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Acronyms:
- ISTAT: (Istituto Nazionale di Statistica) National Institute of Statistics
- AMD: Associazione Medici Diabetologi) Association of Italian Diabetologists
- OSDI: (Operatori Sanitari di Diabetologia Italiani) Italian Healthcare Professionals on Diabetes
- MD: medical doctor
- Prof: Professor
- HN: Head Nurse
- APSS: Provincial Health Services
- ASL: Local Health Services
- LD: Lipodystrophy
- LA: lipoatrophy
- LH: lipohypertrophy
- SSN: National health system

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INTRODUCTION

Local skin injection-related side effects have been well-known complications of injection therapy for a long time but are still a neglected chapter in the field of therapy.

They are have been mostly related to insulin treatment but sporadic reports have recently appeared in the literature on skin alterations due to other hypoglycaemic injecting drugs as well.

In addition to droplet leakage after insulin shots (dripping), a number of local skin reactions have been described within injection sites, including pain, burning, itching, local inflammation, bleeding, bruising, hardening, loss of sensation and lipodystrophy.

Cutaneous lipodystrophy is by far the most frequent local complication of needle injections, and the most widely described in the literature as well. Nevertheless, despite its heavy impact upon metabolic control, it continues to occur in a high percentage of insulin-treated subjects. This fact is evidence per se of a lack of both efficient health personnel training and patient education at the start of insulin treatment and/or during follow-up.

In this chapter we will try to address as many as possible clinical aspects related to local complications of injection therapy and their consequences for patients, caregivers and the healthcare system, and to suggest some methods to prevent and / or treat them.

LIPODYSTROPHY

Definition

Lipodystrophy (LD), a disorder of the adipose tissue, is one of the most common complications of subcutaneous insulin injections and may present as either lipohypertrophy (LH) or lipoatrophy (LA). The latter is defined as a large, often deep, retracted scar on the skin that results from a serious damage to subcutaneous fatty tissue [1]. Several features suggest an immunological etiology for LA [2]: (1) it is more frequent in patients with type 1 diabetes, and mostly affects women - who often have other signs of autoimmunity; (2) it is often characterized by the presence of mast cells and eosinophils in biopsy specimens and may be responsive to topical 4% cromolyn sodium preparations (an inhibitor of mast cells); (3) it seems to be the result of a lipolytic reaction to impurities or other components in some insulin preparations, as its prevalence has dropped to only 1-2% with increasing purified insulin utilization [3,4].

LH in turn is a thickened ‘rubbery’, mostly firm tissue swelling which, especially when occasionally soft, is easily missed during a standard medical examination. Although the exact etiology of LH is unclear, several local factors appear to be at play, including both the insulin molecule per se - with its strong growth-promoting properties - and the repeated trauma caused by poor injection habits, such as infrequent/missed injection site rotation and/or frequent needle reuse [5]. However, a large body of evidence also lends support to a significant association between LH and many other factors, including female sex, low socioeconomic level, high body
mass index, as well as long disease duration and/or longstanding insulin treatment [1]. LH lesions are generally correctly identified whenever physical examination is accurate, although in various published series identification steps followed by the authors were not fully documented [6-8].

LH can present under various forms which may let them elude any fast, careless examination as, despite its habitual hard-elastic texture, it can be either small or large, either flat or protruding (Figures 1-6). Thus a lot of experience and a systematic approach are needed to identify it by periodic inspection, palpation and pinching, as documented by various studies [9,10].

LH may result from the incorrect use of pen or syringe needles and of insulin pump infusion sets as well (Figure 6). The mechanism behind is always the same [1], i.e. an educational defect mostly attributable to diabetes care teams. The latter in fact tend to underestimate patients’ inability to retain specific educational messages for a long time even after successfully completing a dedicated training course [10]. Papers published on this topic so far show great differences in its prevalence in insulin treated patients (see Table 1). This is probably due to the lack of a well-structured diagnostic flow-chart despite the world-wide availability of suitable ultrasound and radiological methods [1,5,7,8,11-18].

Table 1: Lipohyperthrophy prevalence variability among different case studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Publication Year</th>
<th>Prevalence (%)</th>
<th>Reference #</th>
<th>DM Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hauner</td>
<td>1996</td>
<td>28.7</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Partanen</td>
<td>2000</td>
<td>34.5</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Raile</td>
<td>2001</td>
<td>27.1</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Kordonouri</td>
<td>2002</td>
<td>48.0</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Seyoum</td>
<td>1996</td>
<td>31.0</td>
<td>7</td>
<td>1+2</td>
</tr>
<tr>
<td>Vardar</td>
<td>2007</td>
<td>48.8</td>
<td>14</td>
<td>1+2</td>
</tr>
<tr>
<td>Hajheydari</td>
<td>2011</td>
<td>14.5</td>
<td>15</td>
<td>1+2</td>
</tr>
<tr>
<td>Teft</td>
<td>2002</td>
<td>57.0</td>
<td>16</td>
<td>1+2</td>
</tr>
<tr>
<td>Blanco</td>
<td>2013</td>
<td>64.0</td>
<td>1</td>
<td>1+2</td>
</tr>
<tr>
<td>Grassi</td>
<td>2014</td>
<td>49.0</td>
<td>17</td>
<td>1+2</td>
</tr>
<tr>
<td>Mcnally</td>
<td>1988</td>
<td>28.0</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Hauner</td>
<td>1996</td>
<td>3.6</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Al Ajlouni</td>
<td>2015</td>
<td>37.3</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

An ever increasing number of reports in the literature points to poor insulin administration habits, indeed, as the main cause of LH lesions and of their metabolic consequences, including wide blood glucose variability and even severe unexplained hypoglycaemic episodes.
Many papers in fact provide recommendations on how to properly inject insulin [1,19]. Despite this, once again, the extremely wide variation in LH rate reported so far in insulin-treated patients proves that no systematic educational programs have been fully and repeatedly implemented worldwide to have people with diabetes correctly inject insulin [2,17]. It reflects the fact that the literature is still lacking a clear, explicit and standardized methodology describing how to recognize and diagnose LH lesions. Quite recently we were able to show for the first time that a specific training involving repeated and well codified maneuvers let inexperienced health professionals (HPs) easily acquire diagnostic accuracy in identifying LH lesions independently of site, size, shape, and even BMI [9]. This kind of training also granted a 97% consistency rate among HPs as compared to the gold standard represented by skin ultrasonography (USS), while the lack of training was associated with a wide variability and inconsistency of identification results. Therefore the extremely wide variability in LH frequency reported by the literature might really depend on the lack of a clear definition of suitable procedures for lesion identification per se [7,8].

**Figure 1:** Schematic representation and morphological features and the identification features of four major types of lipodystrophy.

<table>
<thead>
<tr>
<th>Type</th>
<th>Definition</th>
<th>Visibility</th>
<th>Palpable</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Small nodule</td>
<td>easily seen silhouette / better with tangential light</td>
<td>easily</td>
<td>harsh-elastic</td>
</tr>
<tr>
<td>B</td>
<td>Large nodule</td>
<td>clearly visible silhouette / better with tangential light</td>
<td>easily</td>
<td>harsh-elastic</td>
</tr>
<tr>
<td>C</td>
<td>Flat plastron</td>
<td>hardly visible</td>
<td>not easily palpable / better by pinching</td>
<td>usually elastic</td>
</tr>
<tr>
<td>D</td>
<td>Flat nodule</td>
<td>not visible</td>
<td>difficult to identity, easier by deep palpation or pinching</td>
<td>usually elastic</td>
</tr>
</tbody>
</table>
Figure 2: Unusual sites of LH. Panels a) and b) distal third of the arm; c) upper thigh; d) both thighs; e) proximal forearm.

