PILOT STUDY FATTY LIVER AND OBESITY: MR FAT QUANTIFICATION AND MR ELASTOGRAPHY DURING A VERY LOW-CALORIE-KETOGENIC DIET (PRONOKAL METHOD®) VERSUS A LOW CALORIE DIET.

A multicenter randomized controlled clinical trial

STUDY PROTOCOL

Protocol Code: PNK-FATLIV-2016-04

(Version 2.0 dated, 21 April, 2016)

Principal investigators

Dr. Walmir Coutinho

Dr Guilherme Moura da Cunha

1 ABSTRACT

1.1 Type of study

Prospective, multicenter, comparative, open-label, randomized and controlled pilot study

1.2 Sponsor's identification

Protein Supplies S.L.
C/ Roger de Llúria, 58.
08009 Barcelona. España
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1.3 Title

Pilot study fatty liver and obesity: MR fat quantification and MR elastography during a very low-calorie-ketogenic diet (Pronokal Method®) versus a low calorie diet

1.4 Protocol Code

PNK-FATLIV-2016-04

1.5 Principal investigators

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Dr Guilherme Moura da Cunha
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1.6 Coinvestigators

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1.7 Study locations

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Rua Moncorvo Filho, numero 90
Centro, Rio de Janeiro, Brasil

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Rua Ataulfo de Paiva, numero 699, Leblon,
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1.8 Study design

Prospective, multicenter, open-label, randomized and controlled study of the effects of weight loss and reduced visceral fat on non-alcoholic fat infiltration into liver after a very low calorie ketogenic diet (VLCK diet) (Pronokal® Method) versus a hypocaloric diet, with a 6-month follow-up.

1.9 Study objectives

- To evaluate the effects of body weight loss after a very low-calorie-ketogenic diet (Pronokal Method®) versus low calorie diet, on visceral fat reduction, liver fatty infiltration, using a new MRI technique of fat liver quantification (Ideal IQ**).
- To correlate the degree of liver fat infiltration and liver stiffness using MR Elastography
- To investigate the hypothesis of a linear correlation between adrenal gland size and variables commonly associated to insulin resistance such as BMI, visceral fat and liver steatosis.

1.10 Condition in study

Fat infiltration into the liver (nonalcoholic fatty liver disease)

1.11 Study treatment

A very low-calorie-ketogenic diet (Pronokal Method®)

1.12 Outcome Measures

- Body weight loss
- Visceral fat (measured by the area in squared centimeters at the level of the 3rd vertebrae, using MRI technique)
- Porcentaje de contenido graso del hígado
- Liver stiffness using MR Elastography
- Linear measurements of the adrenal glands
- Salivary cortisol levels

1.13 Study population and number of subjects

Obese patients of either sex with a BMI over 30 kg/m^2.

1.14 Follow-up and duration of the study

The follow-up will be 6 months.

1.15 Funding

We were awarded a grant from PronoKal Group® to partially fund this study, where MR image acquisition and post-processing costs will be covered. The leading investigators, free of charge, will do all data analysis and manuscript writing.

1.16 Schedule

Study onset: May, 2016.
Completion of study: May, 2017.

2 INDEX

3 GENERAL INFORMATION

3.1 Title

Fatty Liver and obesity: MR Fat Quantification and MR Elastography during a very low-calorie-ketogenic diet (Pronokal Method®) versus a low calorie diet. A multicenter randomized controlled clinical trial.

3.2 Protocol code

PNK-FATLIV-2016-04

3.3 Type of study

Prospective, multicenter, open-label, randomized and controlled study of the effects of weight loss and reduced visceral fat on non-alcoholic fat infiltration into liver after a very low calorie ketogenic diet (VLCK diet) (Pronokal® Method) versus a hypocaloric diet, with a 6-month follow-up.

3.4 Study treatment

A very low-calorie-ketogenic diet (Pronokal Method®)

3.5 Sponsor

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3.7 Expected duration

The expected duration of the study is 10 months (4 months of recruitment and 6 follow-up months).

4 INTRODUCTION AND RATIONALE OF THE STUDY

The liver histology in obese people can be altered by an accumulation of intrahepatic triglyceride (IHTG), i.e., steatosis, which is the hallmark feature of nonalcoholic fatty liver disease (NAFLD). Nonalcoholic fatty liver disease (NAFLD) is the liver manifestation of the complex metabolic derangements associated with obesity, being an emerging epidemic in Western countries and affects all ages and ethnicities. Individuals with hepatic steatosis alone were thought to have a benign long-term prognosis. However, up to 25% of these patients may develop nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis in susceptible individual (5). Because early NAFLD may be reversible, screening and early intervention may be indicated (1-2).

