



Severe Lipoatrophy in a Patient With Type 2 Diabetes in Response to Human Insulin Analogs Glargine and Degludec: Possible Involvement of CD4 T Cell–Mediated Tissue Remodeling

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CASE SUMMARY

- A female patient age 69 years with >10-year history of type 2 diabetes (T2D), on a therapy of premixed aspart insulin, presented with poor glycemic control (HbA_{1c} 8.7%).
- Change of therapy to three injections of short-acting aspart and once-daily glargine improved glycemic control but resulted in severe lipoatrophy at all sites of injection, a rare complication of insulin therapy almost exclusively associated with type 1 diabetes (T1D).
- The patient had C-peptide levels within normal range and lacked autoantibodies against GAD, islet antigen 2 (IA-2), and tissue transglutaminase (tTg), excluding T1D.
- Glargine injection was replaced by degludec, but this did not prevent formation of new indentures.
- Histological analysis of tissue biopsies revealed strong tissue remodeling at affected sites including fibrosis, reduction of adipocyte size, and increased vascularization.
- Immunohistochemical staining showed a strong influx of CD4 T cells in affected sites but no apparent signs of T cell–mediated cell death.
- Flow cytometry of peripheral blood leukocytes did not show an overt effector cell profile of CD4 T cells, indicating that the response was mediated locally.
- Our findings indicate that insulin-induced lipoatrophy in the context of T2D is distinct from that seen in T1D and appears to depend on CD4 T cell–mediated tissue remodeling.

CASE NARRATIVE

Lipoatrophy in response to insulin therapy is a rare condition resulting in a loss of subcutaneous adipose tissue at the site of injection. Since the introduction of humanized insulins, insulin-induced lipoatrophy (IIL) is almost exclusively associated with T1D. IIL was suggested to be an aspect of autoimmunity (1,2), but its underlying pathophysiology is poorly understood. We investigated a unique case of a patient with T2D with severe IIL in response to two different basal insulin analogs. In accordance with national and international guidelines, the patient provided signed informed consent for the acquisition and publication of data regarding her condition before initiation of the study.

Our patient is a 69-year-old Caucasian woman with a >10-year history of T2D. She presented with poor glycemic control (HbA_{1c} of 8.7%) on a regimen of three injections of premixed aspart insulin (NovoMix 30) per day. Her therapy was therefore intensified to a basal-bolus regimen with three applications of short-acting insulin aspart and one daily injection of basal insulin glargine. Glycemic control improved, with less glycovariability during the day and a reduced number of hypoglycemic events, though levels of HbA_{1c} remained high (8.6%). In addition, the patient

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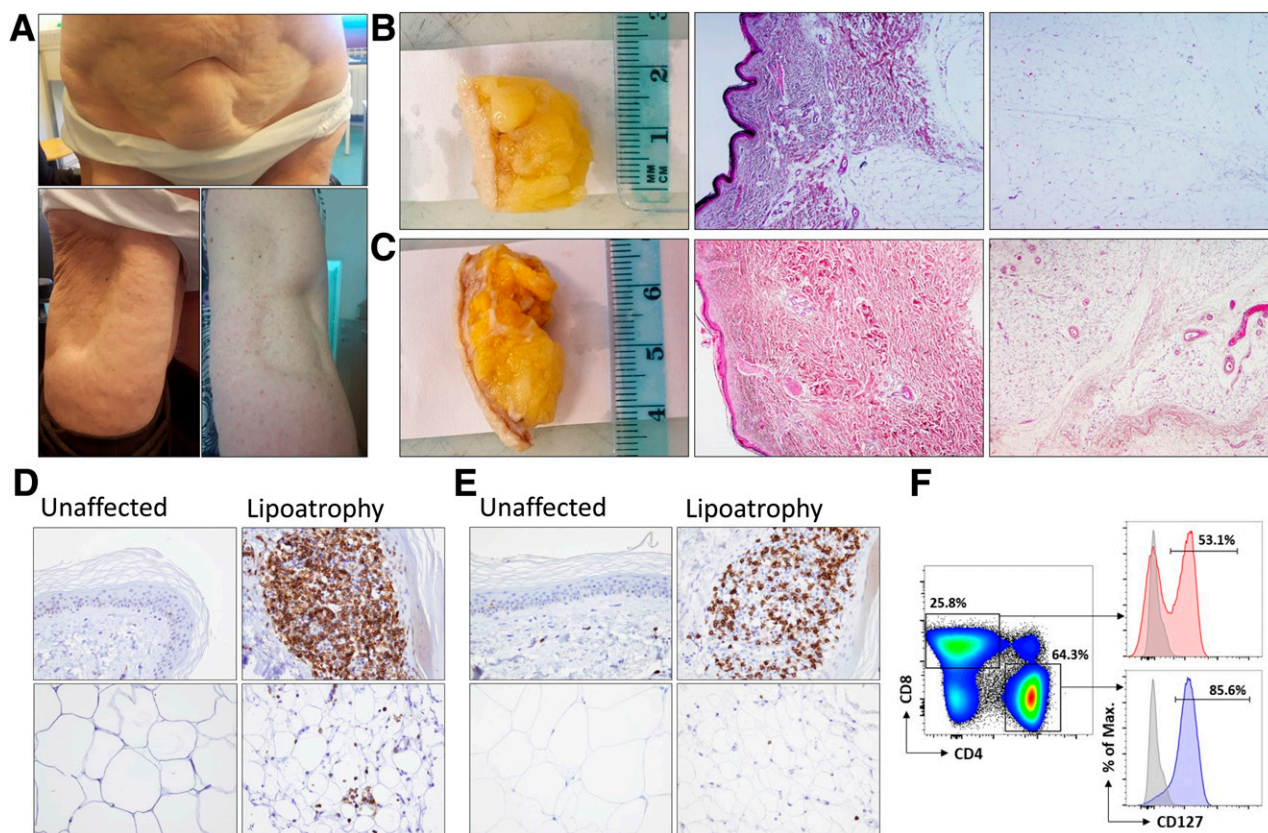