Figure 3: Clear effect of close shots within a small skin area (enlarged detail at the top right).
**Figure 4:** Moderate swelling of the abdominal wall below the umbilicus, preferred place for insulin injections (panel a); right hand fingers pinch a thick fold in the presence of large LH plates while only a thin skin fold results from the left hand squeezing the area systematically spared by the patient for insulin shots (panel b).

**Figure 5:** An example of pinching on (left panel) and externally to(right panel) a lipodystrophic oval nodule.
Figure 6: LH areas in insulin pump users.

Ultrasound Appearance

Despite their fully accepted role in the diagnosis of LH, ultrasound scans (USS) are too costly to be proposed for routine clinical examinations [20-22], still it’s feasible to use them for methodological studies or for individual cases [9,10].

The ultrasound appearance of LH lesions is that of a localized thickening of subcutaneous adipose tissue where a nodule may be detectable by palpation. In their context fibrous “striae” are often found. In flat, plastron-like lesions a more or less uniform thick tissue is observed while thickness progressively decreases at the periphery of protruding (rounded or oval) lesions.

Figure 7: Ultrasound scans of an abdominal LH nodule. Fibrous striae are easily seen within the subcutaneous adipose tissue in all three figure panels. In the first and third ones, however nodule borders are linear and sharp while in the second panel rounded borders are seen (narrower peripherally than in the center).
Histopathology

The information available on the histopathology of lipohypertrophic nodules are primarily derived from a case described by a Japanese group, in which two large abdominal lesions were surgically removed [23].

Surgical excision was performed for cosmetic purposes under local anesthesia. The en bloc-resected surgical specimen was 24.2 × 9.9 × 3.0 cm, and weighed 405 g. The lesions were composed entirely of a yellowish material, were not encapsulated by fibrous tissues and displayed decreased vascularity, with consequent delayed insulin absorption [24].

The gross findings were compatible with benign LH. Light and electron microscopy examinations were carried out on the LH area and the surrounding normal adipose tissue. Hematoxylin-eosin (HE) staining showed an excess of mature adipocytes within the dermal reticular layer of sections from LH lesions.

Adipocytes in the LH area were significantly larger (diameters ranging 100 to 200 μm) than those of the normal surrounding area (diameters ranging 70 to 90 μm). Approximately 10% of LH adipocytes contained numerous lipid droplets at their periphery, showing stimulated lipogenesis. Some heterogeneity in the size of adipocytes was observed in the lipohypertrophic area. A few adipocytes though, surrounded by hypertrophic ones, were as small as 20 μm in diameter.

Insulin-induced LH is generally thought to be the result of insulin lipogenic effects. The enlarged adipocytes are impressive but the large volume of lipohypertrophic mass is only partially explained by the degree of cell hypertrophy. In human obesity, when body weight exceeds 170% of ideal, a maximum adipocyte size twice as normal is achieved while hyperplasia becomes increasingly relevant with greater disease severity [25]. It is well known that insulin stimulates proliferation and differentiation of pre adipocytes [26,27]. A possible asymmetric cell division pattern of mature human adipocytes has also been reported [28,29]. Small adipocytes seen in the lipohypertrophic area would then represent newly formed adipocytes caught during their maturation phase and originated by either differentiation or proliferation. Taken together, lipohypertrophy is therefore assumed to be the result of maximum adipocyte hypertrophic changes accompanied by insulin-stimulated hyperplasia.
Figure 8: Hypertrophic adipocytes. Scanning electron microscopy (SEM) of the insulin-induced lipohypertrophic area (higher left panel) and adjacent normal subcutaneous adipose tissue (higher right panel) at 100 x magnifications). Lower panels show SEM images of exposed lipid droplets underneath adipocyte plasma membrane (modified from [5]).

Figure 9: Heterogeneous adipocyte sizes in a lipohypertrophic area (magnification: x 200). Arrows indicate small adipocytes. Fibrous striae appear on the left (modified from [5]).
Figure 10: In the left panel a great variability of blood sugar levels is displayed as occurring in a person with T1DM injecting insulin into LH nodules. In the right panel significantly lower blood glucose fluctuations are seen in the same subject injecting insulin into healthy skin areas.

In the first case the patient used 125 insulin IU per day, getting down to 96 UI per day in the second case instead.

**CLINICAL CONSEQUENCES OF LIPODYSTROPHIES**

A missed diagnosis of LD may have major clinical consequences. The injection of insulin into parts of the body affected by LD may cause wide glycaemic oscillations, including inappropriately high glucose levels and a high rate of unexplained hypoglycaemic episodes, both of which are scarcely responsive even large changes in insulin dose [1,30]. Programs aimed specifically at educating patients with LD on proper injection techniques has proven to be effective in significantly reducing glucose oscillations [31].

First of all, though, despite LH and LA being sometimes improperly referred to as synonyms [5], the two concepts would better be kept separate. Most studies suggest that insulin absorption from areas affected by LH may be both delayed and erratic, leading to the need for ever increasing doses of insulin and worsening metabolic control [32-36]. This in turn causes unacceptable glucose oscillations due to a high rate of serious hypoglycaemic episodes followed by rebound glucose spikes whenever patients suddenly switch from affected injection sites to normal ones. Under these conditions, the economic burden of the disease increases for both patients and the healthcare system. Therefore, it is crucial that as many LH areas affected as possible are systematically identified in order to teach patients how and why to adhere to best insulin injection practice. The reported prevalence of LH in patients receiving insulin injections varies widely in published studies [9], possibly due to the lack of a well-structured diagnostic flow-chart despite the world-wide availability of suitable ultrasound and radiological methods [1,20-22,27-39]. We recently published a methodological paper on a palpation technique that enables the clinician to identify skin lipohypertrophic lesions in diabetic patients receiving insulin [9,10]. We therefore suggest medical institutions to educate diabetes teams to systematically follow our simple LH
diagnostic procedure at all insulin injection sites and then implement and hopefully progressively refine this procedure in large-scale studies. In particular, unexplained variations in glucose levels and/or unexplained hypoglycaemic episodes may alert healthcare providers to look for LH in diabetic patients receiving insulin injections.

Implementing proper injection techniques and avoiding to inject insulin into LH nodules produces beneficial effects such as: i) a significant reduction in hypoglycaemic episodes; ii) a marked improvement in glycaemic variability (Figure 10), (iii) a 25-30% decrease in insulin requirement [17,40-41], and iv) a rapid improvement in glycaemic control [1,17,19].

![Figure 11: Needle length and risk of intramuscular injections [42].](image)

In fact major causes known to precipitate hypoglycaemic events are dosage errors, inadvertent insulin preparation mix-up, imbalance between ingested food and insulin bolus dose, alcohol abuse, vomiting, diarrhea, etc. Still, needle length and subcutaneous adipose tissue thickness (which differs from one area of the body to another) are the main factors responsible for a large number of unexplained hypoglycaemic reactions. These concepts are extensively discussed in the literature [42-46], still long needles are still used and in case insulin is injected into lipoatrophic areas hypoglycaemia is predicted for sure while in case insulin is injected into LH nodules hypoglycaemiamight be expected to occur rather frequently.

