The genetics of lipid storage and human lipodystrophies.

Ann L. Robbins, David B. Savage

University of Cambridge Metabolic Research Laboratories, Wellcome Trust–Medical Research Council Institute of Metabolic Science, University of Cambridge, UK.

Corresponding author:

Dr D.B.Savage

Wellcome Trust–Medical Research Council Institute of Metabolic Science,

University of Cambridge

Addenbrooke's Hospital

Cambridge CB2 0QQ, UK

Tel: +44 1223 767923

Email: dbs23@cam.ac.uk

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Abstract

Life depends on securing sufficient energy intake to enable growth, movement, and reproduction. Throughout evolution, life-forms have struggled to ensure adequate energy intake and this remains a major challenge for many species. Modern humans are particularly well adapted to store surplus energy efficiently, but they are considerably less well adapted for coping with sustained access to energy dense food. Here we briefly review the evolution of adipocytes and the metabolic consequences of suboptimal energy storage, focussing on insights derived from rare human monogenic disorders. From the evidence presented, we argue that a mismatch between the capacity for nutrient storage and the burden of excess energy intake is an important factor in the development of some forms of human insulin resistance.

Evolution of neutral lipid storage within lipid droplets and adipocytes

Throughout most of evolution, living organisms have contended with challenging environments and struggled to ensure adequate energy intake. As a result, mechanisms have evolved in which nutrients can be stored in times of plenty to later be used when food is less readily available. Although the evolution of efficient energy storage has ensured survival when nutrients become unavailable, sustained access to an appetising supply of energy challenges these very systems that evolved in man.

Nutrient storage in the form of triacylglycerol (TAG) has two advantages over carbohydrate (glycogen) based forms of energy: the more highly reduced carbons of fatty acids yield more energy per gram than carbohydrates (9 kcal/g compared to 4 kcal/g, respectively) and the hydrophobicity of lipids allows for anhydrous 'lighter' storage. For the same mass of carbohydrate, TAG yields more than 6 times the amount of energy [1]. Due to the aqueous nature of the cell cytosol, TAG storage within the cell inevitably occurs as some form of droplet; typically a hydrophobic neutral lipid core is surrounded by a monolayer of phospholipids. Phospholipids, an amphipathic species comprised of a polar head group and a non-polar 'tail,' are uniquely suited for bridging the barrier between the lipid droplet and surrounding cytosol, as the polar head groups interact with the aqueous cytosol while the non-polar tails interact with the hydrophobic TAG or steryl esters (SE) of the lipid droplet core. Release of energy from the neutral lipids at the core of the droplet is achieved through lipolysis, a process which breaks down the TAG or SE, and ultimately yields the constitutive backbone (glycerol or sterol) and fatty acids. In the case of TAG, lipolysis proceeds through three successive steps involving the release of a single fatty acid chain and the generation of the intermediate species of diacylglyercol (DAG) and monoacylglycerol (MAG).

Storage of excess energy in the form of lipid droplets (LD) has been conserved from primitive eukaryotes such as *Saccharomyces cerevisiae*, and even some prokaryotes, through to *Homo sapiens* [2] (Figure 1). In *S. cerevisiae*, the basic machinery of LD synthesis and lipolysis is roughly analogous to that found in higher mammals, making yeast a very useful model organism for understanding the formation of lipid droplets through synthesis of TAG, as well as the degradation of lipid droplets by lipases [3]. Yeast models have been particularly useful in the study of the enzymatic pathways leading to TAG formation, with the two major acyl transferases found in yeast, Dga1 and Lro1, having homologues found in humans, diacylglycerol O-acyltransferase 2 (*DGAT2*) and Lecithin-cholesterol acyltransferase-like 1 (*LCAT1*) respectively [4-7].