Weight loss alone by dietary changes has been shown to lead to histological improvement in fatty liver making nutrition therapy to become a cornerstone of treatment for NAFLD. Studies show that those who achieved a minimum of 7% weight loss had improvements in their liver histology (3). In recent years the VLCD diets, especially in combination with intensive life interventions (ILI), are gaining recognition as a suitable alternative to conventional low-calorie diets for weight loss (6-8), with greater weight loss at short and long term (9-11). PronoKal® method is a commercially available weight loss program, which includes a dietary regime (very low-calorie-ketogenic diet based on a high-biological-value protein preparations diet and natural foods followed by low calorie diet), lifestyle and behavioral modification support. This method has proved more effective for weight loss compared with a standard low-calorie diet, achieving a reduction of more than 10 % of the initial weight at 2 months in 96 % of the patients, and in 100% of the patients at 4 months (11).

Liver biopsy is the gold standard in diagnosing NAFLD and the most accurate tool for grading fibrosis. However, is invasive and carries the risk of complications (3). Also, histological examination of biopsy samples is subject to sampling error due to histological heterogeneity. Scoring is semi-quantitative, limiting its ability to detect modest changes, and scoring results vary between reports precluding direct comparisons. Various imaging techniques are proposed for liver fat quantification. Liver fat fraction (FF) can be calculated using fat and water MRI signal intensities in chemical-shift images (in-phase [IP] and out of phase [OP] sequences). Typically, the fatty liver shows increase in signal intensity (SI) on IP images and shows diffuse signal drop in OP image. The fat fraction can then be determined by calculating the loss of SI in OP images as compared to IP images. From congruent sets of IP and OP images,

acquired within the same breath-hold sequence, the fat fraction can be calculated pixel-wise, and misregistration errors can be avoided (4). MRI multiecho method estimates T2* from six different echo times (TE). This method resulted in improved diagnostic specificity compared with that of the triple-echo method which caused systematic underestimation at high FFs (1). New noninvasive methods including MR elastography have been developed for o distinguish necroinflammation and mild fibrosis from simple steatosis (4). Given that the spectrum of fatty liver disease ranges from simple steatosis, through stages of liver cell injury (steatohepatitis), to fibrosis, and eventually to cirrhosis, it is appropriate to ask whether hepatic stiffness as a biomarker can be used to identify the presence of liver cell injury prior to the onset of fibrosis.

One of the leading extra-hepatic causes of liver steatosis is metabolic syndrome (MS). Although, multiple definitions of MS exists; the essential components of MS include insulin resistance, increased levels of stress hormones, such as cortisol, and visceral obesity. In the obese population, the hallmarks of a metabolic syndrome are highly prevalent, being peripheral insulin resistance and increase levels of stress hormones the leading causes of organ injury. In such individuals higher levels of cortisol are expected, secondary to an over activated hypothalamic-pituitary-adrenal (HPA) (13, 14). A linear correlation between adrenal gland volume and metabolic syndrome has been demonstrated, with increased adrenal volume in patients under a metabolic syndrome status. However, once these patients are engaged on a treatment regime, leading to weight loss and diminished insulin resistance, the increase in adrenal volume tends to be reversible. Adrenal volume measurements can be achieved using cross-sectional imaging and semi-automated softwares. In clinical practice, adrenal gland dimensions are generally assessed using limb linear measurements on multidetector row computed tomography or magnetic resonance imaging (15,16).

We conducted a prospective longitudinal clinical study to correlate the effects of a very low-calorie-ketogenic diet (PRONOKAL method*), body weight loss and visceral fat reduction, with liver fat fraction, using a new MRI model of fat liver quantification (Ideal IQ**). Additionally, the degree of liver fat and its evolution, during the diet program, will be correlated with liver stiffness using MR Elastography. Adrenal gland measurements will also be made using the linear technique described by Carsin-Vu A. (16) to address its correlation with weight loss, visceral fat and liver steatosis.

5 STUDY OBJECTIVES

5.1 Primary aim

- To evaluate the effects of body weight loss after a very low-calorie-ketogenic diet (Pronokal Method®) versus low calorie diet, on visceral fat reduction, liver fatty infiltration, using a new MRI technique of fat liver quantification (Ideal IQ**).