The theoretical risk of injecting insulin into muscle depending on the length of the needle was calculated in a medium body size non-obese subject and was estimated as very low even with medium sized needles (figure 11), still1 hypo out of 5 is possibly linked to IM injection [47].
CAN’T WE REUSE NEEDLES AT ALL?

The title of this section may look strange and provocative after clearly stating that a major cause of lipodystrophy is needle reuse.

Most people with diabetes reuse needles in fact, mainly for economic reasons and personal convenience. Currently, however, best practice guidelines for insulin injection recommend using a fresh needle for each injection [48-50].

Actually, new disposable devices have generated a lively debate among health providers regarding cost, appropriateness, and accuracy. One of the most controversial issues was needle reuse [51-54], arguing a possible relationship with numerous health risks, including infection, cutaneous lipodystrophy, more painful punctures and loss of accuracy in insulin dose administration [55-58]. This problem now comes from a recently published meta-analysis [59] raising several doubts on that. It is therefore necessary to analyze this issue and to point out in greater detail that the understandable desire to spare money by reusing needles can not lead us to ignore the strong risk of developing LH nodules and the deriving long-term socio-economic burden in terms of deteriorated metabolic control, acute and chronic complications, and overall treatment cost increase.

Data collected in the aforementioned meta-analysis underline that reusing needles was not associated with local infection, whereas the second most studied side effect analysed in 8 out of its 25 selected studies was LH; however, contributing studies were cross-sectional and showed inconsistent results themselves. Only one study [14] included multivariate analysis. The meta-analysis carried out with five of these studies showed a statistically significant association between the presence of LH and needle reuse; however, these studies were highly heterogeneous, making the available evidence inconclusive.

Three cross-sectional studies and three trials assessed the relationship between reusing needles and pain associated with the injection. Only one of these studies [60] showed a statistically significant association between increased pain perception and needle reuse.

Table 2: Theoretical risk of intramuscular injection according to needle length [47].

<table>
<thead>
<tr>
<th>Needle length (mm)</th>
<th>IM injections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.7</td>
<td>45.3</td>
</tr>
<tr>
<td>8.0</td>
<td>15.1</td>
</tr>
<tr>
<td>6.0</td>
<td>6.2</td>
</tr>
<tr>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td>4.0</td>
<td>0.4</td>
</tr>
</tbody>
</table>
Blood glucose control was the least evaluated result. Only three cross-sectional studies evaluated the effect of reusing needles and glycaemia; they found no association.

Given the lack of solid scientific evidence, some experts express no objections to individual patients reusing their own needles within a limited time period [61]; however, most best-practice guidelines recommend against any reuse because of eventually occurring adverse effects [50,62,63]. Nonetheless, many of these adverse effects can be explained by other factors, such as the duration of insulin therapy, type of insulin, characteristics of the needles, used injection technique, or inappropriate rotation of injection sites [5,8,64].

Some criticisms on the meta-analysis arise by the authors themselves however, because: (i) selected studies have a high risk of bias; (ii) the methodological quality of 23 studies was considered “poor”; (iii) only two cross-sectional studies were rated as “fair” and none were classified as having “good” methodological quality and, in addition, most of the studies included fewer than 100 participants; and, what perhaps is even more relevant, (iv) many studies were outdated being performed in the 80s, 90s and in the early 2000s, using old technologies and limited scientific information.

Conversely, more recent data from other authors and ourselves underline the close correlation between repeated needle use (plus other known factors) and lipodystrophy [1,2,9,10,17,65,66].

In developing countries with limited economic resources it is understandable today to save on the consumption of needles (as stated in the NICE Guideline 2015) [67], still we wonder what will be the impact of such choice on national health systems tomorrow. There will certainly be a heavier burden, carried along by long-duration LH-related unsatisfactory glycaemic control and the consequent much higher rate of acute (e.g. hypoglycaemia) and chronic complications (e.g. increased number of hospitalized people suffering from amputation, blindness, dialysis, etc.). In addition, the economic burden is bound to increase further per se because of the world diabetes epidemic.
Figure 12: Ultrasound scan of a LH nodule (red areas in the template to the right). Note the different thickness between central panels (panels b and, slightly less, c) and peripheral ones (a and d). Fibrosis is present in all scanned areas.

This patient did not rotate injection sites, his HbA1c was 11.5%, daily insulin dose was 86UI, average daily glycaemic excursions were 270mg/dL, with about 12 hypoglycaemic events per month. After 6 months of proper rotation he suffered from no hypoglycaemic events any more, his LH nodule got significantly smaller, his HbA1c went down to 7.9%, daily insulin dose decreased to 70 UI and glycaemic excursions averaged down to 168mg/dL.

**UNEXPLAINED HYPOGLYCAEMIA AND LARGE GLYCAEMIC VARIABILITY: SKIN LIPOHYPTERTROPHY AS A PREDICTIVE SIGN**

In a series of 387 insulin-treated diabetic subjects (n. 81 Type 1) we recently showed lipohypertrophy to be a possible marker of increased risk for unjustified hypoglycaemic events (i.e. those occurring independent of any common iatrogenic factors or patient errors), as well as, for unexplained cases of high glycaemic variability [66].

Hypoglycaemia is an acute complication known to increase diabetes morbidity and mortality. It is also responsible for a heavy - still not fully defined - economic burden. However it is by far the strongest limiting factor in terms of tight glycaemic control in insulin-treated patients [68,69]. In people with T1DM predictable triggers explain many hypoglycaemic events (Hypos) [70,71], including skipped meals, strenuous exercise, high insulin doses, chronic renal or liver disease, as well as defective glucose counter-regulation and established hypoglycaemia-associated autonomic failure [72-74].

Nevertheless, apparently inexplicable hypoglycaemic episodes may be related to incorrect insulin injection techniques including the use of long needles - eventually allowing insulin injection into the subcutaneous muscle tissue – or patients’ incorrect habit to inject insulin into LH nodules [32,33,74].

In the series of patients in the Hypos-1 study [75,76] a high rate of severe Hypos, non severe Hypos and glycaemic variability (GV) was reported (28%, 70% and 56%, respectively). Accordingly, our data documented an overall 62% Hypos rate, 16.8% in fact being inexplicable and showing a strong correlation with the presence of LH, as well as, with all known associated factors.
The profile of people with diabetes displaying inexplicable Hypos / GV included the presence of LH nodules, as well as, poor metabolic control, high micro-/macrovascular complication rates, longer/larger needles, missed injection site rotation, low socio-economic level and solitary life. The risk for LH to be deemed as responsible for unexplained Hypos or GV rose to 4.38 in the case of simultaneously occurring above mentioned factors and LH.

The presence of LH was able to predict about 77.1% of unexplained Hypos / GV itself.

Indeed, factors known to be associated with LH were also present at a high rate in subjects with such phenomena. However, the remaining 22.9% people without any LH nodules experienced unexplained Hypos / GV too. It might depend on the fact that identifying subtle skin lesions is very difficult so that “no identified LH” cannot be considered as equivalent to “absence of any LH”. In fact we feel like stressing what was already pointed out by other authors [1,47,77]: education on injection techniques is still inadequate and diabetes specialists and dedicated nurses should make their best to prevent patients from causing themselves any skin damage through wrong habits.

Indeed, in the presence of unexplained Hypos / GV, injection sites should be systematically and thoroughly explored in search of LH areas. Furthermore, appropriate educational activities should be implemented to improve patients’ behaviour, including periodical investigation on their injection habits.

