variety of lipid species. Later on, through the actions of lipid remodelling enzymes such as phospholipases and lysophospholipid acyltransferases (LPLATs) a specific membrane lipid composition can be maintained. However, a remaining challenge is to determine how these mechanisms can be regulated to match the specific needs of the membranes. In our opinion the most efficient way to solve this biological problem would be via double levels of regulation: (a) A long loop based on adaptive transcriptional responses aiming at producing a substrate pool and (b) A short loop able to select/produce the specific lipid species from the pool to provide the specific needs. As a proof of concept of the existence of allostatic mechanisms contributing to the membrane quality control in this review, we will focus specifically on the first level of control through the action and clinical relevance of transcriptional networks controlling the level of unsaturation (for further information on elongases and elongation of lipids please see reviews by [11,12]).

The regulation of desaturases will ultimately affect lipid composition of the cell, as their activity will determine the ratio of unsaturated to saturated lipids within the cell. In mammals, there are two classes of desaturases. Stearoyl-CoA desaturase (SCD) is a Δ-9 desaturase ER bound membrane rate-limiting enzyme involved in the conversion of saturated fatty acids to MUFA. SCD is associated with nicotinamide adenine dinucleotide phosphate (NADPH), cytochrome b5 reductase, and cytochrome b5 and in the presence of molecular oxygen introduces a single double bond (between carbons 9 and 10), into saturated fatty acyl-CoAs [12]. The other class of desaturases is comprised of Δ-5 (FADS1) and Δ-6 (FADS2) desaturases that are responsible for the production of PUFAs or HUFAs (highly unsaturated fatty acids) such as arachidonic acid (AA) and EPA which are the precursors for omega-3 and omega-6 fatty acids (for review see [12]).

3. Why is it so important to control the balance of unsaturated versus saturated fatty acids? Insights from model organisms lacking desaturase activity

The importance of the desaturase programme and the resulting physiological complications arising from unsaturated fatty acid deficiency has been demonstrated using mutant model organisms such as *Caenorhabditis elegans* (*C. elegans*) and murine models. For instance *C. elegans* mutants lacking crucial components of the fatty acid desaturation pathway demonstrate the vital function that unsaturated fatty acids play in overall physiology of organisms. *C. elegans* mutants that lack Δ-6 desaturase activity have delayed growth and altered behaviour. This is likely to be a direct result of defects in the synaptic vesicle recycling and thus impairment in neurotransmission. They also exhibit neuromuscular defects, cuticle abnormalities, reduced innate immune response, impaired sensory signalling, altered biological rhythms and reduced fertility [13]. Fertility problems are more evident in the Δ-6 desaturase mutants at low temperatures where embryogenesis is impaired, most likely through impairment in oogenesis. At higher temperatures reproduction success is higher suggesting that it is a defective adaptation to low temperatures that is compromised in these mutants. An inability to adapt to low temperatures is not surprising as it has been well documented that in poikilotherms, bacteria and plants in response to lower temperatures there is an induction in desaturase activity, suggesting a direct link between membrane fluidity and desaturase activation [6].

In higher organisms such as mice, the role and function of the desaturases have also been extensively investigated. Mice lacking Δ-9 desaturase activity (SCD1 KO) have reduced body adiposity, increased insulin sensitivity and are resistant to diet induced obesity [14]. This alteration in body fat composition is a result of increased energy expenditure that appears to be an adaptive activation in response to disruption of the skin epidermal lipid barrier in these mice [15]. SCD1 KO mice have a reduction in lipid biosynthetic genes corresponding with decreased triglyceride accumulation, a reduction in cholesterol/wax esters and sebaceous gland hyperplasia [16]. These mice also suffer from "dry eye syndrome" as a result of reduced sebaceous lipids. With these alterations in the lipids of the skin and retinal barriers it is not surprising that as observed with the *C. elegans* mutants, mice which that have lack of SCD1 only in the skin have a reduced capacity to adapt to low temperatures and are cold intolerant [16]. Mice lacking Δ-6 desaturase activity (FADS2) are relatively normal phenotypically but again, similar to the *C. elegans* mutants have fertility problems with a large proportion of the mutants being sterile. In humans, a mutation related to impairment in Δ-6 desaturase activity has been associated with impairment in growth and skin disease [17].

All in all, the data from various organisms highlight at least three main physiological abnormalities that correspond with a reduction in lipid unsaturation: (1) Neurological function, possibly through altered synaptic transmission between neurons and propagation of electrical signals. (2) Alterations in the skin barrier. (3) Inability to adapt to low temperatures. Composition of the bilayer may affect electrical properties and the properties of proteins and receptors embedded in the membrane. The association of a broad range of diseases with dysregulation of lipid metabolism and membrane composition underlines the important need for tight regulation of lipid composition of the cell and biological membranes.

4. How are phospholipids being synthesised and remodelled?

Fatty acids are activated by acyl CoA synthetases (ACS) yielding fatty acyl-CoA molecules that are used in the biosynthesis of phosphatidic acid (PA) and subsequently in phospholipid biosynthesis. ACS provide a first level of regulation since they seem to have a certain degree of fatty acid substrate specificity and contribute to sort specific fatty acids to specific compartments and functions [52]. As mentioned previously, the nature of the fatty acids incorporated during the biosynthetic process is an important determinant of the biophysical properties of the membrane. The glycerol backbones of phospholipids are generated from the catabolism of glucose and pyruvate (glyceroneogenesis). The precursor molecule glycerol-3-phosphate is acylated and incorporates the activated fatty acids via the action of glycerol 3-phosphate acyltransferase (GPAT) producing 1-acyl-glycerol 3-phosphate (lysophosphatidic acid, LPA). LPA is subsequently converted to PA via the enzyme acylglycerol 3-phosphate acyltransferase (AGPAT). Depending on substrate needs PA can either be dephosphorylated to generate a diacylglycerol molecule (DAG) or is converted to cytidine 5-diphospho-diacylglycerol (CDP-DAG) to be used in the generation of phosphoinositides, phosphoglycerol and cardiolipin.

DAG molecules can then be further utilised to generate triacylglycerol (TAG), PC or PE species. DAG production from PA is catalyzed by the enzyme phosphatidate phosphatise 1 (PAP1/Lipin). PAP1 transcription is upregulated by fatty acids mediated by activation of PPARα and potentially through PPARγ [25]. Upon activation (by fatty acids/insulin) PAP1 translocates from the cytosol to the ER where it utilises PA to generate DAG, the precursor of TAG and phospholipids. The flux of DAG towards either TAG synthesis or phospholipid synthesis is largely influenced by the activity of the CTP:phosphocholine cytidylyltransferase (CCT) and the requirement for phospholipids. There seems to be in place tightly regulated systems that allows prioritisation of lipid species into specific biosynthetic pathways. In fact when mechanisms of quality control sense that phospholipid synthesis requirements are low, DAG is converted into TAG by the enzyme diacylglycerol-acyl CoA acyltransferase (DGAT) and TAG is stored as lipid bodies/droplets within the cell.