5.2 Secondary aims

- To correlate the degree of liver fat infiltration and liver stiffness using MR Elastography
- To investigate the hypothesis of a linear correlation between adrenal gland size and variables commonly associated to insulin resistance such as BMI, visceral fat and liver steatosis.

6 STUDY DESIGN

6.1 Type of study

Prospective, multicenter, comparative, open-label, randomized and controlled pilot study

6.2 Randomization process

Patients will be randomized at the time of inclusion, in accordance with an automatically generated randomization list. One group will start treatment with a very low calorie and ketogenic diet (VLCK diet group) while the other group will start a low calorie diet (control group). The patient ratio in both groups will be 1: 1.

6.3 Type of control

The control group will consist of patients who follow the hypocaloric diet.

6.4 Masking Techniques

Not applicable. This is an open-label study

7 SUBJECT SELECTION

7.1 Study population

Obese patients of either sex with BMI over 30 kg/m^2, who agree to participate in the study.

7.2 Inclusion criteria:

- Patients of either sex, older than 18 years

- Obese patients with BMI equal or over 30kg/m^2
- Patients who agree to participate and sign the Informed Consent

7.3 Exclusion criteria:

- Pregnant or lactating women.
- Patients weighing over 140 kg (by limitation of the MRI device)
- Patients on anti-obesity drugs (eg, sibutramine, orlistat and liraglutide) or weight-interfering medications (eg, topiramate). In such cases a wash out period of 3 months is required.
- Patients with alcohol intake > 20 g/day in women and > 30 g/day in men.
- Patients with liver failure or with pathologies that may affect the liver
- Patients with other causes of liver steatosis: chronic viral hepatitis and/or drug intake (corticosteroids, estrogens, NSAIDs, calcium antagonists, amiodarone, tamoxifen, tetracyclines, chloroquine, antiretrovirals, perhexiline).
- Patients with eating disorders.
- Patients with any psychiatric disorder (eg schizophrenia, bipolar disorder, substance abuse, severe depression).
- Patients receiving dicumarinic anticoagulants (Sintrom®).
- Patients taking corticosteroids.
- Patients with severe kidney failure (gfr <30).
- Patients with type 1 and type 2 diabetes mellitus.
- Patients with hemopathies, including coagulation disorders
- Patients with cardiovascular or cerebrovascular disease (of heart rate disorders, recent infarction [<6m], unstable angina, decompensated heart failure, recent stroke [<6m]).
- Patients in acute attack of gout.
- Patients with renal lithiasis verified by ultrasound.
- Patients with cholelithiasis verified by ultrasound.
- Patients with electrolyte imbalance, according to medical criteria.
- Patients with orthostatic hypotension.
- Patients with cancer or history of cancer who have not been discharged from oncology
- Patients who are not expected to attend follow-up visits

7.4 Number of subjects

A total of 40 patients will be included: 20 patients randomly assigned to a very low-calorie-ketogenic diet (VLCK diet group) and 20 patients randomly assigned to low calorie diet (control group).

7.5 Rationale of sample size

Exploratory pilot study to test a concept, where it has been estimated that 20 patients per group will be sufficient to analyze results.

7.6 Recruitment period

We expect a 4-month recruitment period

7.7 Criteria for withdrawal or dropouts

Patients who do not attend the control visits and/or have not undergone at least two MRIs (baseline and control after the end of ketosis or at month 6) will be considered as dropouts.

8 TREATMENT DESCRIPTION

8.1 Weight Loss Program: PronoKal® Method

All patients in the study group will undergo the same standardized weight loss program (PronoKal® Method) with progressive diet based on commercial high-biological-value protein preparations, dietary supplements, physical exercise plan and emotional support, structured in 3 stages: active stage, food re-education stage and maintenance stage

<table>
<tr><td colspan="3">80% of target weight loss</td><td colspan="2">20% of target weight loss</td><td>Long-term mantenance of weight loss</td></tr>
<tr><td colspan="6">Multidisciplinary team (Dietary counseling/physical activity/psychological support)</td></tr>
<tr><td colspan="5">Weight Loss Moment</td><td rowspan="2">My New Lifestyle Moment</td></tr>
<tr><td>Step 1</td><td>Step 2</td><td>Step 3</td><td>Step 4</td><td>Step 5</td></tr>
<tr><td colspan="3">Low-fat ketogenic diet
(very low-calorie-ketogenic diet)
600-730 kcal/day</td><td colspan="2">Low calorie diet
(800-1500 kcal/day)</td><td>Controlled diet
adapted</td></tr>
<tr><td colspan="3">Supplementation
Vitamins, trace elements
OMEGA BALANCE: 700 DHA/500 EPA</td><td colspan="2">Supplementation
Vitamins, trace elements
DHA VITA: 500 DHA/100 EPA</td><td>DHA VITA: 500 DHA/100 EPA</td></tr>
</table>

a) Treatment schedule

Active stage: The diet will consist of a protein diet, ie a very low-calorie ketogenic diet (VLCK diet) (between 650 and 730 kcal/day) based on PronoKal® commercial preparations and low-glycemic-indexvegetables. Patients will start with 5 commercial

preparations a day. Two of them will be replaced progressively by natural proteins (meat or fish). This active stage will be maintained for 2 months.