A recent Meta-Analysis of Population Based Studies [78] including 46 publications (n = 532,542) found Hypos to occur quite frequently among people on insulin: mild / moderate Hypos were in fact about 50% with an incidence of 23 episodes per person-year while severe episodes were about 21% with an incidence of 1 event per person-year.

Table 3: Multivariate analysis of subjects’ characteristics potentially associated to lipohypertrophy. All differences were statistically significant (p<0.001).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.91 (0.76-1.10)</td>
<td>0.338</td>
</tr>
<tr>
<td>Type 1 diabetes (%)</td>
<td>0.84 (0.39-1.80)</td>
<td>0.656</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>2.00 (1.85-2.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DM duration (years)</td>
<td>1.07 (0.80-1.44)</td>
<td>0.627</td>
</tr>
<tr>
<td>Insulin dose (IU/day)</td>
<td>2.88 (2.56-2.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypos / GV</td>
<td>4.85 (4.02-5.30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes complications</td>
<td>2.99 (2.34-3.51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Living status</td>
<td>3.15 (2.89-3.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Marital status</td>
<td>3.22 (2.97-3.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pen needle length (mm)</td>
<td>2.98 (2.56-2.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gauge (G)</td>
<td>3.56 (2.60-3.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Needle reuse</td>
<td>3.78 (3.06-4.12)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Failure to rotate injection sites</td>
<td>4.22 (3.78-4.86)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Figure 13: Complication rates in subjects with (dark green bars) or without (light green bars) Hypos / GV; 57% had more than one complication and 32% more than two complications. All differences were statistically significant (p<0.001) except for PA.CV=Cardio-CerebroVascular complications; R=Retinopathy; Neph=Nephropathy;

PA=Peripheral Artery Disease; Per Neuro=Peripheral Neuropathy; Aut Neuro= Autonomic N.
Figure 14: Relationship between needle length and lipo hypertrophy. * p < 0.001.

Figure 15: Relationship between needle gauge and lipo hypertrophy. * p < 0.001.
Should we ideally select people with unexplained Hypos from the extremely large number of patients mentioned above, we would eventually deal with an impressive cohort: in that case the systematic utilization of a suitable LH identification protocol followed by patient rehabilitation would be expected to dramatically reduce the prevalence, incidence and health consequences of Hypos, as well as, the costs related to such frightening, yet neglected, complication of insulin treatment.

Further studies are still warranted to assess whether educational activities like those reported in our study may result into a decreased burden of diabetes in terms of unexplained Hypos.

**TREATMENT**

The web hosts plenty of proposals of creams, massages, topic cortisone derivatives, as well as, cosmetic surgical interventions against most evident lipo-hypertrophic nodules. All these remedies are not supported by scientific evidence and therefore are not supposed to be used. Avoiding to inject insulin into LH or LA areas is generally enough: after adopting such tactic for several months, in fact, skin gets back to its normal consistency and shape [1]. The time needed varies on an individual basis and according to nodule’s size. Surgery is indicated only in rare cases, as those, for instance, characterized by very large, non-responsive, disfiguring nodules [23].

**OTHER MINOR SIDE EFFECTS ASSOCIATED WITH SUBCUTANEOUS INJECTIONS**

Another original aspect of insulin injection-related skin lesions is bruising at the level of the injection site, as shown in Figure 1. Bruising is mentioned in several published studies on insulin injections [47,79-81]. It is a really problematic, mostly unsolved insulin injection side-effect disturbing people with diabetes due to the resulting blemishes. Unfortunately, in terms of both patient and healthcare provider perspectives, injection-related lesions negatively affect the overall number of shots diabetic patients are willing to take [81]. In fact, half patients in some studies reported mentioning injection-related problems to their healthcare providers without getting any good advice from them against the associated pain and bruising [77,81]. In a series of 780 insulin-treated adults with type 2 (n = 556) or type 1 (n = 223) diabetes mellitus on a multiple daily injection regimen (4 shots/day), we found that 46.2% had LH areas, with a mean lesion diameter of 4.8 ±3.1 cm, against a low LA rate (3.2%) (unpublished data). In the same series, 33.2% patients showed bruising, either associated with LH (n = 178, 53.9%) or isolated (n = 156, 46.6%), independent of the use of antiplatelet and/or anticlotting agents [65].

Possible explanations for bruising may be how deep and how long a pen has been pressed onto the skin at the time of injection, as well as, the choice to repeatedly choose the same area for the injection instead of rotating sites but systematic studies are still warranted to clarify this issue.
It has to be realized that injection site-related adverse events, such as pain, redness, bruising, and bleeding, are significant barriers to patient adherence to treatment regimens involving multiple daily injections. This is especially important when physicians and/or healthcare providers are not sufficiently experienced or possess insufficient knowledge or when doctor-patient relationship is unsatisfactory [77]. Such conditions in fact occur rather frequently as these phenomena are subtle and there is no scientific literature rigorously and systematically addressing “minor” injection complications.

To fill this gap, during the last few years an interesting exchange of experiences has developed among patients through various networks, beginning with the American Diabetes Association Community [82]. Such forums have enabled diabetic patients themselves to propose several interesting solutions, including a sufficiently long injection time, very thin and short needles, and a careful injection site rotation technique. However, specific investigations are still warranted to assess reasons behind such complications and to identify scientifically sound solutions expected to eventually improve patient adherence to insulin therapy.
CONFOUNDING FACTORS: OTHER, GENETIC OR ACQUIRED LIPODYSTROPHIES, INCLUDING THOSE ASSOCIATED TO HUMAN IMMUNODEFICIENCY VIRUS

After taking into consideration all above mentioned concepts, LD has to be interpreted as part of a group of different clinical pictures associated with injection-related skin lesions rather than as a single complication of insulin treatment. In fact LDs are heterogeneous, genetic, or acquired disorders of skin fat. It is well known that exogenous proteins may induce local inflammatory reactions and that, when injected, different medications may cause local adverse events.

A typical expression of the latter concept is the most prevalent subtype of non-insulin treatment-related LDs occurring in human immunodeficiency virus-infected patients on long-lasting protease inhibitor-containing, highly active antiretroviral therapy. This type of LD likely results from lipid and/or glucose metabolic disorders, with the latter ranging from fasting hyperglycaemia to insulin resistance/hyperinsulinemia [32,33].

**Pegvisomant**

Another reversible LD reported to date is LH occurring after pegvisomant injections in patients with acromegaly. Several cases have been published [34] and, interestingly enough, this kind of LH was reported to regress in all patients discontinuing medication or performing injection site rotation regularly according to a structured educational program [35].
Exenatide Long-Acting Once-Weekly Formulation

Palpable, yet often invisible subcutaneous nodules are also reported to be a frequent consequence of subcutaneous shots of the long-acting, once-weekly formulation of exenatide (EQW), a glucagon-like peptide-1 receptor agonist (GLP-1RA) [36]. This adverse event was reported in registration studies, together with other injection site-related adverse events [37], among which itching was the most common, although its rate appeared to wane over time from 11.0% between weeks 4 and 6 to 4.6% between weeks 28 and 30 [38]. An informal communication from the DURATION-1 study staff indicated that nodules were generally 0.5–0.75 cm in diameter, and their incidence seemed to decline over time and slowly vanished, even in patients with several earlier similar episodes [37-39]. As EQW uses a PLG [poly (D,L-lactide-co-glycolide)] microsphere technology, LH lesions were thought to be the result of a typical foreign body local reaction [39], implying the migration of polymorphonuclear leukocytes, monocytes/macrophages, and lymphocytes [40].

