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From Scarcity to Solutions: Therapeutic Strategies to Restore Adipose Tissue Functionality in Rare Disorders of Lipodystrophy

Running title

Treatments for rare disorders of lipodystrophy

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Conflict of interest

M.T. has no conflicts of interest to declare. G.D.M. is co-inventor on a patent application for the use of gene therapies designed for the treatment of lipodystrophy disorders.

Novelty Statement

What is already known?

- Lipodystrophy is a rare and life-threatening disorder.
- Genetic lipodystrophies may affect 1 in 7000 people.
- Complications include severe type 2 diabetes and fatty liver disease.
- Current treatments for lipodystrophy are limited and inadequate.

What this study has found?

- This review brings to light novel approaches to restore adipose tissue functionality in lipodystrophy.
- Effective and diverse interventions include adipose transplantation, leptin therapy, lipolysis inhibition, and cutting-edge gene/cell therapies.

What are the implications of the study?

- The therapies discussed offer significant potential, opening new opportunities for treatment.
- The findings highlight the need for further research and collaborative partnerships to expedite the development of effective treatments, providing hope and improved outcomes for affected individuals.

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Abstract

Aims: Lipodystrophy is a rare disorder characterised by abnormal or deficient adipose tissue formation and distribution. It poses significant challenges to affected individuals, including

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development of severe metabolic complications like diabetes and fatty liver disease. These conditions are often chronic, debilitating, and life-threatening, with limited treatment options and a lack of specialised expertise. This review aims to raise awareness of lipodystrophy disorders and highlight therapeutic strategies to restore adipose tissue functionality. Methods: Extensive research has been conducted, including both historical and recent advances. We have examined and summarised the literature to provide an overview of potential strategies to restore adipose tissue functionality and treat/reverse metabolic complications in lipodystrophy disorders. Results: A wealth of basic and clinical research has investigated various therapeutic approaches for lipodystrophy. These include ground-breaking methods such as adipose tissue transplantation, innovative leptin replacement therapy, targeted inhibition of lipolysis, and cutting-edge gene and cell therapies. Each approach shows great potential in addressing the complex challenges posed by lipodystrophy. Conclusions: Lipodystrophy disorders require urgent attention and innovative treatments. Through rigorous basic and clinical research, several promising therapeutic strategies have emerged that could restore adipose tissue functionality and reverse the severe metabolic complications associated with this condition. However, further research and collaboration between researchers, clinicians, patient advocacy groups, and pharmaceutical companies, will be crucial in transforming these scientific breakthroughs into effective and viable treatment options for individuals and families affected by lipodystrophy. Fostering such interdisciplinary partnerships could pave the way for a brighter future for those battling this debilitating disorder.

Keywords

Lipodystrophy, Rare disorders, Adipose transplantation, Leptin replacement therapy, Lipolysis, Gene therapy, Cell therapy

1. Introduction

Rare disorders, also known as rare diseases or orphan diseases, refer to a medical condition affecting a relatively small number of individuals. The European Union defines a rare disorder as having a prevalence of fewer than 1 in 2,000 individuals.¹ It is estimated there are more than 6000 different rare disorders, which collectively may affect 446 million people, representing nearly 6% of the world's population.² They can be genetic or non-genetic in nature, and often have diverse and complex manifestations. Due to their rarity, they typically present unique challenges in terms of diagnosis and treatment. Many rare disorders are chronic, debilitating, and life-threatening conditions. Each disorder may only have a small number of affected individuals, but the overall impact is significant due to the cumulative number of rare disorders and their effects on individuals, families, and communities.

An indicator of disease burden is the disability-adjusted life-years (DALYs), however DALYs are difficult to estimate for rare diseases. Initiatives including the Global Burden of Disease, the World Health Organization's Global Health Estimates and Orphanet aim to provide information on rare diseases. Unfortunately, large gaps exist in the available data which prevent the calculation of DALYs. This has recently been addressed by the European Burden of Disease Network through the launch of a Burden of Rare Diseases Task Force.³ No outcomes are available at present and specialists with expertise in rare diseases, epidemiology, health technology or population health metrics are encouraged to join.

Due to the scarcity of rare diseases, a lack of scientific research and understanding of such disorders often contributes to delayed or inaccurate diagnoses, further reducing their visibility.¹ Additionally, limited therapeutic treatment options are available after diagnosis, which contributes to unfavourable prognoses. Efforts are being made by patient advocacy groups, healthcare professionals, researchers, and policymakers to raise awareness, improve diagnosis and treatment, and support research and development of therapies for rare disorders.

