Hospitalaria

Artículo especial

The reduction of the metabolyc syndrome in Navarra-Spain (RESMENA-S) study; a multidisciplinary strategy based on chrononutrition and nutritional education, together with dietetic and psychological control

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Abstract

Introduction: **The high prevalence of metabolic syndrome (MS) in Spain requires additional efforts for prevention and treatment.**

Objective: **The study RESMENA-S aims to improve clinical criteria and biomarkers associated with MS though an integral therapy approach.**

Methods: **The study is a randomized prospective parallel design in which is expected to participate a total of 100 individuals. The RESMENA-S group (n = 50) is a personalized weight loss (30% energy restriction) diet, with a macronutrient distribution (carbohydrate / fat / protein) of 40/30/30, high meal frequency (7 / day), low glycemic index/load and high antioxidant capacity as well as a high adherence to the Mediterranean diet. The control group (n = 50) is assigned to a diet with the same energy restriction and based on the American Heart Association pattern. Both experimental groups are under dietary and psychological control during 8 weeks. Likewise, for an additional period of 16 weeks of self-control, is expected that volunteers will follow the same pattern but with no dietary advice.**

Results: **Anthropometrical data and body composition determinations as well as blood and urine samples are being collected at the beginning and end of each phase. This project is registered at www.clinicaltrials.gov with the number NCT01087086 and count with the Research Ethics Committee of the University of Navarra approval (065/2009).**

Conclusions: **Intervention trials to promote the adoption of dietary patterns and healthy lifestyle are of great importance to identify the outcomes and nutritional mechanisms that might explain the link between obesity, metabolic syndrome and associated complications.**

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Key words: *Metabolic syndrome. Weight loss. Inflammation. Oxidative stress. Mediterranean diet.*

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EL ESTUDIO RESMENA-S: REDUCCIÓN DEL SÍNDROME METABÓLICO; UNA ESTRATEGIA MULTIDISCIPLINAR BASADA EN LA CRONONUTRICIÓN Y LA EDUCACIÓN NUTRICIONAL, JUNTO CON CONTROL DIETÉTICO Y PSICOLÓGICO

Resumen

Introducción: **La alta prevalencia del síndrome metabólico (SM) en España requiere de esfuerzos adicionales para su prevención y tratamiento.**

Objetivo: **El estudio RESMENA-S tiene como objetivo mejorar criterios clínicos de SM y biomarcadores asociados a través de un tratamiento integral.**

Métodos: **El estudio consiste en un ensayo aleatorizado de diseño paralelo y prospectivo en el que está previsto participen un total de 100 individuos. El grupo RESMENA-S (n = 50) sigue una dieta personalizada de pérdida de peso (restricción energética 30%), con una distribución en macronutrientes (hidratos de carbono/grasas/ proteínas) de 40/30/30, elevada frecuencia de ingestas (7/día), bajo índice/carga glucémica y elevada capacidad antioxidante y adherencia a la dieta Mediterránea. El grupo control (n = 50) sigue una dieta con la misma restricción energética y basada en la Asociación Americana del Corazón. El estudio tiene una duración de 8 semanas bajo control dietético y psicológico en ambos grupos. Durante un periodo adicional de 16 semanas de auto-control, los voluntarios siguen el mismo patrón dietético pero sin ningún asesoramiento específico.**

Resultados: **Datos antropométricos y de composición corporal, así como muestras sanguíneas y de orina están siendo recogidas al inicio y al final de cada fase. Este proyecto está registrado en www.clinicaltrials.gov con el número NCT01087086 y cuenta con la aprobación del Comité de Ética de Investigación de la Universidad de Navarra (065/2009).**

Conclusiones: Las intervenciones que favorezcan la adopción de patrones dietéticos y de estilo de vida más saludables, son de elevada importancia para identificar los mecanismos que podrían explicar el nexo de unión entre obesidad, SM y complicaciones asociadas.

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Palabras clave: *Síndrome metabólico. Pérdida de peso. Inflamación. Estrés oxidativo. Dieta mediterránea.*

Abbreviations

Tumor necrosis factor alpha (TNF-alpha); interleukin (IL); plasminogen activator inhibitor protein-1 (PAI-1); protein C reactive (PCR); complement factor 3 (C3); retinol binding protein 4 (RBP4); malondialdehyde (MDA); cardiovascular disease (CVD); calorie restriction (CR); dual-energy X-ray absorptiometry (DEXA); State-Trait Anxiety Inventory (STAI); homeostasis model assessment-estimated insulin resistance (HOMA-IR),

