

Brown adipose tissue transplantation as a promising approach for insulin-independent reversal of type 1 diabetes: animal studies and clinical perspectives

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Keywords: Type 1 diabetes, T1D, Brown adipose tissue, Transplantation.

ABSTRACT

Type 1 diabetes (T1D) is a serious autoimmune disease characterized by progressive destruction of pancreatic beta cells, leading to lifelong dependence on exogenous insulin. Available therapies focus primarily on insulin replacement, either directly or through transplantation/regeneration of insulin-secreting cells. Despite tremendous advances in recent decades, the major limitations in insulin replacement therapy have not been overcome yet. The ideal therapeutic approach for T1D should produce physiological and long-lasting glucose homeostasis without the need for invasive surgery, immunosuppression or long-term administration of any exogenous agent. A promising approach that meets these requirements is subcutaneous transplantation of embryonic brown adipose tissue (BAT). In mouse models of insulin-dependent diabetes, BAT transplants produce long-term euglycemia independent of insulin and without immunosuppression. Reversal of diabetes is accompanied by decrease of inflammation, recovery of healthy adipose tissue, suppression of glucagon and secretion of beneficial adipokines, with no detectable increase in endogenous insulin production. It appears that a combination of alternate hormones arising from adipose tissue establishes a new physiological equilibrium compensating for the lack of insulin. Existing data point to

a critical role for insulin-like growth factor 1 (IGF-1), both in the early survival of transplants and their continued function. Adult BAT transplants alone cannot correct T1D. However, temporary administration of exogenous IGF-1 enables adult BAT transplants to correct T1D in mice, confirming the importance of this hormone and providing a plausible path for clinical translation of this approach. While the underlying mechanisms are not fully documented, the consistent efficacy and lack of undesirable side effects of BAT transplantation attest to its therapeutic potential.

BACKGROUND

Brown adipose tissue (BAT) is a type of mammalian adipose tissue, located in several specific depots in the body. It is distinct from white adipose tissue (WAT) in morphology, function and developmental origin^{1,2}. Unlike WAT which is principally a fat storage organ, the main functions of BAT are to metabolize fat, generate heat via non-shivering thermogenesis and increase overall metabolism. BAT contains large amounts of mitochondria and uncoupling protein 1 (UCP-1), which are considered the defining morphological markers for BAT. It is also more vascularized and innervated than WAT, and brown adipocytes contain small multilocular lipid droplets as opposed to the large unilocular droplets found in white adipocytes. Newborns have large quantities of BAT, which decrease to a few local depots in adulthood. Adult humans have several distinct BAT de-



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pots within cervical, supraclavicular, paravertebral, mediastinal and para-aortic areas, in addition to diffuse clusters within skeletal muscle tissue. BAT originates from dermatomyotomal precursor cells in common with skeletal muscle, and has an interchangeable developmental relationship with skeletal muscle, whereas WAT originates from mesodermal stem cells^{1,2}. Increased BAT activity negatively correlates with obesity, and is generally associated with improved health¹. Even though WAT can be harmful under certain circumstances such as insulin resistance and metabolic syndrome, situations where BAT and WAT functionally complement each other can produce unique benefits including correction of metabolic disease, as would be described in this review.

Type 1 diabetes (T1D) is a serious autoimmune disease of increasing prevalence, requiring constant glucose management. The major characteristic of T1D is progressive immune-mediated destruction of pancreatic beta cells, resulting in lifelong dependence on exogenous insulin. Traditional treatments largely focus on insulin replacement by direct subcutaneous injection, allotransplantation of insulin-secreting tissue, or beta-cell replacement by other means. Each approach has its own advantages and limitations.

Besides inconvenience, exogenous insulin administration poses the risk of life-threatening hypoglycemic episodes. While transplantation of whole pancreas is generally effective in producing long-term insulin independence, it requires major surgery with a high risk of complications³⁻⁶. Islet transplantation is a safer and less invasive alternative towards insulin-independence. However, long-term success rates are considerably low, and this procedure is further limited by the need for large amounts of donor islets⁷⁻⁹. Additionally, the need for lifelong immunosuppression is a major limitation in transplantation of allogenic tissue or organs.