Lipodystrophy is one example of a rare disorder. This complex group of conditions has been extensively reviewed in an excellent publication by Lim and colleagues.⁴ This condition is characterised by abnormal or deficient fat (adipose tissue) formation and distribution in the body. Lipodystrophy is a heterogeneous group of conditions that can affect both children and adults. It is considered an ultra-rare disease, however due to clinical heterogeneity that complicates diagnosis, the true prevalence is thought to be greatly underestimated. Through the examination of electronic health records of more than 1.3 million adults, recent estimates indicate that the prevalence of genetic forms of lipodystrophy may affect as many as 1 in 7,000 individuals in the general population.⁵ The loss or abnormal distribution of adipose tissue in lipodystrophy can have significant health implications. Individuals with lipodystrophy often face severe metabolic complications, such as insulin resistance, type 2 diabetes, hypertriglyceridemia, and hepatic steatosis. These metabolic disturbances can lead to various associated health problems and cardiovascular risks.⁴

2. Types of lipodystrophy

Lipodystrophies are categorised into two main types, congenital (inherited) and acquired, and further sub-classified depending on the severity of adipose tissue loss.⁴ Inherited forms of lipodystrophy typically present from birth or early childhood and are caused by genetic mutations. Adipose tissue loss is either generalised or partial in nature. Congenital generalised lipodystrophy (CGL) represents the most severe phenotype within the spectrum of this disorder. CGL is characterised by an almost complete absence of white adipose tissue throughout the body, leading to a very lean and muscular appearance.^{4,6} CGL type 1 is associated with genetic mutations in the *AGPAT2* gene,⁷ which encodes the enzyme 1-acylglycerol-3-phosphate O-acyltransferase 2 required for the biosynthesis of triglycerides and phospholipids. CGL type 2 on the other hand is attributed to mutations in the *BSCL2* gene,⁸ which encodes the protein seipin, and is critical for lipid droplet formation and adipose tissue development. Mutations in *AGPAT2* and *BSCL2* represent the most common forms of CGL and have been observed in individuals from diverse ethnic backgrounds. CGL type 3 is characterised by mutations in the *CAV1* gene,⁹ which encodes a caveolae structural protein that is abundant in adipocyte membranes and facilitates the translocation of fatty acids into lipid droplets. Finally, CGL type 4 results from mutations in the *CAVIN1* (also known as *PTRF*) gene,¹⁰ which controls caveolae biogenesis and regulates the expression of caveolin 1 and 3.

Familial partial lipodystrophies (FPLD) on the other hand are characterised by a loss of adipose tissue in the limbs, buttocks, and hips, leading to a more pronounced fat accumulation in the face and neck. Whilst the genetic cause of Köbberling-type lipodystrophy (FPLD type 1) remains unclear,⁴ mutations in numerous genes have been identified that are responsible for causing FPLD. These include *LMNA/C* and *ZMPSTE24* (FPLD type 2), involved in nuclear lamina formation; *PPARG* (FPLD type 3), the master transcription factor of adipogenesis; *PLIN1* (FPLD type 4), a lipid-droplet-associated protein

involved in adipocyte lipolysis; *CIDEA* (FPLD type 5), which promotes lipid droplet formation in adipocytes; and *LIPE* (FPLD type 6), which encodes the lipolytic protein hormone-sensitive lipase. There are also several unclassified forms of lipodystrophy which are also inherited.⁴

Acquired forms of lipodystrophy usually occur later in life and are often associated with autoimmune conditions or other underlying medical conditions. They are divided into three main types, acquired generalised lipodystrophy, acquired partial lipodystrophy and acquired localised lipodystrophy.⁴ Acquired generalised lipodystrophy or Lawrence syndrome (named after Dr RD Lawrence, co-founder of Diabetes UK) involves a progressive loss of adipose tissue throughout the body. Whilst some adipose depots can be spared, the severity of adipose tissue loss results in severe metabolic complications including type 2 diabetes similar to that observed in CGL.⁴ Acquired partial lipodystrophy or Barraquer-Simons syndrome typically begins during childhood and primarily affects the face, upper body, and arms but the lower parts of the body are typically spared. Acquired partial lipodystrophy also includes the treatment-induced form of lipodystrophy in human immunodeficiency virus (HIV) infected individuals receiving highly active antiretroviral therapy (HAART).⁴ Unlike general or partial acquired lipodystrophies, acquired localised lipodystrophies result in adipose tissue loss in small and specific regions of the body. Due to the limited loss of adipose tissue, this form of lipodystrophy does not typically lead to the development of metabolic complications.⁴