Introduction

Obesity rates are currently achieving epidemic proportions worldwide.[1,2] The metabolic syndrome describes a clustering of metabolic abnormalities that increase the cardiovascular and diabetes risk, which not only includes the obesity as a process characterized by an excess of body fat conditioned by genetic and environmental determinants, but also other related diseases sharing risk factors and most likely mechanisms of action.[3-6] Inflammatory markers, such as tumor necrosis factor alpha (TNF-alpha), interleukin 6 and 18 (IL-6; IL-18), plasminogen activator inhibitor protein-1 (PAI-1), protein C reactive (PCR), complement factor 3 (C3), retinol binding protein 4 (RBP4), intercellular adhesion molecules (ICAMs), vascular cell adhesion molecules (vCAMs), asymmetric dimethylarginine (ADMA), ceruloplasmin and leptin have been negatively correlated to clinical features of the metabolic syndrome.[7-11] In addition, the evaluation of several markers of the oxidant/antioxidant status such as malondialdehyde (MDA), oxidized LDL and the total antioxidant capacity of the plasma might be also relevant to understand the mechanisms behind the development of clinical metabolic syndrome features.[12] Further research in both, the role of inflammation and oxidative stress in the metabolic syndrome is therefore needed to elucidate those mechanisms.[13] For this purpose, new nutritional interventions and complementary approaches for effective prevention and treatment of metabolic syndrome must be designed.

The Mediterranean diet, a dietary pattern that has attracted considerable interest because of its potential advantages in the prevention of chronic diseases[14,15] contains many food compounds with putative anti-inflammatory and/or antioxidant effects.[16] A Mediterranean diet supplemented with virgin olive oil or nuts has for example recently shown an anti-inflammatory effect reducing serum CRP, IL-6 and endothelial and monocytary adhesion molecules and chemokines in comparison to a low-fat diet in subjects at a high cardiovascular risk in the PREvención con DIeta MEDiterránea (PREDIMED) Study.[17] A regular weekly consumption of legumes within a hypocaloric diet also resulted in a specific reduction in CRP and C3 and a clinically significant improvement of the lipid profile and blood pressure in overweight and obese subjects.[13] The application of nutrigenomics techniques for large-scale profiling of genes, proteins and metabolites recently showed that a short-term double-blind, crossover study with a dietary mix containing resveratrol, alpha-tocopherol, vitamin C, n-3 polyunsaturated fatty acids and tomato extract, all of them naturally present in the Mediterranean diet, was able to modulate inflammation of adipose tissue, improve endothelial function, and increase liver fatty acid oxidation in overweight men with mildly increased C-reactive protein concentrations.[18]

Other dietary factors have been shown to be effective in metabolic syndrome and related pathologies.[19] For example, high protein diets might guarantee a satiety effect and a lower recovery of the lost weight according to the results derived from The DIet, Obesity and GENES (DIOGENES) study due to the higher thermogenic effect of this nutrient.[20-22] Low glycemic load diets have proved to positively affect gene expression in subcutaneous adipose tissue in persons with metabolic syndrome[11,23] and a daily consumption of 3 portions of whole-grain foods has recently demonstrated to significantly reduce CVD risk in middle-aged people mainly through blood pressure-lowering mechanisms.[24]

In addition to the quantitative and qualitative composition of the diet, other factors related to eating behavior habits might significantly influence the success of a weight-loss nutritional intervention.[24-28] Factors such as the meal frequency, the size of the eating portions, the distribution of the portions along the day, the subjective feelings of hunger and the quality of life and mood of the subject might be taken into account in current and future interventions.[29]

A decrease in energy intake by means of dietary restriction has also shown to lower the risk of CVD in obese populations.[30] The most common form of dietary restriction implemented is daily calorie restriction (CR), which requires individuals to decrease their energy intake by 15-40% of baseline needs each day.[19,31] Preliminary results from the Comprehensive Assessment of the Long-term Effect of Reducing Intake of Energy (CALERIE) Study, aimed to test the effects of 25% CR in 150 non-obese healthy subjects aged 25-45 years, have pointed out significant alterations in energy metabolism, oxidative damage, insulin sensitivity, and functional changes in both the neuroendocrine and sympathetic nervous systems.[32,33] Interestingly, the 6-month CR intervention not only caused favorable physiological responses in body composition, diabetes risk factors, CVD risk, biomarkers of longevity, energy expenditure, endocrinology and physical activity, but also psychological and behavioral responses.[34,35] However, it is still a challenge for most individuals to practice CR in an obesogenic environment so conducive to overfeeding. Other dietary and non-dietary factors must be also considered in the design of new nutritional strategies involving psychological, health-care and social support to deal with the problem of obesity and overweight.[36,37] Behavioural

therapy based on the Mediterranean diet has been recently reported as a useful tool for obesity treatment.[38] Behavioural therapy in relation to body weight management is based on the principles of "conditioning", which indicate that eating is frequently associated with external events closely linked to ingestion.[39] There are different techniques used in behavioural therapy, such as stimulus control, self-monitoring, positive reinforcement, or cognitive restructuring.[38] A healthy lifestyle needs planning, skill in the choice of alternatives and in estimating portion sizes, and compliance in recording food intake and energy expenditure. All this needs time to be learnt and maintained, which is one of the objectives of applying behavioural therapy techniques in nutritional studies dealing with weight loss and maintenance. Subjects can then develop skills in order to adopt proper habits and attain their healthiest weight, learning to establish realistic goals and evaluating their progressin modifying eating and physical activity habits.

