

Integrated Approach to Cardio-Vascular Risk in Insulin-Treated People with Diabetes based on the "Heart Project" and on Metabolic Complications Expected from Incorrect Injection Practice-Related Lipohypertrophy

Sandro Gentile^{1,2}, Ersilia Satta^{1,3,4}, Maurizio Capece⁶, Giuseppina Guarino^{1,2}, Teresa Della Corte^{1,2}, Carmine Romano¹, Giampiero Marino², Maria Pasquarella¹, Carmelo Alfarone¹, Fabrizio Loiacono⁵, Rossella Lamberti⁷ and Felice Strollo⁸, AMD-OSDI Study Group on Injection Technique and Nefrocenter Research & Nyx Start-Up Study Group

¹Nefrocenter Research s.c. ar. l and Nyx Start-Up, Naples, Italy

²Department of Internal Medicine, Campania University "Luigi Vanvitelli", Naples, Italy

³Polispecialistic Nephrological Center CNP Srl, Nefrocenter Network srl. Frattamaggiore, Italy

⁴Emodial Center s.r.l. Nefrocenter Network, Naples, Italy

⁵Nefrocenter Research s.c. ar. l, Naples, Italy

⁶Vomero Center Crisci Bersabea & C S.N.C., Nefrocenter Network, Naples, Italy

⁷Metelliano Medical Center, Nefrocenter Network, Naples, Italy

⁸Endocrinology and Diabetes, IRCCS San Raffaele Pisana, Rome, Italy

SUMMARY

Type 2 diabetes (T2DM) is known to be associated with increased cardiovascular (CV) morbidity and mortality. Over time, the CV Risk of patients with T2DM has been assessed according to various methods, often borrowed from different populations. Nevertheless, never have been evaluated changes in insulin absorption due to improper injection technique and responsible for further, well recognized CV-R factors as high glycemic variability, frequent hypoglycemia, and unsatisfactory glycemic control. Aim of the study was to intensify diabetes and its own comorbidities treatment, and in addition reduce the impact of injection technique of insulin to evaluate possible improvement in the CV-R score and NHYA score in 4499 insulin-treated T2DM subjects with (LH+) and without lipohypertrophy (LH-) due to improper injection habit. The educational training and the treatment intensification significantly reduced glycemic control, hypoglycemia rate and the mean amplitude of glycemic variability, especially in LH+ subjects, adding new knowledge to a global intervention addressed to reduce the devastating impact to the increased cardiovascular risk of diabetic people, inclusive of the risk due to LHs.

*Corresponding author

Sandro Gentile, Department of Internal Medicine, Campania University "Luigi Vanvitelli", Nefrocenter Research s.c. ar. l and Nyx Start-Up, Naples, Italy. E-Mail: s.gentile1949@gmail.com

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Introduction

Type 2 diabetes (T2DM) is known to be associated with increased cardiovascular (CV) morbidity and mortality [1]. Patients with T2DM display two to four-fold increased risk of coronary heart disease and ischemic stroke and 1.5 to 3.6-fold increased mortality [1]. In general, patients with diabetes face a 4-8-year lower life expectancy than metabolically healthy individuals [2]. The risk for cardiovascular disease is also increased in subjects with type 1 DM and is 3.6-7.4 times greater the earlier the onset of diabetes is [3]. Despite great advances in prevention and treatment resulting in significant reductions in diabetes-related CV mortality, combined CV morbidity and mortality still remains high in most patients with diabetes. Considering the growing number of survivors of cardiovascular events and the global epidemic of T2DM, the number of T2DM patients at higher cardiovascular risk (CVR) is expected to increase, posing a major challenge to healthcare systems around the world. Therefore, the implementation of cost-effective strategies for CVR reduction in such a population is urgent [4]. Diabetes has long been considered an "equivalent of CVR". This claim is based on the results of a Finnish study, in which patients with T2DM without coronary artery disease

(CHD) had coronary artery mortality similar to that of non-diabetic patients who had a previous coronary event [5]. Diabetes also increases coronary death rates, worsening patients' prognosis after the first event of coronary heart disease [6]. These arguments have led to the recommendation that diabetic patients be treated as a high-risk category [7]. However, recent evidence indicates that the risk of CHD in T2DM is highly heterogeneous [8, 9].

