(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 November 2020 (12.11.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/225129 A1

(51) International Patent Classification:
A61K 38/28 (2006.01) *A61N 1/32* (2006.01)
A61P 3/10 (2006.01) *A61N 1/36* (2006.01)

(21) International Application Number:
PCT/EP2020/062132

(22) International Filing Date:
30 April 2020 (30.04.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
19172548.0 03 May 2019 (03.05.2019) EP

(71) Applicant: **DIAVEIN** [FR/FR]; 227 route de la Chapelle, 74540 SAINT FELIX (FR).

(72) Inventor: **L'HERMITE, Etienne**; Route de Converney 87, 1093 LA CONVERSION (CH).

(74) Agent: **CABINET BECKER ET ASSOCIES**; 25, rue Louis le Grand, 75002 PARIS (FR).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2020/225129 A1

(54) Title: METHOD FOR TREATING DIABETES AND ASSOCIATED DISORDERS WITH VASCULAR ELECTRICAL STIMULATION THERAPY

(57) Abstract: The invention relates to the prevention and the treatment of insulin resistance and hyperglycemia in a subject, in particular in a subject at risk or, or suffering from, diabetes, which comprises treating the subject with vascular electrical stimulation therapy (VEST). The invention also relates to the treatment, the alleviation or the prevention of diabetes and associated disorders with VEST.

METHOD FOR TREATING DIABETES AND ASSOCIATED DISORDERS WITH VASCULAR ELECTRICAL STIMULATION THERAPY

Field of the invention

The invention relates to the prevention and the treatment of insulin resistance and hyperglycemia in a subject, in particular in a subject at risk or, or suffering from, diabetes. The invention also relates to the treatment, the alleviation or the prevention of diabetes and associated disorders.

Background of the invention

Insulin is an hormone produced by beta cells of the pancreatic islets. Insulin regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of carbohydrates, especially glucose from the blood into liver, fat and skeletal muscle cells. In these tissues, the absorbed glucose is converted into either glycogen via glycogenesis or triglycerides via lipogenesis, or, in the case of the liver, into both.

Insulin resistance is considered as a pathological condition in which cells fail to respond normally to insulin. As a consequence, pancreas releases more insulin to help blood glucose to enter the cells. As long as pancreas is able to secrete enough insulin to overcome cells' weak response, blood glucose levels remain in the normal range.

However, over time, pancreas may become enable to secrete enough insulin to promote sufficient absorption of blood glucose by cells. This leads to hyperglycemia, namely to an excessive amount of sugar in blood plasma. Temporary hyperglycemia is often benign and asymptomatic. However, chronic hyperglycemia is characterized by the so-called hyperglycemia triad of polyphagia, polydipsia and polyuria and can produce a very wide variety of serious disorders over time, including neurological damages, cardiovascular damages, retinopathy, damages to kidney, legs and feet. Long-term hyperglycemia may be also associated with diabetic neuropathy and an increased susceptibility to certain infections. Insulin resistance and hyperglycemia are often associated with prediabetes and can lead to the development of 2-type diabetes and may play a part in the development of fatty liver diseases.

There are a multiplicity of risk factors that can contribute to insulin resistance or prediabetes such as a family history of diabetes, a history of heart disease or stroke, health conditions such as polycystic ovary syndrome, high blood pressure, abnormal cholesterol levels and/or obesity, age, ethnicity, and lifestyle habits such as physical inactivity and high glucose/sweetener content diet. Along with these risk factors, other conditions may contribute to insulin resistance

such as certain medicines (e.g. glucocorticoids, antipsychotics), hormonal disorders (e.g. Cushing's syndrome) or sleep disorders (e.g. apnea).

As of today, prediabetes and type 2 diabetes are major health concerns worldwide. In the US, more than 84 million of adults would have prediabetes, which represent 1 out of every 3 adults. It is estimated that about 50% of people having prediabetes will develop 2 type-diabetes within 10 years. Prediabetes and type-2 diabetes are not limited to high income countries. Indeed, Diabetes has become a new priority in Africa. Surveys carried out recently in the African Region indicate that up to 15% of adults aged 25 to 64 have diabetes and it is estimated that its prevalence will rise to 23.9 million cases by 2030. The risk factors for insulin resistance and type-2 diabetes are the same as those in high income countries and include physical inactivity, overweight and obesity, and consuming foodstuffs that are high in bad fats and calories. Diabetic subjects often lack access to proper treatment and diabetic medications, especially insulin, resulting in avoidable complications such as neurological, vascular or visual disorders, heart disease, stroke, lower limb amputation, kidney failure and many other chronic conditions. As of today, there are few treatments to reverse insulin resistance and prediabetes and thus prevent or delay subsequent type 2-diabetes. The main recommendations providing by physicians are being more active, losing weight and eating healthier. Turning to medication, the main option is metformin, a first-line agent to treat type 2 diabetes. A large US study conducted by the NIH (the Diabetes Prevention Program) showed that metformin can delay diabetes in subjects with prediabetes, especially in young adults, people with obesity and in women with a history of gestational diabetes.

Thus, there is a need of alternative methods to treat and prevent insulin resistance and hyperglycemia.

Summary of the invention

The invention relates to a method for treating, decreasing or delaying the onset of hyperglycemia or insulin resistance in a subject in need thereof, which comprises subjecting the subject to vascular electrical stimulation therapy (VEST). Typically, the subject has a capillary glycemia in fasting conditions of at least 1.00 g of glucose per liter of blood, before being subjected to VEST treatment. The subject may be diabetic or prediabetic or/and at risk of developing type-2 diabetes. For instance, the subject may suffer from type-2 diabetes and has been diagnosed with a capillary glycemia in fasting conditions of at least 2.00, preferably at least 2.50 g of glucose per liter before VEST treatment. The subject may further suffer from a diabetic foot syndrome, a lower limb ulcer or wound, and/or from peripheral neuropathy. In

some embodiments, the subject is not treated with any antidiabetics drug during the treatment with VEST. In other embodiments, in particular if the subject has a capillary glycemia in fasting conditions of at least 2.50 g of glucose per liter, VEST may be combined with antidiabetic drugs such as insulin or insulin sensitizer.

The invention also relates to a method for improving the general condition in a subject suffering from diabetes or prediabetes, wherein the subject is subjected to a long-term treatment with VEST, preferably over at least three months.

The invention also relates to insulin for use in combination of VEST for treating diabetes, preferably type-2 diabetes, in a subject, e.g. for regulating glycaemia in a diabetic subject. Typically, the subject has been diagnosed with a capillary glycemia in fasting condition of at least 2.00, preferably of at treat 2.5 g glucose per liter of blood, and may further suffer from a diabetic neuropathy and/or diabetic foot syndrome, and optionally from a lower limb ulcer or wound. Insulin may be daily administered to the subject, preferably at a daily dose which is at least 20%, preferably at least 25, 30, 35, 40, 45, 50 or even 55% lower than the daily dose to administer to the subject to lower the capillary glycemia in fasting conditions at a level of about 1.10 g of glucose per liter in the absence of VEST treatment.

For all the objects of the invention, the subject is treated with VEST means that the subject is administered with an electrical periodic current having a frequency from 0.01 Hz to 15 Hz. The periodic current is typically composed of electric pulses, the duration of each pulse being of at least 0.5 ms with a rise duration (T_1) of at least 0.25 ms and a decrease duration (T_2) of at least 0.25 ms. The pulses may be unidirectional positive pulses, unidirectional negative pulses or streams of pulses which are alternatively negative or positive.

In some embodiments, the electrical periodic current administered to the subject is composed of pulses and has at least one of the following features:

- the extinction duration (T_1) of the pulse is from 0.2 to 5-fold the rise duration (T_2)of the pulse,
- The rise duration (T_1) is of at least 0.25 ms and the decrease duration (T_2) is of at least 0.25 ms
- the peak amplitude of the pulse is from -130 V to 130 V,
- the base width of the pulse is from 0.5 ms to 30 ms, and
- the period of the electrical current is from 100 ms to 5000 ms.

The pulses of the electrical periodic current may further have an exponential-type waveform.

The electrical periodic current may be administered to the patient by transcutaneous route, typically by the mean of a medical device for electrotherapy, comprising at least two electrodes

disposed on the skin of the patient. The medical device for electrotherapy may comprise two electrodes, each electrode being positioned on a skin area selected from the forearms, the wrists, the calves and the feet of the subject. VEST is preferably applied to the subject at least every two days and each VEST session lasts from 30 min to 1 hour. The invention also relates to an electrical periodic current as described above for use in treating, decreasing or delaying the onset of hyperglycemia or insulin resistance in a subject in need thereof, by VEST

Figures

Figure 1A shows one pulse waveform according to the invention comprising an exponential rise part and an exponential decrease part. U_{max} : refers to the amplitude of the pulse in V, T_0 refers to the base width of the pulse (namely the duration of the pulse), T_1 refers to the rise duration of the pulse and T_2 refers to the extinction (decrease) duration of the pulse. τ_1 and τ_2 are the time constants of the exponential curves. X-axis: time (t) in ms. Y-axis: voltage (V) in volts.

Figure 1B shows an example of positive and negative electrical pulses according to the invention for an electrical resistance of 2 kΩ. The pulses have a base width of 2.8 ms, and an amplitude of +22 V or -22 V. X-axis: time (t) in ms. Y-axis: voltage (V) in volts.