Objective

The main aim of the present study is to reduce body weight and to manage the oxidative and inflammatory impaired status of Spanish obese adults with metabolic syndrome features by means of a controlled parallel nutritional intervention based on caloric restriction personalized diets accompanied by dietary counseling and psychological control.

The specific objectives of the study were the following:

1. To diagnose subjects with clinical features of metabolic syndrome based on medical history and measurements of anthropometry and biochemistry.
2. To evaluate the dietary intake and non dietary habits (alcohol intake, smoking habits, and physical activity) of the study participants through validated questionnaires.
3. To gather psychosocial information that might influence the selection of foods (socio-economical factors) and meal habits (meals outside home per week, eating rate, etc.) of the subjects.
4. To estimate the resting energy expenditure of each study participant to design a personalized diet adjusted to his own intake real needs.
5. To design a personalized diet characterized by variety, a high adherence to the Mediterranean Diet, a high intake of dietary antioxidants from natural sources, a low glycemic index and a macronutrient distribution by energy of 40/30/30 (carbohydrate/fat/protein) distributed with a high meal frequency (7 meals a day) that compete to conventional AHA-recommendation based diets.
6. To promote nutritional education, firstly by means of an informative session for the study participants and their families at the beginning of the study, emphasizing in selecting and distributing foods thorough the day; and secondly by attending dietary counseling with dietitians every 15 days during the intervention period.
7. To evaluate the body composition through bioimpedance and dual-energy X-ray absorptiometry (DEXA), at the beginning of the study, after two months of intervention and at the end of the study.
8. To measure representative markers of the oxidative and inflammatory status related to adiposity, CVD and insulin resistance, at the beginning and after two months of the intervention.
9. To capture changes in the lipid, glucose and hormone metabolism of the subjects due to the nutritional intervention by applying non-targeted metabolic profiling approaches.
10. To elucidate gene-diet interactions due to the nutritional intervention.
11. To evaluate the effects of the nutritional intervention on anxiety and psychological traits related metabolism.

Subjects and methods

Inclusion and exclusion criteria

The inclusion and exclusion criteria for the study are shown in table I. The criteria for metabolic syndrome were based on those established by the International Diabetes Federation.[40]

The study 065/2009 was approved by the local ethical committee (Research Ethics Committee of the University of Navarra). All study participants signed an informed consent document after verbal and written instructions and according to local legislation (see document). This trial is registered at www.clinicaltrials.gov as NCT01087086.

Recruitment of participants

The recruitment of the participants is being carried out with the help of the Department of Endocrinology of the Health Department of Navarra and the Department of General Medicine of the University Hospital of Navarra. Advertisements (poster approved by the Ethical Committee), internet, interviews to local press and to the University of Navarra information office, and databases from previous studies in the Department have been used for recruitment.

Sample size estimations

Calculations were based on findings of previous studies.[41,42] A group size of 40 was estimated to be necessary

Table I
Inclusion and exclusion criteria

Inclusion		*Exclusion*
Age: 35-65 years old		Subjects with difficulty for changing dietary habits
Central obesity (WC[1] > 94 cm males and > 80 cm females)		Subjects with psychiatric or psychological disorders
plus any two of the following four factors:		Subjects with eating disorders (bulimia; test of Edinburgh)
Raised triglycerides	≥ 150 mg/dL or specific treatment for this lipid abnormality	Subjects with weight instable for 3 months before the beginning of the study
Reduced HDL cholesterol	< 40 mg/dL in males < 50 mg/dL in females or specific treatment for this lipid abnormality	Subjects under any pharmacological treatment
Raised blood pressure	Systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension	Subjects with chronic diseases related to the metabolism of energy and nutrients (gastric ulcer, disorders of the digestive system, hyperthyroidism, hypothyroidism)
Raised fasting plasma glucose	≥ 100 mg/dL or previously diagnosed type 2 diabetes	Peri- and postmenopausal women Subjects on special diets
	If above 100 mg/dL , OGTT is strongly recommended but is not necessary to define presence of the syndrome	Subjects with food allergies or intolerances

[1]WC: waist circumference; BP: blood pressure; OGTT: oral glucose tolerance test.

in order to obtain a significant ($P < 0.05$) difference in the reduction of the waist circumference of 4.3 ± 6.8 cm with a power of 80%. Given an estimated dropout rate of 25%, the sample size was fixed to 50 subjects in each group (intervention and control group).