Potential alternative approaches include microencapsulation of transplanted islets^{10,11}; stimulating regeneration of endogenous beta cells including conversion of other cell types into beta cells by reprogramming¹²⁻¹⁶; and immunomodulation targeting the islet-specific autoimmune response, using cell-based therapies, drugs or nutritional interventions¹⁷⁻²⁴. These techniques, while promising, pose various complications such as inadequate insulin production *in vivo* and potential pathogen transfer associated with encapsulation; neoplastic transformation, low conversion rate of insulin-producing cells, and continued immune-mediated destruction

of newly generated beta cells associated with beta-cell regeneration/reprogramming; and continued deficiency of beta cells and poor resistance to the autoimmune response in the long-term with immunomodulation¹⁰⁻²⁴. Thus, despite much success in animal models, so far there is insufficient data on the applicability of these approaches to human patients.

INSULIN-INDEPENDENT THERAPIES FOR T1D

The ongoing need for insulin remains a major limiting factor in traditional therapies. While insulin is the primary physiological regulator of glucose homeostasis, numerous extra-pancreatic hormones originating mainly from adipose tissue²⁵⁻³¹ and gut³²⁻³⁵ also exert a powerful influence on glucose regulation. Some of these hormones actively decrease blood glucose, either by enhancing insulin secretion and function or in an entirely insulin-independent manner. Such hormones include but are not limited to: glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) from the gut; adiponectin, leptin, and apelin from adipose tissue; and insulin-like growth factor 1 (IGF-1) that is secreted primarily by the liver upon growth hormone (GH) stimulation, although it also originates from a variety of extrahepatic tissues including lung, kidney, heart, skeletal tissue, cartilage and adipose tissue³⁶⁻⁴¹. Metabolic diseases generally correlate with imbalance of one or more of these hormones^{25-27,42-44}. It is also worth outlining the pathophysiologic importance of hyperglucagonemia in T1D. In healthy subjects, glucose-responsive beta cells normally regulate juxtaposed glucagon-secreting alpha cells through paracrine and juxtacrine signaling. Other endocrine cells in the pancreatic islet may also play a role in the regulation of glucagon secretion. In subjects with T1D, disruption of such intercellular control from neighboring endocrine cells leads to glucagon hypersecretion by alpha cells. Therefore, it has been suggested that the resulting uncontrolled hyperglucagonemia may account, at least partly, for exaggerated postprandial hyperglycemia and glucose variability observed in subjects with T1D^{45,46}.

Many of the aforementioned hormones or their analogs have been proposed as therapeutic agents for diabetes. Rodent studies show the potential of some non-insulin therapies in long-term correction of T1D. A supraphysiological dose of leptin or hyperleptinemia induced by adenoviral transfer

can produce dramatic reversal of hyperglycemia and clinical signs of T1D in an insulin-independent manner, principally through suppression of glucagon and increased production of hepatic IGF-1⁴⁷⁻⁴⁹. The benefits of glucagon inhibition in T1D are further confirmed by adjunct therapy with pramlintide, a synthetic analog of human amylin, in subjects with T1D⁵⁰. Blockade of glucagon action by glucagon receptor knockout⁵¹ or antibodies⁵² is shown to mimic the anti-diabetic effect without the undesirable side effects of leptin. Thus, glucagon receptor antagonism is a promising approach for correction of T1D without insulin. Clinical trials on patients with type 2 diabetes (T2D) receiving a small molecule glucagon receptor antagonist show significant improvement of glucose homeostasis⁵³. However, long-term use of glucagon receptor antagonists is limited by perturbations in lipid metabolism and blood pressure^{54,55}, and malignant transformation of alpha cells as reported in previous rodent studies⁵⁶.

Adiponectin and apelin stimulate glucose uptake and utilization in peripheral tissues, by virtue of their insulin-sensitizing effects as well as insulin-independent mechanisms^{25,31,57-63}. Monotherapies with each hormone or their analogs have shown some success alleviating hyperglycemia in T2D⁶⁴⁻⁷⁰. Limitations in these approaches include side effects associated with supraphysiological concentrations, and the lack of data on long term effects on metabolic homeostasis. GLP-1 receptor agonists and dipeptidyl peptidase-4 inhibitors (which act by increasing the half-life of GLP-1 through inhibition of its degradation by dipeptidyl peptidase-4) are currently used for treatment of T2D³²⁻³⁵. However, their efficacy as non-insulin adjunct therapies in T1D is limited^{71,72}, not surprising considering that the perturbation of the incretin effect typical of T2D is not seen in T1D⁷³. Overall, analysis of comprehensive data on the safety and efficacy of commonly used T2D drugs as add-on therapies to insulin in T1D show their efficacy to be modest, with safety concerns limiting long term use⁷⁴⁻⁷⁶.

