Adipocyte cholesterol balance in obesity

S. Le Lay, P. Ferré and I. Dugail

INSERM U 465, Institut Biomédical des Cordeliers, 15, rue de l’école de médecine, 75006 Paris, France

Abstract
Adipose tissue is specialized in the storage of energy in the form of triacylglycerol. Within the fat cell, triacylglycerols are found in a well-defined structural compartment called the lipid droplet, which occupies the vast majority of the fat cell volume. However, many other lipids are present in the lipid droplet. These include sterols, carotenoids, cholecalciferol and lipophilic toxic pollutants of the environment such as dioxins and tocophersols. The topic of this article is the role of fat cell cholesterol in adipose tissue physiology and its potential implication in pathological states such as obesity.

Adipocyte cholesterol and triacylglycerol storage
The presence of sterols in the adipocyte was recognized more than 25 years ago [1], and adipocyte cholesterol represents around 1–5 mg/g of total lipids. An interesting feature is that adipocyte cholesterol is found in its free, non-esterified form (in humans, less than 6% of adipocyte cholesterol is esterified [2]). This clearly distinguishes the adipocyte from other cell types such as steroid-hormone-producing adrenal cells or cholesterol-laden foam cells of atherosclerotic lesions, in which lipid droplets are filled with cholesteryl esters. Due to their biophysical properties, free sterols do not reside in the lipid core of the lipid droplet, but rather accumulate in a phospholipid monolayer present at the cytoplasmic interphase of the lipid droplet.

Another characteristic of adipocyte cholesterol metabolism is the low activity of the de novo cholesterol synthetic pathway. This is shown by early observations in the literature in which the rate of cholesterol synthesis in fat cells, estimated by the incorporation of labelled precursor acetate into cholesterol, was shown to be only 4% of that of liver [3]. This might illustrate preferential utilization of acetate in the fatty acid biosynthetic pathway in adipocytes. Also, it has been reported that upon incubation of isolated human adipocytes for 3 h with labelled mevalonate, more than 90% of the radioactivity in the non-saponifiable material was mates by the incorporation of labelled precursor acetate into cholesterol, was shown to be only 4% of that of liver [3]. This might explain why intermediates of the cholesterol biosynthetic pathway such as squalenes are also found in adipose tissue [5].

As a consequence of the low synthetic rate, the majority of adipocyte cholesterol originates from circulating lipoproteins. Like all peripheral cells, fat cells express the LDL (low-density lipoprotein) receptor endocytic pathway for cholesterol-rich LDLs. Adipocytes are also very active in the degradation of triacylglycerol-rich lipoproteins via the lipoprotein lipase pathway, and subsequent exogenous fatty acid uptake. During this process, it is likely that some cholesterol might enter the adipose cell through a presently undefined route. Fat cells express several scavenger receptors such as SR-BI and CD36 [6], and their importance for exogenous cholesterol uptake has been documented in other cell types. In 3T3L1 adipocytes, a model system for adipocytes, the rapid accumulation of a fluorescent cholesterol analogue, NDB-cholesterol [22-(N-7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminob-24-bisnor-5-cholen-3-ol], occurs when the analogue is provided to the cells within LDLs or high-density lipoproteins. Furthermore, the rapid targeting of the fluorescent cholesterol to the adipocyte lipid droplet can be easily observed (G. Dagher, unpublished work). Thus at least two cell compartments in which cholesterol accumulates within the fat cell can be defined: the plasma membrane, which represents the ‘functional’ cholesterol, and the lipid droplet, in which cholesterol function is still to be defined.

Noticeably, numerous studies in the literature have highlighted a strong correlation between adipocyte cholesterol content and fat cell size (for a review see [7]). Indeed, the bigger the fat cell, the more cholesterol it contains. First, the cholesterol content of the lipid droplet is closely proportional to the triacylglycerol content. Secondly, total membrane cholesterol content depends upon fat cell size and thus triacylglycerol load. This explains why the ratio of cholesterol to triacylglycerol in adipocytes remains independent of the body mass of the donor subject over a wide range of body mass (from a lean normal mass to massive obesity). In obesity, which is considered a state of overall expansion of triacylglycerol stores, cholesterol accumulation in fat can reach considerable levels, representing several hundred grams of free cholesterol in the adipose tissue. Conversely, when triacylglycerol stores are depleted, adipocytes reduce their size and their cholesterol content also decreases.

Some evidence suggests that excessive triacylglycerol storage in obesity is accompanied by changes in intracellular cholesterol distribution in the adipocytes. First, during adipocyte

Key words: cholesterol traffic, fat cell, gene expression, lipid droplet, sterol-response-element-binding protein (SREBP), triacylglycerol.