Figure 1C shows an example of positive and negative electrical pulses according to the invention for an electrical resistance of 2 kΩ. The pulses have a base width of 3.2 ms, and an amplitude of +62 V or -62 V. X-axis: time (t) in ms. Y-axis: voltage (V) in volts.

Figure 2 shows foot ulcer healing overtime in diabetic patients treated with VEST combined with insulin or a combination of insulin and metformin at day 0, after 6 weeks of treatment and after 12 weeks of treatment. A significant improvement in the foot ulcer was observed after 6 weeks at treatment only, showing an effective healing process. After 12 weeks of treatments, the wounds were significantly reduced and no sign of infection was visible. Lower limb amputation was avoided.

Figure 3 shows diabetic foot ulcer of grade V before (A) and after treatment (B) with VEST. Before the treatment with VEST, the necrosis was severe, resulting in amputation of four limbs. Treatment with VEST promoted recolonization of the ulcer by the epithelium and healing. After few weeks of treatment with VEST, the healing was complete and the foot amputation avoided.

Detailed description of the invention

The Applicant demonstrated that vascular electrical stimulation therapy was effective in the management of hyperglycemia in subjects suffering from diabetes.

As illustrated in the case reports described in Example 1, VEST was effective in decreasing glycemia in subjects suffering from type-2 diabetes and having high hyperglycemia. Noteworthy, a significant decrease of capillary glycemia in fasting conditions was observed after two weeks of treatment with VEST in diabetic patients who did not take any antidiabetic drug or insulin. A drop of about 1.3 g of glucose per liter of blood in capillary glycemia in fasting conditions was observed after one month of treatment with VEST as compared to the initial value.

The Applicant further showed that VEST was useful in the management of diabetic patients suffering from diabetic foot syndrome and treated with insulin or insulin desensitizer such as metformin or a combination of insulin and insulin desensitizer. In these patients, the VEST was shown to significantly reduce the daily intake of insulin or metformin while significantly decreasing glycemia, as evidenced in Example 3. VEST treatment also enabled to promote foot ulcer healing as illustrated in Figure 2. Of note, VEST enabled to avoid foot amputation in a patient with a diabetic foot ulcer of grade V by promoting epithelium recolonization and healing in few weeks.

In Example 4, the Applicant further demonstrated that VEST enabled to potentiate the action of insulin and improve the regulation of glycemia in patients suffering from type-2 diabetes as compared to control patients treated with insulin only.

Accordingly, the present invention relates to the use of vascular electrical stimulation therapy (VEST) to regulate blood glycemia in a subject in need thereof. More precisely, the invention relates to the use of VEST to prevent, delay the onset, or treat hyperglycemia in a subject in need thereof. In an additional aspect, the invention relates to a method for treating hyperglycemia in a subject, wherein the subject is subjected to VEST.

The invention also relates to the use of VEST to prevent, delay the onset of, or treat insulin resistance in a subject in need thereof. Accordingly, the invention also relates to a method for preventing, treating, or delaying the insulin resistance in a subject, which comprises subjecting the subject to VEST. In a particular aspect, the invention relates to the use of VEST to prevent or delay the onset of insulin resistance and prediabetes in a subject at risk thereof. In a further aspect, the invention relates to the use of VEST to reverse or treat insulin resistance and regulate blood glycemia in a subject suffering from, or at risk of developing, diabetes, preferably type-2 diabetes.

In a more general aspect, the invention relates to the use of VEST for treating prediabetes or diabetes in a subject in need thereof. Accordingly, an object of the invention is a method for treating prediabetes or diabetes in a subject which comprises applying VEST to the subject. The invention also relates to the use of VEST to treat, alleviate or delay the onset of symptoms and disorders associated with hyperglycemia, insulin resistance and/or diabetes in a subject. In a further aspect, the invention relates to the use of VEST to decrease the risk of developing diabetes, especially type-2 diabetes, in a subject suffering from insulin resistance or prediabetes. The invention also relates to the use of VEST in combination of insulin or antidiabetics drugs such as insulin sensitizers in the treatment of prediabetes and diabetes. The invention also relates to the use of VEST to potentiate the effect of insulin or insulin sensitizers in the subject. In a particular aspect, VEST enables to reduce the daily dose of insulin or insulin sensitizer to administer to a subject suffering from diabetes in order to regulate his glycaemia.

A further aspect of the invention relates to the use of VEST for treating, preventing, or delaying the onset of one or several disorders associated with diabetes, especially type-2 diabetes such as neurological, vascular or visual disorders, in particular retinopathy and diabetic neuropathy, in particular diabetic peripheral neuropathy, heart disease, stroke, kidney failure and diabetic foot syndrome.

Of note, the Applicant observed that VEST improves (e.g. decreases) symptoms relating to diabetic neuropathy such as numbness, reduced sensitivity, e.g. to pain or temperature changes, sensations such as cramps, pains, tingling or burning sensations, in particular in the hands and feet, and erectile dysfunction.

With respect to diabetic foot syndrome, the Applicant showed that VEST is particularly effective to promote wound healing in subjects suffering from diabetic foot syndrome. In particular, the Applicant showed that VEST is particularly effective to promote healing of diabetic foot ulcerations, even of high grades, such as grade 4 or 5, and avoid amputation. Thus, the invention also relates to the use of VEST for treating, and/or promoting healing in a subject suffering from diabetic foot syndrome. The invention also relates to a method for treating diabetic foot in a subject, more precisely for promoting the healing of a diabetic foot ulceration, wherein the subject is subjected to VEST.

As used herein, "*insulin resistance or resistance to insulin*" refers to a pathological condition in which cells fail to respond normally to insulin. As a consequence, pancreas releases more insulin to help blood glucose to enter the cells. As long as pancreas is able to secrete enough insulin to overcome cells' weak response, blood glucose levels remain in the normal range.

However, over time, pancreas may become enable to secrete enough insulin to promote sufficient absorption of blood glucose by cells. This leads to hyperglycemia, namely to an excessive amount of sugar in blood plasma.

As used herein, "*diabetes*" or "*diabetes mellitus*" refer to a chronic metabolic disorder of multiple aetiology, characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effect of diabetes mellitus includes long-term damage, dysfunction and failure of various organs. Diabetes mellitus is usually divided into two major categories:

- Type 1 diabetes (formerly insulin-dependent diabetes mellitus) usually develop in childhood or adolescence and are prone to ketosis and acidosis. Type 1 diabetes accounts for around 10% of all diabetes.
- Type 2 diabetes (formerly non-insulin-dependent diabetes mellitus) includes the common major form of diabetes which results from defect(s) in insulin secretion, almost always with a major contribution from insulin resistance. Type 2 diabetes accounts for around 90 % of all diabetes.

Diabetes also encompasses "*gestational diabetes*". Gestational diabetes refers to a condition in which a woman without diabetes develops high blood sugar levels during pregnancy.

As used herein, "*prediabetes*" refers to a condition wherein the subject has a blood glucose level higher than normal, but not yet enough to be classified as diabetes. Prediabetes increases the risk of developing a type-2 diabetes.

"*Diabetes*" and "*prediabetes*" can be diagnosed by measuring glycemia.

As used herein, "*glycemia*" refers to the glucose level in plasma. "*glycemia*" is generally expressed as mg of glucose per deciliter of plasma, g of glucose per liter of plasma, or as mmol of glucose per liter of plasma.

Diabetes and prediabetes can be screened based on plasma glucose criteria, either by the fasting plasma glucose (FPG) test, the A1C test, or the oral glucose tolerance test (OGTT). The A1C test measures the amount of hemoglobin with attached glucose and reflects the average blood glucose levels over the past 3 months. The A1C test result is reported as a percentage. The higher the percentage, the higher the blood glucose levels have been. A normal A1C level is below 5.7 percent. A1C test can be affected by certain hemoglobinopathies such as sickle cell anemia. Thus, A1C test is not the blood test of choice for people of African, Mediterranean or Southeast Asian descent. The FPG test measures the blood glucose level in a subject under fasting conditions, namely who has not eaten for 8-12 hours, usually overnight. The OGTT

measures blood glucose level in a subject after fasting overnight and 1 to 3 hours after the intake of a high glucose-containing drink.

According to the American Standard Association (Standards of Medical care in diabetes, 2019), criteria for the screening and diagnosis of Diabetes and prediabetes are as follow:

	prediabetes	Diabetes
A1C	5.7-6.4%	≥ 6.5%
FPG	100-125 mg/dL	≥ 126 mg/dL
OGTT	140-199 mg/dL	≥ 200 mg/dL

In the context of the invention, prediabetes and diabetes are preferably screened by FPG test. However, diabetes and prediabetes can be also screened by random plasma glucose test such as capillary glycaemia test or venous glycemia test, which can be performed regardless the intake of food, or by urine glucose testing.

"*Hyperglycemia*" refers to a condition in which an excessive amount of glucose circulates in plasma. Chronic hyperglycemia can produce a wide variety of serious complications such as neurological damages, cardiovascular damages, retinopathy, damages to kidney, legs and feet. In the context of the invention, hyperglycemia refers to a blood glucose level in the FPG test of at least 100 mg of glucose per deciliter (which corresponds to 1.00 g of glucose per liter). Hyperglycemia associated with diabetes can be defined as a blood glucose level of at least 126 mg/dL in FPG test. Hyperglycemia in a subject having prediabetes can be defined as a blood glucose level from 100 to 126 mg/dL in FPG test .