Therefore, despite many advances in the use of non-insulin agents in the treatment of T2D, leptin is the only insulin-independent agent with consistent efficacy for T1D in rodent models so far⁴⁷⁻⁴⁹. While remarkably effective in rodent models, leptin therapy has significant limitations. For example, its pro-inflammatory properties can accelerate the autoimmune response⁷⁷ and may exacerbate metabolic disease in the long term, as evidenced by

the correlation between metabolic syndrome and leptin/adiponectin ratio⁴⁴. A recent study suggests that the benefits of leptin therapy in T1D are limited to conditions of hypoleptinemia⁷⁸, and may not translate well to human T1D which is not generally a condition of leptin deficiency. T1D patients show elevated leptin levels more often than not⁷⁹⁻⁸¹, reflecting the systemic inflammation associated with this disease. There is little data available on the long-term efficacy of leptin therapy in rodent models of T1D, or its applicability to human patients⁸². Furthermore, monotherapy with any exogenous agent carries the same complications as monotherapy with exogenous insulin. Thus, there is a substantial need for better therapies for T1D.

The ultimate goal in treating T1D is to establish long-term glucose homeostasis. Considering the glucose-lowering effects of many adipokines, and the strong correlation between adipose tissue inflammation and metabolic diseases, improving the health of adipose tissue may automatically correct the defects in T1D. Although it is generally believed that insulin is necessary to maintain adipose tissue health, recent data show that improving adipose tissue function, and subsequently glucose homeostasis, can be achieved without insulin^{83,84}.

INSULIN-INDEPENDENT REVERSAL OF T1D WITH BROWN ADIPOSE TISSUE TRANSPLANTS

The ideal treatment for T1D would produce physiological glucose homeostasis without the need for immunosuppression, invasive surgery or long-term administration of any exogenous agent. A promising approach that meets these requirements is subcutaneous transplantation of embryonic BAT. In multiple mouse models of insulin-dependent diabetes, BAT transplantation has consistently produced long-term euglycemia without the need for insulin or immunosuppression^{83,84}.

Streptozotocin (STZ) treatment causes rapid destruction of beta cells in mice⁸⁵, whereas non-obese diabetic (NOD) mice experience progressive destruction of beta cells through an autoimmune mechanism closely related to human T1D^{86,87}. NOD mice develop spontaneous insulinitis after 12 weeks of age, followed by rapid decrease of plasma insulin levels, severe hyperglycemia, and death within 2-3 months if untreated. BAT transplantation re-establishes normoglycemia and reverses clinical

signs of diabetes in NOD mice and/or STZ-treated mice^{83,84}. Reversal of diabetes occurs with no detectable increase of endogenous insulin, and the progressive decline of plasma insulin characteristic of T1D remains unchanged. BAT transplantation performed under 8 weeks of age also prevents the development of diabetes in NOD mice⁸⁴.

As described in detail in previous publications^{83,84}, the techniques of BAT isolation and transplantation are as follows. For adult BAT: following general anesthesia and aseptic preparation of donor mice, interscapular adipose tissue is exposed through a dorsal midline skin incision. White adipose tissue is gently removed, and the underlying BAT depots are dissected out. The mouse is immediately killed by cervical dislocation while under anesthesia. Freshly isolated BAT is placed in sterile ice-cold Hanks Balanced Salt Solution (HBSS) and transplanted into recipients as quickly as possible. For embryonic BAT: following general anesthesia and aseptic preparation of pregnant female mice, a bilateral subcostal incision is made and extended by a midline transverse incision to expose the abdominal cavity. Uterine horns are exposed one at a time. Starting near the ovary, a longitudinal incision is made along the uterine horn. Embryos are removed and placed in sterile, ice-cold HBSS. The mouse is immediately killed by cervical dislocation while under anesthesia. The embryos are rapidly dissected with Dumont forceps, and the embryonic BAT from the interscapular region is removed, placed in sterile, ice-cold HBSS, and transplanted into recipients as quickly as possible. Transplantation: freshly isolated BAT is transplanted into the subcutaneous space of diabetic or pre-diabetic recipient mice. Following general anesthesia and aseptic preparation, a small (2-5 mm) incision is made in the skin of the dorsal body surface caudal to the endogenous BAT. A subcutaneous pocket is made by blunt dissection using a blunt-ended micro spatula. Donor tissue is introduced into the pocket with Dumont forceps and pushed in with blunt-ended micro spatula. 4-6 units of embryonic BAT or 1-2 units of adult BAT are introduced into each recipient. The incision is closed by gentle pressure with hemostats alone, or with 1-2 simple interrupted sutures as necessary with 5-0 non-absorbable sutures. Mice are allowed to recover with postoperative analgesia and wound care. Blood samples are collected before transplant and at regular intervals post-transplant, to monitor glucose and hormone levels.