Figure 1—A: Severe lipoatrophy on the lower abdomen, arms, and legs. B and C: Macroscopic (left) and hematoxylin-eosin–stained microscopic images of dermis (middle) and subcutaneous fat (right) from adipose tissue biopsies of an unaffected (B) and affected (C) site. D and E: Immunohistochemical staining for CD4 (D) and CD8 (E). Top row shows dermis at site of injection and bottom row shows subcutaneous fat. F: Flow cytometry plots of blood T cells. Left plot is gated for CD45⁺CD3⁺ cells.

developed severe lipoatrophy at all sites of injection, despite proper rotation of injection sites (Fig. 1A). Atrophic sites were not bruised, red, or painful and did not show acanthosis nigricans. Because the patient did not previously show adverse effects against aspart insulin, glargine was replaced with once-daily injection of insulin degludec. Nevertheless, formation of new indentations continued. Five months after initiation of basal insulin therapy we took a tissue biopsy at an unaffected site and at an atrophic site 30 min after insulin injection. We isolated peripheral blood of the patient for immunophenotyping of leukocytes by flow cytometry.

Serum from the patient contained levels of C-peptide that were within normal range and did not show elevated levels of autoantibodies against GAD, IA-2, or tTG, excluding T1D. Tissue biopsies revealed an expansion of the dermis with collagenized connective tissue and increased vascularization in lipoatrophic areas. Subcutaneous fat was

divided into lobules with thick fibrous bands and showed a strong increase of blood vessels, which had a dilated lumen and were surrounded by an abundance of connective tissue. Adipocytes were greatly reduced in size and irregularly shaped. We also observed a strong increase of infiltrating lymphocytes that did not appear to cluster at specific sites (Fig. 1B and C). Notably, we did not see an increase of granulocytes, tissue destruction, or other overt signs of inflammation.

Immunohistochemical analysis revealed that the majority of infiltrating lymphocytes expressed CD4. These cells were abundantly present both in the dermis and in subcutaneous adipose tissue but did not form clusters, with a notable exception at a site of insulin injection (Fig. 1D). Cells staining positive for CD8 were also increased, though considerably less than CD4 T cells (Fig. 1E). CD56⁺ natural killer (NK) cell numbers were not increased and we did not see signs of T cell–

mediated cell death, as affected tissues showed no positive staining for activated caspase-3 (data not shown).

Analysis of peripheral blood leukocytes by flow cytometry did not show a clear increase in any major immune cell populations, including monocytes, macrophages, NK cells, or CD4 and CD8 T cells. Evaluation of cell surface markers showed that the majority of CD4 T cells expressed CD127, indicating that they had not been recently activated (Fig. 1F). These findings suggest that, in the context of IIL, immune responses in affected adipose tissue cannot be detected by analyzing immune cells in peripheral blood.

In patients with T1D, IIL appears to be an autoimmune response characterized by high levels of autoantibodies and abundant eosinophil infiltration in affected tissues (1,3). Our patient had neither autoantibodies nor myeloid cell infiltrates in affected tissues, suggesting that the pathophysiology of lipoatrophy in the context of T2D is distinct from that in T1D. To our knowledge, we

are the first to report lipotrophy beyond a single injection site following injection with different basal insulin analogs, especially in the context of T2D. Notwithstanding, it does illustrate that no insulin therapy is exempt from side effects. As IIL is generally associated with poor glycemic control (4), its occurrence should not be ignored.

The pathophysiology of IIL in this patient is unclear, but a role for CD4 T cells is suspected. Recently, it was shown that human insulin can fuse with other proteins, thus generating a structure that is immunogenic for CD4 T cells (5), and this may have occurred in our patient. Recruitment of CD4 T cells did not drive inflammation, but rather caused strong tissue remodeling. These observations underscore our recent findings in preclinical models that the activated immune system strongly impacts normal endocrine regulation of insulin-sensitive organs (6,7).

In summary, our article shows that IIL 1) can be a complication of insulin

therapy in patients with T2D, 2) can be induced with the insulin analogs glargine and degludec, and 3) is associated with tissue remodeling, most likely by locally activated CD4 T cells.

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References

1. Lopez X, Castells M, Ricker A, Velazquez EF, Mun E, Goldfine AB. Human insulin analog–induced lipotrophy. *Diabetes Care* 2008;31:442–444
2. Holstein A, Stege H, Kovacs P. Lipotrophy associated with the use of insulin analogues: a new case associated with the use of insulin glargine and review of the literature. *Expert Opin Drug Saf* 2010;9:225–231
3. Reeves WG, Allen BR, Tattersall RB. Insulin-induced lipotrophy: evidence for an immune pathogenesis. *BMJ* 1980;280:1500–1503
4. Tsadik AG, Atey TM, Nedi T, Fantahun B, Feyissa M. Effect of insulin-induced lipodystrophy on glycemic control among children and adolescents with diabetes in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. *J Diabetes Res* 2018;2018:4910962
5. DeLong T, Wiles TA, Baker RL, et al. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. *Science* 2016;351:711–714
6. Šestan M, Marinović S, Kavazović I, et al. Virus-induced interferon- γ causes insulin resistance in skeletal muscle and derails glycemic control in obesity. *Immunity* 2018;49:164–177.e6
7. Wensveen FM, Jelenčić V, Valentić S, et al. NK cells link obesity-induced adipose stress to inflammation and insulin resistance. *Nat Immunol* 2015;16:376–385