- Phase 1: Protein diet of 600 kcal/ day (with 5 PronoKal® products and low-glycemic-index vegetables) + Nutritional supplements (vitamins and trace elements) + Cardiovascular physical exercise + Emotional support.
- Phase 2a: Protein diet of 680 kcal/day (with 4 PronoKal® products, a high-protein food [meat or fish] and low-glycemic-index vegetables) + Nutritional supplements (vitamins and trace elements) + Cardiovascular physical exercise + Emotional support.+ Soporte emocional.
- Phase 2b: Protein diet of 780 kcal / day (with 3 PronoKal® products, two high-protein foods [meat or fish] and low-glycemic-index vegetables) + Nutritional supplements (vitamins and trace elements) + Cardiovascular physical exercise + Emotional support.

Patients will remain in Phase 1 until they have lost 40% of the excess weight or the target weight to be lost. Subsequently, they go on to Phase 2a until they have lost 60% of the excess weight and then to Phase 2b until they have lost 80% of the excess weight.

For this study, patients will leave the active Ketogenic Stage within 2 months of treatment, even if they have failed to achieve 80% of the weight loss goal.

Phase of physiological adaptation: Progressive introduction of natural foods and maintaining the intake of 2 commercial preparations per day, with a progressive increase in calorie intake up to 1300-1500 kcal/day and an increased supply of carbohydrates. The diet will be accompanied by with cardiovascular physical exercise and emotional support. The length of the food re-education stage will be 4 months.

Maintenance stage: Upon completion of dietary re-education, the patient will go on a balanced diet of 1500-2200 kcal/day, along with cardiovascular physical exercise and emotional support, until completing the 2-year follow-up.

8.2 Hypocaloric diet

Balanced hypocaloric diet (caloric intake 10% below basal metabolic rate).

The diet will also be accompanied by physical exercise.

9 STUDY PROCEDURES AND OUTCOME MEASURES

9.1 Outcome measures

9.1.1 *Primary variables*

- Liver fatty infiltration, measured by a MRI technique of fat liver quantification (Ideal IQ**).
- Visceral fat, measured by the area in squared centimeters at the level of the 3rd vertebrae, using MRI technique.

9.1.2 Secondary variable

- Liver stiffness detected by MR Elastography
- Adrenal gland linear measurement
- Body weight
- Salivary cortisol levels

9.1.3 End points

- Reduction of fat infiltration into the liver between the baseline visit and 2 months (or end of the ketosis stage) and between the baseline visit and the control visit at month 6 of treatment.
- Visceral fat reduction between the baseline visit and 2 months (or end of the ketosis stage) and between the baseline visit and the control visit at month 6 of treatment.
- Correlation between degree of liver fat infiltration and liver stiffness
- Correlation between adrenal gland size and BMI, visceral fat and liver steatosis.
- Correlation between salivary cortisol levels and BMI, visceral fat and liver steatosis.
- Weight loss between the baseline visit and 2 months (or end of the ketosis stage) and between the baseline visit and the control visit at month 6 of treatment.

9.2 Study procedures

9.2.1 Follow-up of patients

The patient follow-up will be 6 months. The patients will be visited monthly and data will be recorded on 4 occasions: pre-inclusion, baseline visit, final visit Active stage (end of ketosis) and visit at month 6.

Patients will be included after verifying the inclusion and exclusion criteria and after obtaining the signed informed consent.

Screening visit (day -15). In this visit, the patient's medical history will be assessed and a complete blood test will be required to verify the inclusion/exclusion criteria: complete blood count (red blood cells, leukocytes, neutrophils, hematocrit, hemoglobin), fasting blood glucose, Hb1c, insulinemia, lipid profile (total cholesterol, c-HDL, c-LDL, triglycerides), kidney function (serum creatinine, creatinine clearence, glomerular filtration), nitrogen balance (urea, uric acid), thyroid profile (TSH, T4) and ionogram (Na, K, Cl, Ca, Mg)

The pre-inclusion blood test will also include liver function tests as the first screening of hepatic steatosis:

- GOT
- GPT
- Gamma-GT
- Bilirubin
- Albumin
- Prothrombin time
- Ferritin
- Alkaline phosphatase.