PC is the primary phospholipid of eukaryotic cellular membranes. PC can be synthesised either by the via the CDP-choline pathway dependent on CCT as the rate-limiting enzyme. The production of PC by the CDP-choline pathway takes place through three stages. First, there is the phosphorylation of intracellular choline (mediated by choline kinase), followed by conversion of phosphocholine to CDP-choline (mediated by CCT), and finally a phosphocholine moiety is transferred from CDP-choline to DAG thus forming PC [mediated by CDP-Choline: 1,2-diacylglycerol cholinephosphotransferase (CPT)]. CCT is reversibly phosphorylated at multiple serine residues (via casein kinase II, cdc2 kinase, cAMP kinase and PKC) and is activated by associating directly with the cell membrane. It seems that alterations on the membrane structure resulting in changes in membrane bilayer curvature induce elastic stress that governs the degree of CCT association with membranes and its activity [18].

The pool of phospholipids does not only depend on transcriptionally driven de novo biosynthesis. Cell membranes undergo continual remodelling with phospholipids being continually synthesised and hydrolyzed [4]. This second loop of regulation allows acute adjustment of membrane composition in response to environmental demands. Globally considered remodelling of phospholipids is mediated by three different steps: (1). Deacylation (by phospholipases). (2). Fatty acid synthesis (by FAS). (3). Reacylation (by acyltransferases). Phospholipases are a group of enzymes able to hydrolyze phospholipids and contribute to the biosynthesis of many intracellular lipid messengers. Products derived from phospholipids by phospholipases include DAG, inositol triphosphate, arachidonic acid (AA), platelet activating factor and prostaglandins. LPLATs are responsible for the turnover of the fatty acid hydrocarbon at the sn-2 position of phospholipids resulting in a diverse array of phospholipids independent of de novo synthesis. LPLATs transfer activated fatty acids (acyl-CoA esters produced via acyl-CoA synthetase) to lysophospholipids and control the phospholipid levels containing lipid-mediated precursors such as arachidonic acid (for a more detailed review refer to [19]).

In summary, there are a number of key steps as part of a double level of control in the generation of specific phospholipids to be incorporated into biomembranes. Through a concerted effort of desaturases, elongases, genes involved in phospholipid biosynthesis (long loop) as well as phospholipases and LPLATs (short loop) a diverse array of lipids is generated in order to provide an efficient membrane composition. However the efficiency of these loops depends on having proper regulatory allostatic membrane quality control mechanisms.

5. Is there any evidence that human metabolic pathologies are related to altered unsaturated lipid composition?

As aforementioned, the maintenance of an appropriate membrane lipid composition appears to be crucial since differences in the ratio between specific lipids (e.g. saturated/PUFA) can result in differences in membrane fluidity, topology of attached proteins, activity of membrane-bound enzymes (e.g. GTPases), degree of exposure of surface proteins, lateral mobility of receptors (e.g. insulin receptor) and activation of specific signalling pathways (e.g. insulin signalling). In fact the subtle alterations in lipid metabolism and phospholipid composition (in particular levels of PUFAs) can have profound clinical effects. For instance, alterations in the fatty acid composition of membrane phospholipids have been linked to obesity and insulin resistance [20]. The effects of fatty acids on energy balance can also occur at the level of the metabolic rate. This is shown in rats fed a diet rich in saturated fatty acid (directly associated with decreased membrane fluidity) which had decreased beta-adrenergic receptor binding affinity in adipose tissue and brain, leading to a reduction in sympathetic activity and lipolysis in brown adipose tissue and by hence, a reduction in diet induced thermogenic capacity [21]. Whether the alterations in fluidity are directly due to alterations in fatty acid desaturase/elongase/LPLAT activity or redistribution of other membrane lipids has yet to be determined. Diseases such as diabetes is associated with decreased red blood cell membrane fluidity as a result of increased cholesterol content, decreased phospholipids and decreased protein levels [22]. Altered phospholipid composition has also been linked to insulin resistance in skeletal muscle in human and rodent models [23]. In the same line, it has already been established that lipid microdomains are critical for proper compartmentalisation of insulin receptors and the mediation of downstream signalling [24]. Conversely, increased PUFA concentration (and presumably increased fluidity of membrane) seems to improve insulin action. The distribution of phospholipid classes in the adipocyte membrane has also been associated with insulin resistance. In human obese females, the amount of sphingomyelin and cholesterol content within the adipocyte membrane negatively correlated with insulin sensitivity suggesting that these changes may affect structural properties and subsequently affecting insulin action [22].

6. How dysregulated unsaturated fatty acids may influence adipose tissue expansion and obesity complications?

The need to maintain a proper level of fatty acid desaturation is fundamental to prevent the deleterious effects of the accumulation

of excessive long chain saturated fatty acids (and not unsaturated fatty acids). This surplus of saturated fatty acids has been associated with lipid induced toxic effects such as the generation of reactive oxygen species (ROS), de novo ceramide synthesis, and activation of the apoptotic cascade [25], ultimately leading to insulin resistance and impairment of mitochondrial structure/function. MUFA such as oleic acids are preferentially incorporated into triglycerides and in doing so they may have a less lipotoxic effect than saturated fatty acids as the TAG pool may help to buffer the saturated fatty acids [26,27]. Thus, the improved ability to store excess lipids should be considered as a crucial buffering process sequestering away potentially toxic fatty acids to ensure the optimum functionality of the cell. Illustrating this concept, ablation of the key triglyceride biosynthetic enzyme, DGAT-1 results in impaired triglyceride synthesis and DAG accumulation promoting the development of lipotoxicity in cells.

The accumulation of TAG stores in the form of a neutral lipid droplet provides a reservoir of lipids for maintenance of cellular lipid homeostasis, energy storage and production of signalling lipids under stress conditions such as food deprivation. Adipose tissue is a vital organ as it is able to provide safe storage capacity of energy and indirectly prevents ectopic energy storage. Under conditions of overnutrition and excess of influx of fatty acids into peripheral organs such as liver, skeletal muscle and pancreas, accumulation of excessive lipids can lead to metabolic complications, such as insulin resistance and inflammation [28]. Therefore, storage and expandability capacity of the adipocyte are important for the partition and storage of fatty acids away from vulnerable tissues.