Despite these reports, until recently no published studies were available on any injection site-related adverse effects involving EQW. Neither is information available on any possible skin reaction-driven changes in EQW pharmacokinetics and pharmacodynamics or on any related potential clinical consequences. In addition, no reports are available in the literature on any injection-related skin lesions associated to other daily or weekly GLP-1 RA formulation. Still we recently analyzed a case series showing that regular injection site rotation is essential to prevent esthetically uneasy nodules in people on EQW (Gentile S. and Strollo F. Subcutaneous Nodules during Treatment with Exenatide Long-Acting Once-Weekly Formulation: An Ultrasound Evaluation. Divers Equal Health Care (2016) 13(4): 313-318, accepted for publication).

Figure 18: Enlarged detail of the USS picture a single nodule with a marked peripheral hyper echoic reactive border, taken from an area of clustered nodules observed 8 weeks after injection.
Figure 19: Appearance of the an area repeatedly used for EQW injections on the right arm.

Anti-Tumor Necrosis Factor

Also anti-tumor necrosis factor (anti-TNF) biologic agents used against Crohn’s disease have been associated with a number of injection site reactions, including redness, itching, bruising, pain, or swelling [41]. The latter have been commonly observed with subcutaneous protein injections even in the combined safety trial [42-44]. However, also in the case of anti-TNF agents no published reports are available on lipodystrophic injuries similar to those occurring after insulin injections, and therefore specific studies are warranted on this issue as well.

METHODOLOGICAL CONSIDERATIONS

All these different types of skin lesions can generate some confusion, especially when no clear-cut differentiation is made between well-defined lesions with different morphology and pathogenesis. Specifically, LH and LA are still occasionally confused with each other [9], even though the latter is a scarring lesion and is therefore quite different from LH in terms of both morphology (see Figure 2) and pathogenesis. Indeed, mistaking one lesion type with the other might have been justified back in 2002, when a letter to the editor was published in Diabetes Care proposing a three-stage classification for insulin-related LDs despite little being admittedly known about the nature and causes of described phenomena [3,7,23]. However, given the state of current scientific knowledge, no misclassification is acceptable any more, as the two lesions have totally different appearance and metabolic consequences. In conclusion, too little information is still available on possible clinical consequences of local injection-related side-effects of subcutaneous
non-insulin based medications (exenatide and other GLP-1-RA), acromegaly (pegvisomant), or Crohn’s disease (anti-TNF). Based on evidence showing that insulin shots are the most frequent factors associated to skin lesions, we suggest that precautions recommended for insulin injections may be adopted for all other subcutaneously injected drugs as well. In particular, local damage may be minimized through the use of very short and thin needles and careful injection site rotation methods [45]. However, dedicated studies are needed to support this hypothesis.

LIPOATROPHY

LA is another skin lesion less frequent than LH [18,46,82-84] but no less dangerous, although much less frequent. No cases of LA associated with subcutaneous use of drugs other than insulin have been reported to date. However LA lesions are quite rare even in insulin-treated patients, since the most numerous patient series described in the literature adds up to 24 subjects [4]. In fact, LA can cause unexpected and seemingly inexplicable hypoglycaemic events, because of thin subcutaneous tissue allowing even short needles to get directly into the muscle [1,2, 9,10,19,32]. As for pathogenetic mechanisms, these lesions have been suggested to be related to the use of animal insulins [1], and actually their prevalence (previously given as 10-55%) [18,46,82-84] has declined considerably - without going down to zero, unfortunately - along with the introduction of new improved purity medications and insulin analogs.

So, LA continues to be reported with insulin analogs [85] and poses a clinical challenge owing to erratic insulin absorption from affected areas and distressing cosmetic issues. A high prevalence of degranulatingtryptase/chymase-positive mast cells was demonstrated in biopsies from insulin-induced LA sites and in a small series of patients cromolyn sodium treatment (4% cromolyn sodium in petrolatum solvent for topical administration twice a day) was reported to be both effective and devoid of considerable side effects [46].

Unmet needs and Future Perspectives

Usually patients express psychological aversion to insulin therapy and perceive that injections are painful and inconvenient. These factors contribute to patient and healthcare provider reluctance to initiate insulin therapy [86-87] and subsequently to poor adherence and persistence [15-17]. In the last 10-15 years a lot of expectations have been placed in the possibility of eliminating, or at least limiting, the use of injection systems for insulin therapy but the results of the trials investigating effectiveness and safety of jet-insulins have been disappointing [88]. Therefore this path cannot be confidently beaten to solve the injection problem at the moment.

Alternative noninvasive insulin delivery systems such as inhaled insulin have not achieved widespread acceptance because of safety concerns, low bioavailability, lack of precise dosing control, and cumbersome device design [89-92]. These limitations highlight the need for alternative insulin delivery methods. stainless steel micro needles (MN) with lengths of 1.25-1.75mm and an external diameter of approximately 260 mm were utilized for intradermal (ID)
insulin administration. In animal models, micro needles provide highly accurate, reproducible drug delivery into the dermis with pharmacokinetics (PK) more closely resembling intravenous than SC administration [93-97].

Pharmacokinetic studies and pharmacodynamics of rapid insulin analogues injected intradermal with microneedles (MN) are very promising. However, information is still lacking on MN utilization with other types of insulin, especially at high doses, and, above all, on its long-term effects. Nevertheless, this seems to be a promising field of research eventually leading to improved patient acceptance of and adherence to injection therapy [86,87].

**Special Populations and Problems**

**BMI and skin thickness**

Needle lengths designed for subcutaneous injections started out as long as 16 mm in 1985, and 12.7-mm needles were introduced in the early 1990s. Over time, with growing evidence of an increased risk of intramuscular injections and with improved technology, shorter needles (4, 5, 6, and 8 mm) were developed.

When evaluating skin thickness in 388 patients with BMIs varying from 19.4 to 64.5 kg/m², Gibney et al. [41] found that a 10kg/m² difference accounted for a 4-mm difference in subcutaneous tissue thickness. The average subcutaneous thickness ranged from 10.35 mm to 15.45 mm across all injection sites. This study concluded that 1) the thickness did not vary so much between under-, normal-, or over-weight individuals and that 2) statistically significant BMI differences did not cause huge skin thickness variations and, what was even more important, did not have any clinical impact (P<0.001). Skin thickness averaged 1.9-2.4 mm across several different injection sites (i.e., arm, thigh, abdomen, and buttocks), as well as, across several BMI levels. Being top skin thickness values below 2.4 mm, it was logical to assume that a 4-mm needle would be effective in all patients. Therefore, the study further concluded that 4-mm needles would be able to successfully deliver insulin in most adult patients.