In addition, the patients will be informed about the study through the patient information sheet (Attachment 1) and they will be provided with an informed consent (Attachment 2).

At the baseline visit (day 0). The results of the additional tests (blood tests) will be verified to complete the inclusion/exclusion criteria.

Liver function data will be reviewed. Patients with one or more of the following conditions will be considered as suspicious of hepatic steatosis:

- GPT/GOT ratio over 1
- Gamma-GT and alkaline phosphatase increased twice to three times the normal levels
- High ferritin
- Normal bilirubin and albumin in the presence of any of the above changes

The informed consent will be signed and randomized. The corresponding diet will be scheduled depending on the treatment group (VLCK diet group or control group) and the physical exercise program. Patient demographics (sex, age, weight, height, comorbidities and medication) will be recorded. Anthropometric data (weight, BMI, waist circumference) will be recorded. Fat infiltration into liver and visceral fat will be scanned by MRI and hepatic stiffness will be assessed by MR Elastography. The salivary cortisol levels will also be tested.

Control visit at month 2 or at the end of the ketogenic stage: For patients in the VLCK diet group, as soon as the patient loses 80% of the excess weight or within 2 months of treatment, just before moving to phase 3, the patient will undergo a new control. In the case of the control group, this visit will take place at month 2 of the hypocaloric diet.

The patient will undergo a new MRI and MR Elastography. The salivary cortisol levels will also be tested. Anthropometric data are recorded, the patient undergoes a complete blood test including liver function (GOT, GPT, GammaGT, bilirubin, albumin, prothrombin time, ferritin, alkaline phosphatase) and the potential occurrence of adverse effects will be recorded.

Visit at month 6 At month 6 of the study, a new control will be performed, evaluating hepatic steatosis and visceral fat using MRI and liver stiffness using MR Elastography. The salivary cortisol levels will also be tested. A complete blood test including liver function (GOT, GPT, GammaGT, bilirubin, albumin, prothrombin time, ferritin, alkaline phosphatase) will be required and the anthropometric data, the treatment phase, the blood test results and the potential occurrence of adverse effects will be recorded.

9.2.2 Flow chart

	Pre-study	Onset	Visit at month 2 (end of ketosis)	Visit at month 6 (Final visit)
Review of inclusion and exclusion criteria.	X	X		
General blood test with liver function	X		X	X
Patient information	X			
Informed consent		X		
Comorbidities and medication		X	X	X
Anthropometric data (weight, BMI, WC)	X	X	X	X
MRI + MR Elastography		X	X	X
Salivary cortisol test		X	X	X
Adverse reactions			X	X
Drop outs and end of study			X	X

10 STATISTICAL ANALYSIS

10.1 Descriptive statistics

A descriptive statistics will include all variables collected in the Data Collection Notebook. Frequency tables will include the nominal variables and measures of central tendency and dispersion for the continuous variables. 95% confidence intervals (95% CI) will be estimated for the latter.

10.2 Analysis of objectives

For the analysis of the primary objective and the secondary objectives, the differences between visits and between groups will be studied for the changes in the ratio between liver fat and the degree of steatosis (measured by MRI), changes in the degree of liver stiffness, changes in cortisol levels in saliva, weight changes, BMI and waist circumference, and changes in the amount of visceral fat among visits. The qualitative variables will be compared using Fisher's exact test, and the quantitative variables will be compared by means of the Student's t test for paired data. If the conditions to apply the Student's t test are not met, we will use the non-parametric Mann-Whitney U test. In the case of comparing paired data, we will use the tests described above, in their version for comparing this type of data.

The Pearson correlation coefficient or Spearman's correlation coefficient if the application conditions are not met will be used to study the following: the association between reduction of hepatic steatosis and weight loss and loss of visceral fat; the association between hepatic steatosis and liver stiffness; and the association between adrenal gland size and salivary cortisol levels with weight changes, BMI, hepatic steatosis and visceral fat.

For all tests, the significance level will be set at $p < 0.05$.

