# A CLINICAL RESEARCH, WITH A LABORATORY EVALUATION, TO ASSESS THE EFFICACY AND HARMLESSNESS OF A WEIGHT REDUCING TREATMENT

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Summary
A well known obesity center, located in Rome but with several branches in many Italian Regions, after more than twenty years of excellent results has asked us to settle a research protocol to assess the cellular and molecular mechanisms on which their results are based. We have developed a clinical research, authorized by the ethical committee of the University of Rome, respecting all the good clinical practices even if the protocol did not include pharmacological treatments but a combinated surface electrophoresis and dietetic treatment.
Our results indicate that the treatment is harmless and improves several metabolic markers. Anyway, the reduced dimension of the sample does not allow to draw definitive conclusions about the mechanism of action of the treatment, so that further work is needed – with a larger and more homogeneous sample – to achieve definitive answers.

## Introduction

The World Health Organization (WHO – OMS) has recently defined obesity as a “global epidemy”. In Europe, the percentage of overweight (BMI>25) and of obese (BMI>30) individuals has reached the values of 40% and 15%, respectively. Together with the increase of the number of obese people and of BMI values, an increase of secondary pathologies such as type II diabetes (NIDDM), glucose intolerance, insulin resistance, hyperlipidemia, hypertension, that concur to increase the cardiovascular risk has been observed. At the same time, many people feel obesity as an unaesthetic condition and do everything to reduce weight. Both conditions have lead to the growth of many “obesity centers” that promise more or less miraculous results. The major part of these centers act only on “experience”, in certain cases really of few time, and hardly can be found a scientific basis for the treatments they perform.
One of these centers, DCD, active since more than twenty years with excellent results, has asked us to settle a research protocol able to give scientific answers to their results. The treatment performed by DCD is based on two arms: the New Electrosculpture and the Dietetic Treatment.
The New Elettrosculpture DCD is a machinery for aesthetic medicine used since several years by the group DCD as a support to improve the loss of the weight and especially regionalized lipodystrophy. The treatment with the New Elettrosculpture is based on a total body surface electrophoresis that uses a continuous low amperage flow (from 2,2 to 6,5 milliampere). The treatment is carried out by “wrapping” the subject with bands of pure anallergic cotton moisturized with water and covered by special bands of grafitated rubber to allow electric conduction. Each session of the treatment lasts 30-35 minutes, divided into two stages: in the first one, the galvanic current runs from the upper part of the body to the legs for 15 minutes; subsequently the polarity is inverted and the current travels from the low part to the upper part of the body for 15 further minutes. The base treatment is constituted by three sessions per week for a total of 20 sessions

(eight weeks), but it can be repeated without problems when weight or lipodystrophy are more serious. With a cycle of 20 sittings, an average weight loss of 6-10Kg in 40/60 days can be obtained. To achieve a correct weight loss, the association of the New Elettrosculpture treatment to the Chrono Dissociated Normocaloric Diet (CADN) is mandatory.
The CADN allows an easy weight loss to healthy patients as well as to patients bearing different pathologies, obesity-related and resistant to other kinds of dietetic treatment. This result is achieved without the use of drugs and without perceiving the symptom of hunger, without psychological stresses and with a remarkable compliance, this latter due to the rewarding administration of carbohydrate amount and proteins.
This kind of feeding has been set up in 1986 and more than 30.000 cases have been resolved. The advantages of DCD method are: 1) Remarkable improvement of the circulation (noticeable already after 2-3 sittings); 2) Remarkable improvement of the skin and connective tissue with reduction of the thickness of the adipose tissue and therefore of "cellulitis"; 3) Improvement of the risk factors related to obesity as well as of hypertension and normalization of biochemical markers.