Lipodystrophy disorders clearly highlight the significant role played by adipose tissue in the maintenance of metabolic homeostasis. Adipose tissue is of critical importance for various physiological functions including energy storage, insulation/thermoregulation, hormone secretion/regulation, protective cushioning and nutrient absorption/distribution.¹¹ Adipose tissue deficiency can therefore be associated with a number of metabolic complications, including impaired glucose tolerance, insulin resistance, elevated triglyceride levels, fat accumulation in the liver, and renal dysfunction, which together contributes to the development of complex diseases such as type 2 diabetes and cardiovascular disease.⁴ Bone marrow adipose tissue is also known to play a critical and specific role in the regulation of haematopoiesis, skeletal development, and bone mass. Curiously, bone marrow adipose tissue is completely absent in some, but not all subtypes of CGL, which results in skeletal phenotype differences including osteosclerosis, advanced bone age, and cortical thickening.¹² Adipose tissue is also an important endocrine organ responsible for the release of important metabolic hormones such as leptin and adiponectin, which help to regulate metabolism, maintain energy balance, and facilitate inter-organ communication.¹¹ These adipokines are often significantly depleted in individuals with severe forms of lipodystrophy. Therefore, the ability to develop and maintain appropriate quantities of functional adipose tissue is of critical importance to safely store lipids, prevent ectopic fat accumulation in peripheral tissues and maintain metabolic health.

Despite significant advances in the field, treatment options for lipodystrophy are currently limited. Existing therapeutic strategies mainly focus on treating metabolic consequences that arise due to lipodystrophy, rather than addressing the root cause. In this review, we aim to explore and summarise both the historical literature and recent research which have examined the restoration of adipose tissue functionality as a potential therapeutic approach to treat lipodystrophy disorders. This includes both basic and clinical research investigating the use of adipose tissue transplantation, leptin replacement therapy, inhibition of lipolysis and the use of gene and cell therapies as novel treatment strategies (Figure 1).

3. Therapeutic strategies to restore adipose tissue functionality

3.1 Adipose transplantation

Metabolic disorders associated with excessive adiposity (obesity) or its scarcity (lipodystrophy) reveal the critical importance of developing and maintaining appropriate quantities of functional adipose tissue.¹¹ Adipose tissue deficiency in individuals with severe inherited generalised lipodystrophy has been strikingly revealed through whole-body magnetic resonance imaging.⁶ Therefore, a logical therapeutic strategy would be to restore adipose tissue functionality through transplantation.

Gavrilova and colleagues were the first to experimentally address the therapeutic potential of adipose transplantation for lipodystrophy using the A-ZIP/F-1 mouse.¹³ Transgenic A-ZIP/F-1 mice express a dominant negative protein, specifically in adipose tissue. This inactivates the C/EBP and JUN families of B-ZIP transcription factors, which are critical for adipose tissue formation. A-ZIP/F-1 mice fail to develop white adipose tissue, like that observed in individuals with inherited generalised lipodystrophy. Consequently, A-ZIP/F-1 mice manifest severe metabolic complications including insulin resistance, glucose intolerance, hepatic steatosis, hypertriglyceridemia and hypoleptinemia. When subcutaneous white adipose tissue was transplanted from wild type mice donors, this was sufficient to reverse hyperglycaemia, lower insulin levels and improved muscle insulin sensitivity. Circulating leptin levels, hepatic steatosis and organomegaly were also partially or completely reversed. These beneficial effects were shown to be dependent on the quantity of adipose transplanted, however, transplantation of either subcutaneous or parametrial adipose tissue depots was found to be equally effective.¹³

Numerous studies have replicated the beneficial effects of adipose tissue transplantation to metabolic dysfunction that develops in alternative transgenic models presenting with a lipodystrophic phenotype. These include the aP2-nSREBP-1c transgenic line,¹⁴ the *Kbtbd2*^{-/-} knockout mouse with altered insulin signalling,¹⁵ the adipocyte-specific MDM2-KO model, which disrupts apoptotic and senescent programs¹⁶ and mice with adipose-specific mevalonate pathway-disruption through knockout of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase.¹⁷ Inducible lipodystrophy models have also been generated. When mice harbouring the primate diphtheria toxin receptor under control of a STOP-flox cassette are crossed with mice expressing adiponectin-Cre, postnatal white, brown and marrow adipocytes are eliminated. Curiously, white adipose tissue transplantation effectively normalised serum blood glucose levels and hepatic steatosis, however failed to rescue osteosclerosis observed in this model.¹⁸ This data suggests that marrow adipocyte ablation may be specifically responsible for influencing osteosclerosis and bone mass in congenital forms of lipodystrophy.