The lipid droplet is a subcellular compartment with a specific and unique lipid and protein composition distinct to other cellular organelles [29]. The lipid droplet is composed of a monolayer of phospholipids and hydrophobic proteins surrounding a hydrophobic core of neutral lipid species such as TAGs and cholesterol esters. Under conditions where fatty acids are needed, lipids are mobilised from the lipid droplet by lipases. There is evidence that lipases are recruited and interact directly with the lipid droplet membrane in response to lipolytic stimuli such as the beta-adrenergic pathway. A number of proteins have been found embedded in the lipid droplet membrane such as perilipin and adipophilin [30]. These proteins are believed to be important in the coordination and recruitment of lipases and other proteins to the lipid droplet. Lipid droplet compositional analysis in the human liver cell line HepG2 has revealed that PC is the most abundant phospholipid present and that interestingly the acyl chains of the phospholipid are highly unsaturated [31]. This unique membrane lipid composition is likely to prove vital to the functioning of the lipid droplet. Thus the ability and efficient accommodation of lipid storage within a lipid droplet may be highly dependent of the generation of MUFA. Unsaturated fatty acids (acyl chains C 12-18 in length) but not saturated fatty acids induce the formation of lipid droplet or increase the size of pre-existing lipid droplets [32]. Impairment in desaturase activity is correlated with a decreased ability to generate lipid droplets and expansion of lipid droplets to facilitate triglyceride storage [33]. Hence, a defect in the fatty acid desaturation programme may compromise the adipose tissue capacity to store excess of dietary lipid inertly as triglyceride within the lipid droplet, leading to the accumulation of toxic saturated lipids within the cytoplasm.

In summary, the importance of maintaining an appropriate lipid composition is stressed by the fact that a broad range of diseases are associated with dysregulation of lipid metabolism and membrane composition. The importance of maintaining tight control of the lipid membrane characteristics requires that the cell membranes have exquisite sensors able to detect subtle perturbations coupled with effector mechanisms that actively maintain the optimal membrane lipid composition. An important effector of this allostatic response is SREBP, a transcription factor that regulates unsaturated fatty acid biosynthesis (membrane fluidizer) and cholesterol biosynthesis (membrane rigidifier). The fact that SREBP1 is a strong regulator of desaturase activity (Δ-9, Δ-5 and Δ-6) and that the transcription and activation of the SREBP1 protein is itself regulated by the degree of saturation of lipids [34–36], along with evidence produced via studies as discussed below, makes the SREBP1 regulatory network an attractive candidate to be the mechanism of lipid membrane quality control.

7. SREBP: a lipid transcription factor that mediates the allostatic response to maintain unsaturated lipid and cholesterol content in cells

7.1. Structure of SREBPs

SREBPs belong to the basic helix loop helix leucine zipper (bHLHLZ) family of transcription factors. SREBPs can bind to promoter sequences containing sterol regulatory elements (SRE-5′-TCACNCCAC-3′) and E-box sequences (5′-CANNTG-3′) .Three isoforms of SREBPs encoded by two genes have been identified SREBP1a and SREBP1c (also known as ADD1) are produced from a single gene, *srebf-1*, whereas SREBP2 is produced from the gene *srebf-2.* SREBP2 is approximately 50% identical to the SREBP1 isoforms. SREBP1a and SREBP1c result from the use of two transcription start sites that produce two separate 5-prime exons, each of which is spliced to a common exon 2 [37]. SREBP1 and SREBP2 preferentially regulate triglyceride and cholesterol biosynthesis, respectively [5,38]. SREBPs are synthesised as ER precursor proteins that require proteolytic cleavage to be transcriptionally active. Immature precursor SREBPs are orientated in the ER membrane in a hairpin configuration with the carboxyl- and amino-terminus facing towards the cytosol and are anchored in the ER as part of a complex with SREBP cleavage activating protein (SCAP) and an ER embedded protein Insig (insulin induced gene) until SREBP activation is required [37,38 and references within].

7.2. SREBP1 expression profile

SREBP1a and SREBP1c differ in their tissue distribution. In general SREBP1c is the predominant isoform in vivo, and is found in liver, adrenal gland, adipose tissue, brain, kidney, muscle and pancreas, with its highest expression being in liver and adipose tissue [39]. SREBP1a is the predominant isoform in vitro and in proliferative tissues such as spleen, testis and ileum. In terms of gene expression the ratio between SREBP1c and SREBP1a in tissues varies, with SREBP1c being nine- and three-fold higher than SREBP1a in liver and adipose tissue, respectively [39]. As SREBP1a has higher transcriptional activity compared with SREBP1c, the baseline ratio of SREBP1a and SREBP1c may be particularly important in regulation of gene transcription.

7.3. Regulation of SREBP cleavage

In response to stimuli to increase SREBP activity (e.g. low sterol levels; saturated fatty acids), SCAP escorts SREBP1 from the ER to the Golgi apparatus. SCAP/SREBP complex exits the ER via COPII vesicles that bud from the ER membrane. Proteolytic cleavage of SREBP1 occurs on the Golgi apparatus membrane by site-1-protease (S1P) and site-2-proteases (S2P) releasing the amino terminus of SREBP1 (64kDa), which is able to enter the nucleus and transcriptionally up-regulate SREBP1 target genes [37]. Under conditions where SREBP1 is to be repressed (e.g. high sterol levels; unsaturated fatty acids) the retention of the SREBP–SCAP complex is facilitated by Insig. SCAP contains a sterol-sensing domain (SSD). Cholesterol has been shown to induce a conformational change in

SCAP facilitating its binding to Insig [40], which prevents the translocation of the SREBP–SCAP complex from the ER and therefore preventing up-regulation of SREBP1 target genes.

7.4. SREBP1 target genes

A promiscuous nature of SREBP1 is beginning to emerge with SREBP1 regulating a diverse range of lipid and non-lipid pathways illustrating that SREBP1 is not just involved in lipid biosynthesis and accumulation. Recent studies, using bioinformatic tools have identified 1141 promoters with putative binding sites for SREBP1 in HepG2 cells after insulin and glucose stimulation [41]. Overexpression of SREBP1 revealed significant alteration in genes encoding mitochondrial proteins and proteins involved in mitochondrial respiration and oxidoreduction [42]. Thus these findings suggest that SREBP1 may also modulate mitochondrial fatty acid oxidation.

SREBP1 is a fundamental regulator of fatty acid desaturation, elongation and phospholipid biosynthesis, suggesting it could be a key allostatic point of control of membrane lipid homeostasis capable of strongly influencing the lipid composition of membranes. As illustrated, the SREBP1/Insig allostatic network exerts a great influence on the production of specific lipid species starting either from de novo lipogenesis or from modulation of dietary lipids. These lipid species can subsequently be incorporated into triglycerides and phospholipids in a wide variety of possible combinations (Fig. 2).

SREBP1 regulates a wide array of genes involved in lipid biosynthesis, including genes involved in de novo lipogenesis (FAS, acetyl co-A carboxylase (ACC1)), fatty acid reesterification (diacylglycerol acyltransferase (DGAT), Glycerol-3-phosphate acyltransferase (GPAT), lipoprotein lipase (LPL)), fatty acid desaturation and elongation [stearoyl Co-A desaturase (SCD), elongation of very long chain fatty acids (ELOVL)] and also phospholipid biosynthesis (CCT). As regulation of these pathways is crucial for the metabolic function of the cell, SREBP is an important central figure that must be tightly controlled (Fig. 3).