Research Goal

The positive effects of the method observed since more than 20 years has led us to wonder which is the real biochemical mechanism by which the new electrosculpture acts at a cellular and molecular level and in particular to answer to the following questions: 1) Ionophoresis itself has a lipolytic effect? 2) Are there metabolic or humoral factors that justify some clamorous results?
The strategy used to answer these questions is based on three points: 1) To confirm the harmlessness of the method; 2) To characterize which metabolic and/or humoral components are activated by the electrophoretic treatment; 3) To inquire on the eventual recovery of the metabolism due to the diet and the electrophoresis treatment in order to confirm our hypothesis, that is witnessed by the maintenance of the new weight in the subjects that have followed the method correctly. These patients generally do not regain their weight even increasing the caloric intake. The same subjects, prior to the treatment, gained weight even if a great decrease of the caloric intake was done, thus demonstrating a slow and not reactive metabolism.

Materials and Methods

In order to answer the questions, a protocol including medical examinations and laboratory reports has been performed.
Primary objective was the identification of the eventual metabolic modifications in the subject enrolled into the study and undergoing electrophoresis (Method DCD) and diet (CADN) for a period of 8 weeks. Secondary objectives were the identification of the eventual persistence of metabolic modifications after 4 weeks from the conclusion of the treatment (follow up). Control of the profile of the treated subjects (ECG, laboratory parameters, recording of adverse events) after 4, 8 and 12 weeks.

Diagram of the study

| Procedures | Visit 1 | Visit 2 | Visit 3 | Visit 4 |
|---|---|---|---|---|
| Informed consent | X | | | |
| Pathological anamnesis | X | X | X | X |
| Medical examination | X | X | X | X |
| ECG | X | | X | |
| Vital signs | X | X | X | X |
| Antropometric measurements | X | X | X | X |
| BMI | X | X | X | X |

| | | | | |
|---|---|---|---|---|
| Evaluation of fat mass | X | X | X | X |
| Evaluation of the inclusion/exclusion criteria | X | | | |
| Hematochemical markers | X | X | X | X |
| IGF-1 | X | X | X | X |
| Leptin | X | X | X | X |
| Urine analysis | X | | X | |
| Adverse events | | X | X | X |

Experimental period

All the subjects observed a dietetic regimen (CADN – see appendix 1) throughout 8 weeks; during the same period they have been treated with electrophoresis (DCD method) three times per week.

*Visit 1 (zero time control).*

Every subject, after delivering the informed consent, has undergone to a complete medical examination, the collection of pathological anamnesis and the information about the eventual concomitant therapies, ECG, blood collection for biochemical and metabolic markers, urine analysis, determination of BMI and the percentage of fat mass. If the subject met the inclusion criteria, he was enrolled in the study and begun the dietetic and electrophoretic treatment.

*Visit 2 (intermediate control).*

After 4 weeks of treatment (week 4) subjects underwent to a second blood drawing in addition to the antropometric measurements. Adverse events and/or variations of the concomitant therapies have been annoted.

*Visit 3 (end of treatment).*

After 8 weeks of treatment (week 8) subjects underwent to ECG, blood drawing, urine collection and antropometric measurements; information of eventual adverse events and/or variations of the concomitant therapies have been noticed.

*Visit 4 (Follow-up).*

After 4 weeks from the end of the treatment (week 12) subjects underwent to blood drawing and to antropometric measurements; information of eventual adverse events and/or variations of the concomitant therapies have been noticed.

Sample

30 subjects, males and females have been recruited; all of them respected the following criteria of inclusion and exclusion:

Inclusion criteria: Males or females > 18 years; BMI between 30 and 40; Presence of fat mass > to 30%; Signature of the informed consent.

Exclusion criteria; Familiar Dyslipidemia; Type I Diabetes; Greater cardiovascular events in the last 6 months; Endocrine diseases; other Clinical Conditions requiring the exclusion of the patient.