Study design

The study was designed as a 6-month weight-loss caloric restriction trial divided in 2 consecutive phases. The first phase consisted of an 8-week controlled parallel intervention period, which was followed by a 16-week self-control second phase. At the beginning of the study, subjects were randomized either to a moderately high protein weight-loss diet (group A, n = 50) or to a weight-loss diet based on the American Heart Association recommendations (Group B, n = 50). Diets in both groups (table II) were designed on a daily caloric restriction of 30% of the subjects total energy baseline needs. Group A diets were characterized by a macronutrient distribution of 40/30/30 (carbohydrate/lipid/protein), a high adherence to the Mediterranean dietary pattern, an intake of low glycemic index carbohydrates, a higher supply of energy from protein at the end of the day, a high total antioxidant capacity, and a meal frequency of 7 meals/day. A weekly intake of at least 3 portions of wholegrain pasta, 3-4 portions of legumes, 3 portions of fatty fish and 6 fruits/vegetables portions was mandatory. Group B diets were characterized by a macronutrient content of 55/30/15 (carbohydrate/lipid/protein) distributed in 3- 5 meals/day.

Table II
Examples of 1-day diet for each dietary group (1,300 kcal diet)

	Group A	*Group B*
Breakfast	Orange (175 g) 2 low-fat yogurts (2 x 125 g)	Orange (175 g) 2 low-fat yogurts (2 x 125 g) 1 slice of refined white bread (15 g)
Morning snack I	1 low-fat yogurt (125 g)	Apple (125 g) 1 low-fat yogurt with sugar (125 g)
Morning snack II	2 thin slices of ham (45 g) 2 slices of whole-grain bread (20 g)	
Lunch	Vegetables (cooked; 250 g) Whole-grain pasta (cooked; 45 g) Lean fish (cooked; 140 g) Apple (125 g)	Vegetables (cooked; 240g) Pasta (cooked; 90 g), 1 slice of refined white bread (15 g) Lean fish (40 g) Melon (250 g) 1 low-fat yogurt (125 g)
Afternoon snack I	1 low-fat yogurt (125 g)	Banana (75 g)
Afternoon snack II	Walnuts (10 g) Low-fat cheese (60 g)	
Dinner	Salad (200 g) 1 slice of whole-grain bread (30 g) Lean meat (cooked, 80 g) Pear (150 g)	Salad (200 g) 1 slice of refined white bread (30 g) 2 slices of ham (60 g) Pear (150 g)

During the first phase of the study, subjects received dietary counseling by qualified nutritionists every 15 days, while during the second phase of the study sub-

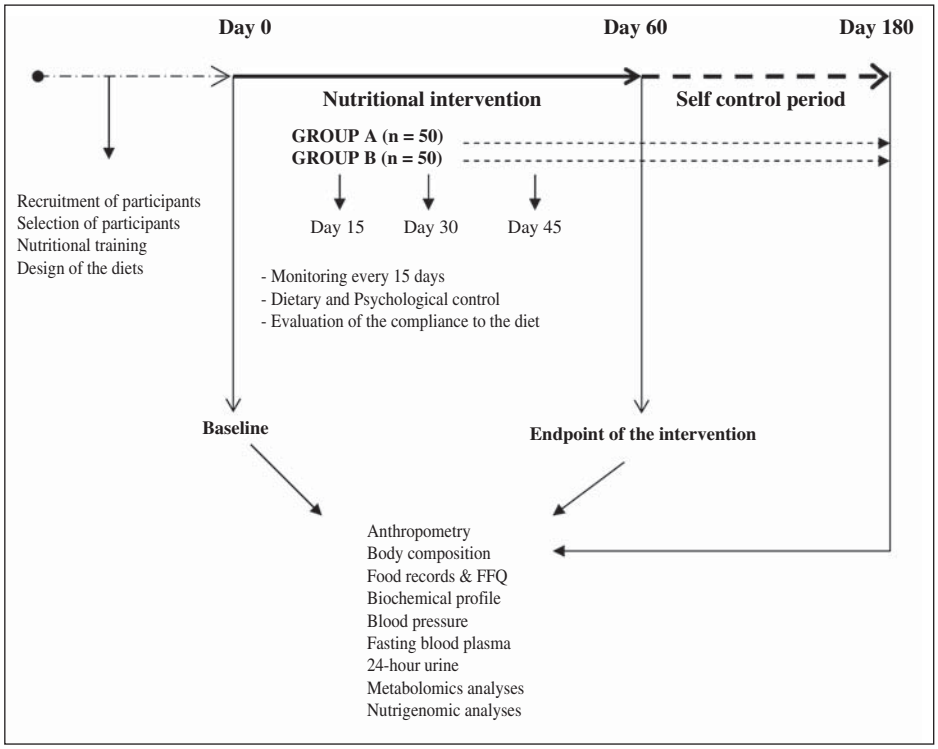

Fig. 1.—Experimental design of the RESMENA-S study.