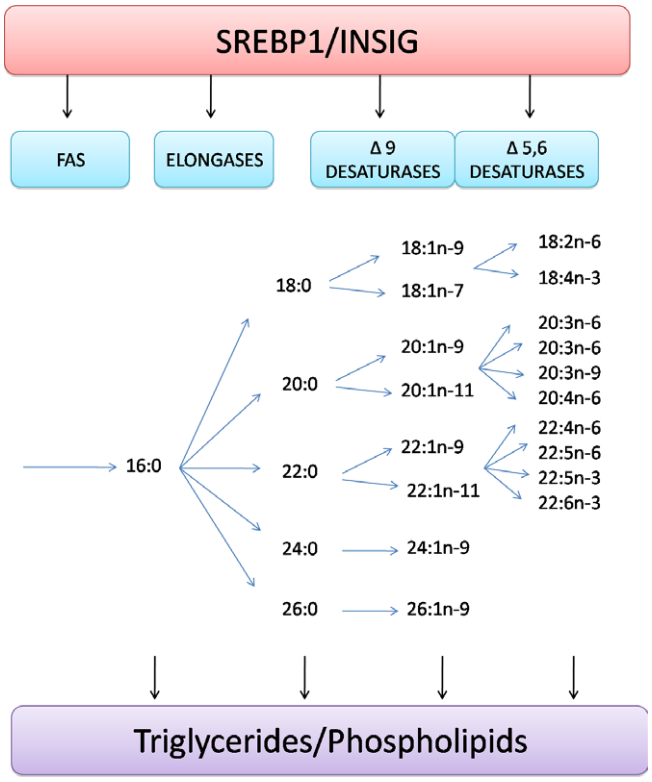

Fig. 2. Fatty acids are numerically represented C:D, where C is the number of carbon atoms present and D is the position of double bonds relative to the methyl end of the fatty acid. SREBP1/Insig regulates synthesis of the major long chain fatty acids that can be incorporated into triglycerides and phospholipids in any combination at multiple points along the path.

Although it is obvious that a fundamental function of SREBP1 is to regulate fatty acid biosynthesis and TAG storage; SREBP1 is also involved in the regulation of non-lipid pathways. For instance, SREBP1 upregulates the transcription and gene expression of GIRK1/4 (a G protein coupled inward rectifying potassium channel) in heart and sulfonylurea and potassium channels in the beta cells of islets as a result of lipid lowering [43,44]. Therefore, this is a direct evidence of how SREBP1 modulation affects physical properties of biomembranes influencing ion channel function. As well as acting as a transcriptional activator SREBP can also act as an inhibitor. SREBPs for example can impair insulin signalling in the liver via inhibition of IRS-2 gene transcription, and thus may play a crucial role in hepatic insulin sensitivity [45].

7.5. Transcriptional SREBP regulation

At transcriptional levels, promoter analysis of human and mouse SREBP1 revealed the presence of SRE and RXR/LXR DNA binding sites [38]. Glucose, insulin and liver X receptor (LXR) upregulate the transcription of SREBP1c [38,43]. Activation of SREBP1c stimulates its own promoter in an autoregulatory manner and thus amplifies the initial proteolytic signal [38]. Insulin increases transcription, protein levels and proteolytic cleavage of SREBP1 [43]. Action of insulin on SREBP1 is counteracted by glucagon that inhibits SREBP activation via production of cAMP levels.

7.6. Posttranslational SREBP regulation

SREBPs are posttranslationally modified through various mechanisms including phosphorylation, sumoylation, ubiquitination and acetylation. Lysine residues on nuclear SREBPs are ubiquitinated and degraded by the 26S proteosome [44]. Ubiquitination can be blocked through the acetylation of lysine residues enhancing the stability of SREBP1. Sumolyation represses transactivational capacity of SREBP1a and SREBP2 [44]. SREBP1 is negatively regulated by phosphorylation by GK3-beta and protein kinase A [44]. The phosphorylation status of SREBP1 appears to be important during cell division as nuclear SREBP1a and SREBP1c are regulated in mitotic cells by hyperphosphorylation [45]. It is hypothesised that phosphorylation stabilises the SREBP protein and maintains SREBP levels throughout cell division.

The addition of excess cholesterol inhibits both SREBP1 and SREBP2 proteolytic cleavage in vitro and in vivo [39]. However, physiologically the posttranslational regulation of SREBPs appears to be isoform specific, as in vivo studies suggest that whilst sterol depletion activates SREBP2 cleavage, sterol depletion does not regulate SREBP1 cleavage [40,46], suggesting that SREBP1 may not be regulated by cholesterol. Also, stimulation of SREBP1c cleavage by insulin occurs in the absence of variations in sterol [46]. These suggest that SREBP1c maturation under physiological conditions is predominantly regulated in response to nutritional status rather than by cholesterol. In fact changes in nutritional status have been shown to be important in SREBP1 regulation in adipose tissue and liver [43]. SREBP1 nuclear protein levels are increased in liver in response to a high fat diet (HFD) and a fasting/refeeding protocol whereas SREBP2 is not. Its physiological relevance is confirmed as induction of lipogenesis by HFD is prevented in SREBP1c KO mice [38].

7.7. Fatty acid regulation of SREBP

In vitro and in vivo studies have shown that SREBP1 regulation is mediated by fatty acids in animal cells. Transcription and