As used herein, "*VEST decreases or regulates glycemia*" means that treatment with VEST enables to decrease the blood glucose level in the subject suffering from hyperglycemia, preferably in a subject with prediabetes or type 2 diabetes. More precisely, the treatment with VEST enables to decrease the blood glucose level in the subject by at least 5%, e.g. at least 10, 15, 20, 25, 30, 35, 40, 45 or 50% as compared to the blood glucose level in the subject before being treated with VEST. Preferably, the VEST enables to decrease capillary glycemia level at a level which is at least 0.70 g of glucose per liter.

As illustrated in the Examples, a significant decrease of the glycemia can be observed after two weeks of treatment with VEST only. Preferably, the follow-up of glycemia is performed by measuring capillary glycemia with a blood glucose meter such as Accu-Check® by Roche Diagnostic, in fasting conditions, typically in the morning. Thus, in the context of the invention glycaemia generally refers to capillary glycaemia in fasting conditions

As used herein, "*VEST decreases the daily dose of insulin to administer in a subject suffering from diabetes*" means that the daily dose of insulin to administer in the subject is at least decreased by 5%, for instance by at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60% as compared to the daily dose of insulin needed to decrease the blood capillary glycaemia in fasting conditions under 1.1 g of glucose per liter of blood, in the absence of VEST treatment, in the patient.

In some embodiments, VEST may even enable to stop the administration of insulin in the patient.

As used herein, "*the patient or subject*" according to the invention may be any human being of any gender and of any age. The patient may be a male or a female of at least 18 years old, in particular of at least 35 or 40 years old. Typically, the patient of interest is selected from the group consisting of subjects suffering from insulin resistance and/or hyperglycemia, subjects having prediabetes, subjects suffering from diabetes, in particular type 2 diabetes, and patients at risk of developing type-2 diabetes. The subject may have a blood capillary glycemia in fasting conditions of more than 1.00 such as more than 1.26, 2.00 or 2.50 g of glucose per liter of blood.

In a particular embodiment, the subject suffers from prediabetes or type-2 diabetes.

In a further particular embodiment, the subject suffers from type-2 diabetes and has been diagnosed with a capillary glycemia in fasting conditions of at least 2.50 g glucose per liter of blood.

In an additional embodiment, the patient has type-2 diabetes and suffers from diabetic foot syndrome. The patient may further have been diagnosed as being diabetic with a capillary glycemia in fasting conditions of at least 2.00, preferably of at least 2.50 g glucose per liter of blood For instance, the patient may be diabetic with a diabetic foot ulcer of grade III, IV or V.

In another embodiment, the subject suffers from diabetes such as type-2 diabetes, but does not experiment any ulceration or wound, such as those present in diabetic foot syndrome. In a further embodiment, the patient suffers from prediabetes or type-2 diabetes without experimenting a diabetic foot syndrome.

As used herein, "*subjects at risk of developing type-2 diabetes*" refers to a subject having prediabetes and/or having one or several of the risk factors such as a family history of diabetes, a history of heart disease or stroke, health conditions such as polycystic ovary syndrome, high blood pressure, abnormal cholesterol levels and/or obesity, age, ethnicity, and lifestyle habits such as physical inactivity and high glucose/sweetener content diet.

As used herein, "*diabetic foot*" refers to foot disorders resulting from type-2 diabetes. The diabetic foot syndrome encompasses a variety of foot pathologies which can be observed in

diabetic subjects such as lower limb infection, lower limb ulcer and neuropathic osteoarthropathy. In some embodiments of the invention, the diabetic subject with diabetic foot syndrome experiments a foot ulceration, such as a foot ulceration of grade III, IV or V.

As used herein, "*vascular electrical stimulation therapy (VEST)*" refers to a therapeutic treatment based on the external and non-invasive administration of low-frequency electrical pulses able to stimulate vascular smooth muscle and/or vascular endothelium in the subject. As used herein, a low frequency electrical pulses refer to an electrical periodic signal having a frequency from 0.01 to 15 Hz, preferably from 0.1 to 10 Hz such as a frequency from 0.4 to 8.5 Hz, or from 0.5 Hz to 4Hz. An appropriate frequency is for instance 1.6 ± 0.2 Hz.

It should be noted that "*vascular therapy electrical stimulation therapy*" is distinct from "*transcutaneous electrical nerve stimulation (TENS)*" in terms of delivered electrical pulses and the stimulated tissue. In the case of VEST, the target tissue is the vascular system, in particular vascular smooth muscle through the sympathetic nervous system and/or the vascular endothelium. By contrast, in TENS, the target tissue is the skeletal striated muscles through the somatic nervous system.

An electrical current appropriate to provide VEST in the subject according to the invention refers to a periodic current having a frequency and a pulse waveform able to stimulate the smooth muscle and/or the endothelium of the vascular tissue.

In some embodiments, the electrical signal does not induce a significant stimulation of motor and/or sensitive nerves.

The periodic electrical current delivered to the subject can be composed of unidirectional positive pulses or, unidirectional negative pulses. Alternatively, the periodic current is in the form of streams of pulses that are alternatively positive or negative. In some embodiments, the current delivered to the subject is composed of identical pulses.

Without to be bound by any theory, the Applicant is of the opinion that alternating unidirectional positive pulses groups and unidirectional negative pulses groups may improve the efficacy in VEST.

In preferred embodiments, the electrical current delivered to the subject consists in alternatively positive and negative streams of pulses. Each stream comprises from 2 to 20, for instance from 6 to 10 identical pulses. As an example, the periodic electrical current administered to the subject may be composed of the repetition of 8 positive pulses followed by 8 negative pulses.

The waveform of the pulses may be of any type with proviso that the duration of the pulse is of at least 0.5 ms with a rise duration (T_1) of at least 0.25 ms and a decrease duration (T_2) of at least 0.25 ms. As used herein, T_1 refers to the duration of the rise part of the pulse from the

baseline to the pulse maximum peak. T_2 refers to the duration of the decrease part of the pulse from the pulse maximum peak to the baseline. Thus, the waveform of the pulses is not squared or rectangular.

In some embodiments, the electrical periodic current delivered to the subject is characterized by one or several of, preferably all, the following features:

- the pulse has a duration (namely a base width – T_0) from 0.5 ms to 30 ms. Preferably the pulse duration is from 0.5 to 5 ms, for instance from 1.5 ms to 4 ms, or from 2.0 ms to 3.5 ms, such as 2.5 ms.
- the extinction (or decrease) duration (T_2) of the pulse is from 0.2 to 5-fold, preferably from 0.5 to 1.5-fold the rise duration (T_1) of the pulse. Preferably the two durations are equal.
- The rise duration (T_1) of the pulse is at least 0.25 ms, preferably at least 0.5 ms, such as at least 0.6, 0.8, and 1.0 ms.
- The decrease duration (T_2) of the pulse is at least 0.25 ms, preferably at least 0.5 ms, such as at least 0.6, 0.8, and 1.0 ms.
- the peak amplitude of the pulse (Umax) is from -130 V to 0 V or from 0 V to 130 V, preferably from -100 V to -10 V or 10 V to 100 V, such as from -10 V to -60 V or from 10 V to 60 V.
- the period of the electrical current is from 100 ms to 5 000 ms, for instance from 120 ms to 2200 ms such as a period from 250 ms to 2000 ms. For instance, the period of the electrical current may be about 560 ms to 720 ms such as about 625 ms

For instance, an appropriate electrical periodic current to provide VEST may be characterized by:

- a pulse duration from 1.5 ms to 4.0 ms
- a ratio of T_2 to T_1 from 0.5 to 1.5,
- a T_1 of 0.5 ms to 2.0 ms
- a T_2 of 0.5 ms to 2.0 ms
- a Umax from – 60 V to -10 V or from 10 V to 60 V, and
- a period from 560 ms to 720 ms.

In some embodiments, Umax is from 20 V to 50 V or from -50 V to -20 V. For instance, Umax is from 25 to 40 V or from -40 to -25 V such as from 30 to 35 V or from -35 to -30V.

In some additional or alternate embodiments, the maximum intensity of the current (namely the intensity at the pulse peak) is at most 100 mA. Preferably, the peak intensity is at most 90 mA,

preferably at most 80 mA. Typically, the peak intensity may range from 0.5 mA to 60 mA, more preferably from 1 to 20 mA such as from 5 to 15 mA or from 8 to 12 mA.

In a preferred embodiment, the waveform of the pulse is of the exponential type. This means that the pulse comprises an increase part and a decrease part which correspond to exponential curves. An example of a pulse of the exponential type is shown in Figure 1A. In a preferred embodiment, the electrical pulses of the current administered to the subject has :

- an rise part of formula (I) :

$$U = Umax\left(1 - e^{-\frac{t}{\tau 1}}\right) \text{ (I)}$$

- a decrease (or extinction) part of formula (II)

$$U = Umax\left(e^{-\frac{t-T1}{\tau 2}}\right)$$

wherein U refers to voltage, t refers to time, Umax is the amplitude peak of the pulse, T_1 is the duration of the rise part of the pulse and τ_1 and τ_2 refer to the time constants. In some embodiments, τ_1 is equal to τ_2. In certain embodiments, τ_1 and τ_2 is from about 0.1 to about 0.8 ms.

Preferred Umax is from 25 to 40 V, such as from 30 to 35 V.

