Juanola-Falgarona, Martí; Ibarrola-Jurado, Núria; Salas-Salvadó, Jordi; Rabassa-Soler, Antoni; Bulló, Mònica

DESIGN AND METHODS OF THE GLYNDIET STUDY; ASSESSING THE ROLE OF GLYCEMIC INDEX ON WEIGHT LOSS AND METABOLIC RISK MARKERS

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Design and methods of the GLYNDIET study; assessing the role of glycemic index on weight loss and metabolic risk markers

Martí Juanola-Falgarona1,2, Núria Ibarrola-Jurado1,2, Jordi Salas-Salvadó1,2,3, Antoni Rabassa-Soler1 and Mònica Bulló1,2


Abstract

Background: Glycemic index and/or glycemic load have been explored as an alternative for the prevention and/or management of obesity, cardiovascular disease, type 2 diabetes mellitus, and cancer.

Objective: The purpose of the manuscript is to describe the design and methods used in the GLYNDIET Project, a study designed to simultaneously address the questions related to the exact role of low glycemic index carbohydrates has on weight loss.

Methods: This study was designed as a 6-months randomized, parallel, controlled clinical trial aiming to evaluate the effect of the dietary glycemic index on weight-loss, satiety, glucose and insulin metabolism, lipid profile, inflammation and other emergent metabolic risk markers. Eligible subjects were community-dwelling men and women aged between 30 and 60 years, with a body mass index between 27 and 35 kg/m². Subjects were randomly assigned to three different dietary intervention groups (low glycemic index diet, high glycemic index diet or low-fat diet), that were isocaloric, and did not differ in the amount of dietary fibre. Monthly, study subjects were scheduled for control visits where anthropometry, blood pressure, dietary habits, satiety and physical activity were assessed. Blood, urine and subcutaneous adipose tissue samples were collected at baseline and at the end of the study to further molecular and biochemical measurements.

Discussion: The GLYNDIET study was designed to determine if there is a greater effectiveness of a carbohydrate restricted diet with low glycemic index compared to an isocaloric diet with carbohydrates of high glycemic index or low-fat diet on weight loss in middle long-term.

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Key words: Glycemic index. Weight loss. Inflammation. Satiety.

Correspondence: Mònica Bulló.
Human Nutrition Unit.
Faculty of Medicine and Health Sciences.
Universitat Rovira i Virgili.
C/ Sant Llorenç, 21.
43201 Reus. Spain.
E-mail: monica.bullo@urv.cat

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DISEÑO Y MÉTODOS DEL ESTUDIO GLYNDIET; EVALUANDO EL PAPEL DEL ÍNDICE GLUCÉMICO SOBRE LA PéRDIDA DE PESO CORPORAL Y MARCADORES DE RIESGO METABÓLICO

Resumen

Introducción: El índice glucémico y la carga glucémica se han postulado como una alternativa para la prevención y/o el manejo de la obesidad, enfermedades cardiovasculares, diabetes mellitus tipo 2 y cáncer.

Objetivo: Describir el diseño y los métodos utilizados en el proyecto GLYNDIET, un estudio diseñado para evaluar el papel del índice glucémico sobre la pérdida de peso corporal, la saciedad, la inflamación y marcadores de riesgo metabólico.

Métodos: Ensayo clínico, en paralelo, controlado, aleatorizado y de 6 meses de duración realizado en hombres y mujeres de entre 30 y 60 años, con un índice de masa corporal de entre 27 y 35 kg/m². Los sujetos fueron asignados aleatoriamente a una de las 3 intervenciones (dieta con carbohidratos de bajo índice glucémico, dieta con carbohidratos de alto índice glucémico o dieta baja en grasa). Los sujetos fueron citados mensualmente para realizar visitas control en las que se recogían datos a antropométricos, de presión arterial, hábitos dietéticos, sensación de saciedad y grado de actividad física. Al inicio y al final del estudio se recogieron muestras sanguíneas, urinarias y de tejido adiposo subcutáneo mediante biopsia abdominal.