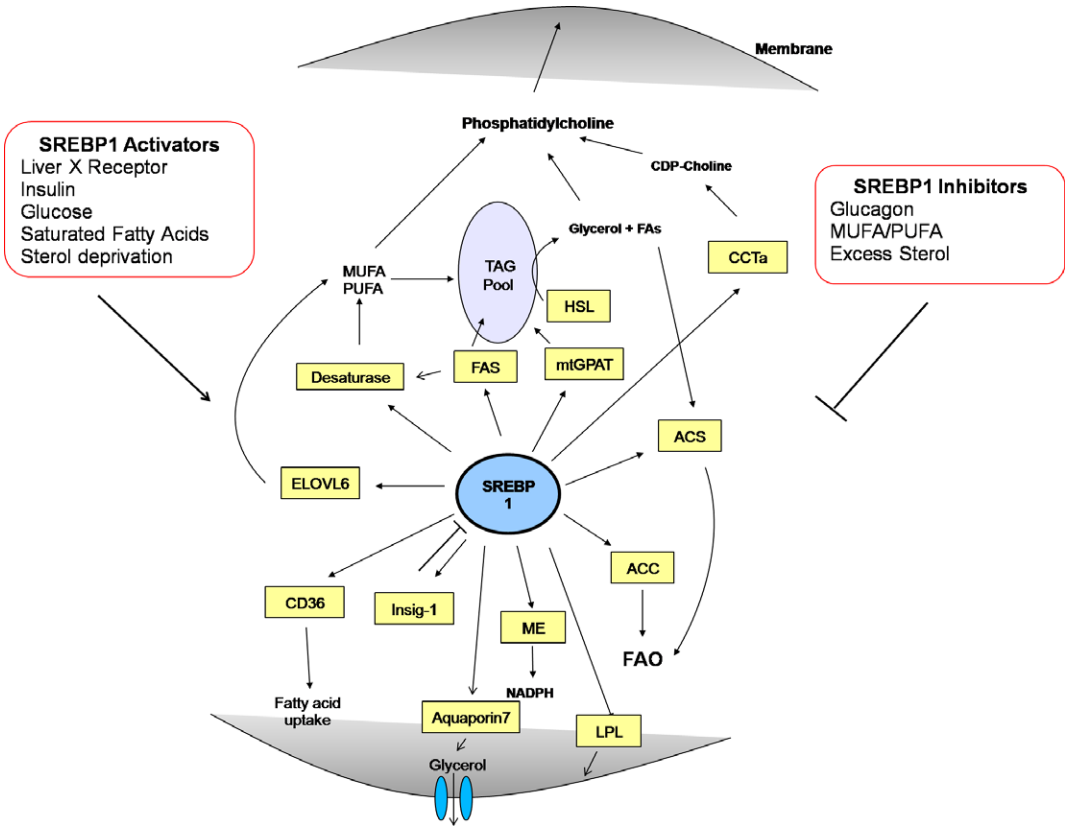

Fig. 3. SREBP1 is a central node linked to a wide variety of genes and thus has the capacity to influence many pathways. SREBP1 activity/inhibition is regulated depending on the environmental demands of the cells. ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; CCT, CTP:phosphocholine cytidylyltransferase; CD36/FAT, fatty acid translocase; ELOVL, elongation of very long chain fatty acids; FAS, fatty acid synthase; HSL, hormone sensitive lipase; Insig, Insulin-induced gene; LPL, lipoprotein lipase; ME, malic enzyme; mtGPAT, mitochondrial glycerol-3-phosphate acyltransferase; SREBP1, sterol regulatory element binding protein; TAG, triacylglycerol.

processing of SREBP1 is negatively regulated by MUFA and PUFA [34–36]. In contrast, human and mouse cultured cells grown in medium lacking unsaturated FA have increased activation of SREBP1a and SREBP1c [34–36]. The potency of inhibition by unsaturated fatty acids increases with chain length and the degree of unsaturation in cultured human embryonic kidney (HEK)-293 cells [34]. The effect of MUFA and PUFAs on SREBP1 transcription may be mediated through blockage of ligand dependent activation of LXR. In addition, the PUFA, arachidonic fatty acid has been shown to prevent SREBP processing by stabilising Insig-1 via blockage of ubiquitination sites on Insig-1 in a Chinese hamster cell line [47]. Sterols are able to prevent the degradation of Inisg-1 by inducing a conformational change that blocks the Insig-1 ubiquitination sites. Unsaturated fatty acids delay the degradation of Insig-1 by preventing the extraction of the ubiquitinated Inbsig-1 from the ER membrane. This extraction process is mediated by valosin-containing protein and Ubxd8 protein. Unsaturated fatty acids prevent the interaction between Ubxd8 and Insig-1 [47]. Whether saturated fatty acids also exert an effect on SREBP activation via Insig regulation requires further examination. As aforementioned, SREBPs are known to regulate desaturase gene expression [48,49]. Therefore, fatty acids appear to regulate SREBP1 activation in a feedback loop, controlling the ratio of unsaturated and saturated fatty acids.

Another interesting aspect of SREBP1 regulation comes from the studies performed in drosophila. The bHLHLZ domain of SREBP1, including the tyrosine residue in the basic domain is evolutionary conserved. Interestingly, there is an ortholog of SREBP and SCAP present in drosophila (dSREBP). SREBP proteolytic cleavage by SIP and S2P occurs in drosophila similar to mammals, despite drosophila being unable to synthesise sterols. In fact, in this model, it is the phospholipid phospatidylethanolamine (PE), which coordinates regulation over SREBP activity [50,51]. In this model increased PE content decreases SREBP activity. The SSD of the SCAP protein is thus sensitive to alterations in PE rather than exclusively to sterols Whether this property of the sterol-sensing domain has been conserved in higher animal organisms is unknown at present and it is an important aspect that may reveal the primary fundamental role of SREBP. The insertion of cholesterol and PE into the membrane exerts similar phase transitions in the membrane as both are cone shaped and promote the transition of lipid bilayers to an inverted hexagonal phase. This suggests that the SSD of SCAP is capable of responding to the physical properties (membrane thickness or fluidity) or phase transitions of the membranes rather than just sterol content [51]. As the SSD of dSCAP shares approximately 47% amino acid sequence identity with human SSD of SCAP, it may be the case that human SCAP monitors lipid composition responding to changes in the physical state of the membrane rather than sterol content alone. This places the SREBP network as a possible lipid sensor/effector network able to respond and activate appropriate lipid biosynthetic pathways when optimal lipid composition/physical properties of membranes are compromised.

8. SREBP network as allostatic mediators of lipid membrane composition

It has been demonstrated that activation of SREBP1 and SREBP2 occurs in response to general membrane demand as well as by

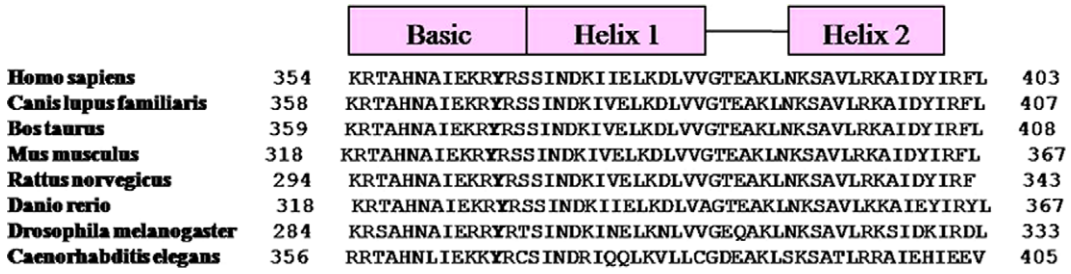

Fig. 4. SREBP1 conservation through organisms – amino acid sequences of basic helix loop helix leucine zipper were aligned using NCBIs HomoloGene database (http://www.ncbi.nlm.nih.gov/homologene). Conserved tyrosine residue is highlighted in bold. Gene reference sequences are NP_001005291.1 (*Homo sapiens*, XP_864642.1 (*Canis lupus* familiaris), XP_001790652.1 (*Bos Taurus*), NP_035610.1 (*Mus musculus*), XP_213329.4 (*Rattus norvgicas*), NP_001098599.1 (*Danio rerio*), NP_730450.1 (*Drosophila melanogaster*), XP_311076.4 (*C. elegans*).