A later multisite crossover study by Hirsch et al. [98] supported these conclusions with similar findings. Patients in this study had A1C levels ranging from 5.5 to 9.5% and were randomly divided into two groups: 1) 32G, 4-mm/31G, 5-mm pen needles (further separated into low [≤20 units] or regular [21-40 units] insulin doses) or 2) 32G, 4-mm/31G, 8-mm pen needles (also further separated into low or regular insulin doses). The patients within each group used the two assigned needles for a 3-week period each. The study did not find any statistically significant fructosamine differences (11-13 mmol/L) as referred to needles or BMI (note that fructosamine is an indirect measure of glucose concentrations over the previous 2-3 weeks). One criticism that can be raised against this study is that, although fructosamine is a short-term marker of glucose control, the time lag is too short to assess all possible effects on lipodystrophy, which generally requires a longer period to fully develop. Other studies have also found that patients were able to maintain similar glycaemic control with longer (12.7 mm) or shorter (5-8 mm) pen needles.
[99,100]. A controlled, multicenter, crossover study by Kreugel et al. specifically evaluated the use of a 31G, 5-mm compared to a 31G, 8-mm pen needle, as well as needle preferences in 130 patients with type 1 or type 2 diabetes and a BMI range of 30.1-62.5 kg/m² [99]. Patients were divided into two groups, and each group used a specific needle length for 3 months before switching to the other for an additional 3 months. Although a favorable (0.12%) statistically significant (p=0.02) HbA1c difference was found between the 5- and 8-mm pen needles, there was no statistical difference between the two groups as for fructosamine levels, hypoglycaemic episodes, site bruising, or pain perception. Unique to this study was the evaluation of 1,5-anhydroglucitol for possible differences in postprandial elevations, which could not reveal any changes anyway. The patients in this study did not express any needle length preference (46% for 5 mm and 41% for 8 mm). In a multicenter, crossover study of 62 patients with type 1 or type 2 diabetes and a BMI range of 30-64 kg/m², Schwartz et al. also demonstrated that glycaemic control could be maintained with either a 31G, 6-mm or a 29G, 12.7-mm needle [10]. Similar to the study by Kreugel et al. [99], their patients used one needle length for 3 months before switching to the other for an additional 3-month period. No statistical differences were found in the final HbA1c levels between the two needle lengths (7.6% with the 5-mm and 7.9% with the 12.7-mm needle). The study concluded that the two needle sizes provided comparable glycaemic control.

Patients in the above reported studies - except for [99] - rated the shorter pen needles as much less painful and, not surprisingly, mostly preferred over longer pen needles [5,10]. In the Schwartz study (10), patients also completed several surveys, including the World Health Organization Diabetes Treatment Satisfaction Questionnaire; Insulin Treatment Satisfaction Questionnaire; and, specifically related to needles, The Needle Handling Questionnaire and the Needle Preference Questionnaire. Their results show that patients reported greater satisfaction (p<0.001) and greater preference for shorter needles (89%, p<0.001) and rated shorter needles more favorably with regard to ease of use, perceived pain, and glycaemic control (p<0.001).

Pain

According to Hirsch et al. [98] patients experienced less pain with 4-mm than with 5-mm needles (pain score 11.9 mm less on a 150-mm visual analog scale ranging from -75 mm to +75 mm with zero as the mid-point indicating “no difference”) and even less than with 8-mm needles (23.3 mm less, p <0.02). All of this evidence supports the argument that the longer 12.7-mm needle is obsolete. After all, why would a patient intentionally select a long needle when shorter, less painful needles work just as well [98-100].

Insulin Leakage

Some worries were present concerning the possibility for shorter needles to be more apt to cause insulin leakage, especially with larger doses of insulin or with obese patients, but the evidence contradicts them [48, 98,99,100,101]. In addition to evaluating glycaemic control with a 4-mm pen needle, Hirsch et al. [98] also looked at insulin leakage and concluded that there
were no differences between 4-, 5-, and 8-mm needles in the amount of leakage. This study found a higher proportion (58%) of reported leaks with 5- and 8-mm than with 4-mm pen needles. Similarly, the study by Kreugel et al. [99] found no correlation between insulin leakage, BMI, and insulin dosage with the 5- or the 8-mm needles. No comparable differences either were found in leakage volume by Schwartz et al. between the 6- and 12.7-mm needles (2.7 vs. 3.2 mm) [100].

However, a statistically significant, though minimal and clinically irrelevant, leakage difference, was found with 5-mm needles when using a vertical as opposed to an angled injection technique (65% vs. 59%, p<0.001) [2]. Additionally, in absolute terms, Wittmann et al. [101] found increasing leakage with larger doses and shorter needles when various amounts of medium were injected into pork rind. But, once again, when comparing the percentage of leakage against the larger volume, such difference was not found to be significant. It can be concluded that shorter pen needles can be used without any significant increase in insulin leakage.

ACKNOWLEDGMENTS

SG and FS wrote the document, which was approved by all authors after a review process involving also the National Executive Councils of AMD and OSDI Scientific Societies.

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APPENDIX

RECOMMENDATIONS ON APPROPRIATE INJECTION TECHNIQUES

Was a Consensus Document on Injecting Techniques really needed?

According to data from ISTAT (National Institute of Statistic) 2011 report [1] about 3 million people with diabetes mellitus (DM) are living in Italy nowadays, mostly in the South of our country, with a 5.1% prevalence of type 1 DM (T1DM). ARNO 2011 report, on the other side, witnessed in favour of a 10-year lasting ever increasing utilization of newer insulin preparations endowed with a more physiological pharmacokinetics profile, such as rapid-acting and basal analogues, as well as, of innovative therapeutic regimes [2]. In spite of technological progresses, and a general improvement in the quality of care, the 2012 AMD Annals [3] reported that only 22.2% T1DM and 43.8% T2DM patients reached HbA1c levels below 7% (53 mmol/mol), and T2DM patients above 9% (75 mmol/mol) accounted for 25.7% monitored people.

Many factors related to insulin administration, storage and handling might contribute to poor metabolic control. Therefore, a major goal of health care teams is to educate patients and caregivers to the best possible injectable drug administration techniques and the most skilful utilization of related available devices.

In order to let them reach that goal, we collected all available scientific evidences on this issue and organised it according to well-defined hierarchically distributed criteria targeted at patient’s self-management / empowerment [4], and based on National Guideline System [5] with structured education on top. Our Consensus Document on Injecting Techniques represents in fact the final result of this strong clinical effort.

References

RECOMMENDATIONS CONCERNING INJECTABLE DRUG ADMINISTRATION

1. A correct injection technique is essential for insulin and other subcutaneously administered medications to ensure optimal effects in people with diabetes mellitus (DM). It implies the ability to (i) choose the best needles, (ii) rotate injection sites regularly, (iii) handle and store insulin appropriately, (iv) insert needles correctly into the skin, (v) let pen needles inside the skin long enough to get the whole dose be absorbed and (vi) manipulate the skin before and after injection.

   (Level of evidence III, Strength of recommendation B)

2. Insulin must be injected into intact subcutaneous tissue and intramuscular shots have to be avoided, which would result into fast absorption and consequent risk of hypoglycaemia.

   (Level of evidence II, Strength of recommendation B)

3. Insulin effects are not affected by injection depth, provided the latter falls within the subcutaneous tissue.

   (Level of evidence V, Strength of recommendation B)

4. Injection site rotation within large surfaces, needle disposal after each shot and pinch technique utilization at a 45° angle with respect to the skin whenever needles longer than 6 mm are chosen are essential factors to ensure optimal insulin absorption and prevent skin injury.

   (Level of evidence II, Strength of recommendation A)

5. An effective rotation scheme consists of dividing injection site into quadrants and regularly spacing shots 1-2 cm apart from one another within each quadrant in order to avoid causing repeated traumas to the same site.

   (Level of evidence I, Strength of recommendation A)

6. Rapid acting insulin analogues can be injected anywhere as their absorption is not site-dependent. On the opposite, regular human insulin is preferentially injected into the abdomen where absorption is faster and rather stable.