Discusión: El estudio GLYNDIET se diseñó con el objetivo de determinar si el consumo de una dieta con carbohidratos de bajo índice glucémico muestra una mayor efectividad sobre la pérdida de peso corporal y la modulación de factores de riesgo metabólico en comparación a una dieta con carbohidratos de alto índice glucémico o una dieta baja en grasas.

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Correspondencia: Mònica Bulló.
Human Nutrition Unit.
Faculty of Medicine and Health Sciences.
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C/ Sant Llorenç, 21.
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Background

Overweight and obesity are one of the major public health concerns because the prevalence and its rapidly increasing worldwide. Moreover, obesity has been associated with the incidence of multiple co-morbidities such as type-2 diabetes (T2DM), hypertension, cardiovascular disease and cancer. The most reliable explanation of this situation is changes occurred in lifestyle (i.e. dietary habits) of modern industrialized societies.

Traditionally, low-fat diets have been widely recommended for weight control. Nevertheless, the interest on the amount and quality of dietary carbohydrates has been of a growing interest. In a meta-analysis of randomized controlled trials encompassing a total of 447 subjects, evidence was found to support the use of low-carbohydrate diets for weight reduction in short to medium term (up to 6 months). However, the results of longer-term trials in terms of body weight reduction and metabolic benefits are highly controversial.

Despite of that, dietary carbohydrates provide the most frequently and important source of energy worldwide, reaching between 45 and 60% of total energy intake. In 1998, FAO recommended to classify carbohydrates according to their glycemic effect. Since then, the control of glycemic index (GI) and/or glycemic load (GL), have been explored as a dietary alternative for the prevention and/or management of obesity, cardiovascular disease, T2DM, and cancer. In the scientific community there is a growing consensus on the protective effect of low GI/GL diets on the risk of chronic conditions such as T2DM, coronary heart disease and some types of cancer. However its effect on obesity and satiety are less conclusive and recently, the European Foods Safety Agency has considered insufficient the evidences to make recommendations for or against the use of glycemic index on obesity treatment.

The knowledge of the mechanisms underlying the potential beneficial role of carbohydrates according to their GI classification could be of great interest in terms to design effective therapeutically strategies on obesity and its comorbidities. GI has been involved in fuel partitioning, although the magnitude of this effects seems to be not sufficient to modify body composition. Increasing satiety has also been proposed as a potential mechanisms induced by low-GI foods for the control of weight-gain. However, the effect on satiety was observed only in acute clinical trials, whereas studies conducted in the short/medium (1-12 weeks) or in the long-term (12 months or more) do not found any effect of the GI or GL on satiety control. Finally, inflammation rise as an alternative mechanism underlying the beneficial effects of low-GI foods on obesity control and its metabolic derangements. Nonetheless, most of these studies have been conducted in a reduced number of subjects, are of shortly duration and without control of dietary potential confounders. For these reasons the exactly role of GI on inflammation is still a matter of debate.

The GLYNDIET Project was designed to simultaneously address the questions related to the exactly role of low glycaemic index carbohydrates has on weight loss, and its underlying molecular mechanisms.

Methods/design

Study design

The GLYNDIET study has been designed as a 6-months randomized, parallel, controlled clinical trial aiming to evaluate the effect of the dietary glycemic index on weight-loss, satiety, glucose and insulin metabolism, lipid profile, inflammation and other emergent metabolic risk markers. The second objective is to assess the acute postprandial effects of breakfasts differing in its GI foods on satiety, glucose and insulin metabolism, lipid profile and systemic inflammation response. Thirdly, in a subgroup of patients, we evaluate chronic effect of the dietary glycemic index/load on adipose tissue expression of several biomarkers of stress.

Fig. 1.—Study design. Intervention period and scheduled visits.