Over time, the CVR of patients with T2DM has been assessed according to various methods, often borrowed from populations other than the Italian one and, therefore, characterized by different lifestyles and health care organization systems [10, 11]. However, some fifteen years ago the Italian National Institute of Health launched the so called Progetto Cuore, encouraging the utilization of an individual 10-year-risk assessment score for cardiovascular events in the diabetic population (I-10-y RS) [12]. Being adapted to the characteristics of the Italian population, the latter can provide a key to reading this complex problem within our context, by evaluating simple parameters as reported below under "Methods". The stratification of people with diabetes into different CVR groups allows for the identification of those

who could benefit most from more intensive CV prevention. Therefore, it may be ethically and economically reasonable and useful to develop rational strategies to detect and treat patients at higher risk earlier and more intensively. Add to this complex and varied scenario some typical traits of diabetic disease, such as the classic triad of independent metabolic CVR factors represented by high HbA1c, as well as fasting and post-meal glucose levels contributing to the extent of glycemic variability (GV) understood as individual variations in time spent at glucose levels higher or lower than the accepted range [13-15]. So far, almost no one took into due account the fact that a significant proportion of subjects treated with insulin are even more exposed to otherwise avoidable hypoglycemic episodes and high GV as a result of incorrect insulin treatment practice involving the utilization of lipodystrophic areas for injections, mostly motivated by the denervation-related painless nature of these areas [16-18]. Indeed, correcting such errors could prevent wide variations in insulin pharmacokinetics and related metabolic consequences including recurrent, unexplained, and sometimes severe hypoglycemic episodes endowed with an even increased inner risk for CV events and for a more frequent use of common NHS services and emergency areas, expected per se to further increase CVR [19-32].

For the first time, based on the abovementioned considerations, as the primary outcome we aimed to assess whether or not a global, i.e., drug- and education-based, approach could have a better impact on a large cohort of people with T2DM undergoing insulin treatment than another one based on controlling only risk factors traditionally included in risk maps, such as blood pressure, dyslipidemia, cigarette smoking, glycemic control. The secondary outcome of our study was to evaluate the share of metabolic events, such as hypoglycemia and the wide glycemic variability, avoidable by correcting the errors in insulin injection technique.

Methods

Ours was designed as a two-arm, open-label, multicenter, case-control study realized in an outpatient, real-life setting, and was approved by the Ethical and Scientific Committee of the Reference center (University "Luigi Vanvitelli" Naples, Italy (Trial registration no. 126/10.09.2015) and by the Institutional review board (IRB Min No 6226 dated 05.12.2015). Before enrollment occurring under the inclusion criteria, all involved subjects with T2DM regularly referring to Diabetes Centers (DCs) and to the Cardiology Center (CC) involved in the study, gave their informed consent to participation.

In greater detail, the DCs and CC were part of the Nefrocenter Research Network from Southern Italy – a private consortium convention-supported by the National Health System and having an agreement with the "Vanvitelli" University also in terms of Ethics Committee –, sharing the same electronic record system, diagnostic/therapeutic procedures, and operating standards by adhering to the national program for continuous quality improvement. Their healthcare professionals (HCPs) proved to be specifically trained to follow the study procedures appropriately, after attending several clinical trials, some of which are listed in the present paper's reference section.

A web-based clinical record form (eCRF) served as a privacy-compliant repository for clinical data related to those signing the informed consent to anonymous data utilization for better disease control and improved patients' quality of life (QoL).

Data collected were: age, weight, height, BMI, disease, and insulin treatment duration, daily insulin dose (DID) and number of injections, HbA1c level, serum creatinine level, blood pressure (BP), glucose, total, low-density lipoprotein and high-density lipoprotein cholesterol (TC, LDL-C and HDL-C, respectively), triglycerides (Tg) and serum uric acid (UA), diabetes complications/comorbidities as reported in Table 2, including GV and number of severe or symptomatic Hypos (SeHs and SyHs, respectively) experienced within three months before enrollment as previously described, and chronic non-communicable diseases including over-5-year-inactive cancer [33-36].