The adipose transplantation studies described above are extremely convincing as a therapeutic treatment for lipodystrophy. However, these transgenic mouse models do not represent any recognised form of lipodystrophy in humans.⁴ Identification of the genetic causes of inherited generalised lipodystrophy⁷⁻¹⁰ has allowed researchers to generate transgenic mouse models of these disorders. Two recent studies have shown that adipose tissue transplantation is effective in *Bsc12*/seipin knockout (SKO) mice, the causative gene responsible for congenital generalised lipodystrophy type 2 (CGL2). Liu and colleagues revealed that subcutaneous adipose transplantation rescued insulin resistance and low plasma leptin levels in SKO mice and effectively ameliorated renal injury.¹⁹ Wang and colleagues similarly revealed that healthy adipose tissue from wild type mice transplanted

into SKO mice dramatically increased levels of plasma leptin. Severe hepatic steatosis, insulin resistance and dyslipidaemia were also ameliorated four months after adipose transplantation.²⁰

Whilst adipose tissue transplantation in rodent models appears to be highly effective, translating these therapeutic benefits to individuals with lipodystrophy is likely to be challenging. Mouse studies use immune-matched donors and recipients, therefore immunosuppression would be required to prevent graft rejection in humans. A study of 20 individuals with CGL2 revealed reduced lifespan by more than thirty years, with infectious disease listed as the cause of death in one third of individuals.²¹ Additionally, although fat transplantation has been extensively performed for reconstructive and cosmetic surgery, a recent review of the literature by Davis and colleagues identified no studies reporting on the metabolic outcomes of human-to-human adipose tissue transplants.²² Side effects of transplant can also include significant bleeding, infection, graft reabsorption/loss, peritonitis, bowel adhesions and fat embolism.²²

Additionally, further elegant transplantation studies in lipodystrophic A-ZIP/F-1 transgenic mice raised the question as to whether adipose restoration was even strictly required to improve metabolic health in lipodystrophy. Curiously, transplantation of adipose tissue from leptin-deficient *ob/ob* mice had no beneficial effect to glucose, insulin or triglyceride levels of lipodystrophic A-ZIP/F-1 transgenic mice.²³ This was despite the fact that adipose tissue grafts from both wild type and *ob/ob* mice expanded similarly in recipients, indicating adipose tissue storage functionality. These results suggested that leptin replacement therapy could be sufficient to correct insulin resistance and glucose intolerance observed in conditions of adipose tissue deficiency in lipodystrophy disorders.

3.2 Leptin replacement therapy

Transgenic mouse models have also been utilised to establish the effectiveness of leptin replacement therapy as a treatment for lipodystrophy. Shimomura and colleagues first revealed that systemic infusion of recombinant leptin (5 µg/day for 12 days) reduced food intake and normalised circulating insulin, glucose and hepatic triglyceride levels in aP2–nSREBP-1c mice.²⁴ Using a similar dosage (5 µg/day for 4 weeks), this effect was not initially observed in the more-severe lipodystrophic A-ZIP/F-1 mouse.²⁵ However, both a higher dose of leptin (30 µg/day for 6 days) and a longer duration of treatment (30 µg/day for 2 weeks) effectively improved metabolic health in A-ZIP/F-1 mice.²³ Similar beneficial effects have also been observed in the double transgenic LepTg/1:A-ZIPTg/1 line, which lacks adipose tissue but has elevated circulating leptin levels through hepatic overexpression of leptin.²⁶ Leptin therapy has also been shown to be effective in both the *Bscl2* and *Agpat2* knockout mouse models of severe congenital generalised lipodystrophy.^{19,27}

In 2002, Oral and colleagues published the first examination of methionyl human leptin therapy in nine women participants with lipodystrophy and low serum leptin levels (<4 ng/ml). Subcutaneous injections twice daily for four months improved glucose and insulin tolerance and decreased average triglyceride levels and liver volume by 60 and 28 percent respectively.²⁸ A follow-up study in three of these participants confirmed leptin therapy improved liver and muscle insulin sensitivity using hyperinsulinemic-euglycemic clamp studies.²⁹ Further long-term studies which include multiple different subtypes of lipodystrophy have also confirmed the effectiveness of leptin replacement therapy for lipodystrophy.³⁰ In many cases, the effects of leptin therapy are so robust that frontline

diabetes medications prescribed to individuals with lipodystrophy are often decreased or even discontinued entirely.

The positive metabolic effects of leptin treatment are largely thought to be due to its ability to decrease food intake. However, when *aP2-nSREBP-1c* or *A-ZIPTg/1* mice were severely food restricted or pair-fed, glucose intolerance and insulin resistance did not improve. This indicates that leptin may have beneficial effects independent of food intake.^{24,26} A recent nonrandomised, crossover design trial has investigated this in individuals with lipodystrophy, aiming to determine if metreleptin could improve metabolic health even when food intake was held constant.³¹ Hyperinsulinemic-euglycemic clamp studies indicated that peripheral insulin sensitivity decreased 41% when metreleptin was withdrawn and increased 32% after metreleptin initiation. Fasting glucose levels also decreased 11% and liver fat decreased from 21.8% to 18.7% even when food intake was strictly maintained.³¹ Recent elegant studies in humans³² have revealed that central leptin signalling increases very-low-density lipoprotein triglyceride (VLDL-TG) secretion and reduces hepatic lipid content independent of food intake through a brain-vagus-liver axis. In fasted lean men, metreleptin increased VLDL-TG secretion and reduced hepatic lipid content, which was not replicated in metabolically healthy liver transplant recipients. Vagal stimulation by modified sham feeding also replicated the effects of metreleptin on VLDL-TG secretion.³² Targeting this pathway could therefore lead to the development of novel therapies to alleviate hepatic steatosis both in lipodystrophy as well as non-alcoholic fatty liver disease.