Treatment

Total body surface electrophoresis at low amperage (from 2,2 to 6,5 milliampere) on continuous flow. Before the treatment the subject is bandaged with pure anallergic cotton moisturized with water, covered with a further bandage of electroconductive, grafitated rubber. The treatment lasts 30/35 minutes. The current runs for 15 minutes from the upper part of the body to the lower; then the polarity is inverted and the current runs from the lower to the upper part of the body for further 15 minutes. In combination, the patient followed a dietetic regimen called CADN.

Chrono Dissociated Normocaloric Diet (CADN)

At raise. Two Evian water glasses (400 ml).

Breakfast. Coffee + 150ml of semi skimmed milk or Lemon Tea. Do not use sugar but Fructose, Aspartame or Saccharin. As a second choice: squeezed grapefruit or orange or mandarin or centrifugate of apple, pineapple, carrot or mixed (without bananas). 6-8 spoons of corn flakes. During the day 3 coffees are allowed.
Lunch. 60/80/100gr of pasta with tomato or vegetables, without parmesan (not noodles); or 60/80/100gr of rice (better if integral) with lemon or tomato or added to 200g of salad. As a second choice, 50/100gr of maize with 50gr of integral rice with the salad.
Always: A raw oil spoon at every meal.
Always: 100/150gr of mixed salad (leaves) flavored with lemon or vinegar ad libitum.
Supper. 120/150/200gr of red meat or 200/250gr fish of whichever type, comprised crustaceans and shellfish. Fruits must be consumed at least 1h after the meal. For water-melon and melon, a maximum of 2 slices are allowed.

Antropometric and Vital signs

Evaluation of the BMI, of fat mass and vital signs has been performed at all 4 visits. ECG was performed at visits 1 and 3. The compliance to the treatment has been estimated at the end of the study analyzing all the information collected on the appropriate CRF foreseen for the study, according to the protocol.

Laboratory tests for the evaluation of the Metabolic Status

Laboratory markers to evaluate the eventual metabolic modifications as a result of the treatment in study were the following: Haemochrome, Ureic nitrogen, Creatinine, Blood glucose, AST, ALT, ALP, gamma GT, CPK, Electrolytes, Tryglycerides, Cholesterol, Urine analysis, Protein electrophoresis, Assay of the leptin, Assay of the IGF-I. These latter two analytes were performed only at visit 1 and 3. Leptin has been investigated since its circulating levels are tightly related to sex and fat mass (Cuillard et al, 1997; Nagy et al, 1997); IGF-1 has been investigated because almost every cell in the human body is affected by IGF-1, including adipocytes with an insulin like effect.
All hematochemical parameters were obtained by standardized routinary methods, except Leptin and IGF-1, that need particular methods.

*Assay of Leptin Plasma Levels*

Leptin plasma concentration has been measured by the Assay Designs, Inc. Kit as previously described (Magni et al, 2003). Briefly, the assay kit contains a policlonal antibody to immobilize Leptin in the wells of the microtiter plate. After a brief incubation, 1h a 37°C, the exceeding sample is removed and a policlonal antibody for Leptin, bearing horseradish peroxydase is added. After a new incubation period - 1h a 37°C, the exceeding labeled antibody is removed and the substrate for peroxydase is added. The colour is read at 450nm and is directly proportional to the concentration of Leptin in the sample.

*Assay of IGF-1*

IGF-1 levels were determined according to the method described by Broussieres (Broussieres et al, 2000).

Concomitant therapies

No pharmacological treatments interfering with the evaluation of the effects of the treatment have been allowed. Eventual preexisting therapies were allowed provided that dosages were not modified.
the experimentation.

Suspension/Interruption of the experimental treatment

No correlated adverse effects were reported by any patient. No one has withdrawn the informed consent or showed pathologies that could modify the results of the study.

Adverse events

The clinical tolerability of the treatment has been evaluated by means of the recording of the appearance of adverse events or reported adverse experiences from the patient to the Researcher. All the manifested adverse events during the clinical study must be noticed on the cards attached to the CRF. The adverse events will be documented and collected during all the period of research. The Researcher will have to follow all the adverse events begun during the period of treatment, also after the end of the experimentation, until the definition of the outcome. All the relative information to the adverse events will have to be recorded on the CRF.