Abbreviations used: LDL, low-density lipoprotein; SREBP, sterol-response-element-binding protein; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; SCAP, sterol-closteve activating protein.

1To whom correspondence should be addressed (e-mail idugail@ibdc.jussieu.fr).
differentiation of 3T3F442A adipocytes, which is characterized by the transition from a lipid-poor fibroblast-like state to a triacylglycerol-enriched adipocyte, the fluidity of the plasma membrane, measured by fluorescence polarization declines, together with the cholesterol/phospholipid molar ratios in plasma membranes [8]. Secondly, the membrane composition of hypertrophied adipocytes is altered in obese rats, and contains less cholesterol than those from normal weighed control animals [9]. These data indicate that adipocyte cholesterol status is profoundly altered by triacylglycerol storage. These changes resemble those of a cholesterol-deprived state of the plasma membrane (Figure 1).

The adipocyte SREBP2 (sterol-response-element-binding protein 2) pathway is activated in obesity

The sensing of the cholesterol status of cell membranes is a complex process, which involves the SCAP (sterol-cleavage activating protein)/SREBP pathway [10]. The SREBP transcription factors are conditional transcriptional activators that reside in the endoplasmic reticulum, and which can be activated by proteolytic cleavage when cell cholesterol supply is low. Through their ability to regulate the transcription of the genes required for the uptake and synthesis of cholesterol, such as those encoding the LDL receptor and HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA reductase), SREBP proteins achieve efficient control of cell cholesterol supply. SREBP transcriptional activity is strictly dependent on cholesterol sensing through SCAP.

The SREBP pathway is ubiquitously expressed, and thus it can be assumed that the cholesterol-deprived state of the plasma membrane in triacylglycerol-engorged adipocytes should lead to SREBP activation. Indeed, in the fa/fa rat adipocyte, the nuclear-active form of SREBP2 was found to be elevated in obese compared with lean controls [11]. Moreover, among the SREBP isoforms, ADD1/SREBP1c, which is unrelated to cholesterol but rather functions as a transcriptional regulator of lipogenesis [12,13], was unaffected (Figure 2).

In agreement with these data, showing adipocyte-specific SREBP2 activation in obesity, several studies in the literature report increased expression of SREBP2 target genes in the adipose tissue of obese rodents or humans. In vivo experiments showed that cholesterol synthesis was increased in adipose tissue from obese rodents or humans [7]. Also, an early study in ob/ob mice (a widely used model of obesity) has reported higher activities of HMG-CoA reductase, a SREBP2-regulated gene, in the adipose tissue of young obese mice compared with their lean littersmates [14]. Such an increase contrasted with the lower activity of this enzyme in the liver of obese mice and their higher plasma cholesterol levels [14], and first suggested an adipocyte-specific regulation of cholesterol metabolism in obesity. In agreement, recent studies on a wide-ranging analysis of adipose tissue gene expression using microarrays also identified HMG-CoA reductase among the adipocyte genes differentially expressed in ob/ob versus lean control mice [15]. Finally, we also reported elevated levels of the HMG-CoA reductase and LDL receptor mRNAs in hypertrophic adipocytes isolated from two other models of obese rodents: fat cpe−/− mice and Zucker rats [16]. Interestingly, we found normal adipocyte expression of SREBP2 target genes in a transgenic model of hyperplastic obesity in which fat cell size was normal [17]. These data imply that adipocyte
SREBP2 activation is not a general characteristic in all obesity models, but rather is linked to the presence of adipocyte hypertrophia. To summarize, some experimental evidence in the literature suggest that the SREBP pathway is activated in the enlarged fat cells that compose obese adipose tissue. The direct demonstration that SREBP-2 activation is brought about by cholesterol storage in the engorged lipid droplet is still lacking.

**Cholesterol balance affects fat cell function**

The importance of cholesterol for cell signalling from the plasma membrane has been well established since it was recognized that local inhomogeneities in lipid composition exist at the cell surface (for a review see [18]). A particular subtype of such microdomains, the flask-shaped caveolae, are particularly abundant in adipocytes, because these cells express extremely high levels of the cholesterol binding protein caveolin [19], which is the structural organizer of caveolae [20]. In many cell types, the dynamic location of signalling components in caveolae greatly influences signal transduction. With regard to adipocyte metabolism, signalling from the insulin receptor and the G-protein-coupled adrenergic receptors, both of which are located in caveolae [21], is of particular importance. In two papers, the disruption of these domains by cyclodextrin treatment was shown to impair insulin action in isolated adipocytes, strongly decreasing insulin-stimulated glucose transport and metabolism [16,22]. This suggests that the decreased plasma membrane cholesterol content in enlarged adipocytes might be important in the establishment of the insulin-resistant state that is a hallmark of the obese fat cell.