The success rate of this procedure, i.e. the percentage of diabetic recipients that achieve euglycemia following BAT transplants, ranges from 50-65%

depending on the recipient strain. The failed cohorts become rapidly hyperglycemic similar to diabetic controls, whereas the successful cohorts consistently achieve euglycemia within 1-2 weeks of transplant placement, and remain euglycemic for varying periods from 6 to 18 months. Euglycemia and reversal of clinical signs of diabetes occur without immunosuppression, while both transplant recipients and diabetic controls show rapidly declining insulin levels. Insulin immunostaining in pancreatic islets is severely deficient in diabetic controls, and undetectable in euglycemic transplant recipients.

The general association of inflammation with diabetes is now well known⁸⁸. In addition to insulinitis, T1D is associated with systemic inflammation as well as WAT inflammation and sometimes loss of WAT⁸⁹⁻⁹³. Successful BAT transplants reverse these effects, leading to progressive decreases in plasma levels of the pro-inflammatory cytokines monocyte chemoattractant protein 1 (MCP-1) and interleukin 6 (IL-6), decrease of insulinitis in pancreatic islets, and robust recovery of healthy WAT characterized by smaller adipocytes, increased vascularization, increased numbers of M2 anti-inflammatory macrophages and decreased expression of the inflammatory markers tumor necrosis factor alpha (TNF-alpha) and IL-6. Within 2-3 months after transplantation, the transplanted tissue blends into the surrounding WAT, loses some of its BAT characteristics such as high UCP-1 expression and multilocular lipid droplets, and becomes undetectable by histology^{83,84}. Thus, reversal of diabetes does not require the survival of transplanted tissue in its original form, instead correlating with the health status of adipose tissue and the presence of insulin-mimetic adipokines. Euglycemia is associated with suppression of glucagon secretion and progressive increases in plasma adiponectin, IGF-1 and sometimes leptin. From these data, it appears that a combination of alternate hormones arising from adipose tissue establishes a new physiological equilibrium compensating for the lack of insulin.

MECHANISMS OF GLUCOSE HOMEOSTASIS FOLLOWING BAT TRANSPLANTS

The most remarkable effect of BAT transplantation is insulin-independent reversal of T1D with rapid and long-lasting euglycemia, accompanied by decrease of systemic inflammation, regeneration

of healthy WAT, suppression of glucagon and increased secretion of beneficial adipokines^{83,84}. This effect is unique to embryonic BAT, not shared by adult adipose tissue transplants or other embryonic tissue types. Many studies in rodent models report the ability of adult BAT and WAT transplants to alleviate obesity and T2D, and to improve metabolism⁹⁴⁻¹⁰⁵. However, they do not produce complete reversal of insulin-dependent diabetes as seen with embryonic BAT transplants. Similarly, transplantation of other embryonic tissue types does not reverse T1D, except for embryonic pancreatic buds under limited circumstances¹⁰⁶. Thus, a combination of characteristics unique to both BAT and embryonic tissue seem necessary for reversal of T1D.

Embryonic tissue is immune privileged, and contains growth factors with anti-inflammatory, regenerative and adipogenic properties. BAT promotes non-shivering thermogenesis and energy expenditure¹⁰⁷, and is characterized, at least in animals, by a high glucose utilization¹⁰⁸. Indeed, ¹⁸F-fluorodeoxyglucose (FDG) positron emission tomography combined with computed tomography (PET/CT) has become the gold standard for the detection of metabolically active BAT in humans¹⁰⁹. Endogenous BAT content is inversely related to body mass index in humans, suggesting the value of BAT activation or induction as a therapeutic approach for obesity¹¹⁰. The benefits of BAT in combating metabolic disease is now widely reported^{1,111}. Transplantation of adult BAT can decrease obesity and insulin resistance while improving glucose regulation in rodent models⁹⁴⁻¹⁰⁰. Activation of endogenous BAT through cold exposure and/or stimulation of beta-3 adrenergic receptor agonists improves resting metabolic rate, insulin sensitivity and glucose metabolism in humans, with modest effects on decreasing fat mass¹¹²⁻¹¹⁹. Another promising approach for the treatment of obesity and related metabolic diseases may be the stimulation of the “WAT browning” process, which consists of the acquisition of BAT features by WAT upon specific stimuli, such as cold exposure, activation of beta-3 adrenoreceptors, irisin, or use of certain drugs, among others¹²⁰⁻¹²⁴.