Whether replacement of additional adipose-tissue-secreted factors, such as adiponectin, could be of benefit has not been extensively explored to date. One report treated *Ppar γ ^{+/-}* mice with an RXR antagonist to induce lipoatrophy. In this model, insulin resistance was completely reversed by combining physiological doses of adiponectin and leptin. This contrasts with the partial reversion observed by either adiponectin or leptin alone.³³ Further work will be required to explore whether a combination of adipose secreted factors can be achieved and provide enhanced efficacy as a treatment for lipodystrophy.

Leptin replacement therapy is currently the “gold standard” therapeutic treatment for certain lipodystrophies and has been approved for use in multiple countries.³⁴ However, metreleptin is not always widely available or approved for all forms of lipodystrophy. It is expensive and adverse effects including the development of neutralising antibodies can occur, which may blunt its metabolic efficacy.³⁵ Whilst being highly effective as a treatment, leptin replacement therapy does not restore adipose tissue development or functionality. Therefore, development of additional therapeutic strategies is clearly warranted.

3.3 Inhibition of lipolysis

Whilst investigating the effects of chronic leptin treatment in individuals with severe generalised lipodystrophy, Petersen and colleagues observed significantly increased rates of basal glycerol turnover.²⁹ This finding indicated that increased rates of lipolysis were present in these individuals. These observations have also been replicated in several mouse models relevant to human forms of lipodystrophy.

Examination of mouse embryonic fibroblasts and stromal vascular cells from global and adipose tissue-specific SKO mice revealed uncontrolled lipolysis was present and responsible for the failure of terminal adipocyte differentiation.³⁶⁻³⁸ This was found to be due to uncontrolled cyclic AMP (cAMP)-dependent protein kinase A (PKA)-activated lipolysis, leading to lipid droplet depletion and the downregulation of the adipogenic transcription

cascade. Intriguingly, Chen and colleagues revealed that inhibition of lipolysis was able to correct defects in adipogenesis *in vitro*, an effect that was not replicated with the use of a peroxisome proliferator-activated receptor gamma agonist, the master regulator of adipogenesis.³⁶ In 2019, additional research from the Chen laboratory confirmed that pharmacological inhibition of lipolysis, using Atglistatin, could rescue adipocyte differentiation *in vitro*. They also revealed that genetic inactivation of lipolysis *in vivo* was able to rescue lipodystrophy in SKO mice.³⁹ Heterozygous deficiency of adipose tissue triglyceride lipase (ATGL) in SKO mice resulted in restoration of approximately 30% adiposity, whilst complete ATGL inactivation restored adipose tissue development to levels greater than those observed in wild type mice. Impressively, the partial inactivation of ATGL was sufficient to reverse whole body insulin resistance, modestly elevate circulating leptin levels and significantly reduce hepatomegaly in SKO mice.³⁹

Elevated rates of lipolysis have also been reported in *Lmna* knockout mice, a model of FPLD type 2.⁴⁰ Treatment of *Lmna* knockout mice with rapamycin can significantly extend the lifespan of this model. The rapid weight loss normally observed in these mice was suppressed with drug treatment and *Lmna* knockout mice showed significantly increased and prolonged accumulation of total fat content as assessed by Echo-MRI. Liao and colleagues observed that ATGL was elevated in both adipose tissue of *Lmna* knockout mice and *ex vivo* preadipocyte cultures purified from adipose tissue, along with increased levels of circulating serum free fatty acids. Treatment with rapamycin was able to suppress these effects, resulting in the prevention of adipose tissue loss, which the authors reveal was due to occur specifically through inhibition of mTORC1.⁴⁰

Increased levels of lipolysis have also been observed in *Plin* knockout mice⁴¹, *Cidec* knockout mice⁴² and *Zmpste24* knockout mice.⁴³ These knockout mice represent models to examine disruption to genes that are thought to be responsible for FPLD disorders including FPLD type 4, FPLD type 5 and FPLD type 2 respectively.⁴ However, it is currently unclear if increased rates of lipolysis are a general phenomenon that occurs in all subtypes of lipodystrophy. For example, mice deficient for *Cav1/Ptrf* represent a model for CGL type 4 in humans. Basal rates of lipolysis were unchanged or lower than control mice and isoproterenol stimulated lipolysis was found to be blunted both *in vivo* and in isolated adipocytes from this knockout model.⁴⁴ Therefore, inhibiting lipolysis may be of therapeutic potential to certain subtypes of lipodystrophy, but possibly not all. However, these findings reinforce the concept that restoring small amounts of functional adipose tissue could be particularly beneficial to metabolic health, especially in the most severe subtypes of this rare disorder.