11 REPORT OF ADVERSE EVENTS

All adverse events shown in patients during the time they are in the study will be recorded in the Data Collection Notebook, specifying:

- Type of reaction
- Intensity: mild (no limitation of daily living activities, patient may have mild discomfort), moderate (cause some limitation of daily living activities, patient may experience increased discomfort), severe (inability to perform daily living activities; patient may report intolerable pain or discomfort).
- Length
- Causality with treatment and with QEEG scans: definitive, likely, possible, unlikely, unrelated, unknown.
- Therapy administered
- Resolution

For any serious adverse reaction (see box below), the physician should complete a specific form (Attachment 6) with all available data and report it immediately (if possible within a maximum period of 24 hours) by fax, telephone or e-mail to the sponsor, who will implement the corresponding Pharmacovigilance procedures in accordance with current legislation:

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Tel +34 931 833 310 or +34 666 988 021.

SERIOUS ADVERSE REACTION: is any adverse reaction that can be classified in one or more of the following categories:

- Mortal
- Life threatening
- It causes significant or persistent discapacity/ disability
- It causes hospitalization or prolongs hospitalization

Congenital anomalies/birth defects and serious adverse clinical consequences associated with use under conditions other than those set out in the Summary of Product Characteristics (SPC), overdose or abuse are also included.
Medical judgment should be applied when deciding whether an event or reaction is serious in other situations. Major adverse reactions or events that do not pose immediate threat to life, or do not cause death or hospitalization but may endanger the patient, should be deemed serious.

12 ETHICAL ISSUES

12.1 General considerations

This study will be conducted in accordance with current regulations, international accepted ethical standards of Good Clinical Practice (CPMP/ICH/135/95) and the principles provided in the latest version of the Declaration of Helsinki.

12.2 Evaluation by an Authorized Clinical Research Ethics Committee

Prior to its inception, this study will be submitted to the Ethics Committee of the IEDE - Instituto Estadual de Diabetes e Endocrinologia

to obtain their approval.

12.3 Patient information and informed consent

The patient will be informed about the study issues for their knowledge and written informed consent will be requested for participation in the study.

12.4 Data confidentiality

The patient's identity will appear coded in the study forms. The clinical investigator of the clinical trial will maintain a separate confidential record listing the patient identification codes and patient record number. The data of the subjects participating in the study will be saved in an automated file, property of Protein Supplies S.L .

13 PRACTICAL CONSIDERATIONS

13.1 Workplan

The investigators will be in charge of recruiting patients. Once the patients have confirmed their willingness to participate in the study, the investigator should verify if they meet the inclusion/exclusion criteria of this study. If they are eligible, they will be requested the additional tests (blood test with liver function) and will be informed on every issue of the study through the Patient Information Sheet (Attachment 1).

At the baseline visit, once the complementary tests have been reviewed and verified to meet inclusion criteria, the physician must confirm that the patient agrees to participate in the study and obtain the patient's signature of the Informed Consent (Attachment 2). Upon signing, the investigator will verify the assigned group according to the randomization list.

At this baseline visit, the physician should request MRI and MR Elastography to assess the degree of hepatic steatosis, the amount of visceral fat and liver stiffness. The physician will also perform the first salivary cortisol test on the patient. Also, he should record the clinical data requested in the Data Collection Notebook and schedule the treatment.

During the weight loss treatment, patients in the study group will be monitored by the investigator fortnightly during the protein diet (Ketogenic Active Stage), although at these additional visits no data are expected to be collected for the study. Upon completion of the first two months of follow-up, the study group will end the ketogenic stage and from that moment on, the patients will be controlled outside the center by dietitians specialized in the Pronokal® Method. Patients in the control group (hypocaloric diet) will undergo controls at the center monthly, although data will only be recorded in the visits arranged in this protocol (screening, baseline, month2 and month 6).

At the moment the patient completes the two months of follow-up, the data requested in the DCN will be recorded, the salivary cortisol test will be performed and an MRI and a MR Elastography will be requested.

The investigator, apart from monthly visits or any additional visits deemed necessary, will have an appointment with the patient at month 6 of treatment for a new clinical assessment, will record the anthropometric data, and request a Control MRI and MR Elastography.

At the end of the 6-month follow-up, the study will be completed.

In case of drop out or discontinuation of the study, the investigator will submit the DCN visit sheets and complete the study completion sheet, specifying the reason for discontinuation.

13.2 Disseminating the study results

The overall data will be used by the whole project, not individually and will be owned by Protein Supplies S.L.

With the results obtained, we will issue the final study report.

With the overall data we will try to maximize the exploitation of the study results in the form of publications or reports in conferences, always mentioning the study.

14 REFERENCES

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