It goes without saying that the invention also relates to the use of an electrical periodic signal having a frequency of at most 15 Hz, preferably from 0.1 to 10 Hz, to treat a subject in need thereof by vascular electrotherapy, so as to prevent, delay the onset, or treat hyperglycemia and/or insulin resistance in said subject. As described above, the subject may be selected from the group consisting of subjects suffering from insulin resistance and/or hyperglycemia, subjects having prediabetes, subjects suffering from diabetes, in particular type 2 diabetes, and patients at risk of developing type-2 diabetes. Said electrical periodic signal may be as defined above.

In the context of the invention, VEST may be performed with any medical device for delivering an electrical current able to stimulate the human vascular smooth muscle and/or the vascular endothelium in the subject though electrodes disposed on specific body area. The medical device comprises at least two electrodes, because at least two electrodes should be positioned to enable the flow of electrical current through the subject's body. The administration of the electrical pulses is preferably performed by transcutaneous route. The medical device comprises at least two electrode pads which are positioned on appropriate skin area of the subject's body. For instance, the electrode pads can be positioned on each calf or each foot of

the subject. Alternatively, the first electrode pad can be positioned on the wrist (or the hand palm) and the second electrode pad can be positioned on the opposite calf or plant arch in the subject. In another embodiment, the first electrode pad can be positioned on the wrist (or the hand palm) and the second electrode pad can be positioned on the homolateral calf or plant arch in the subject. When the subject suffers from a foot ulceration, one of the electrode is positioned on the foot with ulceration, if possible on the plant arch.

During the VEST sessions, the subject may be in a lying position, in particular in supine position.

Any medical electrotherapy device can be used in the context of the invention, with proviso that the device can deliver an appropriate electrical current as defined above. For instance, the medical device may be as described in EP 0 137 007 or in US 5,725,563, the disclosure of which being incorporated herein by reference. An appropriate medical electrotherapy device is also available on the market, namely DIAVEIN, marketed by CT Sciences SA, DIAVEIN Sàrl,

Typically, the medical device for administering the vascular electrical stimulation according to the invention comprises an electrical current generator unit including (i) a mean for generating an electrical pulse current and (ii) a mean for controlling the generated electrical current, said electrical current-generating unit being connected to at least two electrodes or electrode pads adapted to be placed on the subject, preferably on the skin, and delivering electrical stimulation to said subject. The mean for controlling the generated electrical current may enable to adjust the intensity, the shape, the frequency and/or the voltage of said current. Said controlling mean may comprise a selector unit enabling to select the frequency, the pulse waveform, the pulse duration, the pulse amplitude, the intensity and/or the energy of the current, and/or preset programs able to control the delivery of preset electrical currents to the subject.

Optionally, the medical device may comprise a measuring unit for measuring the subject-dependent electrical parameters, such as impedance, and a processing unit in communication with said measuring unit and the electrical current generator unit, whereby the electrical current delivered to the subject can be optionally adjusted depending on the measured subject-dependent electrical parameters.

The electrical current to deliver to the subject, may be adjusted depending on the therapeutic effect to achieve (e.g. the regulation of glycemia in a subject suffering from prediabetes or diabetes), the severity of the disorder to treat to be treated and the sensitivity of the subject to the electrical stimulation

In particular, the pulse peak amplitude (Umax) may vary from one subject to another, depending on the subject impedance and the subject sensitivity to electrical stimulation. The subject

impedance may vary from 0.5 kΩ to 4 kΩ, typically from 1.5 to 2.5 kΩ. The higher impedance, the higher Umax. For instance, Umax of the electrical pulse is typically less than 50V for an impedance of 2 kΩ measured between the electrodes. Preferably, a therapeutic electrical current corresponds to an electrical current able to induce a small muscle tremor at each electrode disposed on the subject. The physician may determine the appropriate voltage of the current to deliver to the subject by gradually increasing the voltage of the electrical current while observing the reaction of the subject. At the beginning of the treatment, the physician may gradually increase the intensity of the electrical stimulations until the subject feels them. Then, the physician may let the subject subjected to said current during few minutes, for instance 10 minutes, to check that the subject does not feel any disorder or significant discomfort. The physician may then increase the stimulation until a very slight muscle tremor is observable at the skin area in the contact with the electrodes. If the subject complains about the stimulation, the physician decreases the energy level so as to obtain an electrical stimulation which is comfortable for the subject while providing a small muscle tremor at the electrodes

At the second session, the physician can adjust the energy level of the electrical stimulation at the level previously identified as providing muscle tremor in subjects while being comfortable.

The regimen of the treatment depends on the therapeutic effect which is sought.

Typically, in order to treat hyperglycemia, decrease insulin resistance and/or to treat a pathological disorder associated with diabetes such as diabetic foot syndrome, the subject is subjected to at least one VEST treatment session every days or every two days, during at least one week, such as during at least 2, 3, or 4 weeks and even during at least 1, 2, 3 or 4 months, andor until the therapeutic effect sought is achieved.

The daily duration of VEST depends on the therapeutic effect which is sought. The total duration of VEST therapy per day may vary from a subject to another. However, a total daily VEST duration of at most 4 hours, such as a duration of at most 3 hours or 2 hours may be sufficient for most of the subjects.

Typically, the subject is subjected to one or several VEST sessions per day. Each treatment session may last from few minutes to few hours, typically from 10 minutes to four hours. In some embodiments, the subject is subjected to one treatment session daily or every two days, said session lasting from 10 minutes to two hours, for instance from 30 min to 60 min, such as about 45 min. However, longer treatment sessions and/or higher frequency of treatment can be used for the treatment of diabetic foot syndrome, especially in case of foot ulceration of high

grade. A typical treatment protocol to implement the instant invention comprises a VEST session of about 30 min to 1 hour every day or every two days during at least 4 weeks.

The positions of the electrodes can change at each treatment session.

For illustration, the following regimen can be performed:

The VEST treatment may comprise three treatment sessions per week. The treatment sessions are performed every two days. Each treatment session lasts from 30 min to 60 min, e.g. 45 min.

In the first treatment, the first electrode is placed on the calf or the foot (such as the plant arch), and the second electrode is positioned on the contralateral calf or foot of the subject.

In the second treatment session, the first electrode is positioned on the wrist or the foot (e.g. the plant arch), and the second electrode is positioned on the contralateral forearm or wrist of the subject.

In the third treatment session, the first electrode is positioned on the other calf or foot, and the second electrode is positioned on the contralateral forearm or wrist of the subject.

This treatment cycle can be repeated over several weeks (2, 3 or 4) and even over several months (e.g. 1, 2, 3 or 4 months).

This treatment regimen may be used to treat subjects suffering from diabetes, in particular type-2 diabetes, mainly in order to regulate or decrease blood glycemia and/or decrease insulin resistance in the subject. This treatment regimen can be also used to treat subjects with prediabetes or at risk of developing prediabetes or diabetes, but regimen with shorter VEST sessions and/or with a lower frequency of sessions can be used as well.

The duration and the frequency of VEST sessions can be also adapted during the treatment depending on the response of the subject to the patient. For instance, the duration and/or the frequency of VEST sessions can be increased, in order to improve the regulation of glycemia in the subject.

When the subject has a wound or an ulceration on a foot, the first electrode is preferably positioned on this foot, typically on an intact skin area of the plant arch. In such a case, the patient is typically a diabetic patient suffering from a foot wound or ulceration such as diabetic foot. In such a case, the VEST treatment also enables to promote healing. Accordingly, the VEST treatment of the invention is also used for treating a wound or an ulceration in a subject suffering from diabetes. Typically wounds or ulcerations are foot wounds and ulcerations as observed in diabetic foot syndrome.

As illustrated in Example 1, VEST is able to decrease hyperglycemia in patients with diabetes who were not treated with any antidiabetic drugs. In some other embodiments, the VEST is the sole treatment applied to the subject to treat or prevent insulin resistance, hyperglycemia, and/or

diabetes or prediabetes in said subject. In other word, the subject is not administered with any antidiabetic drug such as metformin or insulin during the treatment period with VEST. In a particular embodiment, said patients have been diagnosed with a capillary glycemia in fasting conditions of more than 2.0, such as more than 2.5, e.g. more than 3.0 g of glucose per liter. When used alone, VEST is not likely to expose the patient to a risk of hypoglycemia contrary to insulin therapy. Thus, VEST alone can enable to regulate glycaemia while avoiding the daily control of glycemia, which is a real benefit for the patients, in particular those who do not have access to glucose meter.

In some other embodiments, depending on the severity of prediabetes, diabetes and associated disorders in the subject, VEST may be combined with drug therapy. Drug therapy encompasses, without being limited to, alpha-glucosidase inhibitors such as acarbose and miglitol, biguanides such as metformin, DPP-4 inhibitors such as sitagliptin, linagliptin or alogliptin, meglitinides such as repaglinide and nateglinide, sodium glucose transporter 2 inhibitors such as dapagliflozin or empagliflozin, sulfonylureas such as glimepiride and thiazolidinediones such as rosiglitazone and pioglitazone, and combinations thereof. VEST can be also combined with insulin administration.

In certain embodiment, VEST is used in combination with an insulin sensitizer, preferably selected from a biguanides or a thiazolidinedione, for instance metformin, to treat a subject suffering from diabetes, preferably type-2 diabetes or prediabetes.

In another embodiment, VEST is used in combination with insulin for treating a subject suffering from diabetes, preferably type-2 diabetes.

In an additional embodiment, VEST is used in combination with insulin or biguanide for treating a diabetic subject having a foot wound or ulceration such as a diabetic foot of grade III, IV or V.