T2DM diagnosis relied on criteria defined by the ADA Standards of Medical Care in Diabetes 2019 (confirmed in the case of patients enrolled before 2019) [37]. The International Classification of Diseases, Clinical Modification (ICD-9-CM, V82.9 2014) was used to classify comorbidities and complications related or unrelated to DM [38].

The common biochemical parameters were measured by high standard auto-analyzers in public laboratories successfully participating in nationwide quality control programs. All electro-medical devices used to evaluate patients were certified and periodically validated in accordance with the International Standards Organization (ISO) directive 15189/2012 [39]. Kidney function was assessed by both serum creatinine and urinary albumin excretion rate measurements. Estimated glomerular filtration rate (eGFR) was calculated for each patient based on a standardized serum creatinine assay and the chronic kidney disease epidemiology collaboration (CKD-EPI) equation [40]. Only patients having at least one serum creatinine measurement and concordant eGFR values during the last 3 months were included in the study.

Inclusion Criteria were:

- o type 1 and type 2 diabetes mellitus on insulin treatment for at least one year,
- o age between 39 and 65 years
- o blood pressure recordings lower than 200 mmHg for systolic and 90 mmHg for diastolic values
- o at least 2 injections of insulin per day
- o systematic assessment of the insulin injection sites for the presence / absence of skin insulin injection-related lipohypertrophy
- o absence of previous cardio-vascular events
- o regularly specialized structures attendance
- o signed informed consent

Exclusion criteria:

- o exclusive treatment of diabetes with drugs other than insulin
- o Total Cholesterol over 320 mg/dL or below 130 mg/dL, HDL below 20 mg/dL or over 100 mg/dL
- o presence of solid and non-solid, debilitating neoplastic diseases affecting various organs and systems
- o advanced liver or renal failure
- o dementia
- o poor adherence to treatments and clinical-diagnostic procedures
- o subjects in class IV according to the NHYA classification [41].

The study started in January 2016 and ended in December 2020 and enrolled all subjects meeting the study inclusion criteria over the years. All of them underwent a thorough search for lipodystrophic lesions at the injection sites, in accordance with the standardized methodology widely used by us in the past and further validated recently [32,42,43].

The individual I-10-y RS was assessed using the calculator from the CUORE project. The latter was an epidemiological and ischemic heart disease prevention project launched in 1998 [12]. Its score, validated in patients 35 to 69 years of age without previous major cardiovascular accidents, allows to estimate the probability of experiencing any CV events (mainly myocardial infarction or stroke) for the first time over the next 10 years based on eight risk factors including age, gender, systolic BP (SBP), Total Cholesterol (TC), HDL-C, diabetes mellitus, smoking habit, hypertension or use of antihypertensive medications [44-47]. It cannot be used in case of extreme risk factor values, including systolic blood pressure (SBP) higher than 200 mmHg or less than 90 mmHg, TC higher than 320 mg/dL or less than 130 mg/dL, HDL less than 20 mg/dL or higher than 100 mg/dL. A calculated risk value > 20% is considered high, if between 3% and 20% it is considered medium, and if <3% low.

Major adverse cardiac events (MACE) were defined as the composite of total death, MI, stroke, hospitalization for heart failure (HF), and revascularization, including percutaneous coronary intervention, and coronary artery bypass graft [48]. anyone having smoked at least 100 cigarettes in his/her life or still smoking or having stopped smoking for less than six months was defined as smoker [49].

Assessment of the severity of HF was classified on the basis of NHYA four-class staging [50].

Individual I-10-y RS and NHYA classification were reevaluated at baseline and annually after that. All subjects underwent a structured educational training on correct injection techniques in accordance with the methodology we already described elsewhere, independently of eventually identified lipohypertrophy (LH) [36, 42, 43]. In accordance with the Italian Standards of Care AMD-SID 2018 and with the indications of the National Health System [51]. Blood glucose, lipid and pressure management was periodically readapted according to a treat-to-target strategy and the need to give up smoking and to increase physical activity to overcome at least the 3-MET-per-week threshold was strongly emphasized at each visit.