   (Level of evidence I, Strength of recommendation A)

7. Areas of lipodystrophy can occur as a consequence of missed injection site rotation and needle reuse, especially when certain insulin preparations are chosen.

   (Level of evidence III, Strength of recommendation B)

8. The choice of needle length (for either pens or syringes) is crucial to ensure optimal insulin absorption.
9. Injections performed with shorter and thinner needles cause less pain and discomfort and ensure better therapy acceptance and adherence.

(Level of evidence II, Strength of recommendation A)

10. Injection with a pen and the use of shorter and thinner needles ensure optimal insulin absorption in thin patients and children. This approach does not require the pinch (or skin fold) technique to be used and is therefore easier to follow and to teach. It also causes less fear and pain, thus ensuring better therapy acceptance and adherence.

(Level of evidence II, Strength of recommendation A)

11. When injected into lipodystrophic areas, insulin gets altered in terms of pharmacokinetics and pharmacodynamics, which causes variable and unpredictable absorption and affects glycaemic control.

(Level of evidence II, Strength of recommendation B)

12. When moving from a lipodystrophic area to a healthy zone, insulin dosage should be reduced because of expected improved absorption. Per cent reduction varies from person to person and should be guided by intensified self-monitoring blood glucose.

(Level of evidence II, Strength of recommendation A)

13. All patients should be provided with appropriate education and training sessions by the diabetes care team before starting any treatment regimens based on drug injections.

(Level of evidence II, Strength of recommendation A)

14. Inspection and palpation of injection sites should be carried out systematically in all patients on subcutaneous therapy and education concerning proper injection techniques and injection site self-palpation should be systematically reinforced.

(Level of evidence II, Strength of recommendation A)

15. In the hospital pen needles and syringes have to comply with safety criteria to minimize any risks of accidental injuries and to keep health care providers, patients and relatives far from any hazards during all utilization phases including disposal.

(Level of evidence II, Strength of recommendation B)

16. Home utilization of safety needles and syringes should be made available also to special populations like AIDS, HBV or HCV infected patients

(Level of evidence II, Strength of recommendation B)

17. According to safety rules aimed at minimizing the risk for any infection transfer among patients, each insulin pen should be utilized in the hospital for a single individual only.
18. Insulin storage should comply anywhere with approved producer's leaflet. This should also be the object of careful patient education.

Comments

In order to ensure that injected insulin profile keeps in line with expected pharmacokinetics and pharmacodynamics, thus yielding predictable biological effects, it is necessary to perform a correct injection technique [1-4] as avoidable errors might modify insulin action [5,6]. Since they have to deal with a daily treatment, patients with DM are at risk for progressively increasing careless and incorrect habits which in turn are expected to increase glycaemic variability over time [5].

To get optimal absorption, insulin must be injected into the subcutaneous tissue, not into the dermis or into the muscle. That's why the choice of needle length is crucial. The latter is greater with syringes than with pens and therefore, when allowed to, people with DM choose pens. Nevertheless, it has been proven that, when a proper injection protocol is followed, both effectiveness and safety of the two devices are identical, allowing comparable metabolic control and complication risk [7,8].

Thin adult and children skin displays an average thickness of 2.23 mm in the arms, 1.87 mm in the thighs, 2.15 mm in the abdomen and 2.41 mm in the buttocks, both in [9-11]. As accidental intramuscular shots might cause hypoglycaemia [11-15], it is necessary to inject insulin beyond 2.5 mm without getting into the muscle. This is possible with short (4 mm) needles, which reduce the risk of intramuscular injection and do not increase insulin backflow even when inserted at 90° without pinching the skin [12,17]. The use of a 4 mm needle is suitable for adults, irrespective of BMI [5], as well as for children and adolescents [13,14,19] but the pinch technique may still be required in very thin subjects [18]. In the transition from a longer needle to a shorter one, there might be differences in insulin absorption, therefore monitoring blood glucose levels is recommended under these circumstances [19,20].

Subcutaneous tissue thickness varies significantly depending on sex, body mass index, age, ethnicity, diabetes type, morphology of the diabetic individual, as well as, on pressure exerted during injection [3,6,9]. The risk of intramuscular injections depends on that all and has been estimated to be 15.3% with 8 mm, 5.7% with 6 mm and 0.4% with 4 mm needles [9,21-23].

Injection site rotation within large surfaces, needle disposal after each shot and 45° angle pinch technique utilization with needles > 6 mm are essential factors to ensure optimal insulin absorption and prevent skin injury [6,10,17,24,25,29].

With the advent of insulin analogues, absorption varies much less both between and within individuals and is therefore more predictable and easier to handle by the patient [25]. However,
despite technological advances allowing newer and newer insulin analogue availability, certain absorption / action affecting factors still remain, such as exercise, counter-regulatory hormones, high dosage, incorrectly mixed preparations or even the habit to pull out needles from the skin too early after pen pistons reaching their end stroke positions [24,25]. Another example of how the injection technique may affect insulin pharmacokinetics is given by glargine [26]: its long acting effect is based on its ability to precipitate within the subcutaneous tissue at pH 7, and cannot be exploited in case of intramuscular injections, which thus cause unexpected hypoglycaemic episodes [25].

Insulin absorption does not vary as a function of how deep it reaches into the subcutaneous tissue. However, the use of the same anatomical region for shots performed at the same time of day and the choice of abdomen before meals [19] are recommended only for different types of human insulin preparation [1-3], which also of course require to regularly inject the drug at a distance of about 2 cm from previous shots within each region in order to avoid repeated traumas.

So, to summarize all the above, the choice of delivery devices and needle length has been proven to affect insulin absorption and, independently of using pens or syringes, the needle influences correct techniques the most. Nowadays insulin syringes with needles less than 8 mm are not available in our country and their use without pinching the skin and injecting at a 45° angle increases the risk of intramuscular injections. Therefore we suggest using pens with short needles (4 mm) just to minimise the risk of intramuscular injections. Also needle gauge (G) and sharpness are important factors in terms of patient acceptance and satisfaction [27-30]. 4 mm x 32G needles provide both obese and non-obese patients with comparable degrees of metabolic control as 5 mm x 31G and 8 mm x 31G needles but are perceived as less painful and better accepted [29,31-34].

Injecting insulin by shorter and thinner pen needles (4 mm and 32G) with triple bevels, in compliance with the UNI EN ISO 7864 standards, cause less pain and discomfort [34,35] and ensure better therapy acceptance and adherence. In fact, the greater the outer diameter, the greater the resistance the needle has to face to penetrate the skin and, vice versa, the smaller the outer diameter, the less the feeling of discomfort at injection.

One of the most common complications of such treatment is the development of cutaneous lipodystrophy, also described with continuous insulin infusion systems [36-38]. Its exact a etiology is not entirely clear yet, although several causative factors are implicated, such as repeated traumas into very limited areas, needle re-use, insulin per se as a growth factor, especially at a high doses, and old protaminated / human insulin [17,39]. The risk of lipohypertrophy is 31% higher in subjects using the same needle several times than in the others [6].

Lipodystrophies are widespread. Vardar and Kizilci [40] reported a prevalence of 48.8% in
215 Turkish patients treated with insulin for at least two years; according to Hauner et al [41], in 233 German patients with DMT1 the prevalence was 28.7%. More recently, Blanco et al. [6] documented lipodystrophy in as many as 64.4% investigated patients, with a strong relationship to incorrect site rotation habits. In addition, 39.1% patients with lipohypertrophy showed unexplained hypoglycaemia and 49.1% had high glycaemic variability. Several studies show that insulin absorption from lipodystrophic areas can be delayed or become unpredictable [42-45], thus representing a potential factor of worsening glycaemic control [44-53], while the use of very short needles is preferred by patients [30,32,33] and is less traumatic per se [34,37].