Without to be bound by any theory, the Applicant is of the opinion that VEST potentiates the effect of insulin and insulin sensitizers, whereby the daily doses of such drug to administer to the subject can be decreased.

In particular, the Applicant is of the opinion that VEST increases the efficacy of insulin, by decreasing the resistance to insulin in diabetic patients. The Applicant is also of the opinion that VEST enables to promote the healing of wounds and ulcers in diabetic patients.

Such effects are shown in Examples 2 and 3.

Examples 3 shows that the use of VEST in combination with insulin significantly improves the control of glycemia, as compared to insulin alone, in particular in subject with severe glycemia.

Example 2 shows that the insulin daily dose to use to control glycemia can be significantly reduced in diabetic subjects.

Thus, in a particular aspect, the invention relates to the use of insulin in combination with VEST to treat a diabetic subject and/or to regulate glycaemia in a diabetic subject.

The diabetic subject may have an initial capillary glycemia of more than 2.00, e.g. of more than 2.50 g of glucose per liter in fasting conditions.

The diabetic subject may further suffer from diabetic foot syndrome and/or diabetic peripheral neuropathy.

Typically, VEST may enable to decrease the daily dose of insulin by at least 30%, especially in diabetic patients diagnosed with an initial capillary glycemia of more than 2.00, e.g. of more than 2.50 g of glucose per liter.

For instance, the physician can first determine the daily dose of insulin needed to regulate capillary glycemia in fasting conditions at a level of about 1.1 g of glucose par liter of blood in the patient. Then, the patient is being administered with a daily dose of insulin reduced by at least about 30% while being subjected to VEST treatment, as described above. For instance, the subject may be subjected to VEST session of about 30 min to 1 hour every day or every two days. The glycemia is controlled once a week. If the glycemia is stable or decreased overtime, the dose of insulin is reduced and can even be stopped, the subject being treated with VEST only. As illustrated in Example 2, the VEST enables to compensate the decrease of the daily dose of insulin and promotes healing in diabetic subjects with lower limb ulcer.

Once the therapeutic effect is achieved, e.g. when the glycaemia is decreased and stabilized and/or when a diabetes-associated disorder such as diabetic foot syndrome is improved, the subject may be subjected to a maintenance VEST treatment. In the maintenance treatment, the same regimen as that used in the therapeutic phase can be performed. Alternatively, the frequency and/or the daily duration of VEST can be decreased during the maintenance treatment. For instance, the subject can be subjected to one or two VEST sessions every weeks, during at least 1, 2, 3, or 4 months. In some embodiments, the subject is not administered with an antidiabetic drug therapy, e.g. insulin, during the maintenance treatment. In other embodiments, the antidiabetic drug, e.g. insulin, is also administered in the maintenance treatment, but at a lower dose.

The daily dose of insulin to administer to the subject during the first treatment and/or the maintenance treatment may be from 5 IU to 40 IU, e.g. from 8 IU to 35 IU. The daily dose to administer to the subject is to determine in view of the general condition of the subject and is to be reassessed in view of the responsiveness to VEST during the treatment.

Without to be bound by any theory, the Applicant is of the opinion that VEST may treat or reduce one or several additional disorders associated with hyperglycemia and diabetes.

In that respect, the Inventors is of the opinion that treatment with VEST in subjects suffering from prediabetes and diabetes enables to improve their general condition by treating, alleviating or delaying the onset of damages on several organs generally caused by hyperglycemia, such as neurological damages, cardiovascular damages, retinopathy, damages to kidney, legs and feet.

Accordingly, the invention also relates to a method for improving the general condition, or for treating one or several disorders associated with hyperglycemia, in a subject suffering from diabetes, wherein the subject is subjected to a long-term treatment with VEST.

As used herein, a long-term treatment with VEST, means that VEST is chronically administered to the subject during at least 3 months, preferably during at least 6 months, 12 months and even several years.

Typically, the subject is subjected to a VEST treatment in particular as described above, at least once a month, preferably at least once a week. In preferred embodiments, the subject is subjected to one session of 10 min to 1 hour, preferably of 20 min to 50 min, every two days.

In some embodiments, the long-term treatment is for treating, alleviating, or for delaying the onset of one or several disorders caused, or associated with, diabetes or hyperglycemia, such as retinopathy, nephropathy, microvascular or macrovascular disorders, neuropathy including peripheral neuropathy, diabetic foot syndrome and combinations thereof.

At last, the invention also relates to insulin or an insulin sensitizer for use in combination with VEST for treating diabetes, especially type-2 diabetes. As explained, VEST enables to potentiate the effect of insulin and/or to reduce insulin resistance in the subject, whereby the daily dose of insulin or insulin sensitizer to administer to regulate glycemia is decreased in the subject. VEST treatment is performed as described above, for instance by subjecting the subject to VEST session of about 30 min to one hour every day or every two days. In some embodiments, the subject has been diagnosed as suffering from a type-2 diabetes with a capillary glycemia in fasting conditions of at least 2.00, e.g. of at least 2.50 g of glucose per liter of blood.

In some further embodiments, the subject also suffers from a lower limb or foot ulcer or wound, such as a diabetic foot ulcer of grade III, IV or V, and/or from diabetic neuropathy symptoms such as numbness, reduced sensitivity, cramps, pains, tingling or burning sensations, in particular in the hands and feet, and erectile dysfunction.

The daily dose of insulin can be from 5 IU to 40 IU, e.g. from 8 IU to 35 IU. The daily dose to administer to the subject is to determine in view of the general condition of the patient and is to be reassessed in view of the responsiveness to VEST .

The following examples are provided by way of illustration only and not by way of limitation.

EXAMPLES

Material and method for implementing VEST

a. General protocol of Vascular Electrical Stimulation Therapy (VEST)

The Vascular Electrical Stimulation Therapy was performed by using a conventional medical device for vascular electrical stimulation therapy comprising four electrodes (Diavein). The subjects are subjected to VEST every two days. Each VEST session lasts 45 min. During the treatment sessions, the subject is in supine position.

The clinical dose of electrical stimulation refers to an electrical stimulation sufficient to induce a small muscle tremor at each electrode while being comfortable for the subject. The treatment is carried out very gradually as regards to the increase in energy. At the beginning of the treatment, the physician gradually increases the electrical stimulations until the subject feels them. Then, the physician lets 10 minutes elapse to ensure that the subject feels no disorder or significant side effects. The physician then increases the stimulation until a very slight muscle tremor in tissues located in the contact of the electrodes is observable. If the subject complains about the stimulation, the physician decreases the energy level so as to obtain an electrical stimulation which is comfortable for the subject.

At the second session, the physician can adjust the energy level of the electrical stimulation at the dose identified as providing muscle tremor in subjects while being comfortable (clinical dose).

b. Medical device and electrical current delivered to the subject

The medical device used to deliver the Vascular Electrical Stimulation Therapy is a Diavein device commercialized by the company CT Sciences SA (Switzerland). Typically the current delivered to the subject ranges from 30 to 35V at a 1.6Hz on a 2000 to 2500Ω impedance measured between the electrodes. The intensity is from 8 to 12 mA. The electrical pulse of the signal has an exponential-type waveform as defined in Figure 1A. The duration of the pulse is from 2.8 ms to 3.5 ms. The electrical current comprises unidirectional positive pulses group followed by unidirectional negative pulses group.

EXAMPLE 1: Treatment with VEST in diabetic patients upon detection of hyperglycaemia

Three patients previously diagnosed with type-2 diabetes but who did not take any medical treatment were treated with VEST upon detection of hyperglycemia by capillary glycaemia test in fasting conditions with a glucose meter (Accu-Check Roche Diagnostic).

The VEST protocol comprised a cycle of three treatment sessions, which are repeated:

- session 1 (on day 1): each electrode was positioned on a calf
- session 2 (on day 3): the first electrode was positioned on the right calf and the second electrode was positioned on the left wrist in the subject, and
- session 3 (on day 5): the first electrode was positioned on the left calf and the second electrode was positioned on the right wrist in the subject.

During the protocol, the glycaemia was measured with capillary glycaemia test in fasting conditions using a glucose meter (Accu-Check Roche Diagnostic), if possible once a week.

VEST was the sole treatment used to regulate glycaemia in the patients. The patients were not treated with antidiabetics drug or insulin. The treatment with VEST protocol began on the day on which the first measure of glycaemia was performed.

As shown below, VEST significantly decreased the glycaemia in the patients. The decrease was detectable after two weeks of treatment only.

- Patient n°1: male of 42 years old

The variation of capillary glycaemia in fasting conditions overtime is shown in the below table:

Date	Capillary glycaemia (g/l)
Feb. 21, 2019	3.32
March 18, 2019	2.75
March 28, 2019	2.19
March 31, 2019	1.93
Total variation of glycemia at the end of the protocol: -1.39 g/l (- 42%)	

Table 1: Glycaemia variation in patient 1

- Patient n°2: male of 38 years old

The variation of capillary glycaemia in fasting conditions overtime is shown in the below table:

Date	Capillary glycaemia (g/l)
March 6, 2019	4.48
March 13, 2019	4.45
March 20, 2019	3.38
March 37, 2019	3.19
Total variation of glycaemia at the end of the protocol: - 1.29 g/l (29%)	

Table 2: Glycaemia variation in patient 2

- Patient n°3 : male of 64 years old

The variation of capillary glycaemia in fasting conditions overtime is shown in the below table:

Date	Capillary glycaemia (g/l)
March 7, 2019	3.45
March 14, 2019	3.21
March 21, 2019	2.19
March 28, 2019	2.15
Total variation of glycaemia at the end of the protocol:-1.30 g/l (- 39%)	

Table 3: Glycaemia variation in patient 3

EXAMPLE 2: Treatment of patients suffering from diabetic foot of high grade with VEST

- Protocol of VEST

The VEST protocol comprises a cycle of three treatment sessions, which are repeated at least until an effective healing of diabetic foot is observed:

- session 1 (on day 1): the first electrode is positioned on the plantar arch and the second electrode is positioned on the contralateral calf in the subject,
- session 2 (on day 3): the first electrode is positioned on the plantar arch and the second electrode is positioned on the homolateral forearm in the subject, and
- session 3 (on day 5): the first electrode is positioned on the plantar arch and the second electrode is positioned on the contralateral forearm in the subject.