Unlike with BAT-mediated alleviation of obesity and insulin resistance which result in significant decrease of fat mass^{94-100,117-124}, T1D recipients who achieve euglycemia following BAT transplants show weight gain and an increase of WAT content^{83,84}, suggesting different mechanisms. Our studies consistently show that BAT transplants re-

verse the inflammation of WAT and loss of healthy functional WAT that generally accompany T1D. Thus, it appears that the thermogenic properties of BAT combined with the adipogenic and anti-inflammatory properties of embryonic growth factors may improve the health and function of WAT in diabetic animals, enabling it to secrete beneficial adipokines that compensate for the lack of insulin.

The deficiency of such growth factors is a likely reason for the inability of adult BAT transplants to reverse T1D. Embryonic tissue expresses several growth factors including IGF-1, fibroblast growth factor 21 (FGF21), and adiponectin, all of which have adipogenic and anti-inflammatory properties¹²⁵⁻¹⁴¹. Previous data point to IGF-1 as the most likely candidate influencing the early stages of transplant survival and function^{83,84}. IGF-1 is abundantly expressed in the transplanted embryonic BAT and the regenerated WAT of the recipient, in contrast to little or no expression in the adipose tissue of normal or diabetic controls. IGF-1 levels in plasma rapidly increase following transplant placement and continue to stay up for several months, in negative correlation with the levels of pro-inflammatory cytokines, glucose and glucagon. Interestingly, reversal of diabetes occurs only with donor embryonic BAT from healthy C57BL/6 mice, and not with donor BAT from either NCr nude mice or NOD mice who are consistently deficient of IGF-1. Even with C57BL/6 donors, only 50%-65% of embryonic BAT transplants result in long-lasting euglycemia, whereas the failed cohorts not only lack this increase of IGF-1 but also show a progressive decrease of IGF-1. Based on these data, we hypothesized that the presence of IGF-1 during the early stage is essential for transplant survival and function.

Recent data confirm this hypothesis¹⁴², demonstrating that adult BAT transplants temporarily supplemented with exogenous IGF-1 correct diabetes in NOD mice, in contrast to no effect observed with IGF-1 injections alone or adult BAT transplants alone. The success rate was 57%, with 12 out of 21 diabetic mice achieving long-lasting euglycemia following adult BAT transplants supplemented with 7 daily injections of IGF-1. As previously seen with embryonic BAT transplants, long-term euglycemia occurs only in those recipients who maintain plasma IGF-1 levels at or above 200 ng/ml. These results indicate that IGF-1 is critical for early transplant survival and may have a continued role in graft function.

The best-known function of IGF-1 is to stimulate somatic growth of all cells as part of the GH/IGF axis. IGF-1 is highly expressed in embryonic tissue and is known to suppress inflammation and to stimulate tissue regeneration and adipogenesis^{125-127,134-140}. In addition to its primary effects on growth and development, IGF-1 also exerts several metabolic effects beneficial for glucose regulation. IGF-1 is reported to stimulate insulin-independent glucose uptake into peripheral tissue¹⁴³⁻¹⁴⁸, and the affinity of IGF-1 to the insulin receptor (although low compared to that of insulin) may enable IGF-1 to occupy insulin receptors and promote peripheral glucose uptake^{149,150}. There is strong correlation of plasma levels of IGF-1 with metabolic status in humans, showing significant decreases with insulin resistance, T2D and obesity¹⁵¹⁻¹⁵⁴. T1D is consistently associated with dysregulation of IGF-1, in both children and adults¹⁵⁵⁻¹⁵⁹. Circulating IGF-1 levels tend to go down with onset of T1D, and continue to show a negative correlation with the progression of autoimmune diabetes, while improving in response to insulin therapy. These data show the value of circulating IGF-1 levels as a predictor of metabolic disease, and generally suggest the therapeutic potential of exogenous IGF-1 in correcting the metabolic perturbation in diabetes. IGF-1 monotherapy has been attempted in human patients with insulin resistant diabetes due to genetic defects, where it improved insulin sensitivity and glucose homeostasis¹⁶⁰⁻¹⁶⁴. When used as an adjunct to insulin in T1D patients, recombinant human IGF-1 (rhIGF-1) has produced remarkable improvement in blood glucose and glycated hemoglobin (HbA1c) levels while decreasing the insulin requirement¹⁶⁵⁻¹⁶⁷. However, rhIGF-1 administration does not produce insulin-independence, and as with any exogenous agent, it has been limited by serious adverse effects, such as edema, jaw pain, tachycardia and early worsening of retinopathy^{166,167}.