Adverse events: definitions

Every undesired and/or unexpected medical event that is taken place in a patient during the experimentation, must be recorded like adverse event, even if not relation with the procedures of the study is evidence of one. The adverse events include: a not intentional or unexpected modification of anatomical, physiological or metabolic functionality; insorgence of elapsing diseases; abnormality of the laboratory values or the vital signs. The adverse events are classified like “serious” or “not serious” according to the following definitions. Serious Adverse events (Serious Adverse Events, SAE): events that cause death or danger for life for that specific patient, that require a shelter, that cause meaningful and persistent inability, a congenital defect in the offspring, a medical emergency that prevents one of the previous conditions. Not Serious Adverse Events (Not Serious Adverse Event, NSAE): events that do not satisfy some of the criteria that define the serious adverse events.

This study has been conducted following all the rules of good clinical practice, for ethical and administrative procedures and all the rules for the collection of data and to their access. A qualified clinical monitoring and a quality control has been assured throughout the study.

Results

No one of the patients enrolled in the study showed clinical modifications or reported any adverse effect. ECG and vital signs were always within the reference limits.

Table 1 shows all the values of weight loss, of BMI and fat mass as well as laboratory data. The mean of the total weight loss was of 11Kg, with a partial score of 3,5Kg per sitting. However, the variability was very high, since the standard deviation is $\pm$ 4Kg. Such a result is obviously due to the poor homogeneity of the sample. Also the fat mass and BMI present a large variability that does not allow to draw a precise map, even if the final result is clearly interesting, since the decrease of both is evident. All other parameters investigated resulted within the reference limits, including Leptin and IGF-1.