Although lipid droplets can form in many different cell types in multicellular organisms, the evolution of specialized cells dedicated to the storage of nutrients as lipid in times of energy excess, and the release of nutrients as fatty acids in the state of energy deficit, attests to the more complex energy needs of more highly evolved organisms. In *Drosophila melanogaster*, the primary metabolic tissue is the fat body, an organ capable of storing nutrients as TAG and SE. Despite the name, the fat body does not function solely as an adipose analogue, as it also stores nutrients as glycogen and is responsible for other processes such as amino acid metabolism [8]. Adipose as a tissue capable of TAG storage and secretion of the satiety hormone leptin can be found intra-abdominally in bony fish and amphibians; with the evolution of homeothermy (endothermy) also came the development of subcutaneous and visceral adipose depots in birds and mammals [9, 10].

The evolution of these tissues at a macroscopic level parallels, to some extent at least, the evolution of the lipid droplet associated proteins at the microscopic level. In *S. cerevisae*,

Caenorhabditis elegans and *Drosophila*, lipid droplets are multilocular and much smaller than the classic mammalian adipocyte lipid droplet, which is unilocular and occupies as much as 90% of cell volume (up to 100 μM in diameter, whereas yeast LDs are typically less than 1 μM in diameter; Figure 1). TAG storage in a single, unilocular lipid droplet originated with vertebrates and required specific protein machinery beyond that found in invertebrates.

In addition to the phospholipid monolayer, lipid droplets are coated with many proteins regulating lipid synthesis and traffic [11]. PAT proteins (named after three family members: Perilipin (*PLIN1*), Adipose differentiation-related protein (*ADRP*), and *TIP47*) are among the most studied LD proteins, and the presence of PAT family members on the surface of lipid droplets found in a variety of organisms, from flies to man, underscores the importance of their role in regulating LD metabolism [12]. Just as important are the evolutionary differences in protein structure and function. Although PAT protein analogues exist in flies, mammals express a larger complement with differences in tissue expression, intracellular localization, and constitutive presence on the lipid droplet. Some of these differences have been attributed to variations in C-terminus domains of PAT family members, which allow for more refined regulation of TAG metabolism and lipid trafficking [13]. The more complex energy needs of mammals necessitated this adaptation from that of *Drosophila*, a two protein system controlling LD growth and metabolism in the fat body [14].

A more diverse array of PAT proteins is not the only evolutionary difference seen in mammalian LD bearing tissues. In adipocytes, expression of cell death-inducing DNA fragmentation factor a-like effector c (*CIDEC; Fsp27* in mice) enables the formation of a

unilocular LD, which enables the cell to optimize energy storage within a confined space [15, 16]. Furthermore, in the absence of this protein, multiple smaller lipid droplets form, and the increased surface area to volume ratio has significant implications for the protein-regulated processes on the surface of the lipid droplet such as lipolysis [17].

What happens when it all goes wrong: insights from rare human monogenic disorders of fat storage

The consequences of disruption of this energy storage system have been studied in many different model organisms and are reviewed elsewhere [18-20]. Here we focus on insights derived from human monogenic disorders. These can be broadly classified as those leading to excess lipid storage and those resulting from impaired energy storage in adipocytes. For the most part, the former class of diseases manifest themselves as excess lipid accumulation in tissues other than fat, for example neutral lipid storage disease (NLSD). In NLSD, mutations in adipose triglyceride lipase (ATGL) or its co-activator *CGI-58* (also known as Abhydrolase domain containing 5; *ABHD5*) result in a failure to properly hydrolyse TAG in the cytoplasm, which leads to a build-up of TAG, most noticeably in non-adipose tissues. Despite the ability of many cell types to form LD, the accumulation of TAG in tissues that did not evolve for the primary purpose of lipid storage tends to result in adverse metabolic effects. When ATGL is mutated in NLSD with myopathy (NLSDM), patients exhibit increased fat content in the pancreas and in the skeletal and cardiac muscles, consequently increasing their risk of pancreatitis, type 2 diabetes mellitus (T2DM), and cardiomyopathy [21]. Pathogenic mutations in CGI-58 lead to NLSD with ichthyosis (NLSDI or Chanarin-Dorfman Syndrome), which is characterized by central nervous system complications, ichthyosis, and

a milder skeletal muscle myopathy as compared to NLSDM [22]. In addition to these symptoms, patients with NLSD may also suffer from other complications, such as hepatic steatosis and developmental delays [23].