The number of annually enrolled subjects increased regularly through the years, as more and more DCUs joined the original study group.

Hypoglycaemia

According to ADA guidelines 2019, we defined (i) severe hypoglycaemia (SeH) as an episode leading to unconsciousness or requiring assistance by a third person or associated to blood glucose levels <54 mg/dL (3.0 mmol/L) or in the 56-70mg/dl range [37]; (ii) symptomatic hypoglycaemia (SyH) as an episode characterized by at least one of the following symptoms resolving with food or sugary drink ingestion: palpitations, tremors, sweating, shakiness, irritability, concentration troubles, dizziness, hunger, blurred vision, confusion, tachycardia, or difficulty moving [37,51,52]; and (iii) frequent unexplained hypoglycaemia (UH) as the occurrence of hypoglycaemic episodes at weekly intervals at least, in the absence of any identified precipitating events, such as changes in insulin dosage, diet composition or amount of physical activity [52-54].

Glycaemic Variability

All electro-medical devices used to evaluate BG were ISO-directive certified and periodically validated and verified by DCUs' diabetes nurses [55]. In the absence of any user-friendly unanimously accepted clinical method, GV was investigated through a validated questionnaire and defined as high when BG levels swung consistently, inexplicably, and unpredictably from < 60 to > 250 mg/dL at least once a week over the three months preceding enrollment and at least for three weeks within the first and second trimester of the study [12, 14, 54, 56].

LH Identification Training Protocol

Only trained HCPs with at least three years of specific experience performed the protocol, using a US jelly to enhance fingertip sensitivity as previously described and validated [17, 18, 42, 43, 57]. It consisted of (i) the inspection of each interested area using direct and tangential light against a dark background, taking into account patient body position during injection, and (ii) a thorough palpation technique involving slow circular and vertical fingertip movements followed by repeated horizontal attempts on the same spot. For abdominal examination, patients were lying down and stood up after that; for thigh examination, they sat keeping bent legs and feet on the floor [58]. HCPs gently touched the skin at the beginning and progressively increased finger pressure after that. When perceiving a harder skin, they performed a pinch maneuver to compare the thickness of suspected to that of surrounding areas and repeated all maneuvers mentioned above in the case of smaller and flatter lesions [14]. According to US features, we classified LH areas as described elsewhere [59].

Treatment Intensification

1. All subjects underwent educational training on nutrition, aimed at reducing calories in overweight subjects by reading the nutritional labels of pre-packaged foods (-20% of the basal requirement, with fractionated carbohydrate intake and choice of foods with a low glycemic index and 30g of fiber each 1000 calories) and paying special attention to saturated-fat- or sodium-chloride-rich food avoidance;
2. The number and dosage of daily insulin injections were intensified at clinician's discretion;
3. particular attention to self-monitoring of blood glucose (SMBG) was suggested in subjects shifting from lipohypertrophic injection sites to healthy skin, who are generally recommended to reduce usual doses by 20% to avoid hypoglycemia [17,18];

4. glucagon-like peptide-1 receptor agonists (GLP1-ras) or sodium/glucose transporter inhibitors (SGLT-2is) were added according to the indications of the regulatory bodies and to the standards of care [51];
5. Treatment with lipid lowering agents of all subjects with altered and untreated lipid values, or intensification of the dosage or association of synthetic statins with ezetimibe. Intolerant subjects used vegetable statins.
6. antihypertensive treatment was also re-evaluated as needed, by modifying the dosage and possibly changing or associating different molecules in order to achieve the 130/80 mmHg target in as many subjects as possible;
7. At each visit we strongly emphasized the need to increase physical activity to overcome the 3-MET-per-week threshold at least.

Statistical Analysis

Data are presented as mean values ± standard deviation (M±SD). Categorical variables are given as frequencies and percentages. Repeated measures ANOVA was applied for intergroup and intragroup comparisons at the three chosen time points. p values <0.05 were taken as statistically significant. All analyses were performed using the STATA software, version 14 (Stata-Corp LP, College Station, Tex).