| | Visit 1 | Visit 2 | Visit 3 | Visit 4 |
|---|---|---|---|---|
| **Blood Nitr. (mg/dL)** | 29,5±7,68 | 33,2±6,47 | 28,1±7,5 | 29,6±5,99 |
| **Bilirub-Tot (mg/dL).** | 0,57±0,27 | 0,5±0,33 | 0,7±0,46 | 0,58±0,34 |
| **Bilir – Direct (mg/dL)** | 0,17±0,08 | 0,15±0,04 | 0,2±0,09 | 0,17±0,01 |
| **Chol - Total. (mg/dL)** | 170,2±40,61 | 159,2±30,29 | 149,7±26,7 | 162,1±31,22 |
| **Chol. HDL (mg/dL)** | 58,6±17,82 | 52,5±13,66 | 53,2±14,9 | 62,1±16,79 |
| **Chol.Tot/ HDL** | 3±0,84 | 6,7±0,99 | 2,9±0,93 | 2,8±0,86 |
| **Chol. LDL (mg/dL)** | 94,9±30,13 | 96,4±29,21 | 87,4±24,2 | 83,8±36,46 |
| **Tryglyc (mg/dL)** | 83,5±51,16 | 51±20,28 | 60,9±27,2 | 81,1±40,18 |
| **Creatinine (mg/dL)** | 0,8±0,14 | 0,9±0,13 | 0,9±0,11 | 0,9±0,1 |
| **RBC x10^6/uL** | 0,46 | 4,7±0,38 | 4,6±0,4 | 4,6±0,43 |
| **Ht (%)** | 40,9±1,88 | 4,8±2,66 | 39,6±2,31 | 40,1±2,3 |
| **Hb (g/dL)** | 13,8±0,7 | 14,1±0,78 | 13,5±0,82 | 13,4±0,88 |
| **MCV (fl)** | 87,8±5,55 | 88,9±4,32 | 86,6±4,91 | 88±5,7 |
| **MCH (ug)** | 29,7±1,76 | 29,9±1,4 | 29,5±1,4 | 29,5±1,3 |
| **MCHC (g/dL)** | 33,8±0,67 | 33,6±0,78 | 34,1±0,58 | 33,6±0,8 |
| **WBC x10^3/uL** | 7,4±3,01 | 6,4±2,18 | 6,6±2,36 | 6,8±2,5 |
| **N** | 62±9,78 | 57±6,93 | 59,3±7,51 | 59,2±7,5 |
| **E** | 0,7±0,84 | 0,6±0,84 | 0,9±0,52 | 0,7±1,22 |
| **B** | 0 | 0 | 0 | 0 |
| **L** | 33,3±10,17 | 38,8±7,05 | 36±7,8 | 36,1±16.48 |
| **M** | 4±1,64 | 3,5±1,02 | 3,8±1,97 | 3,9±2,3 |
| **Platelets** | 247,8±64,23 | 226,8±60,92 | 194,8±44,3 | 22,6±51,3 |
| **Blood gluc. (mg/dL)** | 91,8±7,99 | 87,8±6,61 | 92±,04 | 95,7±10 |
| **Gamma GT** | 24,4±11,54 | 17,4±8,49 | 16,1±7,43 | 17,2±9,8 |
| **Weight Loss** | 3±1 | 4±1,5 | 3±1 | 1±0,3 |
| **BMI** | 33±3 | 30±1,5 | 29±2 | 27±0,7 |
| **Fat (%)** | 30 | 26 | 25 | 23 |
| **AST (U/L)** | 18±3 | 18±3 | 18±3 | 18±3 |
| **ALT (U/L)** | 14±2 | 14±2 | 14±2 | 14±2 |
| **ALP (U/L)** | 44±4 | 44±4 | 44±4 | 44±4 |
| **CPK (U/L)** | 101±6 | 101±6 | 101±6 | 101±6 |
| **Electrophoresis** | No pathol evid | No pathol evid | No pathol evid | No pathol evid |
| **Urine** | No pathol evid | No pathol evid | No pathol evid | No pathol evid |
| **Na (mmol/L)** | 140±4 | 140±4 | 140±4 | 140±4 |
| **K (mmol/L)** | 4±1 | 4±1 | 4±1 | 4±1 |
| **Mg (mmol/L)** | 2±0,2 | 2±0,2 | 2±0,2 | 2±0,2 |
| **Cl (mmol/L)** | 106±4 | 106±4 | 106±4 | 106±4 |
| **Ca (mmol/L)** | 6±1,5 | 6±1,5 | 6±1,5 | 6±1,5 |
| **Leptin (ng/mL)** | 6,5±1.3 | 6,2±1.1 | 6,4±1.3 | 6,5±1.1 |
| **IGF-1 (ng/mL)** | 70±5 | 77±4 | 72±4 | 80±6 |

## Discussion

Circulating Leptin levels are tightly related to sex and fat mass (Cuillard et al, 1997; Nagy et al, 1997), but is clear that these two factors alone cannot explain the large variability of the circulating Leptin concentration. It happens, in fact that subjects presenting similar BMI have quite different levels of plasma Leptin (Baumgartner et al, 1999). Leptin has been determined in the plasma in the fasted state in females and was 6.7 ± 2.1 ng/ml. It decreases after glucose challenge. In males 1.3 ± 0.5 ng/ml were detected in the morning and 1.1 ± 0.6 ng/ml postprandial. Leptin is higher in women and this was confirmed in our study group. Clinical studies directed towards examinating the possible involvement not only of the boby mass but also of its distribution gave not comparable results (Wauters et al, 1999). In vitro studies have shown that subcutaneous fat synthesizes Leptin more efficiently than the visceral one (Suzuki et al, 1993; Lefebvre et al, 1998). Leptin synthesis, anyway, does not occur uniquely in the adipose tissue, but a relevant contribution derives by an active secretion at the cerebral level (Wiesner et al, 1999).