In NOD mice, temporary administration of exogenous IGF-1 facilitates insulin-independent reversal of diabetes following adult BAT transplants¹⁴². However, long-term euglycemia cannot be achieved with IGF-1 alone, and requires the combined effects of IGF-1, BAT transplant and healthy un-inflamed WAT. Thus, the principal role of IGF-1 at the early stage may be to stimulate the generation of healthy WAT, which in turn creates a new equilibrium. Once the new equilibrium is established, IGF-1 may also be one of the extra-pancreatic hormones that maintain metabolic homeostasis.

Despite the lack of insulin, successful BAT transplant recipients show normal glucose tolerance, near-normal insulin tolerance⁸³, and remarkably improved glucose uptake and metabolism during hyper insulinemic euglycemic clamps when compared with diabetic controls⁸⁴. Mechanisms may include increased insulin sensitivity and insulin-independent glucose transport through the glucose transporters GLUT1 and GLUT3, as well as occupation of the insulin receptor by non-insulin hormones. Pharmacological inhibition of the insulin receptor in euglycemic BAT transplant recipients partially impairs glucose tolerance, suggesting the use of insulin receptors by another hormone⁸³. IGF-1 is a likely candidate due to the structural homology between the receptors for insulin and IGF-1^{149,150}. Adiponectin and FGF21 are reported to mediate insulin-independent glucose uptake^{57-59,168-170}, as does IGF-1¹⁴³⁻¹⁴⁸. Possible mechanisms include AMPK activation and increased expression of the glucose transporters GLUT1 and GLUT3. These hormones are also well-known to have anti-lipolytic activity¹⁷¹⁻¹⁷⁶ and may well contribute to the insulin-independent replenishment of adipose tissue in BAT transplant recipients. The maximum success rate achieved with either embryonic or adult BAT transplants is at or below 65% so far^{83,84,142}. A consistent observation was that euglycemia occurs only when there are simultaneous increases in plasma levels of IGF-1 and adiponectin together with suppression of glucagon. Control animals and failed transplant recipients, either adult or embryonic, lack this particular hormonal profile, emphasizing the importance of alternate hormones compensating for the lack of insulin.

Long-term survival of transplanted tissue in its original form does not seem to be necessary for the metabolic effects of BAT transplants. Other studies on adult BAT transplants report considerable vascularization and innervation of transplanted tissue, which may not always retain its BAT characteristics⁹⁴⁻¹⁰¹. In our hands, transplanted adult BAT was not always identifiable, and was considerably reduced in size when identifiable^{83,84,142}. Transplanted embryonic BAT soon becomes indistinguishable from surrounding host tissue and cannot be detected microscopically. Despite the fact that transplanted embryonic BAT becomes undetectable after 1-2 months, over 50% of recipients remain euglycemic for many months^{83,84}. Thus, factors secreted from BAT transplants seem to exert a lasting influence

on the metabolic regulation of the recipient. This is confirmed by preliminary data, where administration of media conditioned with BAT produced transient euglycemia in diabetic mice.

While many of the underlying mechanisms of insulin-independent glucose regulation are still unclear, existing data confirm the importance of IGF-1 for both the early survival and continued function

of transplants. They further indicate that secreted factors from BAT transplant impart lasting changes to the recipient's system, enabling physiological glucose regulation despite insulin deficiency. Figure 1 illustrates the mechanisms underlying the insulin-independent reversal of diabetes following subcutaneous transplantation of BAT in murine models of T1D.