lipid deprivation [53]. For instance, SREBPs are activated by phagocytosis: In human HEK-293 cell line, phagocytosis stimulates the proteolytic cleavage of SREBP and enhances transcription of lipogenic genes. Blockage of SREBP inhibits phagocytosis induced transcription and cholesterol and phospholipid biosynthesis. This suggests that SREBPs are activated to generate lipid species to be incorporated into the growing membrane [53]. It appears that the SREBP/Insig regulation is crucial in generating unsaturated lipid species in particular to be incorporated into the membrane. The relevance of SREBP/Insig regulation in generating unsaturated lipid species through desaturase regulation has been demonstrated. For example, the overexpression of SREBP1a results in an enrichment (15–20%) of oleate content in liver [54] due to increased elongase and desaturase gene expression. In addition, the relevance of SCD1 and MUFA generation in altering the membrane organisation has been demonstrated in hepatocytes of SCD1 knockout mice. A lack of SCD1 in mice results in an activation of CCT and PC [55] biosynthesis. The reduction in MUFA content in hepatic phospholipds may result in an increased membrane affinity for CCT thus increasing its translocation and activation. The upregulation of SREBP1 during phagocytosis results in the upregulation of unsaturated fatty acid biosynthesis and redistribution of cholesterol. In addition, cells grown in medium lacking unsaturated FA have increased activation of SREBP1a and SREBP1c [34].

SREBP1s are relatively well conserved across different species (from fission yeast up to humans) (Fig. 4). The *C. elegans* SREBP homolog Sp1 increases unsaturated fatty acid biosynthesis. Reduction of Sp1 using RNA interference in *C. elegans* results in impaired intestinal fat storage, a high saturated fatty acid content, a reduction in Δ-9 desaturase activity, infertility, decreased growth and slow location, suggesting that SREBP is crucial for unsaturation in *C. elegans*. The defects of Sp1 deficient worms could be rescued by dietary addition of oleic acid [56].

In conclusion, these studies indicate that the primary function of SREBP1 may be to coordinate the transcription of membrane biogenesis and in particular, regulating the production of unsaturated fatty acids to maintain optimal membrane composition in response to environmental demands.

9. Concluding remarks

Allostatic programmes are important mechanisms facilitating an adaptive response to maintain physiological normality in response to stress conditions to ensure the survival of the species. Allostatic responses may also be responsible for the strict regulation of biological membrane composition. As detailed earlier membrane fluidity is important in regulating a wide range of biological functions, therefore membrane composition is tightly regulated to maintain cellular viability. Relatively small changes in the concentration of membrane lipids in the order of 10% are significant enough to alter biophysical properties of the membrane [57]. A reduction in temperature results in decreased membrane fluidity and as a consequence unsaturated fatty acid production through the fatty acid desaturation pathway is increased, thus maintaining the biological membrane in a fluid state. This dynamic allostatic response to perturbations results in robust networks that can respond to external and internal stimuli whilst maintaining normal cell function. We postulate that the allostatic adaptation of SREBP1/Insig-1 is sensitive to the degree of fatty acid desaturation occurring within the cell and is likely to be present to maintain the production of unsaturated fatty acids and prevent saturated fatty acid accumulation within the cytosol that can compromise cell function through cell signalling and alterations in the membrane composition.

10. Future studies

Future studies will be needed to further investigate whether the SREBP1/Insig1 network is indeed acting as a lipid membrane quality control mechanism. Particularly relevant to adipose tissue, SREBP1 activation may be crucial in providing unsaturated lipid species as important components of the lipid droplet. Data from the literature suggests a correlation between the supply of MUFA and lipid droplet size. The ability to expand lipid droplets to accommodate and store excess fatty acids is an important process to inertly store away fatty acids. The channelling of excess saturated FA to triglyceride stores is likely an adaptive mechanism against the accumulation of cytosolic fatty acids and flux of excess fatty acids towards peripheral insulin sensitive tissues and protective against lipotoxicity. Therefore, the extent to which the lipid droplet and adipose tissue can expand to accommodate fatty acid influx will determine the extent of the pathological state. In the advent of adipose tissue being no longer able to store excess lipid effectively, excess lipids are diverted to peripheral tissues and have a lipotoxic effect.

In addition, future studies investigating the function of SREBP1 should not be limited to the traditional lipid storage organs such as adipose tissue and liver. Many cell-types contain lipid droplets that store neutral lipids for metabolism, membrane synthesis (phospholipids and cholesterol) and steroid synthesis. Indeed, SREBP1c is found in a variety of tissues, such as brain, skeletal muscle, heart, kidney, pancreas and brown adipose tissue. It may be that SREBPs primary function is to ensure that the appropriate unsaturated fatty acids for phospholipids synthesis are present. Inappropriate accumulation of saturated fatty acids can compromise cell membrane fluidity and function, and in order to maintain an optimum ratio of unsaturated to saturated fatty acids, particularly in biological membrane, SREBPs are activated and upregulate target genes involved in desaturation of fatty acids and phospholipid synthesis.

Acknowledgments

The authors' work is supported by grants from the Medical Research Council (PhD Building Capacity Studentship), Intergrative Pharmacology Fund, British Heart Foundation (pg/08/003/24286) and ETHERPATHS (FP7-KBBE-222639).