The question of the consequences, if any, of the activation of sterol-regulated SREBPs in obesity can be raised. Members of the SREBP family of transcription factors bind to two distinct motifs of DNA sequences, identified as sterol-response element and E-box [23]. Thereby, they can activate the transcription of a subset of genes that bears these motifs in their promoter region. So far, the studies of the effects of SREBP overexpression in different cell systems or transgenic mice have identified at least two groups of genes that are directly controlled by SREBPs. One group is composed of the genes of the cholesterol biosynthetic pathway, in which the LDL receptor gene can be included. The other group includes genes of the lipogenic pathway, which are involved in de novo biosynthesis of fatty acids. SREBPs also exist as three distinct isoforms, SREBP1a, 1c and 2, which do not exert equivalent transactivational effects on SREBP targets, although some overlap is evident. In studies of transgenic mice in which each of the three isoforms were overexpressed, it appeared that SREBP1a and SREBP2 were more active in controlling the cholesterol biosynthetic pathway, whereas SREBP1c was involved in the regulation of lipogenic genes [24]. Such a preference in the physiological effects of the different SREBP isoforms is in agreement with the regulation pattern of expression of the different SREBPs. Indeed, SREBP1c expression is primarily regulated by insulin [12,13,25], the major factor that controls lipogenesis. Also, SREBP2, whose transcriptional activity depends on cholesterol, is most active in the control of cholesterol metabolism [24].

Since adipocyte nuclear SREBP2 is activated in hypertrophied adipocytes, the issue of the existence of some adipocyte genes that might be controlled by SREBP2, directly or indirectly, can be addressed. A partial answer to this question was obtained by the examination, on a large scale, of adipocyte gene expression in cultured cells in which SREBP2 activation was mimicked by cholesterol depletion [16]. For this purpose, 3T3L1 adipocytes were allowed to differentiate in a cholesterol-deprived medium in the presence of mevastatin, an inhibitor of HMG-CoA reductase. Under these conditions the ongoing differentiation process was not significantly altered, but cholesterol depletion resulted in a switch from SREBP1c to SREBP2 as the major SREBP isoform expressed in the cells. Interestingly, the expression of some genes that participate in adipocyte function was found to be altered in these cells. Particularly the expression of adipocyte-secreted products such as angiotensinogen, TNFα and interleukin 6, was strongly stimulated. Interestingly, those gene products have been suggested to participate in the onset of the morbid complications of obesity, such as insulin resistance, hypertension, or atherosclerosis and coronary heart diseases [26–29]. Some metabolic genes were found to be dysregulated in cholesterol-depleted 3T3 L1 cells. Besides fatty acid synthase, a well-known SREBP target [12,13,25,30] that was upregulated, profound changes occurred in the expression of the glucose transporters. GLUT1, the ubiquitously expressed glucose transporter, increased, whereas GLUT4, the isoform expressed essentially in insulin-sensitive tissues, decreased. Impaired GLUT4 expression is a major contributor to altered glucose homoeostasis in all forms of obesity and diabetes [31]. Thus some evidence exists that experimental manipulation of adipocyte cholesterol balance might profoundly alter the gene-expression profile, and consequently modify fat cell metabolism towards that seen in the obese state. In this process, SREBP2 is likely to be a key control point, but it should be mentioned also that other cholesterol-dependent transcription factors, such as liver X receptor, are highly expressed in adipocytes [32], and might also contribute to the remodelling of gene expression in enlarged adipocytes.

**Conclusion**

These features of adipocyte cholesterol distribution and metabolism raise the question of the physiological significance, if any, of the presence of cholesterol in fat cells. An exciting possibility is that cholesterol might serve as an intracellular signal for the size of adipocytes, linked to triacylglycerol stores. The existence of an intracellular sensing process for energy stores is suggested by the dramatic metabolic changes that follow fat cell size enlargement, which primarily occurs in obesity. These changes cover a broad range of adipocyte functions, from the recruitment of newly committed preadipocytes and their subsequent
differentiation, to the production of signalling molecules with various physiological effects at the whole body level (e.g. leptin). The mechanisms by which adipocytes sense the replenishment state of their lipid stores remain completely unknown. Based on the fact that triacylglycerol and cholesterol storage are closely linked in adipocytes, it may be assumed that cholesterol might participate in the intracellular sensing for fat cell size and triacylglycerol content.

References

Received 8 August 2003