When the subject suffers from a foot ulceration or wound, the first electrode is preferably positioned on said foot.

During the VEST protocol, the glycaemia was measured with capillary glycaemia test in fasting conditions using a glucose meter (Accu-Check Roche Diagnostic), if possible once a week.

VEST was used in combination with insulin to regulate glycaemia in the patients. The treatment with VEST protocol began on the day on which the first measure of glycaemia was performed. As shown below, VEST significantly decreased both glycaemia and insulin uptake in the patients.

Furthermore, the combined therapy was effective to promote ulcer healing as shown in Figure 5 which illustrates the healing process overtime in four patients.

- Patient n°4: female of 75 years old

Daily insulin injection of 22 UI in the morning and 16 UI in the evening were given during the first 5 weeks. Insulin injection was reduced from the 6th week to 16 UI in the morning and 14 UI in the evening.

The variation of capillary glycaemia in fasting conditions overtime is shown in the below table:

Date	Capillary glycaemia (g/l)
1st week	3.20
2nd week	2.37
3rd week	2.34
4th week	1.87
5th week	1.51
6th week	1.38
Total variation of glycemia at the end of the protocol between the 2nd and the 6th week: -1 g/l (- 42%) despite the reduction of insulin uptake by 21%	

Table 4: Glycaemia variation in patient 4

- Patient n°5: male of 73 years old

Daily insulin injection of 12 UI in the morning and 10 UI in the evening were given during the first 5 weeks. Insulin injection was reduced from the 4th week to 8 UI in the morning and 8 UI in the evening.

The variation of capillary glycaemia in fasting conditions overtime is shown in the below table:

Date	Capillary glycaemia (g/l)
1st week	3.05
2nd week	2.21

3rd week	2.04
4th week	1.31
5th week	0.82
Total variation of glycemia at the end of the protocol between the 2nd and the 5th week: -1,39 g/l (- 62%) despite the reduction of insulin uptake by 27%	

Table 5: Glycaemia variation in patient 5

- Patient n°6: female of 53 years old

Daily insulin injection of 16 UI in the morning was given during the first 3 weeks. Insulin injection was reduced from the 4th week to 12 UI in the morning and from the 5th week to 8 UI. The variation of capillary glycaemia in fasting conditions overtime is shown in the below table:

Date	Capillary glycaemia (g/l)
1st week	4.89
2nd week	2.93
3rd week	2.47
4th week	1.66
5th week	1.26
6th week	0.84
Total variation of glycemia at the end of the protocol between the 2nd and the 6th week: -2.09 g/l (- 71%) despite the reduction of insulin uptake by 50%	

Table 6: Glycaemia variation in patient 6

- Patient n°7 : patient with a diabetic foot ulcer of grade V

A patient with a diabetic foot ulcer of grade V was treated with VEST in combination with insulin. Figure 3 shows the ulcer before (A) and after treatment (B) with VEST. Before the treatment with VEST, the necrosis was severe, resulting in amputation of four limbs. Treatment with VEST promoted epithelium recolonization of the ulcer and healing. After few weeks of treatment with VEST, the healing was complete and the foot amputation avoided.

EXAMPLE 3: clinical trial to assess the efficacy of VEST in combination with insulin to manage hyperglycemia

This study was conducted on 8 patients divided into two groups :

- Control group: 4 patients receiving insulin only (Control)

- VEST group: 4 patients treated with VEST and insulin. VEST treatment was performed 3 times a week (duration : 45 min) according to the general protocol described in Example 2.

The glycaemia was measured with capillary glycaemia test in fasting conditions using a glucose meter (Accu-Check Roche Diagnostic).

The results are shown in the below tables:

	1st week		2nd week		3rd week		4th week	
Control group	Glycemia	Insulin	Glycemia	Insulin	Glycemia	Insulin	Glycemia	Insulin
C1	169	10	208	10	273	12	206	12
C2	198	12	180	12	189	10	214	10
C3	248	12	224	12	238	12	172	12
C4	281	12	181	12	164	12	221	12
Average	**224**	12	198	12	216	12	**203**	12

Table 7: glycemia (mg/dl) and daily insulin intake (IU) in patients in the control group overtime. The value is an average of capillary blood glycemia performed 3 times a week.

	1st week		2nd week		3rd week		4th week	
VEST group	Glycemia	Insulin	Glycemia	Insulin	Glycemia	Insulin	Glycemia	Insulin
V1	239	30	240	30	159	30	260	22
V2	263	10	190	10	259	10	119	10
V3	346	10	257	10	274	10	114	10
V4	175	34	129	34	155	34	129	34
Average	**256**	21	204	21	212	21	**156**	19

Table 8: glycemia (mg/dl) and insulin intake (IU) in patients in the VEST group overtime. The value is an average of capillary blood glycemia performed 3 times a week.

The average of glycemia was reduced of 100 mg/dl of glucose while the average of insulin uptake was slightly reduced from 21 to 19 units within four weeks in the VEST group. In contrast, the glycemia average was slightly reduced (-21 mg/dl of glucose) with a constant insulin uptake in the control group.

3 out of 4 patients have a regulated glycemia in the VEST group. The glycemia in the fourth patient (V1) is not totally controlled but a significant decrease in insulin uptake is observed in the 4th week.

None of the control patients have a regulated glycemia at the fourth week of treatment despite the fact that the initial average glycemia was significantly higher in the VEST group as compared to the control group (256 mg/dl vs 224 mg/dl in the first week).

Of note, improvements in neuropathy symptoms such as reduced sensitivity, cramps and pains in the feet, legs, arms and hands and erectile dysfunction were reported in the VEST group, but not in the control group.

Claims

1. Insulin for use in combination with vascular electrical stimulation therapy (VEST) for treating diabetes, preferably type-2 diabetes, in a subject.

2. Insulin for use according to claim 1, wherein the subject has been diagnosed with a capillary glycemia in fasting condition of at least 2.00, preferably of at least 2.5 g glucose per liter of blood.

3. Insulin for use according to claims 2 or 3, wherein the subject further suffers from a diabetic foot syndrome, a lower limb ulcer or wound, and/or from peripheral neuropathy.

4. Insulin for use according to any one of claims 1 to 3, wherein insulin is daily administered to the subject, preferably at a daily dose which is at least 20%, preferably at least 25, 30, 35, 40, 45, 50 or even 55% lower than the daily dose to administer to the subject to lower the capillary glycemia in fasting conditions at a level of about 1.10 g of glucose per liter, in the absence of VEST treatment.

5. A method for treating, decreasing or delaying the onset of hyperglycemia or insulin resistance in a subject in need thereof, which comprises subjecting the subject to vascular electrical stimulation therapy (VEST).

6. The method of claim 5, wherein the subject has a capillary glycemia in fasting conditions of at least 1.00 g of glucose per liter of blood.

7. The method of claim 5 or 6, wherein the subject is diabetic.

8. The method of claim 5 or 6, wherein the patient is prediabetic and/or at risk of developing type-2 diabetes.

9. The method of any one of claims 5-8, wherein:
   - The subject is not treated with antidiabetics drug during the treatment with VEST and/or
   - The subject is cotreated with insulin during the treatment with VEST.

10. The method of claim 5, wherein the subject suffers from type-2 diabetes and has been diagnosed with a capillary glycemia in fasting conditions of at least 2.00, 2.50 g of glucose per liter of blood.

11. The method of claim 10, wherein the patient further suffers from diabetic foot syndrome and preferably has a lower limb wound or ulcer, such as a foot ulcer of grade IV or V.

12. The method of claims 10 or 11, wherein the patient is further treating with an antidiabetic drug such as insulin or an insulin sensitizer.

13. A method for improving the general condition in a subject suffering from diabetes or prediabetes, wherein the subject is subjected to a long-term treatment with VEST, preferably over at least three months.

14. The method according to any one of claims 5-13 or insulin for use according to any one of claims 1-4, wherein the patient is administered with an electrical periodic current having a frequency from 0.01 Hz to 15 Hz.

15. The method according to claim 14 or insulin for use according to claim 14, wherein the periodic current is composed of electric pulses, the duration of each pulse being of at least 0.5 ms with a rise duration (T_1) of at least 0.25 ms and a decrease duration (T_2) of at least 0.25 ms.

16. The method according to claim 14 or 15, or insulin for use according to claim 14 or 15, wherein the electrical periodic current administered to the patient is composed of unidirectional positive pulses, unidirectional negative pulses or streams of pulses which are alternatively negative or positive.