A recent publication suggested that pathogenic mutations in hormone sensitive lipase (HSL) in humans result in a milder phenotype than that seen in NLSD [24]. Perturbing the lipolysis of DAG to MAG is, however, not without consequence as patients exhibited ectopic lipid accumulation in the liver with dyslipidemia, insulin resistance, and T2DM [24]. Taken as a whole, these disorders underline the importance of hydrolysing TAG to the component glycerol and fatty acids, and disruption of the enzymes necessary for this process result in accumulation of TAG with ultimately adverse effects in tissues ill-adapted for excess lipid storage.

In contrast to NLSD and HSL deficiency, where ectopic lipid is a consequence of a systemic reduction in the ability to degrade TAG, DAG and possibly other complex lipid species, lipodystrophies are a group of disorders characterized by a primary paucity of functional adipose tissue, which tends to lead to ectopic fat accumulation in many organs including the liver, skeletal muscle, and pancreas. Ectopic storage of lipid in these tissues can result in metabolic complications similar to those seen in obesity, namely dyslipidemia, fatty liver, and severe insulin resistance [25, 26].

Lipodystrophy can be acquired or inherited, and the lack of adipose tissue can be localized, partial, or generalized. Inherited lipodystrophies are broadly categorized from the pattern of fat loss as either familial partial lipodystrophy (FPL) or congenital generalized lipodystrophy (CGL). FPL can be caused by a number of mutations, resulting in a heterogeneous phenotype of fat loss and a range in the severity of symptoms. For poorly understood reasons, fat loss

can be regional and, in specific monogenic subtypes (e.g. FPLD2 associated with lamin A/C (*LMNA*) mutations) can spare selected fat depots, such as those in the face, neck and intra-abdominal regions. These spared regions may even manifest excess fat accumulation [27]. Along with the distressing (particularly in women) morphological consequences, FPL frequently causes serious metabolic complications such as diabetes, dyslipidemia, and coronary heart disease, with women being more severely affected than men [28].

Autosomal dominant FPL has been linked to mutations in the genes encoding lamin A/C (*LMNA*), peroxisome proliferator-activated receptor γ (*PPARG*), v-AKT murine thymoma oncogene homolog 2 (*AKT2*), perilipin 1 (*PLIN1*), or polymerase delta 1 catalytic subunit (*POLD1*) [29-34]. The latter disorder caused by a mutation in *POLD1* is a multisystem disease characterised by male hypogonadism, neurosensory deafness and progeroid features [34]. Autosomal recessive FPL due to mutations in zinc metalloproteinase (*ZMPSTE24*) is also a multisystem condition known as Mandibuloacral dysplasia (MAD) [35, 36]. *ZMPSTE24* is involved in the maturation of the lamin A protein, so it is not surprising that mutations in LMNA have also been associated with MAD. Finally, a homozygous loss-of-function mutation in *CIDEC* has also been shown to cause autosomal recessive FPL [17]. A striking feature of this patient was the presence of many multilocular white adipocytes.