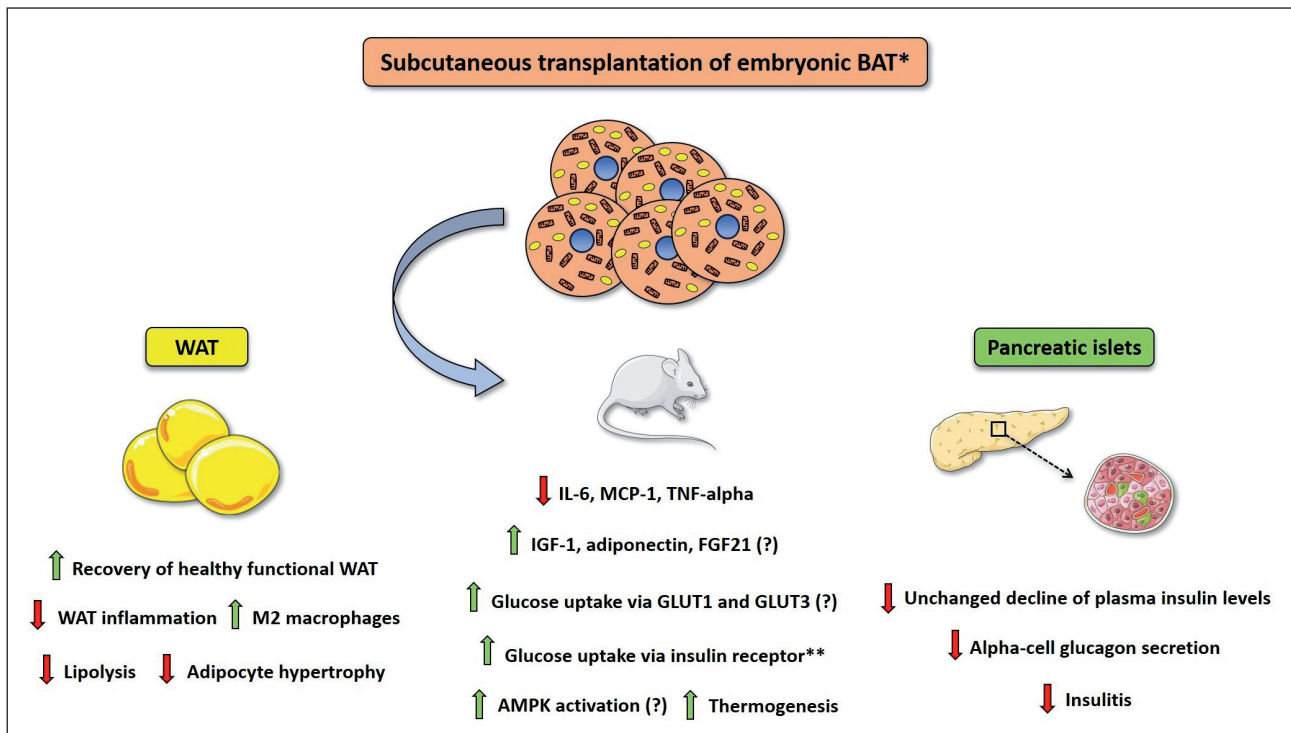


Figure 1. Mechanisms underlying the insulin-independent reversal of diabetes following subcutaneous transplantation of BAT in mouse models of T1D. Subcutaneous BAT transplantation re-establishes normoglycemia and reverses clinical signs of diabetes in NOD mice and streptozotocin-treated mice. Euglycemia and reversal of clinical signs of diabetes occur without the need for insulin therapy and immunosuppression, while plasma insulin levels continue to rapidly decline over time. Detectable improvements in the inflammatory profile include progressive decreases in plasma levels of the pro-inflammatory cytokines MCP-1 and IL-6, decrease of insulinitis in pancreatic islets, and robust recovery of healthy WAT characterized by smaller adipocytes, increased vascularization, increased numbers of M2 anti-inflammatory macrophages and decreased expression of the inflammatory markers TNF-alpha and IL-6. Euglycemia is accompanied by suppression of glucagon secretion and progressive increase in plasma levels of adiponectin and IGF-1. IGF-1, adiponectin and FGF21 are reported to mediate insulin-independent glucose uptake. Possible mechanisms include AMPK activation and increased expression of the glucose transporters GLUT1 and GLUT3. In addition, these hormones exert anti-lipolytic activity, and may well contribute to the insulin-independent replenishment of adipose tissue in BAT transplant recipients. It appears that the thermogenic properties of BAT combined with the adipogenic and anti-inflammatory properties of the aforementioned growth factors and hormones may improve the health and function of WAT in diabetic animals, which compensate for the lack of insulin. *Adult BAT transplants temporarily supplemented with exogenous IGF-1 also promote insulin-independent reversal of diabetes in NOD mice, in contrast to no effect observed with IGF-1 injections alone or adult BAT transplants alone, supporting that the presence of IGF-1 during the early stage is critical for transplant survival and function. **The affinity of IGF-1 to the insulin receptor (although low compared to that of insulin) may enable IGF-1 to occupy insulin receptors and promote peripheral glucose uptake. **Abbreviations:** AMPK, 5' AMP-activated protein kinase; BAT, brown adipose tissue; FGF21, fibroblast growth factor 21; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; IGF-1, insulin-like growth factor 1; IL-6, interleukin 6; MCP-1, monocyte chemoattractant protein 1; NOD mice, non-obese diabetic mice; TNF-alpha, tumor necrosis factor alpha; WAT, white adipose tissue.