In vivo, through the use of ultrasounds, a marked difference in the thickness of adipose tissue of obese women vs. men with a comparable BMI has been evidenced (Shimizu et al, 1997; Minocci et al, 2000); this could support the hypothesis of a tight relationship between Leptin levels and thickness of subcutaneous fat mass but not of the visceral, preperitoneal, one even if it is not sufficient to completely explain the sexual dimorphism of plasma Leptin. Anyway, no difference is present between the two fat types as to Leptin secretion in normal weight men (Alessi et al, 1997). Further studies performed in vivo by means of antropometric measurements suggest that Leptin levels depend only on the fat mass and not on its distribution (Pi-Sunyer et al, 1999).

In conclusion, this study has been able to confirm that the treatment with surface electrophoresis is completely harmless and that does not modify nor vital signs neither ECG; as to the laboratory data, an improvement of plasma cholesterol and triglycerides has been observed, even if the values are at the limit of the significativity. No pathological modification of hematochemical parameters were present. Leptin and IGF-1 measurements were not discriminatory but this may be due to the relatively small sample. Anyway, the wide variability found among the subjects and the consequent wide standard deviation, suggests that this kind of study should be performed with a larger sample, in which to identify a larger number of subpopulations, homogeneous at least for weight, age and sex. In such a larger sample could be easier to identify the eventual variability of laboratory data.

IGF-1 is produced primarily by the liver as an endocrine hormone as well as target tissues in a paracrine/autocrine fashion. Production is stimulated by growth hormone and can be retarded by undernutrition, growth hormone insensitivity, lack of growth hormone receptors, or failures of the downstream signalling pathway post GH receptor including SHP2 and STAT5b. Its primary action is mediated by binding to specific IGF receptors present on many cell types in many tissues. The signal is transduced by intracellular events. IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death. Almost every cell in the human body is affected by IGF-1, especially cells in muscle, cartilage, bone, liver, kidney, nerves, skin, and lungs. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development. IGF-1 binds to cell surface receptors: the IGF-1 receptor (IGFR), and the insulin receptor. The IGF-1 receptor seems to be the "physiologic" receptor - it binds IGF-1 at significantly higher affinity than IGF-1 is bound to the insulin receptor. Like the insulin receptor, the IGF-1 receptor is a receptor tyrosine kinase - meaning it signals by causing the addition of a phosphate molecule on particular tyrosines. IGF-1 activates the insulin receptor at approximately 0.1x the potency of insulin. Part of this signaling may be via IGF1R/Insulin Receptor heterodimers (the reason for the confusion is that binding studies show that IGF1 binds the insulin receptor 100-fold less well than insulin, yet that does not correlate with the actual potency of IGF1 in vivo at inducing phosphorylation of the insulin receptor, and hypoglycemia). IGF-1 is produced throughout life. The highest rates of IGF-1 production occur during the pubertal growth spurt. The lowest levels occur in infancy and old age. IGF-1 levels can be measured in the blood in 10-1000 ng/ml amounts. As levels do not fluctuate

greatly throughout the day for an individual person, IGF-1 is used by physicians as a screening test for growth hormone deficiency and excess. Interpretation of IGF-1 levels is complicated by the wide normal ranges, and variations by age, sex, and pubertal stage. Clinically significant conditions and changes may be masked by the wide normal ranges. Sequential management over time is often useful for the management of several types of pituitary disease, undernutrition, and growth problems. In conclusion, the research presented here and particularly the laboratory findings, confirm that the treatment is harmless, since there are not pathological values. Only a continuous decrease of platelets can be observed during the 16 weeks, even if such decrease remains always within safe limits. This effect cannot be explained in the present paper and needs further investigation. An improvement of the parameters linked to fat mass can be observed: decrease of triglycerides, of total cholesterol and of LDL-cholesterol, increase of HDL-cholesterol. The improvement of the metabolic assessment is witnessed also by a decrease, even if moderate, of gamma glutamyl transpeptidases.

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