Results

Out of 6,280 insulin-treated subjects observed during the study, only 4,499 met the enrollment criteria and were willing to sign informed consent. Table 1 describes the number of subjects enrolled each year, with the indication of the percentage of subjects who presented lipohypertrophy. In fact, out of the total of 4499 cases, 1753 (38.9%) had skin lipohypertrophy (LH+) in the insulin injection sites and 2745 were free (LH-). LH-affected body sites were the abdomen (56%), followed by the thighs (30%), and the arms (14%). LH were present simultaneously at several body sites in 15% of the cases. The clinical characteristics of LH+ and LH- patients are described in Table 2, showing that, compared to LH- subjects, LH+ subjects were older (59.1± 4.2 vs. 57.7±5.3; p <0.05), heavier (BMI: 33.4±4.3 vs. 31.3±3.2 kg/m²; p <0.05), had been on insulin longer (10.4±6.6 vs. 8.3±7.5; p <0.05), had higher fasting blood glucose levels (188±17 vs. 148±13 mg/dl; p <0.01), and larger glycaemic variability (312.34±36.8 vs. 189.32±29.7 mg/dl; p <0.0001), an about three-times-higher rate of severe hypoglycemia (9.8±4.5 vs. 2.6±3.5 n/month; p <0.0001), and an about twice as high rate of symptomatic hypoglycemia (14.6±4.7 vs. 6.8±5.4 n/month; p <0.0001). 38% of LH+ subjects injected insulin directly into the nodules.

Table 1: Patients enrolled divided by year and patients with lipohypertrophy (LH+)

Year	Patients enrolled n.	Patients with LH (LH+)
2016 n. (%)	202	102 (50.5)
2017 n. (%)	1068	373 (34.9)
2018 n. (%)	1086	411 (37.8)
2019 n. (%)	1108	410 (37.0)
2020 n. (%)	1035	458 (44.2)
Total patients	4499	1754 (38.9%)

Table 2: Descriptive Features of the Enrolled Population. Data are expressed as Mean±SD or as n. and Percent Rate in the case of Categorical Variables

	OVERALL	LH +	LH -	P *
Subjects Enrolled (n)	4499	1754 (38%)	2745 (62%)	< 0.001
Sex M/F (n.)	2189/2310	824/930	1225/1520	n.s.
Age (years)	58.5±6.4	59.1±4.2	57.7±5.3	< 0.05
BMI (kg/m ²)	31.5±3.4	33.4±4.3	31.3±3.2	< 0.05
SBP (mmHg)	149.4±14.4	148.6±13.5	148.8±11	n.s.
DBP (mmHg)	82±8	84±6	81±7	n.s.
Diabetes duration (years)	11 ± 9	12±7	10±9	n.s.
Insulin treatment duration (years)	9.5 ± 7.2	10.4±6.6	8.3±7.5	< 0.05
Fasting blood glucose (mg/dl)	156±16	188±17	148±13	< 0.001
Glycemic Variability (mg/dl)	216.18±33.7	312.34±36.8	189.32±29.7	< 0.0001
Severe Hypos (n/month)	4.2±1.3	9.8±4.5	2.6±3.5	< 0.0001
Symptomatic Hypos (n/month)	10.5±3.2	14.6±4.7	6.8±5.4	< 0.001
HbA1c (%)	8.8±1.7	9.6±1.5	8.3±1.3	< 0.05
Patients injecting insulin into LH nodules (%)	38%	38%	0	--
Total Cholesterol (mg/dl)	188.8±22.2	196.4±12.6	175.6±18.4	< 0.05
HDL Cholesterol (mg/dl)	43.5±5.2	41.5±2.3	45.1±4.4	< 0.05
LDL Cholesterol (mg/dl)	109.7±12.5	110.7±11.3	104.3±6.2	< 0.05
Triglycerides (mg/dl)	196.14±16.6	207.15±23.7	189.9±18.8	n.s.
Creatinine (mg/dl)	0.9±0.6	0.9±0.7	0.8±0.5	n.s.
eGFR (ml/min/1.73m ²)	100.6±12.5	107.10±15.7	98.15±12.6	n.s.
Smoking habit (%)	47.6	54.5	41.4	< 0.05
Lipid-lowering treatment&	60.5	58.3	64.4	< 0.05
Antihypertensive treatment&	65.5	64.6	66.5	n.s.
Aspirin	44.5	36.5	46.6	< 0.05
Diabetes-related or associated complications (%)				
Hypertension (%)	65.6	68.7	63.5	n.s.
Dyslipidemia (%)	59.3	61.6	57.4	n.s.
Background Retinopathy (%)	47.7	48.6	45.8	n.s.
Nephropathy **	48.5	51.4	49.4	n.s.
Autonomic Neuropathy	55.5	55.6	55.7	n.s.
Peripheral Neuropathy	44.6	52.4	43.6	< 0.01
Peripheral arterial disease/ulcer	30.3	30.6	29.8	n.s.
individual 10-y RS***				
High risk (% of subjects)	55	69	48	< 0.01
Medium risk (% of subjects)	37	29	40	< 0.01
Low risk (% of subjects)	8	2	12	< 0.01
NHYA SCORE				
I (% of subjects)	88	80	92	< 0.05
II (% of subjects)	12	19	8	< 0.01
III (% of subjects)	1	1	0	--