17. The method according to any one of claims 14-16, or insulin for use according to any one of claims 14-16, wherein the electrical periodic current administered to the subject is composed of pulses and has at least one of the following features :

- the extinction duration (T_1) of the pulse is from 0.2 to 5-fold the rise duration (T_2)of the pulse,

- The rise duration (T_1) is of at least 0.25 ms and the decrease duration (T_2) is of at least 0.25 ms
- the peak amplitude of the pulse is from -130 V to 130 V,
- the base width of the pulse is from 0.5 ms to 30 ms, and
- the period of the electrical current is from 100 ms to 5000 ms.

18. The method according to any one of claims 14-17, or insulin for use according to any one of claims 14-17, wherein the pulses of the electrical periodic current have an exponential-type waveform.

19. The method according to any one of claims 14-18, or insulin for use according to any one of claims 14-18, wherein the electrical periodic current is administered to the patient by transcutaneous route.

20. The method according to any one of claims 14-19, or insulin for use according to any one of claims 14-19, wherein the electrical periodic current is administered to the patient by a medical device for electrotherapy, comprising at least two electrodes disposed on the skin of the patient.

21. The method according to claim 20, or insulin for use according to claim 20, wherein the medical device for electrotherapy comprises two electrodes, each electrode being positioned on a skin area selected from the forearms, the wrists, the calves and the feet of the subject.

22. The method according to any one of claims 5-21 or insulin for use according to any one of claims 1-4 and 14-21, wherein VEST is applied to the subject at least every two days and each VEST session lasts from 30 min to 1 hour.

23. Electrical periodic current as described in any one of claims 14-18 for use in treating, decreasing or delaying the onset of hyperglycemia or insulin resistance in a subject in need thereof, by VEST.

24. The electrical periodic current for use according to claim 23, wherein said current is administered to the subject as described in any one of claims 19 to 22.

25. The electrical periodic current for use according to claim 23 or 24, wherein patient is diabetic, preferably type-2 diabetic, or prediabetic and/or at risk of developing type-2 diabetes

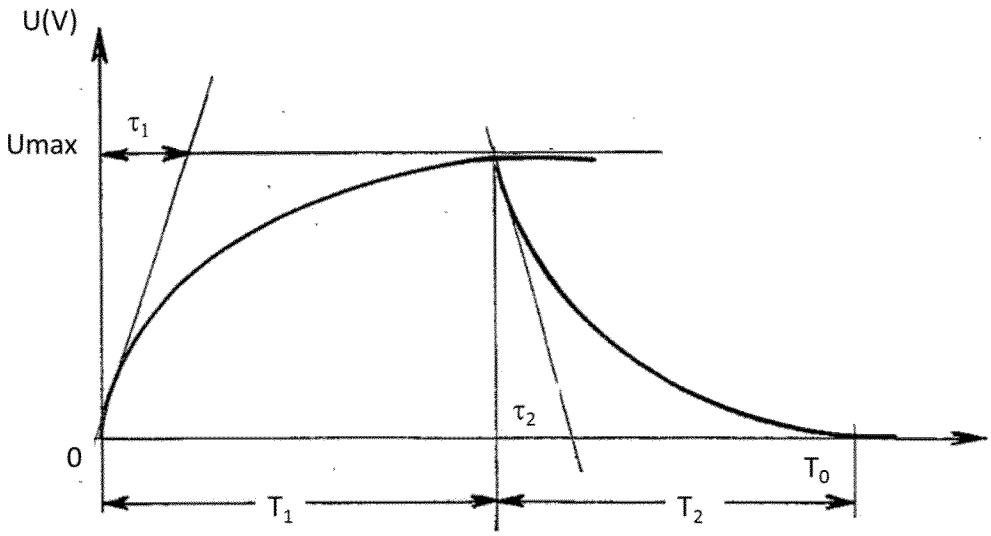


FIGURE 1A

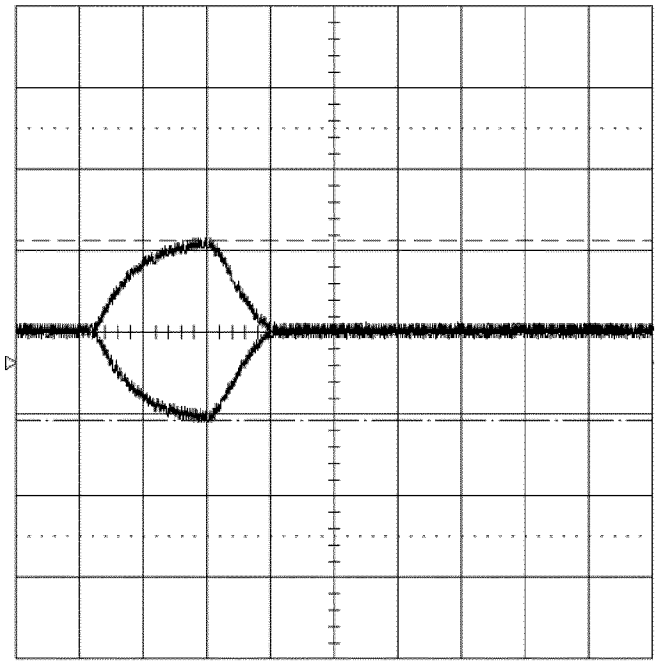


FIGURE 1B

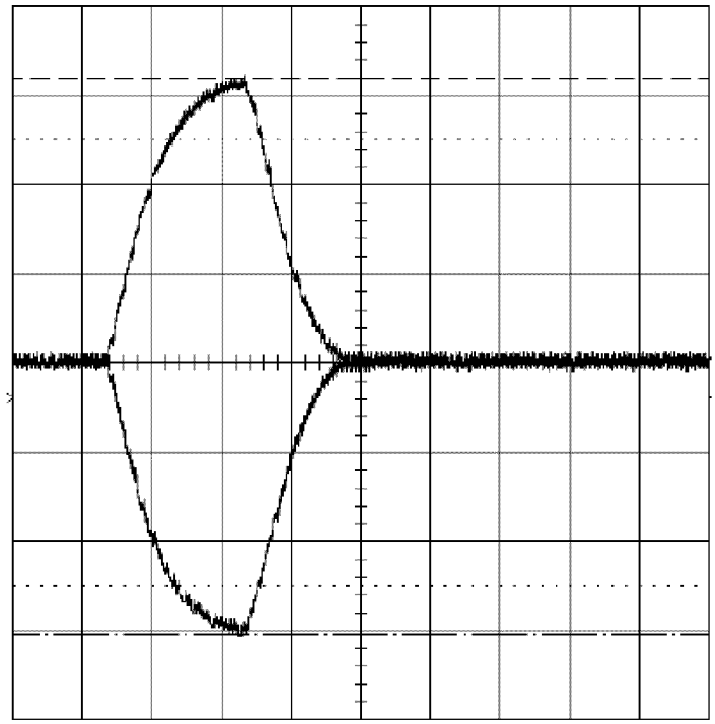
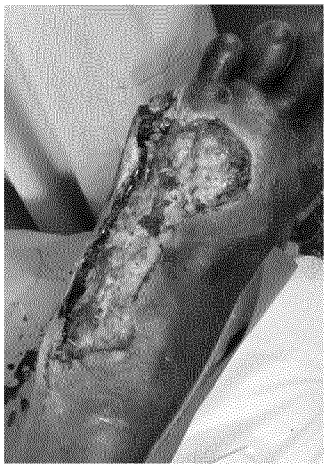
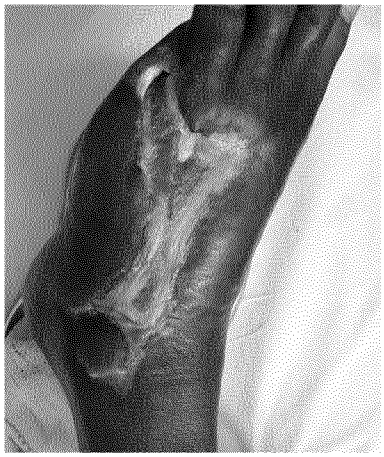