jects were asked to follow the diets without any dietary advice. In addition, subjects were asked to fill in a validated personality test (NEO-PIR) at the beginning of the study. Validated psychological tests (Beck depression Inventory;[43] State-Trait Anxiety Inventory (STAI);[44] and Anxiety thermometer)[45] to evaluate their levels of anxiety during the weight-loss diet intervention were filled at the beginning of the study, every 15 days during the first phase, and at the end of the study. Figure 1 shows the experimental design of the study.

Screening visit

Recruited participants will attend a screening visit in which they will receive a written document with information about the study together with the informed consent to be signed (Appendix). Both documents were approved by the Research Ethics Committee of the University of Navarra. During this visit, any doubt concerning the participation of the subject in the study will be solved by qualified staff. After a medical examination by a physician, anthropometric parameters and blood pressure will be measured and a fasting blood sample will be drawn by a nurse for the biochemical determination of metabolic syndrome clinical features. The subject will be asked to fill in a validated questionnaire concerning food frequency and dietary habits (SUN questionnaire) and a 72-hour food record, data that together with the calculation of the basal metabolic rate of the subject will be used by the dietitian to design their personalized diets.[46] A validated test of personality (NEO-PI-R) will be also filled in by the participant. Those subjects who meet the inclusion criteria will be given an appointment for the first visit of the intervention and for an informative group session.

Study visits

Six visits were planned for each subject in the course of the study at days 0, 15, 30, 45, 60 and 180. Details of the determinations and measurements for each of them are given in table III. The following metabolic determinations and markers will be also calculated at the beginning and end of the study: body mass index, waist-to-hip index, homeostasis model assessment-estimated insulin resistance (HOMA-IR) and atherogenic index.

In addition to the intervention visits, participants from group A were requested to attend an informative group session to reinforce psychological attitude at the beginning of the study. In this session, two qualified nutritionists are explaining the benefits of the dietary pattern to follow in the study, the options to create their own personalized menus with the established personalized diets, as well as emphasized the importance of eating habits and compliance during both the controlled and self-control phases of the study. At the end of the study, participants are receiving a report about the evaluation of their nutritional status in a last dietary counseling visit with the dietitian.

Appendix

HOJA INFORMATIVA PARA EL PARTICIPANTE

TÍTULO: PROYECTO DE INTERVENCIÓN NUTRICIONAL PARA PACIENTES CON SÍNDROME METABÓLICO

Responsable del estudio: Marian Zulet Alzórriz

(Informative document about the study for the participants)

Esta hoja informativa le invita a participar de forma totalmente voluntaria en un proyecto sobre intervención nutricional para pacientes con síndrome metabólico.

El objetivo global del estudio es contribuir a la mejora de su salud siguiendo una dieta saludable, sin recibir ningún tipo de producto dietético adicional.

El estudio se llevará a cabo en la Unidad de intervención nutricional del departamento de Ciencias de la Alimentación, Fisiología y Toxicología de la Universidad de Navarra, y será atendido por un equipo integrado por una enfermera, una médico-dietista, una dietista-nutricionista.

En esta investigación se van a ensayar dos tipos de dietas personalizadas, una dieta control que cumple con las normas establecidas por la Asociación Americana del Corazón (AHA), o una dieta variada y saludable confeccionada a base de alimentos tradicionales, como el consumo de alimentos propios de la dieta mediterránea. La asignación a un grupo dietético u otro se realizará de modo aleatorio.

En esta primera cita, de aproximadamente media hora de duración, se le hace entrega de esta hoja informativa para que usted la lea y pregunte sus posibles dudas sobre el proyecto. A continuación se le hace entrega de la hoja de consentimiento informado, por duplicado y aprobado por el Comité de ética de la investigación de la Universidad de Navarra, para que muestre su conformidad. El estudio comenzará con una breve historia clínica con exploración física llevada a cabo por una Licenciada en Medicina. En tal caso, la enfermera procederá a la extracción de una muestra de sangre. Este procedimiento puede conllevar algunas molestias para usted como ligera molestia en la zona de punción o presencia posterior de hematoma en esta misma zona y en casos excepcionales lipotimias. La finalidad de tomar estas muestras es llevar a cabo análisis bioquímicos de rutina relacionados con el colesterol, la glucosa, y las proteínas para comprobar que usted cumple todos los criterios establecidos para formar parte de este estudio.