BG = background. **Presence of macro-microalbuminuria and/or eGFR < 60 or >90 ml/min/1.73 m². * LH+ vs LH-. SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure. Hypo = hypoglycemic episodes. Please note that Class IV NHYA was an exclusion criterion. &= % of treated subjects vs. those needing treatment according to the diagnosis.

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LH+ subjects had worse values than their counterpart also in terms of CVR parameters, including total cholesterol (196.4±12.6 vs. 175.6±18.4 mg/dl; p <0.05), HDL-Cholesterol (41.5±2.3 vs. 45.1±4.4 mg/dl; p <0.05), LDL-Cholesterol (110.7±11.3 vs. 104.3±6.2 mg/dl; p <0.05), smoking habit (54.5 vs. 41.4%; p <0.05), lipid-lowering and antiplatelet agents utilization (58.3 vs. 64.4% and 36.5 vs. 46.6%, respectively; p <0.05). Regarding renal function, creatinine and eGFR values were substantially overlapping.

Regarding diabetes-related or associated complications, no substantial rate differences were apparent, except for the rate of peripheral neuropathy, which was higher in LH+ subjects (52.4 vs. 43.6%; p <0.01).

Individual I-10-y RS was more frequently elevated (69 vs. 48%; p < 0.01) and less frequently low (2 vs. 12%; p < 0.01) in LH+ subjects, and LH- ones had a medium score more often (29 vs. 40%; p < 0.01).

As regards the HF risk classification based on NHYA score, the lowest class hosted more LH- patients (Class I: 80 vs. 92 <0.05%; p < 0.05), while LH+ subjects were almost double the others in class II (19 vs. 8%; p <0.01), and only 1% vs. no one in class III, being class IV an exclusion criterion.

Table 3. Shows the changes in parameters contributing to the evaluation of the individual I-10-y RS and in those commonly recognized as directly related to CVR.

Table 3: Comparison of the parameters involved in the calculation of the individual 10-y RS and all the other parameters expected to contribute to the overall cardiovascular risk at baseline vs. the end of follow-up in the subjects with (LH+) and without (LH-) lipopertrophy.