3.4 Gene and cell therapy

Of the 6000+ rare disorders that have been identified, 72% of these are hereditary in nature.² Treatments for rare disorders typically only manage the symptoms of the condition. However, advances in gene and cell therapies mean this technology now represents a viable and effective approach to treat, and potentially cure such human disorders. Importantly, the number of gene therapies gaining regulatory approval continues to expand, paving the way to improve the lives of numerous individuals suffering from a wide variety of inherited genetic disorders.⁴⁵ Significant advances have been made to generate gene therapies that target organs such as the eye, muscle, central nervous system and liver.⁴⁵ In contrast however, research into gene therapeutic strategies designed to target adipose tissue remains in its infancy.⁴⁶

Adeno-associated virus (AAV) vectors are emerging as promising delivery tools for gene therapy in the clinic. This is due to the fact they are non-pathogenic, require a helper virus in order to replicate and typically do not integrate into the host genome.⁴⁵ Additionally, the only requirement for packaging DNA into AAV capsids are two palindromic inverted terminal repeats. Therefore, recombinant AAV vector genomes can easily be designed to include promoters, transgenes, and regulatory elements for therapeutic purposes. The only limitation is the AAV packaging capacity of ~4.7 kDa, which can make certain genetic disorders challenging to address.⁴⁶ Targeting adipose tissues using AAV vectors was initially thought to be difficult due to low transduction efficiencies and poor tissue tropism. However, rigorous research efforts have revealed that certain wild type AAV serotypes and novel recombinant AAV vectors can effectively target adipose tissue depots.

Whilst not examining lipodystrophy directly, O'Neill and colleagues revealed that AAV serotype 8 vectors overexpressing human leptin could reverse weight gain, decreased food intake, hyperinsulinemia and improve glucose tolerance in the leptin deficient *ob/ob* mouse model.⁴⁷ These improvements to metabolic health were observed over an 8-week period with a single intravenous injection of 1×10^{12} AAV genome copies. Both leptin mRNA and protein were detectable in adipose tissue and leptin secretion into the circulation reached 7% of age matched wild type mice.⁴⁷ Similar findings were also observed by Huang and colleagues, however their use of the novel engineered hybrid serotype Rec2 AAV vector was able to normalise circulating leptin levels in *ob/ob* mice to similar levels observed in wild type mice and completely reversed metabolic disease including hepatic steatosis.⁴⁸ This was also achieved using single injection at AAV doses approximately 25-fold lower than those required when using the wild type AAV serotype 8 vector.^{47,48} These reports provided proof of concept that AAV vectors could effectively restore a gene deficiency in adipose tissue and correct metabolic dysfunction in a mouse model of a known human disorder.

Research from Uhrig-Schmidt and colleagues also highlighted that AAV mediated gene therapy may be of therapeutic relevance for lipodystrophy disorders.⁴⁹ Once again, this study did not specifically investigate gene therapy as a treatment for lipodystrophy. Recombinant AAV8 vectors were used to deliver *Plin1* (mutations linked with FPLD type 4 and AGL⁴) to adipose tissues using the 2.2 kb minimal murine adiponectin promoter. A single intravenous injection of 1×10^{12} AAV genome copies to C57BL/6NCrl mice significantly decreased serum free fatty acid and glucose levels and increased the respiratory exchange ratio, indicating a shift toward carbohydrate utilisation as a fuel source.⁴⁹ Positive metabolic effects were present after only three weeks post AAV administration. These findings indicate that AAV mediated overexpression of a gene thought to be responsible for certain subtypes of human lipodystrophy can result in significant beneficial alterations to metabolic health. However, this research did not provide any insight as to whether AAV gene therapy would be a viable therapeutic approach in conditions where adipose tissue is absent.

The critical research efforts performed by others⁴⁶ led our laboratory to investigate whether AAV mediated gene therapy could be a viable therapeutic approach to restore adipose tissue development in lipodystrophy disorders. Our research has been the first to report that AAV mediated gene therapy is highly effective in a mouse model of CGL2.⁵⁰ A single systemic injection of AAV serotype 8 overexpressing human *BSCL2* from the cytomegalovirus promoter was able to effectively restore adipose tissue development and reverse metabolic disease in this pre-clinical mouse model. Beneficial metabolic improvements included the reversal of insulin resistance, glucose intolerance and reduction of hepatomegaly, along with significant elevations in circulating leptin and adiponectin