En el caso de que usted cumpla los criterios de inclusión, se le citará para una sesión de grupo en la que se le dará las pautas generales para llevar a cabo la dieta, de una hora de duración aproximadamente. En esta cita, la Dietista le realizará la Historia dietética haciéndole entrega de un cuestionario de hábitos de vida (SUN) y un registro de pesada de 48 horas con las aclaraciones correspondientes acerca de cómo se deben cumplimentar y errores frecuentes que se cometen a la hora de rellenarlos. Igualmente, recibirá citación para comenzar con el estudio de intervención (día 0) de 60 días de duración y se le proporcionará un bote de orina estéril para que recoja orina de primera hora de la mañana el día citado.

En la cita del día 0 se le volverá a recordar en qué consiste su participación y se le tomarán medidas de peso, talla, perímetro cintura y de composición corporal. Al mismo tiempo se le realizará una serie de preguntas relacionadas con el estado anímico y el grado de ansiedad. A continuación se le tomará la tensión arterial y la enfermera le extraerá una muestra de sangre para llevar a cabo análisis bioquímicos de rutina (colesterol, glucosa, etc) y otros más específicos de relacionados con síndrome metabólico, entre ellos un análisis de la expresión de determinados genes. La dietista le proporcionará su dieta personalizada para comenzar el estudio de intervención nutricional. Deberá ajustarse a las pautas que se le establezcan, como la ingesta de alimentos, forma de preparación y a las recomendaciones de estilo de vida.

Durante el estudio, acudirá quincenalmente (días 15, 30 y 45) para comprobar el seguimiento de la dieta, reforzar el cumplimiento de la dieta y resolver las dudas que se le vayan planteando. Además, se le controlará el peso y composición corporal, el apetito y el estado de ánimo y ansiedad; con una duración aproximada de media hora. El día 45 se le proporcionará un bote de orina estéril para que recoja orina de primera hora de la mañana del último día de intervención nutricional (día 60). Además de, un registro de pesada de 48 horas, un cuestionario de hambre y saciedad, y un test de personalidad para su entrega el último día de intervención (día 60).

El estudio de intervención concluirá tras 2 meses con la valoración de la composición corporal, historia dietética, estado anímico y grado de ansiedad y con la extracción de una muestra de sangre.

El estudio continuará con un periodo de autonomía durante 4 meses más. Durante este tiempo usted no recibirá asesoramiento, pero deberá aplicar lo aprendido previamente.

Al finalizar estos 4 meses se le dará cita para acudir a la Universidad de Navarra y que se le evalúe de nuevo su estado nutricional, en una entrevista de una hora aproximadamente. Tras el procesamiento de los datos, se le informará de los resultados de las pruebas realizadas y se mantendrá la confidencialidad propia de todo procedimiento médico.

Toda la información que nos proporcione así como los resultados de los análisis de sangre se tratarán según la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal, utilizando códigos para asegurar la confidencialidad y garantizar el anonimato. Sólo dos miembros del equipo investigador conocerán sus datos personales, ya que serán los encargados de contactar con usted para cualquier evento relacionado con el estudio. El resto de miembros del equipo trabajarán con códigos, ignorando a qué voluntario le corresponde cada código. Usted puede abandonar el estudio en cualquier momento, sin dar explicaciones y sin que esto repercuta en su asistencia médica.

SU PARTICIPACIÓN EN EL ESTUDIO NO ESTÁ REMUNERADA

FORMULARIO DE CONSENTIMIENTO (INFORMED CONSENT IN SPANISH)

Reducción de Síndrome Metabólico en Navarra-Spain (RESMENA-S) mediante una estrategia multidisciplinar e innovadora, basada en la crononutrición y la educación nutricional, junto con control dietético y psicológico

Yo (nombre y apellidos) .....

- He leído la hoja de información que se me ha entregado.
- He podido hacer preguntas sobre el estudio.
- He recibido suficiente información sobre el estudio.
- He hablado con: (nombre del investigador) .....

Entiendo que mi participación es voluntaria.

Entiendo que puedo retirarme del estudio:

1º Cuando quiera.

2º Sin tener que dar explicaciones.

3º Sin que esto repercuta en mis cuidados médicos.

Presto libremente mi conformidad para participar en el estudio.

Fecha Firma del participante

Fecha Firma del investigador

Application of "omics" tools in the study

In addition to traditional determinations, the RESMENA-S study will also apply metabolomics and transcriptomics approaches.