References

[1] McEwen, B.S. and Stellar, E. (1993) Stress and the individual. Mechanisms leading to disease. Arch Intern. Med. 153 (18), 2093–2101.
[2] Sud, M., Fahy, E., Cotter, D., Brown, A., Dennis, E.A., Glass, C.K., Merrill, A.H. Jr., Murphy, R.C., Raetz, C.R., Russell, D.W. and Subramaniam, S. (2007) LMSD: LIPID MAPS structure database. Nucleic Acids Res. 35(Database issue), D527–D532.
[3] Escribá, P.V., Sánchez-Dominguez, J.M., Alemany, R., Perona, J.S. and Ruiz-Gutiérrez, V. (2003) Alteration of lipids, G proteins, and PKC in cell membranes of elderly hypertensives. Hypertension 41 (1), 176–182.
[4] McMahon, H.T. and Gallop, J.L. (2005) Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438 (7068), 590–596.
[5] Sinensky, M. (1974) Homeoviscous adaptation – a homeostatic process that regulates the viscosity of membrane lipids in *Escherichia coli*. Proc. Natl. Acad. Sci. USA 71 (2), 522–525.
[6] Los, D.A. and Murata, N. (2004) Membrane fluidity and its roles in the perception of environmental signals. Biochim. Biophys. Acta 1666 (1–2), 142–157.
[7] Aguilar, P.S. and de Mendoza, D. (2006) Control of fatty acid desaturation: a mechanism conserved from bacteria to humans. Mol. Microbiol. 62 (6), 1507–1514.
[8] van Meer, G., Voelker, D.R. and Feigenson, G.W. (2008) Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. 9 (2), 112–124.
[9] Scaglia, N. and Igal, R.A. (2005) Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells. J. Biol. Chem. 280 (27), 25339–25349.
[10] Lee, A.G. (2004) How lipids affect the activities of integral membrane proteins. Biochim. Biophys. Acta 1666 (1–2), 62–87.
[11] Guillou, H., Zadravec, D., Martin, P.G. and Jacobsson, A. (2009) The key roles of elongases and desaturases in mammalian fatty acid metabolism: insights from transgenic mice. Prog. Lipid Res. doi:10.1016/j.plipres.2009.12.002.
[12] Nakamura, M.T. and Nara, T.Y. (2004) Structure, function, and dietary regulation of Δ-6, Δ-5, and Δ-9 desaturases. Annu. Rev. Nutr. 24, 345–376.
[13] Watts, J.L. and Browse, J. (2002) Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA 99 (9), 5854–5859.
[14] Ntambi, J.M., Miyazaki, M., Stoehr, J.P., Lan, H., Kendziorski, C.M., Yandell, B.S., Song, Y., Cohen, P., Friedman, J.M. and Attie, A.D. (2002) Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc. Natl. Acad. Sci. USA 99 (17), 11482–11486.
[15] Binczek, E., Jenke, B., Holz, B., Günter, R.H., Thevis, M. and Stoffel, W. (2007) Obesity resistance of the stearoyl-CoA desaturase-deficient (scd1−/−) mouse results from disruption of the epidermal lipid barrier and adaptive thermoregulation. J. Biol. Chem. 388 (4), 405–418.
[16] Sampath, H., Flowers, M.T., Liu, X., Paton, C.M., Sullivan, R., Chu, K., Zhao, M. and Ntambi, J.M. (2009) Skin-specific deletion of stearoyl-CoA desaturase-1 alters skin lipid composition and protects mice from high fat diet-induced obesity. J. Biol. Chem. 284 (30), 19961–19973.
[17] Williard, D.E., Nwankwo, J.O., Kaduce, T.L., Harmon, S.D., Irons, M., Moser, H.W., Raymond, G.V. and Spector, A.A. (2001) Identification of a fatty acid Δ-6-desaturase deficiency in human skin fibroblasts. J. Lipid Res. 42 (4), 501–508.
[18] Attard, G.S., Templer, R.H., Smith, W.S., Hunt, A.N. and Jackowski, S. (2000) Modulation of CTP:phosphocholine cytidylyltransferase by membrane curvature elastic stress. Proc. Natl. Acad. Sci. USA 97 (16), 9032–9036.
[19] Shindou, H. and Shimizu, T. (2009) Acyl-CoA:Lysophospholipid acyltransferases. J. Biol. Chem. 284, 1–5.
[20] Faloia, E., Garrapa, G.G., Martarelli, D., Camilloni, M.A., Lucarelli, G., Staffolani, R., Mantero, F., Curatola, G. and Mazzanti, L. (1999) Physicochemical and functional modifications induced by obesity on human erythrocyte membranes. Eur. J. Clin. Invest. 29 (5), 432–437.
[21] Matsuo, T., Sumida, H. and Suzuki, M. (1995) Beef tallow diet decreases beta-adrenergic receptor binding and lipolytic activities in different adipose tissues of rat. Metabolism 44 (10), 1271–1277.
[22] Zeghari, N., Younsi, M., Meyer, L., Donner, M., Drouin, P. and Ziegler, O. (2000) Adipocyte and erythrocyte plasma membrane phospholipid composition and hyperinsulinemia: a study in nondiabetic and diabetic obese women. Int. J. Obes. Relat. Metab. Disord. 24 (12), 1600–1607.
[23] Pan, D.A., Lillioja, S., Milner, M.R., Kriketos, A.D., Baur, L.A., Bogardus, C. and Storlien, L.H. (1995) Skeletal muscle membrane lipid composition is related to adiposity and insulin action. J. Clin. Invest. 96 (6), 2802–2808.
[24] Bickel, P.E. (2002) Lipid rafts and insulin signaling. Am. J. Physiol. Endocrinol. Metab. 282 (1), E1–E10.
[25] Summers, S.A. (2006). Ceramides in insulin resistance and lipotoxicity. Prog. Lipid Res. 45(1), 42–72.
[26] de Vries, J.E., Vork, M.M., Roemen, T.H., de Jong, Y.F., Cleutjens, J.P., van der Vusse, G.J. and van Bilsen, M. (1997) Saturated but not mono-unsaturated fatty acids induce apoptotic cell death in neonatal rat ventricular myocytes. J. Lipid Res. 38 (7), 1384–1394.
[27] Coll, T., Eyre, E., Rodríguez-Calvo, R., Palomer, X., Sánchez, R.M., Merlos, M., Laguna, J.C. and Vázquez-Carrera, M. (2008) Oleate Reverses Palmitate-induced Insulin Inflammation in Skeletal Muscle Cells. J. Biol. Chem. 283(17), 11107–11116.
[28] Unger, R.H. (2005) Longevity, lipotoxicity and leptin: the adipocyte defense against feasting and famine. Biochimie 87 (1), 57–64.
[29] Listenberger, L.L. and Brown, D.A. (2008) Lipid droplets. Curr. Biol. 18 (6), R237–R238.
[30] Martin, S. and Parton, R.G. (2006) Lipid droplets: a unified view of a dynamic organelle. Nat. Rev. Mol. Cell Biol. 7 (5), 373–378.
[31] Tauchi-Sato, K., Ozeki, S., Houjou, T., Taguchi, R. and Fujimoto, T. (2002) The surface of lipid droplets is a phospholipid monolayer with a unique Fatty Acid composition. J. Biol. Chem. 277(46), 44507–44512.
[32] Fujimoto, Y., Onoduka, J., Homma, K.J., Yamaguchi, S., Mori, M., Higashi, Y., Makita, M., Kinoshita, T., Noda, J., Itabe, H. and Takanoa, T. (2006) Long-chain fatty acids induce lipid droplet formation in a cultured human hepatocyte in a manner dependent of Acyl-CoA synthetase. Biol. Pharm. Bull. 29 (11), 2174–2180.
[33] Boutet, E., El Mourabit, H., Prot, M., Nemani, M., Khallouf, E., Colard, O., Maurice, M., Durand-Schneider, A.M., Chrétien, Y., Grès, S., Wolf, C., Saulnier-Blache, J.S., Capeau, J. and Magré, J. (2009) Seipin deficiency alters fatty acid Δ-9 desaturation and lipid droplet formation in Berardinelli-Seip congenital lipodystrophy. Biochimie 91 (6), 796–803.
[34] Hannah, V.C., Ou, J., Luong, A., Goldstein, J.L. and Brown, M.S. (2001) Unsaturated fatty acids down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells. J. Biol. Chem. 276 (6), 4365–4372.
[35] Worgall, T.S., Sturley, S.L., Seo, T., Osborne, T.F. and Deckelbaum, R.J. (1998) Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. J. Biol. Chem. 273 (40), 25537–25540.
[36] Thewke, D.P., Panini, S.R. and Sinensky, M. (1998) Oleate potentiates oxysterol inhibition of transcription from sterol regulatory element-1-regulated promoters and maturation of sterol regulatory element-binding proteins. J. Biol. Chem. 273 (33), 21402–21407.
[37] Brown, M.S. and Goldstein, J.L. (1997) The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89 (3), 331–340.
[38] Eberlé, D., Hegarty, B., Bossard, P., Ferré, P. and Foufelle, F. (2004) SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 86 (11), 839–848.
[39] Shimomura, I., Shimano, H., Horton, J.D., Goldstein, J.L. and Brown, M.S. (1997) Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J. Clin. Invest. 99 (5), 838–845.
[40] Sun, L.P., Li, L., Goldstein, J.L. and Brown, M.S. (2005) Insig required for sterol-mediated inhibition of Scap/SREBP binding to COPII proteins in vitro. J. Biol. Chem. 280 (28), 26483–26490.
[41] Reed, B.D., Charos, A.E., Szekely, A.M., Weissman, S.M. and Snyder, M. (2008) Genome-wide occupancy of SREBP1 and its partners NFY and SP1 reveals novel functional roles and combinatorial regulation of distinct classes of genes. PLoS Genet. 4 (7), e1000133.
[42] Rome, S., Lecomte, V., Meugnier, E., Rieusset, J., Debard, C., Euthine, V., Vidal, H. and Lefai, E. (2008) Microarray analyses of SREBP1a and SREBP1c target genes identify new regulatory pathways in muscle. Physiol. Genom. 34 (3), 327–337.
[43] Iwaski, Y., Iwasaki, H., Yatoh, S., Ishikawa, M., Kato, T., Matsuzaka, T., Nakagawa, Y., Yahagi, N., Kobayashi, K., Takahashi, A., Suzuki, H., Yamada, N. and Shimano, H. (2009) Nuclear SREBP1a causes loss of pancreatic beta-cells and impaired insulin secretion. Biochem. Biophys. Res. Commun. 378 (3), 545–550.
[44] Park, H.J., Georgescu, S.P., Du, C., Madias, C., Aronovitz, M.J., Welzig, C.M., Wang, B., Begley, U., Zhang, Y., Blaustein, R.O., Pattn, R.D., Karas, R.H., Van Tol, H.H., Osbourne, T.F., Shimano, H., Liao, R., Link, M.S. and Galper, J.B. (2008) Parasympathetic response in chick myocytes and mouse heart is controlled by SREBP. J. Clin. Invest. 118, 259–271.
[45] Bengoechea-Alonso, M.T., Punga, T. and Ericsson, J. (2005) Hyperphosphorylation regulates the activity of SREBP1 during mitosis. Proc. Natl. Acad. Sci. USA 102 (33), 11681–11686.
[46] Sheng, Z., Otani, H., Brown, M.S. and Goldstein, J.L. (1995) Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. Proc. Natl. Acad. Sci. USA 92 (4), 935–938.
[47] Lee, J.N., Zhang, X., Feramisco, J.D., Gong, Y. and Ye, J. (2008) Unsaturated fatty acids inhibit proteasomal degradation of Insig-1 at a postubiquitination step. J. Biol. Chem. 283 (48), 33772–33783.
[48] Tabor, D.E., Kim, J.B., Spiegelman, B.M. and Edwards, P.A. (1999) Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. J. Biol. Chem. 274 (29), 20603–20610.
[49] Matsuzaka, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Yoshikawa, T., Hasty, A.H., Tamura, Y., Osuga, J., Okazaki, H., Iizuka, Y., Takahashi, A., Sone, H., Gotoda, T., Ishibashi, S. and Yamada, N. (2002) Dual regulation of mouse Δ-(5)- and Δ-(6)-desaturase gene expression by SREBP1 and PPARalpha. J. Lipid Res. 43 (1), 107–114.