levels. These changes were rapidly observed and remained apparent five months after treatment.⁵⁰ Our findings also indicated that that AAV8 vectors are capable of targeting both visceral and subcutaneous white adipose tissue progenitor cells *in vivo*. Curiously however, only visceral adipose tissue depots (gonadal and retroperitoneal) were restored under these conditions, whilst subcutaneous adipose tissue remained absent.⁵⁰ Further work will be required to determine if additional adipose development can be achieved using AAV mediated gene therapy. This could include the combination of novel recombinant AAV vectors such as Rec2 which efficiently targets adipose tissue,⁴⁸ using tissue-specific promoters to enhance the targeting of adipose tissues⁵¹ and providing a pro-adipogenic environment such as diets that are high in fat, or treatment with thiazolidinedione's to activate *Pparg*, the master regulator of adipogenesis. However, our findings provided proof of concept that adipose tissue development can be restored in a pre-clinical mouse model of severe CGL2 using AAV mediated gene therapy, and this is sufficient to alleviate the severe metabolic complications that arise in this condition.

An alternative therapeutic approach that ought to be considered for lipodystrophy is cell therapy. In 2008, Rodeheffer and colleagues used fluorescence-activated cell sorting (FACS) experiments to identify the presence of adipocyte progenitor cells (Lin⁻:CD29⁺:CD34⁺:Sca-1⁺:CD24⁺) in adipose tissue of mice.⁵² Injecting as few as 50,000 CD24⁺ progenitor cells into lipodystrophic A-ZIP/F-1 mice was able to restore a normal sized white adipose tissue depot and correct the diabetic phenotype of this model. The authors highlighted that the number of progenitor cells used for cell therapy was far lower than those required for transplant experiments¹³ or that were present in the adipose depot that developed from those cells.⁵² This suggests that isolated adipose progenitor cells appear capable of further proliferation *in vivo* before differentiating into functional mature adipocytes. The authors also hypothesised that such progenitor cells from individuals with lipodystrophy could potentially be isolated, their genetic defect corrected *ex vivo* and then autologous transplantation performed to induce functional adipose tissue development.⁵² In a recent study, similar FACS methods have been utilised to identify adipogenic progenitor cells within the bone marrow.⁵³ Ambrosi and colleagues revealed that substantial bone marrow adipocyte development can be achieved when intratibial transplantation of multipotent, adipogenic progenitor or pre-adipocyte cells are used in repopulation assays after a dose of lethal irradiation. This approach may be able to prevent/reverse the development of osteosclerosis and provide a valuable source of the adipokine adiponectin, however further experimentation will be required to confirm this. With the rapid advances in genome editing technology such as homology directed repair or base editing using technologies such as CRISPR, examining whether these therapeutic approaches are viable in individuals with lipodystrophy is a highly exciting prospect and warrants future experimental examination.

One recent study has examined the functionality of adipose derived stem cells (ADSCs) from individuals with APL.⁵⁴ As many as 70% of individuals receiving HAART are reported to have HIV-associated lipodystrophy. Suzuki and colleagues revealed that ADSCs isolated from HIV-infected individuals were capable of *ex vivo* adipocyte differentiation that was indistinguishable to that of ADSCs isolated from non-HIV infected individuals.⁵⁴ It is worth noting that the number of ADSCs recovered from HIV-infected individuals was 90% less than those observed in non-HIV infected individuals. However, despite this, these findings clearly indicate that adipose progenitors from an acquired form of lipodystrophy can be isolated, expanded and retain the capacity to effectively differentiate into adipocytes. Further studies

will be required to establish if this is also possible from individuals with other forms of lipodystrophy.

Both gene and cell therapies show great promise as therapeutic approaches to restore adipose tissue functionality for lipodystrophy disorders. However, more basic research will be required along with the establishment of clinical trials to determine if they can provide viable and long-lasting forms of medical intervention.

4. Support for individuals and families affected by lipodystrophy

Lipodystrophy UK is a charitable organisation (Charity: 1175462) dedicated to supporting individuals and families affected by lipodystrophy. Their mission is to improve the lives of those affected by lipodystrophy through information, support, and advocacy.

Lipodystrophy UK hosts a community forum, providing a platform for individuals to connect with others facing similar challenges. Additionally, Lipodystrophy UK offers educational resources to help individuals better understand and manage the condition. One of the key objectives of Lipodystrophy UK is to raise awareness about lipodystrophy. They strive to educate the public, healthcare professionals, and policymakers about the condition. By increasing awareness, they aim to promote understanding and empathy towards individuals with lipodystrophy. For clinicians, Lipodystrophy UK serves as a valuable resource providing up-to-date information, including diagnosis and management guidelines. The charity actively engages with medical professionals to improve understanding and awareness of the condition.