Metabolites are the cellular endpoints of gene expression and of any physiological regulatory process. Measuring such compounds might therefore offer deeper insights into mechanisms of health and disease as well as might provide a greater understanding the role of lifestyle and dietary factors[47] in relation to specific diseases such as metabolic syndrome. Currently metabolomics is driven by the breathtaking advancements of analytical techniques providing constantly improved sensitivity and larger metabolite panels, but also by advances in chemometrics and bioinformatics. Among the metabolomics platforms, those based on mass spectrometry are increasingly used to characterize the complex meta-

Table III
Determinations and measurements to be performed during the study visits

Visit day	*0*	*15*	*30*	*45*	*60*	*180*
Body weight	x	x	x	x	x	x
Blood pressure (SBP, DBP)	x				x	x
Waist and hip circumference	x				x	x
Skin-folds[(1)]	x				x	x
Body composition by bioimpedance	x	x	x	x	x	x
Body composition by DEXA	x				x	x
Collection of fasting blood[(2)]	x				x	x
24-h urine collection	x				x	x
Fasting plasma glucose	x				x	x
Fasting plasma insulin	x				x	x
Free fatty acids	x				x	x
Cholesterol, HDL-Chol, LDL-Chol	x				x	x
Total proteins	x				x	x
Inflammatory markers[(3)]	x				x	x
Oxidative-stress markers[(4)]	x				x	x
Metabolomics analyses[(5)]	x				x	x
Gene expression analyses[(6)]	x				x	x
Dietary counseling	x	x	x	x	x	x
72 hour-weight food record	x	x	x	x	x	x
VAS questionnaire					x	
State-Trait Anxiety Inventory (STAI)	x				x	x
Beck depression Inventory	x				x	x
Anxiety thermometer	x	x	x	x	x	x

[(1)]Bicipital, tricipital, subescapular suprailiac skin-folds.
[(2)]Plasma, erythrocytes and peripherial blood mononuclear cells sampling.
[(3)]Homocistein, C3, Ceruloplasmin (Turbidimetry COBAS MiraS); Retinol binding protein 4 (RBP4), asymmetric dimethylarginine (ADMA), Protein C reactive (PCR), Interleukins IL-6 and IL-18, TNF-alpha, PAI-1, fibrinogen.
[(4)]Selenium, Oxidized LDL Malondialdehyde, Total antioxidant capacity of plasma.
[(5)]Metabolomics analyses will be performed by non targeted metabolic profiling based on LC/MS.
[(6)]Nutrigenomics gene expression analyses will be performed by Real time PCR.

bolic effects of nutrients and foods.[48] In this sense, in the RESMENA-S study we aim to monitor metabolic changes associated with the 6-month weight loss nutritional intervention by applying non-targeted metabolic profiling approaches to blood and urine samples of the participants at the beginning and end of each phase of the study.[49,50] The application of metabolomics might help to elucidate the effect of the personalized caloric restriction diets on low-grade inflammation, oxidative stress and whole body metabolism with a special interest in the lipid and glucose metabolism. In addition, the metabolomic analyses will be used to investigate the effects that the different diets in the RESMENA-S intervention can have on stress and anxiety trait-related metabolism. In fact, the identification of different metabolic phenotypes and its combination with other phenotypic data to give dietary advice could be envisaged as a potential role of metabolomics in personalized nutrition,[51,52] with a special interest in obesity related diseases such as the metabolic syndrome.

Nutrigenomic profiling and epigenetics studies are envisaged in both dietary groups in order to establish predictive patterns concerning the response to the nutritional intervention as well as prognostic markers of the outcomes.[53] These tasks will be performed using molecular and genetic tools such as microarrays, RT-PCR and sequencing in selected genes related to inflammation, energy homeostasis, and lipid metabolism in order to elucidate the role of gene expression in metabolic syndrome related phenotypes.

Facilities

The Department of Nutrition, Food Sciences, Physiology and Toxicology at the University of Navarra is provided with the facilities and equipment needed for the evaluation and monitoring of the nutritional status of the study participants. This is mainly based in medical data collection, anthropometry and body composi-