	BASELINE			END FOLLOW UP	
	LH +	LH -	p #	LH +	LH -
SBP (mmHg)	148.6+13.5	148.8+11.0	n.s.	132.5+11.5	133.4+10.2
DBP (mmHg)	84+6	81+7	n.s.	80+5	80+8
Fasting blood glucose (mg/dl)	188+17	148+13	< .001	141+15	138+12
Glycemic Variability (mg/dl)	312.34+36.8	189.32+29.7	< .0001	197.25+31.3	185.25+21.6
Severe Hypos (n/month)	9.8+4.5	2.6+3.5	< .0001	2.9+2.5*	2.8+2.5*
Symptomatic Hypos (n/month)	14.6+4.7	6.8+5.4	< .001	5.6+3.5*	5.5+4.4*
HbA1c (%)	9.6+1.5	8.3+1.3	< .05	7.5+1.7*	7.3+1.1*
Patients injecting insulin in LH nodules (%)	38%	0	--	5%	0
Total Cholesterol (mg/dl)	196.4+12.6	175.6+18.4	< .05	176.4+10.6*	170.6+11.4*
HDL Cholesterol (mg/dl)	41.5+2.3	45.1+4.4	< .05	45.7+2.1*	45.3+3.4*
LDL Cholesterol (mg/dl)	110.7+11.3	104.3+6.2	< .05	99.5+5.3	96.3+3.2
Triglycerides (mg/dl)	207.15+23.7	189.9+18.8	n.s.	148.12+20.4*	153.5+18.8*
Creatinine (mg/dl)	0.9+0.7	0.8+0.5	n.s.	0.9+0.5	0.8+0.3
eGFR (ml/min/1.73m2)	107.10+15.7	98.15+12.6	n.s.	104.11+12.8	100.09+10.6
Smoking habit (%)	54.5	41.4	< .05	30.4*°	31.5*°
Lipid-lowering treatment&	58.3	64.4	< .05	91.5*°	93.3*°
Antihypertensive treatment&	64.6	66.5	n.s.	94.3*°	92.5*°
Aspirin	36.5	46.6	< .05	64.3*°	64.3*°

& p vs LH+: * p <0.01 vs LH+ at baseline, and ° p <0.01 vs LH- at baseline. # comparison between LH+ and LH-.

It stands out how, at baseline, all those parameters were significantly worse in LH+ subjects but improved significantly after treatment intensification and appropriate education on injection techniques, getting somewhat similar to those observed in subjects without LH.

Particularly significant was the reduction of the triad consisting of HbA1c, FPG and GV over time in both classes of subjects. Surprisingly, smoking habit also improved significantly and becomes almost superimposable between groups at the end of the follow-up.

However, what appears to be of particularly remarkable importance is the significant decrease of SeH episodes observed at the end of the follow-up in LH+ subjects only (from 111.8 to 34.4 per year) with a substantial stability of the SeH's number at a rate of about 32. episodes 4 per year in the LH- (p <0.0001); SyHs dropped dramatically in LH+ subjects, as well, from 174.2 per year to 67.2, while only slightly decreasing in their counterpart from 81.6 (p <0.01 vs. LH+) to 66.0 per year at the end of the study.

Treatment intensification implied and increased rate of antihypertensive drug utilization in hypertensive patients from some 65% to about 93% in both groups (p <0.01). A similar trend was observed for lipid lowering drugs, utilized initially by 58.3 LH+ subjects and 64.4% LH- subjects (p <0.05) and ending up to a 91.5 and 93.3% utilization rate (p, ns), respectively, after the follow-up period. Interestingly enough, the same occurred with aspirin.

At the end of the follow-up a significant reduction in LH rate from 38% to 10.2% was apparent (Table 4), which was accompanied by a remarkable and statistically significant I-10-y RS and NHYA score reduction in both LH and LH- subjects.

Table 4: Comparison of Individual 10-y RS and NHYA SCORE in subjects with (LH+) and without (LH-) lipohypertrophy at baseline and at the end of follow up.

	BASELINE			END FOLLOW-UP	
	LH+ n. 1754 (38%)	LH- n. 2745 (62%)	p	LH+ n. 416 (10.2%)	LH- n. 4083 (89.8%)
	Individual 10-y RS				
High risk (% of subjects)	69	48	< 0.01	28*	18°
Medium risk (% of subjects)	29	40	< 0.01	48*	54°
Low risk (% of subjects)	2	12	< 0.01	24*	28°
	NHYA SCORE				
I (% of subjects)	80	92	< 0.05	89	96
II (% of subjects)	19	8	< 0.01	7	6
III (% of subjects)	1	0	--	0	0