**Education**

Insulin therapy is a long-lasting patient commitment day after day putting them at risk for more and more careless and incorrect habits in the absence of any proper and consistent educational support. As a consequence of that glycaemic variability and poor metabolic control are expected to increase [5].

Appropriate education on injection techniques is obviously essential, nevertheless it is often lacking [4], as shown by the high rate of skin lesions caused by incorrect injecting habits [6,9,31-35].

Diabetes care teams must promote patient empowerment to let people understand what is best for them day by day. In particular, insulin self-administration requires specific skills to be acquired to perform injections correctly.

Patients need to understand the close relationship between injection technique and metabolic control [56], as well as, changes in pharmacokinetics expected to occur with incorrect injection techniques and consequent increased risk of chronic complication onset / acceleration [5,6,9,10].

All patients starting injections should be accurately educated on proper techniques [18,27,47-49]. Education should also be regularly reinforced and healthcare professionals are expected to perform inspection and palpation interested sites on a regular, systematic basis in all subjects on injection therapy [31,53,55-60] and especially in those facing repeated unexplained hypoglycaemic episodes [6]. Education should not be limited to treatment start, it should rather go on throughout the whole follow-up period with timely reinforcements [61], put special emphasis on the negative effects of getting into the muscle tissue or into altered skin areas.

People with diabetes should also learn that self monitoring blood glucose has to be intensified when moving from longer to shorter needles and/or from healthy to altered skin areas [3,6,45,46].

An appendix on insulin appears in an attachment in “AMD Algorithms Online - The personalisation of therapy in type 2 diabetes” can be consulted on the AMD website: at http://www.aemmedi.it/pages/linee-guida_e_raccomandazioni/.

To try and facilitate the educational approach, several study groups, as well as, needle and
device producing companies prepared several brochures, flyers and small manuals. Scientific societies, volunteer associations and pharmaceutical companies also published other educational materials on the web, addressing various practical topics including injection techniques. Many cartoons are available with sketches describing how to run a proper injection site rotation manoeuvre and how to make further rotation within each individual area, as observed in Figure 20.

However, such drawings are not suitable for all patients (they may be difficult to understand for some of them in fact and others might prove to be too schematic) and in our experience people tend to forget them or to seek and follow the advice provided by peers, and especially to use the most comfortable and fast injection modality. As a result of this, for example, many patients do not explore their own injection areas to avoid taking clothes off (some of them sometimes even inject insulin through clothing!) and therefore tend to repeat the same sequence of gestures, thus ending up using only a few “comfortable” sites and implementing no rotation at all.

Is there an easy way out of it? Not at all when people are not continuously reminded of possible negative consequences of being lazy at adhering to healthcare provider’s suggestions. After catching their attention, though, the most effective and engaging way to have patients follow best injection practice rules is to write down a multiple square drawing using a marker pencil directly on their skin and to explain how to use each square for the injection of insulin in a definite sequence thereafter.

However many issues remain unsolved, mostly concerning the maximum acceptable time lag between educational refreshers. One possible solution is to check systematically (by inspection, palpation and pinching) the injection sites at each annual check of their chronic complications.
**Figure 20:** A sample cartoon showing how to run a proper injection site rotation by changing skin areas and how to further rotate sites within each individual area.

**Figure 21:** Patients often get confused at rotating among areas and sites, so they do not perform properly. The reticular drawing applied directly to the skin is meant at helping them recall what they learned and turn it into good habits. It is also used to highlight wrong choices (right panel).
References


**ADDENDUM 1**

Levels of scientific evidence and the strength of recommendations as classified according to our National Guideline System (S5).

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<th>LEVELS OF EVIDENCE</th>
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<td>Evidence from more than one controlled randomized clinical trials and / or systematic reviews of randomized studies.</td>
<td>The procedure or diagnostic test is strongly recommended. The recommendation is supported by high quality scientific evidence (not necessarily type I or II).</td>
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<td>II</td>
<td>B</td>
</tr>
<tr>
<td>Evidence from one properly designed randomized trial.</td>
<td>There are still doubts concerning whether or not the procedure or intervention should always be recommended, but it is believed that it should be carefully considered.</td>
</tr>
<tr>
<td>III</td>
<td>C</td>
</tr>
<tr>
<td>Evidence from non-randomized cohort studies with concurrent or historical control or their meta-analysis.</td>
<td>Whether or not to recommend the procedure or intervention is still a matter of debate.</td>
</tr>
<tr>
<td>IV</td>
<td>D</td>
</tr>
<tr>
<td>Evidence from retrospective studies, such as case-control, or their meta-analysis.</td>
<td>The procedure is not recommended.</td>
</tr>
<tr>
<td>V</td>
<td>E</td>
</tr>
<tr>
<td>Evidence from case studies (&quot;case series&quot;) without any control group.</td>
<td>The procedure is strongly discouraged.</td>
</tr>
<tr>
<td>VI</td>
<td></td>
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<tr>
<td>Evidence based on expert opinion or authoritative expert committees as indicated in guidelines or consensus conferences, or based on opinions of members of the working group responsible for the present guidelines.</td>
<td></td>
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</tbody>
</table>

**ADDENDUM 2**

1. Insulin injection should be made into the subcutaneous tissue. The top layer of the skin (epidermis + dermis) is never > 2 mm thick, while the subcutaneous fat layer varies depending on patient’s body site and BMI.

No matter how deep the needle penetrates into the subcutaneous tissue, because the absorption does not change as a function of that. Conversely, it is crucial to prevent the needle from getting into subcutaneous muscle tissue to avoid the risk of an immediate absorption (particularly dangerous for any basal analogs, which in this case would behave as fast-acting ones) and of consequent, eventually severe hypoglycaemic events.
2. In lean subjects and in children the pinching technique can be useful even when using short needles (4mm) to avoid the risk of injecting insulin into the muscle. Available evidence clearly indicates that there are no reasons any more to continue using long needles (over 8 mm), which in fact should be reserved for special cases. Another topic of interest for a correct injection technique is where insulin is injected, because subcutaneous tissue thickness varies depending on bodily areas and therefore the risk of performing an intramuscular injection is not the same throughout the skin surface.
Figure 23: The diagram shows how needles of different lengths behave in children, adolescents and adults with and without utilizing the pinching technique.
3. If for special requirements long needles are used (for pens or syringes), in addition to implementing the pinching technique it is useful to tilt the needle at 45° with respect to the skin surface.

**Figure 24:** Schematic representation of bodily areas in lean and obese subjects showing how different the impact of 4, 8 and 12 mm needles can be.

**Figure 25:** Best injection modality when choosing a 90° or 45° angle with respect to the skin surface.
4. The pinching technique must be implemented softly, and care has be taken to avoid the fold to be too thick. This way the underlying muscle is prevented from being lifted into the fold together with the subcutaneous tissue and the risk of an intramuscular injection is avoided. Moreover, when using along needle healthcare providers might risk to pierce the fold, thus accidentally getting injured by the needle stick.

![Schematic representation of a long needle piercing the fold in case of a vigorous pinching manoeuvre.](image)

**Figure 26:** Schematic representation of a long needle piercing the fold in case of a vigorous pinching manoeuvre.