CGL is an autosomal recessive disorder with a striking phenotype from birth. Generalized lack of body fat results in insulin resistance, and higher circulating levels of insulin contribute to prominent musculature, acanthosis nigricans, and pseudoacromegaly. The severity of symptoms depends in part on the nature of the mutation causing the disorder, with some patients suffering total loss of adipose tissue while others retain mechanical adipose depots [27]. Broadly speaking, the metabolic consequences of CGL are more

serious than those seen with FPL, because the severity of symptoms are roughly proportional to the degree of fat loss [37]. Adipose loss in FPL is generally limited to limb and gluteal depots, although truncal depots may also be affected, and as mentioned previously, spared regions may accumulate excess fat [27, 37]. In CGL, a near total lack of body fat removes the possibility of compensatory adipose expansion, resulting in the severe insulin resistance and dyslipidemia which are almost ubiquitous to the condition [37]. The most common causes of CGL are mutations in 1-acylglcerol-2-phosphate O-acyltransferase 2 (*AGPAT2*) and Berardinelli-Seip congenital lipodystrophy 2 (*BSCL2*), although mutations in other genes, such as caveolin 1 (*CAV1*) and polymerase 1 and transcript release factor (*PTRF*), have been identified [28, 38-41].

All the currently known monogenic lipodystrophies are characterised by a degree of fat loss, and, in most, residual adipocyte function is likely to be perturbed. In some cases, this has already been clearly demonstrated [42]. The mechanistic basis for several of the monogenic lipodystrophies is still unclear (Table 1), but mutations in genes such as *PPARG* and *BSCL2* may inhibit the expression of adipogenic genes and impair adipose-tissue differentiation [30, 43, 44]. Mutations in *LMNA* and *ZMPSTE24* appear to lead to abnormal nuclear architecture although exactly if and how this alters adipocyte function remains unclear [36, 45-47]. Pathogenic mutations in genes such as *AGPAT2*, *CAV1*, or *PTRF*, are thought to disrupt adipocyte function as a consequence of altered lipid trafficking or incorporation into TAG [38, 40, 41]. *AGPAT2* encodes an enzyme responsible for synthesizing the precursors to phospholipids and TAG, whereas *CAV1* and *PTRF* have both been implicated in lipid metabolism through their roles in the formation of caveolae [38, 48-50].

Of particular mechanistic interest are mutations in genes that directly impact lipid storage in lipid droplets. Mutations in perilipin yield smaller adipocytes, and *in vitro* experiments suggest disruption of this LD protein increases basal lipolysis [32]. CIDEC mutations result in a striking multilocular LD phenotype; the increased surface area: volume ratio of multilocular LDs, which presumably facilitates greater substrate accessibility by lipases than would be the case with a single droplet, is thought to be a contributing factor to the elevated levels of basal lipolysis seen when the mouse analogue of *CIDEC* is knocked down [17, 51]. In both of these examples of LD protein dysfunction, a smaller LD diameter and *in vitro* evidence of increased basal lipolysis reduces the capacity of adipose tissue to accommodate the energy storage demands typically present in humans, leading to ectopic lipid spill over and metabolic disease.

Defective energy storage as a primary pathogenic factor in human metabolic disease

Whilst protein, carbohydrate (CHO) and fat can all be catabolised to provide energy, living organisms can only store surplus energy in the form of CHO (as glycogen) or as fat (mainly TAG). As mentioned previously, fat is a far more efficient way to store energy in terms of its relative weight and space requirements. It is therefore not surprising that estimates of the total amount of energy that can be stored as glycogen or fat in a lean adult human differ by ~100-fold (fat: 6-800MJ; glycogen: 6-8MJ). Importantly, although the capacity to store surplus CHO is very limited, excess CHO can be converted into fat and stored in that form instead. In contrast, fat cannot be converted into protein or CHO, nor can it be excreted (as far as we know, although we are not aware of studies that have addressed this possibility in detail), so it must be stored or possibly oxidised. The estimates above also highlight the

213 energetic challenge imposed on non-adipose tissues in people with different forms of
214 lipodystrophy, so it is not surprising that they almost inevitably manifest ectopic lipid
215 accumulation in the liver, skeletal muscle, pancreas and other organs.