Lipodystrophy UK also actively supports research initiatives aimed at advancing scientific knowledge and improving treatment options for lipodystrophy. They collaborate with researchers, healthcare providers, and pharmaceutical companies to encourage the development of innovative therapies. Through their advocacy work, the charity also seeks to improve access to quality care and treatments for affected individuals. To support their activities and initiatives, Lipodystrophy UK organises fundraising events and campaigns. These efforts help generate funds for research, support services, and advocacy work.

Lipodystrophy UK is a trusted source of support, information, and advocacy for individuals and families affected by lipodystrophy. For more information, visit the Lipodystrophy UK website (<https://lipodystrophyuk.org/>).

5. Conclusions

In summary, lipodystrophy is a rare and life-threatening disorder that poses significant challenges to affected individuals and their families. The limited treatment opportunities have prompted extensive research efforts to explore therapeutic strategies to restore adipose tissue functionality in lipodystrophy. This review has shed light on various approaches, including adipose tissue transplantation, leptin replacement therapy, inhibition of lipolysis, and gene and cell therapies. These advancements hold great potential for reversing the metabolic complications associated with lipodystrophy. However, further funding, research, and collaboration between academics, clinicians, patient advocacy groups, and pharmaceutical companies are all crucial to develop and establish the efficacy and feasibility of novel treatment strategies. By fostering continued interdisciplinary partnerships, we can pave the way for effective and affordable treatment options that can improve the lives of individuals affected by lipodystrophy.

6. Methods

This review of the literature was compiled through prior knowledge of the field and online searches using the database PubMed. Search terms included the phrase “LIPODYSTROPHY” or “LIPOATROPHIC” in combination with “TRANSPLANTATION”, “LEPTIN” or “LIPOLYSIS” as well as “ADIPOSE” combined with “ADENO-ASSOCIATED VIRUS”. Due to the rarity of lipodystrophy disorders, there were no specific inclusion or exclusion criteria. However, the literature cited mainly focused on rodent and human clinical studies, and only English-language manuscripts were included.

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Figure legend

Figure 1: Therapeutic strategies for lipodystrophy disorders. Experimental research performed by Gavrilova and colleagues in 2000 first demonstrated the potential of adipose tissue transplantation to correct metabolic disorders in mouse models of severe lipodystrophy (1). Additional adipose transplantation studies in transgenic mice indicated that leptin replacement therapy could be of therapeutic benefit. Clinical trials have subsequently confirmed that metreleptin is highly effective in individuals with lipodystrophy, and now represents the “gold standard” treatment for this disorder (2). Continued basic research to identify novel therapies for lipodystrophy has identified that inhibition of lipolysis (3) along with gene and cell therapies (4) show great potential to restore adipose tissue functionality. Currently, limited treatment options are available to manage this life-threatening condition.⁴ These include lifestyle modifications including diet and exercise (5), established diabetes medications (6) and the use of bariatric surgery for certain subtypes of

lipodystrophy (7). Currently, none of the treatments available restore adipose tissue development of functionality. Therefore, the development novel, effective and affordable therapeutic strategies are urgently required.

Accepted Article

THERAPEUTIC STRATEGIES FOR LIPODYSTROPHY DISORDERS

PRE-CLINICAL STUDIES



CLINICAL MANAGEMENT

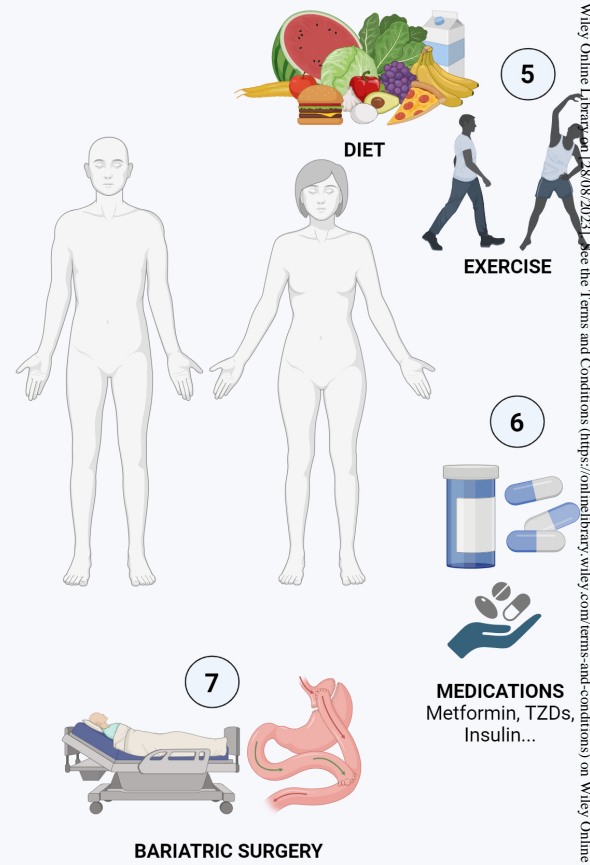


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