*p<0.01 vs LH+ at baseline; ° p<0.01 vs LH- at baseline

Discussion

The results of this real-life study show that a global approach to the problem of the high CVR of people with diabetes represents an all-round commitment of the diabetes team. The commitment to intensify the treatment not only of blood glucose, but also of associated comorbidities such as blood pressure, lipid profile, cigarette smoking and lifestyle undoubtedly contribute to improving cardiovascular prognosis, as also documented by other studies [61-63]. It is not overlooked that even glycemic control alone brings significant benefits but that also the intensive control of other risk factors determines a better cardiovascular prognosis [61-65]. In addition, the comparative evaluation of subjects with and without lipohypertrophy adds new knowledge, clearly showing that the presence of LH describe a cluster of subjects with unsatisfactory glycemic control, large glycemic variability and an excess of hypoglycemic episodes, all factors increasing the already high cardiovascular risk. On the contrary, the educative training significantly reduces the metabolic complications of incorrect injection habit, providing a great improvement of all CV risk factors, reducing in addition the frequency of lipohypertrophies.

Our study for the first time underline that the evaluation of CV risk must be considered globally, taking in consideration all the general and usual parameters, such as hypertension, high levels of vessels, smoking habit, obesity, level of HbA1c, etc, but is needed a more general consideration of all the modifiable factors involved, such as glycemic variability, hypohychemias, unsatisfactory glycemic control all due to changes in pharmacokinetics of insulin improperly injected.

However, the limit of all studies, if so ever, is that each one analyzes only part of the factors that globally concur together to decide the cardiovascular fate of each individual person. Perhaps only some of these large trials analyze multiple factors at the same time but they do so on rather small cohorts of subjects and for a limited period of time. The novelty of our study lies in the fact that for the first time we are also analyzing and treating metabolic alterations resulting from errors in insulin administration. In particular, we refer to excessive glycemic variability, a high HbA1c value and the frequency of hypoglycemia. In the past, by focusing on the benefits produced by a structured training on correct injection techniques, we collected exciting results in terms of clinical improvement and recently we have been able to demonstrate how much a structured and continuous educational action could improve disease outcomes and reduce health care costs [42, 43, 58, 17, 18, 30]. Here, for the first time, we apply this knowledge to cardiovascular risk, adding the beneficial effects of a correct injection technique to the benefits produced by an intensive therapeutic approach, aimed at all the factors that contribute to determining the overall cardiovascular risk.

Limitations

The evident improvements of all parameters used for the evaluation of cardiovascular risk and of quantified 10-year overall risk, as well as of the NHYA score confirm several literature reports describing the efficacy of a systematic, integrated and intensive therapeutic approach. On the other hand, there is no system capable of describing in quantitative terms the benefits that can be obtained from a reduction in dynamic parameters such as blood glucose levels over time, glycemic variability, or hypoglycemic events. A preliminary study from our group, to which we have already referred, showed a strong parallelism between metabolic improvement and less prominent complications on one side and nationwide savings in terms of health resource on the other side. Our findings have prompted us to identify

a single parameter describing health benefits as a whole. When we are ready for that, we expect clinicians to get finally able to see first-hand that improved insulin injection habits, far from being a "child of a lesser god", can contribute to LH prevention as efficiently as innovative treatment strategies including new molecules and high technology.

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Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and gave their approval for this version to be published.: Sandro Gentile, Giuseppina Guarino, Teresa Della Corte, Giampiero Marino, Ersilia Satta, Maria Pasquarella, Carmine Romano, Carmelo Alfrone, P Fabrizio Loiacono, Maurizio Capece, Rossella Lamberti, and Felice Strollo

Authorship Contributions

SG and FS created the paper and wrote it. ES, TDC, GG, GM, CR, CA, MP critically read and approved the paper. All have complied with data collection, critically assessed the results, and approved the final text. All Collaborators critically read and approved the final text.

Compliance with Ethics Guidelines

This study was conducted in conformance with good clinical practice standards. The study was led in accordance with the original Declaration of Helsinki and its later amendments and was approved by Vanvitelli University, Naples, Italy (Trial registration number 118-15.04.2018), and all the ethics committees of the centers participating in the study. Written informed consent was obtained from all participants before enrollment.

Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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