CLINICAL APPLICABILITY

While BAT transplantation has been proven effective in long-term reversal of insulin-dependent diabetes in different mouse models, there are several concerns to address before clinical translation. The greatest efficacy was observed with embryonic BAT transplants so far. As embryonic tissue is not a feasible source for human patients, it is important to establish effective and accessible alternatives. Transplantation of healthy adult BAT temporarily supplemented with IGF-1 presents a promising solution. However, availability of donor tissue still remains a concern, considering that BAT is not as easily accessible or replenishable as WAT, and that standard protocols for procurement are not established as with other organs.

Furthermore, approaches proven to correct diabetes in the NOD mouse model and other animal models do not necessarily translate to human patients. A great benefit observed following BAT transplantation in mice is the lack of rejection. While the tolerance of embryonic BAT allografts is attributable to the immune-privileged nature of embryonic tissue, adult BAT allografts were also well-tolerated¹⁴². Literature shows that, unlike with other organs, rejection is not common with adipose tissue transplants in general⁹⁴⁻¹⁰⁵. A likely reason is the presence of adipose-derived mesenchymal stem cells (ADMSCs) that have anti-inflammatory and immunomodulatory properties¹⁷⁷. The presence of ADMSCs in BAT has not been studied in detail, and the developmental origin of BAT is distinct from that of WAT^{2,178-180}. However, while there are some reports of rejection in WAT allografts¹⁸¹, BAT allografts seem to be well tolerated without immunosuppression⁹⁴⁻¹⁰⁰. Hence, BAT may possess unique immunomodulatory properties that make it particularly suitable for transplantation.

Moreover, BAT may also have a role in the setting of insulin-dependent reversal of T1D. A recent study conducted on diabetic red fluorescent mice suggests BAT as a potential desirable site for islet transplantation, allowing for the viability and biological function of the transplanted ectopic islets¹⁸². The grafted islets retained normal architecture, producing long-term euglycemia. Remarkably, islet-engrafted BAT showed normal structure and morphology without significant immunocyte infiltration. In addition, the anatomical location of BAT offers other remarkable advantages, enabling easy biopsy, removal and re-transplantation of pancreatic islets¹⁸².

It is uncertain whether these advantages would translate to human patients. There are no studies to date on BAT transplantation in humans. If rejection becomes an issue, plausible alternatives include autologous transplantation of stem cell-derived BAT or activation of endogenous BAT temporarily supplemented with IGF-1 and/or other growth factors. The presence of BAT in T1D patients was recently demonstrated for the first time¹⁸³, providing hope for such approaches. In particular, Eriksson et al¹⁸³ used ¹⁸F-FDG PET/CT after cold stimulation to investigate the metabolic activity of BAT (measured as glucose utilization rate) in 11 subjects with T1D. Authors detected cold-induced BAT with a wide range in metabolic activity, and no correlation between BAT activity and insulin requirements or markers of metabolic control (HbA1c and fasting glucose levels) was found. Yet, authors found a tendency towards a positive correlation between the metabolic activity of BAT and plasma IGF-1 levels. Although a major limitation of this study was the small sample size, functional BAT was detected in only 4 out of 11 subjects with T1D, with a prevalence of 36%. Moreover, study participants were patients with long-standing T1D (mean disease duration: ~16.5 years)¹⁸³. Therefore, it cannot be excluded that metabolic activity of BAT is blunted in the majority of patients with established T1D, as it has been observed in subjects with obesity and T2D^{184,185}.

CONCLUSIONS

The ideal therapeutic approach for T1D should produce long-lasting euglycemia without the need for invasive surgery, long-term immunosuppression or administration of any exogenous agent. A promising approach that has met these requirements in animal models of T1D is subcutaneous transplantation of embryonic or adult BAT. In fact, both embryonic and adult BAT transplantation (the latter combined with temporary IGF-1 administration) have been proven effective in promoting long-term and insulin-independent reversal of T1D in different mouse models. Once potential problems are addressed (e.g. availability of donor tissue), further studies are needed to establish whether BAT transplantation would be an effective treatment that bypasses the major limitations associated with insulin therapy and current beta-cell replacement strategies in individuals with T1D.

ACKNOWLEDGMENTS:

Figure 1 was created with images adapted from Servier Medical Art licensed under a Creative Commons Attribution 3.0 (<https://smart.servier.com/>).

FUNDING:

No funding is declared for this article.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest to disclose.

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