[50] Dobrosotskaya, I.Y., Seegmiller, A.C., Brown, M.S., Goldstein, J.L. and Rawson, R.B. (2002) Regulation of SREBP processing and membrane lipid production by phospholipids in Drosophila. Science 296(5569), 879–883.
[51] Seegmiller, A.C., Dobrosotskaya, I., Goldstein, J.L., Ho, Y.K., Brown, M.S. and Rawson, R.B. (2002) The SREBP pathway in Drosophila: regulation by palmitate, not sterols. Dev. Cell. 2(2), 229–238.
[52] Li, L.O., Klett, E.L. and Coleman, R.A. (2009). Acyl-CoA synthesis, lipid metabolism and lipotoxicity. Biochim. Biophys. Acta 8 [Epub ahead of print].
[53] Castoreno, A.B., Wang, Y., Stockinger, W., Jarzylo, L.A., Du, H., Pagnon, J.C., Shieh, E.C. and Nohturfft, A. (2005) Transcriptional regulation of phagocytosis-induced membrane biogenesis by sterol regulatory element binding proteins. Proc. Natl. Acad. Sci. USA 102 (37), 13129–13134.
[54] Shimano, H., Horton, J.D., Hammer, R.E., Shimomura, I., Brown, M.S. and Goldstein, J.L. (1996) Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP1a. J. Clin. Invest. 98 (7), 1575–1584.
[55] Dobrzyn, A., Dobrzyn, P., Miyazaki, M., Sampath, H., Chu, K. and Ntambi, J.M. (2005) Stearoyl-CoA desaturase 1 deficiency increases CTP:choline cytidylyltransferase translocation into the membrane and enhances phosphatidylcholine synthesis in liver. J. Biol. Chem. 280 (24), 23356–23362.
[56] Yang, F., Vought, B.W., Satterlee, J.S., Walker, A.K., Jim Sun, Z.Y., Watts, J.L., DeBeaumont, R., Saito, R.M., Hyberts, S.G., Yang, S., Macol, C., Iyer, L., Tjian, R., van den Heuvel, S., Hart, A.C., Wagner, G. and Näär, A.M. (2006) An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. Nature 442 (7103), 700–704.
[57] Oresic, M., Hänninen, V.A. and Vidal-Puig, A. (2008) Lipidomics: a new window to biomedical frontiers. Trends Biotechnol. 26 (12), 647–652.