FIGURE 1C

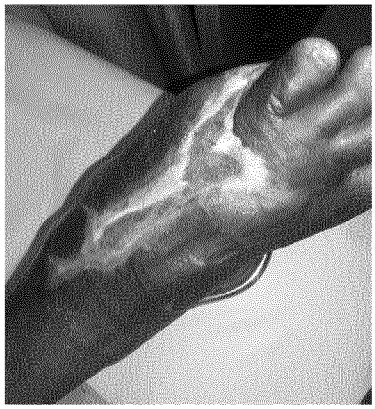
Patient n°1



Ulcer at Day 0

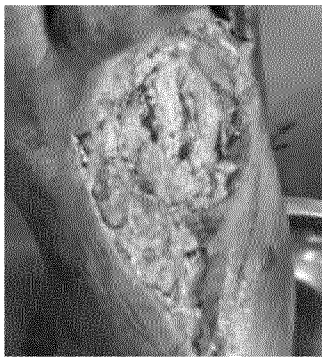


After 6 weeks of treatment

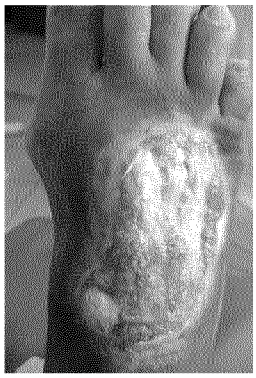


After 12 weeks of treatment

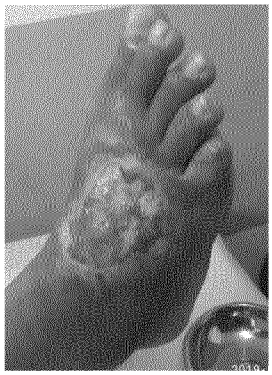
Patient n°2



Ulcer at Day 0



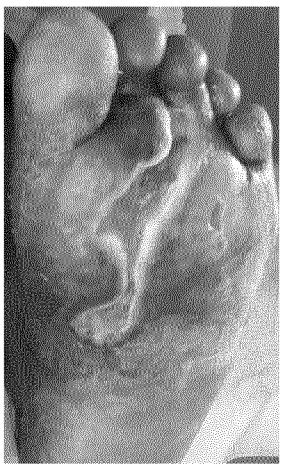
After 6 weeks of treatment



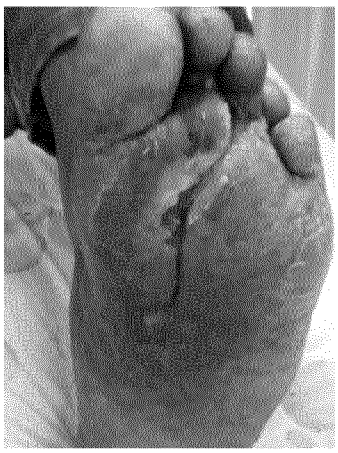
After 12 weeks of treatment

FIGURE 2

Patient n°3

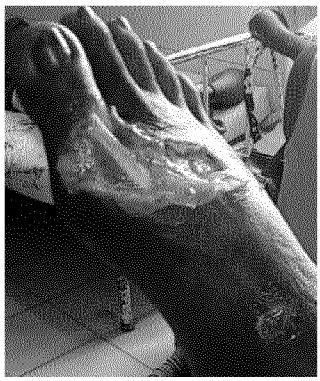


Ulcer at Day 0

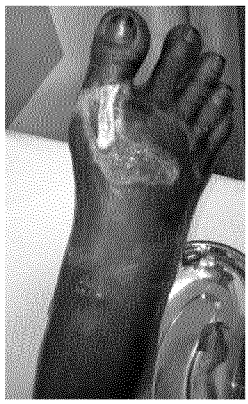


After 6 weeks of treatment

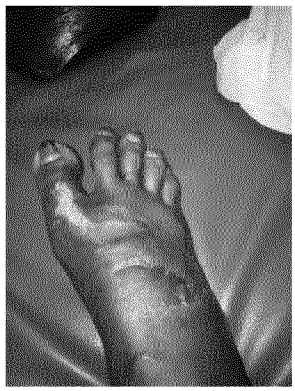
Patient n°4



Ulcer at Day 0



After 6 weeks of treatment



After 12 weeks of treatment

FIGURE 2 (following)

Patient n°7

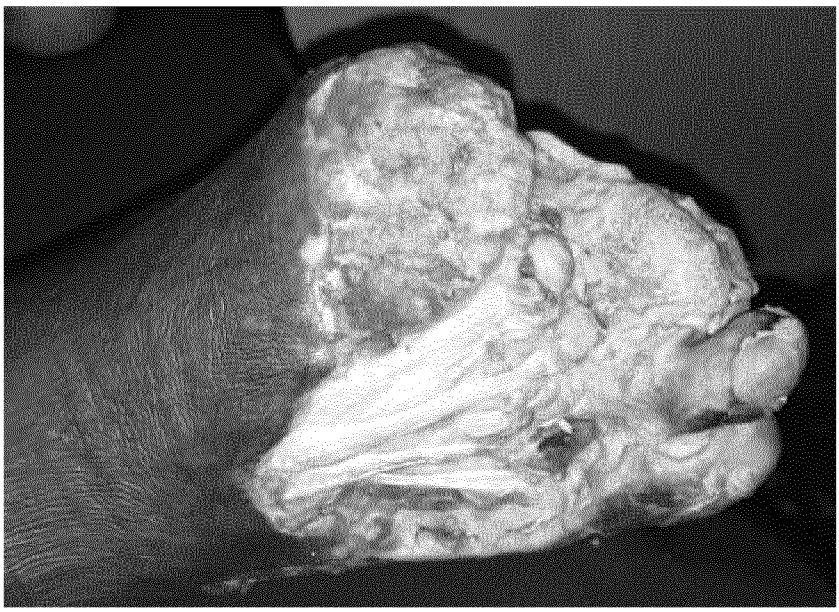


Figure 3A

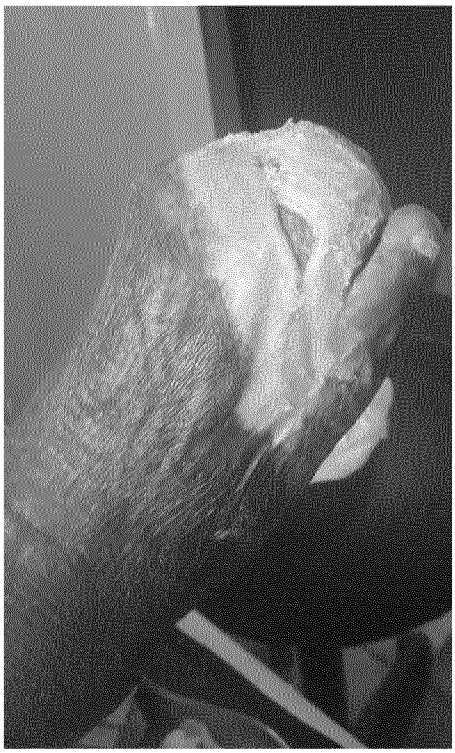


Figure 3B

FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/062132

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/28 A61P3/10 A61N1/32 A61N1/36
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, SCISEARCH, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PETERS E J G ET AL: "The benefit of electrical stimulation to enhance perfusion in persons with diabetes mellitus", JOURNAL OF FOOT AND ANKLE SURGERY, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 37, no. 5, 1 September 1998 (1998-09-01), pages 396-400, XP025985069, ISSN: 1067-2516, DOI: 10.1016/S1067-2516(98)80048-3 [retrieved on 1998-09-01]	13,19
Y	methods, results, discussion ----- -/--	1-25

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2020	07/08/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Venturini, Francesca

3

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TOSHIAKI MIYAMOTO ET AL: "Effect of percutaneous electrical muscle stimulation on postprandial hyperglycemia in type 2 diabetes", DIABETES RESEARCH AND CLINICAL PRACTICE, AMSTERDAM, NL, vol. 96, no. 3, 3 January 2012 (2012-01-03), pages 306-312, XP028490853, ISSN: 0168-8227, DOI: 10.1016/J.DIABRES.2012.01.006 [retrieved on 2012-01-06] results, discussion	1-25
X	L. L. BAKER ET AL: "Effects of Electrical Stimulation on Wound Healing in Patients With Diabetic Ulcers", DIABETES CARE, vol. 20, no. 3, 1 March 1997 (1997-03-01), pages 405-412, XP055717179, US ISSN: 0149-5992, DOI: 10.2337/diacare.20.3.405 results, discussion	3
Y	Shweta Shenoy ET AL: "Effect of electrical stimulation on blood glucose level and lipid profile of sedentary type 2 diabetic patients.", , 11 October 2010 (2010-10-11), XP55717299, DOI: 10.4103/0973-3930.70859.Source: Retrieved from the Internet: URL:https://www.researchgate.net/publication/47671924_Effect_of_electrical_stimulation_on_blood_glucose_level_and_lipid_profile_of_sedentary_type_2_diabetic_patients [retrieved on 2020-07-22] methods, results	1-25
Y	CHRISTIAN ELLUL ET AL: "The Effectiveness of Calf Muscle Electrostimulation on Vascular Perfusion and Walking Capacity in Patients Living With Type 2 Diabetes Mellitus and Peripheral Artery Disease", INTERNATIONAL JOURNAL OF LOWER EXTREMITY WOUNDS, vol. 16, no. 2, 15 June 2017 (2017-06-15), pages 122-128, XP055717337, US ISSN: 1534-7346, DOI: 10.1177/1534734617705253 results, discussion	1-25

-/--

3

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ABRAHAM PIERRE ET AL: "Calf muscle stimulation with the Veinoplus device results in a significant increase in lower limb inflow without generating limb ischemia or pain in patients with peripheral artery disease", JOURNAL OF VASCULAR SURGERY, ELSEVIER, AMSTERDAM, NL, vol. 57, no. 3, 9 January 2013 (2013-01-09), pages 714-719, XP028985511, ISSN: 0741-5214, DOI: 10.1016/J.JVS.2012.08.117	13,19
Y	methods, results -----	1-25
A	WO 2012/055967 A2 (NOVO NORDISK AS [DK]; JOHANSEN THUE [DK]) 3 May 2012 (2012-05-03) claims -----	1-25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2020/062132

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012055967 A2	03-05-2012	AU 2011322495 A1	11-04-2013
		AU 2016202917 A1	26-05-2016
		CA 2815419 A1	03-05-2012
		CN 103167878 A	19-06-2013
		EP 2632478 A2	04-09-2013
		KR 20130139297 A	20-12-2013
		MX 366314 B	05-07-2019
		PL 2632478 T3	31-03-2020
		SI 2632478 T1	29-11-2019
		US 2013261051 A1	03-10-2013
		WO 2012055967 A2	03-05-2012