216 Ectopic fat accumulation is very strongly and consistently associated with insulin resistance
217 and a predisposition to T2DM in humans and in many different rodent models. Mechanistic
218 understanding of this robust association remains the focus of intense research efforts and
219 has been summarised in several comprehensive recent reviews [26, 52-54]. Some of the
220 evidence which has arisen from studies in patients and mouse models of lipodystrophy
221 attesting to the importance of energetic imbalance in the pathogenesis of metabolic disease
222 include the following:

223 1. Severe lipodystrophy is very consistently associated with ectopic fat accumulation
224 and metabolic disease in humans.
225 2. The severity of metabolic disease is generally proportional to the extent of fat loss
226 across the spectrum of human lipodystrophies. This observation is confounded by
227 the fact that the extent of lipodystrophy is also proportional to the relative
228 deficiency of circulating leptin levels and thus the degree of hyperphagic drive
229 experienced by patients. Nevertheless both factors result in a mismatch between the
230 need and capacity to store surplus energy.
231 3. The metabolic consequences of lipodystrophy in humans are generally more severe
232 in women, who under normal healthy circumstances have significantly more body fat
233 (1.5 to 2 fold) than men.

4. Mutations affecting genes encoding proteins almost exclusively expressed in white adipocytes (PLIN1 and CIDEC) and directly involved in TAG storage within lipid droplets can result in severe metabolic disease [17, 32].
5. In mouse models of generalised lipodystrophy, restoring, at least in part, fat mass either by fat transplantation [55] or by transplanting adipogenic precursors [56] significantly alleviates ectopic fat storage and insulin resistance. Furthermore, this effect is to a large extent dependant upon restoring circulating leptin levels and reducing food intake [57].
6. Recombinant leptin therapy dramatically improves metabolic health in lipodystrophic mice [58] and humans [59] primarily by reducing energy intake [57].
7. 'Forcibly' restricting energy intake in a patient with generalised lipodystrophy significantly improved her metabolic status [60].
8. Case reports suggest that bariatric surgery can be a very effective way to improve metabolic parameters in patients with lipodystrophy [61].

Intriguingly, patients with generalized lipodystrophies tend to have low fasting fatty acid levels, so the tendency to accumulate ectopic fat in these patients is not necessarily mediated by high circulating fatty acid concentrations. However, we are not aware of studies that have formally documented 24 hour fatty acid profiles in non-diabetic patients with generalised lipodystrophy, so it is possible that 24 hour circulating levels of fatty acids are increased.

The notion that more prevalent forms of T2DM and insulin resistance might also be a consequence of 'exceeding adipose tissue energy storage capacity' has been around for some time, having been elucidated by Elliot Danforth in a short commentary before being

expanded upon by other authors [62-64]. Admittedly lipodystrophies are an extreme and rare cause of insulin resistance/ metabolic disease. Recent genome wide association data suggest, however, that more prevalent variants associated with hyperinsulinaemia, higher TAG levels and T2DM are also associated with subtle forms of 'lipodystrophy'[65, 66]. Although T2DM typically emerges in the obese, Scott *et al.* highlighted the complex genetic background of T2DM susceptibility by showing associations between insulin resistance and genetic variants independent of BMI; that these variants also associated with decreased subcutaneous adipose mass provides further support to the adipose expandability hypothesis [65].

These recent genetic findings are supported by older studies demonstrating the metabolic benefits of thiazolidinediones, which act by activating PPARG, a key transcriptional regulator of adipogenesis [67]. They are also consistent with a remarkable mouse model generated by the Scherer laboratory, who crossed leptin deficient ob/ob mice with mice transgenically over-expressing adiponectin and found that this led to exaggerated weight gain but improved insulin sensitivity [68].