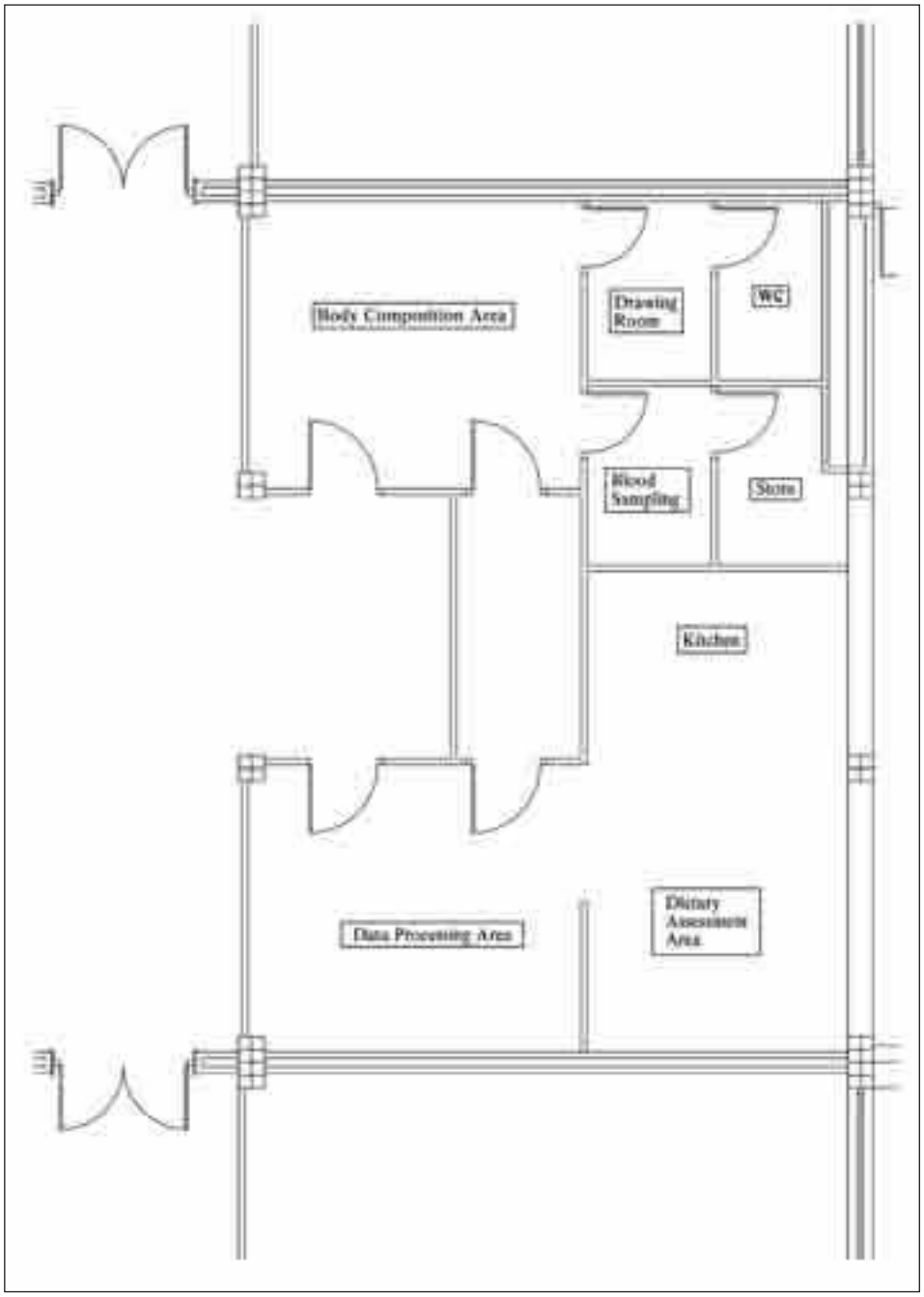

Fig. 2.—Schematic distribution of the metabolic unit.

tion equipments (tapes, scales, tensiometers, skin fold calipers, devices for bioelectrical impedance analysis and DEXA) and dietary measurements (calorimeter, validated food questionnaires, nutrition softwares such as Medisystem, DIAL, Nutriciun). The availability of a recently renovated Metabolic Unit for individual attention to the patient, monitoring and dietary counseling along the intervention study has been of great help (fig. 2). The unit is divided in different areas: an office for medical visiting hours equipped with DEXA and bioimpedance devices; a changing room and a toilet; an area for blood collection; an area for calorimeter measurements; an room for dietary counseling, equipped with a dining room table, microwave, a cooktop, a fridge, and a sink; and an area for participant recruitment formalities, control of medical histories and writing of patient reports.

Novelty of the study and conclusions

The present initiative is based on our traditional diet but from a more personalized point of view. RESMENA-S Study aims to integrate the main results obtained from the latest observational epidemiological

studies and interventional studies in the dietary pattern designed for obese subjects with metabolic syndrome features.[23,54-58] The study will apply the concept of selecting and distributing the foods thorough the day according the physiological needs of each individual, and will not only consider the quantitative and qualitative composition of the diets, but other important factors related to dietary habits. The importance of regular dietary support and psychological control will be also evaluated for a successful loss of weight and improvement of clinical features of metabolic syndrome.

Furthermore, the combination of varied tools generating detailed clinical chemistry, anthropometry, and metabolite and gene data will allow to perform a functional analysis for biological interpretation of the impact of personalized diets based on the Mediterranean dietary pattern and energy restriction in the treatment of metabolic syndrome features.

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