Obesity is strongly and consistently associated with a chronic inflammatory response which is, at least in part, a reaction to adipocyte cell death [69]. Exactly what triggers adipocyte death in this context is unclear, although several plausible hypotheses exist [70]. As with any injury, this is followed by macrophage infiltration as well as an influx of other inflammatory cell types. This response is in turn strongly temporally associated with insulin resistance [71] and, at least in mice, several anti-inflammatory strategies have resulted in significant improvements in insulin resistance [70]. However, confidently attributing insulin resistance in this setting to the inflammatory response is very difficult, whereas the evidence from

lipodystrophic models suggests that the primary abnormality is more likely to be a defect in energy storage within adipocytes, and thus that the primary focus of treatment ought to be alleviating this energetic imbalance. This idea is supported by recent observations in FSP27 deficient mice, which are unable to form large unilocular lipid droplets in adipocytes [51, 72], and when energetically challenged with excess fat intake, do manifest hepatic steatosis and hepatic insulin resistance, despite minimal adipose inflammation [73].

Concluding remarks

All living organisms adapt to surplus energy supplies, at least in part, by generating lipid droplets. In higher organisms, adipocytes have evolved in such a way as to optimise efficient energy storage and release fatty acids from huge unilocular lipid droplets in white adipocytes, enabling birds and mammals to better adapt to fluctuating energy supplies. However, modern humans faced with sustained energy surpluses ultimately fail to accommodate all the fat in adipocyte lipid droplets, instead accumulating fat in other less well-adapted cell types where it impairs insulin action and contributes to metabolic disease. Lipodystrophies robustly attest to the importance of adipose tissue as an essential energy storage depot and to the importance of alleviating the burden of surplus energy imposed upon other insulin target tissues such as skeletal muscle and the liver, in circumstances where the capacity of adipose energy stores are surpassed.

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Figure legend

Figure 1. Evolution of neutral lipid storage within lipid droplets (LDs).

From left to right; multilocular LDs (stained with Bodipy) in a primitive unicellular eukaryote, *Saccharomyces cerevisiae*; multilocular LDs in intestinal (fat storage) cells of a primitive metazoan, *Caenorhabditis elegans*; multilocular LDs (lipid droplets (green), nuclei (blue) and cell membranes (red)) in specialized energy storage cells within the 'fat body' of *Drosophila*; unilocular LDs (stained with LipidTox) within pancreatic visceral adipocytes in *Danio rerio*; unilocular adipocytes in white adipose tissue in *Homo sapiens*. Images are courtesy of Emily Rowe, University of Cambridge (*S. cerevisiae*); Xianglin Ji and Ho Yi Mak, Hong Kong University of Science and Technology (*C. elegans*); Philip Hehlert and Ronald Kühnlein, Max Planck Institute for Biophysical Chemistry (*Drosophila*); James Minchin and John Rawls, Duke University Medical Centre (*D. rerio*); Alison Sleigh & Keli Phillips, University of Cambridge (Human MRI scan and white adipose tissue).

Table 1: Putative mechanisms for monogenic lipodystrophies. In many instances, further work is required to clarify the proposed mechanisms.

	Gene Mutated	Lipodystrophy Phenotype	Protein Function
Lipid Uptake/Synthesis	AGPAT2	CGL	Enzyme synthesizes phosphatidic acid (PA) from lysophosphatidic acid (LPA).
	CAV1	CGL	Required for the formation of caveolae, which may be involved in fatty acid uptake.
	PTRF	CGL	Also involved in the formation of caveolae.
	PCYT1A	CGL/FPL	Rate limiting enzyme in phosphatidylcholine (PC) synthesis.
Defects in Adipogenesis	PPARG	FPL	Nuclear receptor required for adipogenesis.
	BSCL2	CGL	Encodes seipin, a protein of uncertain function, although recent data suggests an important role in LD biogenesis.
	LMNA	FPL	Lamins A and C form part of the nuclear envelope lamina. Exactly how mutations cause lipodystrophy remains unclear.
	ZMPSTE24	MAD/FPL	Involved in pre-lamin to lamin A processing.
Lipid Droplet Associated	PLIN1	FPL	LD surface protein, important in regulating lipolysis.
	CIDEC	FPL	LD protein that facilitates formation of unilocular LDs.
DNA Damage	POLD1	FPL	Polymerase δ catalytic subunit.

Figure 1