Design and methods of the GLYNDIET study


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Eligibly subjects

Eligible subjects were community-dwelling men and women aged between 30 and 60 years, with a body mass index (BMI) between 27 and 35 kg/m². Subjects were excluded if they had one of the following criteria: a) non-controlled T2DM defined as having a HbA1c > 8%; b) systolic blood pressure (SBP) > 159 mmHg or diastolic blood pressure (DBP) > 99 mmHg; c) plasma low-density lipoprotein (LDL) cholesterol > 160 mg/dL; d) plasma triacylglycerol (TAG) concentrations > 400 mg/dL; e) suspicion of secondary obesity; f) presence of any inflammatory or chronic obstructive pulmonary disease, infection, active neoplastic, endocrine or haematological disease at the time of the study; g) leucocyte count ≥ 11 x 10⁶ cells; h) taking anti-inflammatory drugs, steroids, hormones or antibiotics that could affect the parameters analysed in the study; i) changes in medication for lipid profile, diabetes or hypertension in the three months previous of the study; j) active alcoholism or drug dependence, excluding tobacco use; k) having followed a highly restrictive diet for 3 months before the beginning of the study or latest weight loss (more than 5 kg in the last 3 months); l) medical condition that discourages the inclusion in the study; m) problems in to understand the study or anticipated difficulty in making dietary changes according to the Prochaska and DiClemente model.²⁸

Recruitment

Subjects were recruited from the outpatient clinics in obesity of the University Hospital of Sant Joan de Reus and announcements made in the Reus (Spain) primary care centres of the Institut Català de la Salut.

Screening and enrolment procedures

Potential subjects contacted the research staff by telephone or during their clinical visits where they were asked for personal data, anthropometric measures and medical history. Eligible subjects interested in the study were scheduled in a screening face-to-face interview. During this screening interview, the objective and main details of the study were explained, and a signed informed consent was obtained from willing participants that potentially comply with inclusion criteria. Figure 2 shows the workflow of the study.

Interventions

Subjects fulfilling the inclusion criteria were randomly assigned to three equally sized different dietary intervention groups, by using a computer-generated random-number sequence. Subjects were assigned into blocks of 3 participants balanced by sex, age (< 45 years and ≥ 45 years) and anti-diabetic drugs use (yes or no). Subjects were advising on a:

- a) Low-GI diet (40% of energy from fat, 42% from low-GI carbohydrates and 18% from protein).
- b) High-GI diet (40% of energy from fat, 42% from high-GI carbohydrates and 18% from protein).
- c) Low-fat diet (30% of energy from fat, 52% from high-GI carbohydrates and 18% from protein).

Recommended diets were isocaloric, and the amount of dietary fibre, do not differ between the three intervention groups.

Registered dieticians gave personalized advice to each participant with specific recommendations in each group related to the desired frequency of meals, the intake of specific foods with particular emphasis on the type of carbohydrate and cooking methods.

Subjects who were randomized to the low-GI diet were especially encouraged to eat whole grain cereals and pulses as the base of their diet, avoid the rice and potatoes, and were also recommended to select specific type of fruit (apple, orange, peach) and vegetables (courgette, tomato, onion) with low GI, avoiding the ripe pieces. They were advised to reduce the time cooking of carbohydrate rich-foods in order to maintain the low GI of the foods. The principal animal protein sources of the diet were white fish and white meat.

Contrary, participants randomized to the high-GI diet were encouraged to eat refined grain cereals, fruits (banana, kiwi, melon) and vegetables (carrot, green bean, cabbage) with high GI, and avoid pulses. Unlike the low-GI intervention, subjects on high-GI were advised to increase the time cooking in order to rise the GI of the foods. In this intervention group, intake of white fish and white meat were the main animal sources of protein.

Subjects randomized in low-fat diet were also advised to maintain a high-GI diet but with lower fat content. Additionally, daily sugar was substituted by glucose in order to rise GI of this intervention. In this case, they were recommended to avoid red meat and blue fish due its high fat content and also recommended to eat low-fat dairy products.

In order to facilitate the adherence to dietary interventions, we gave to the subjects a dossier containing a leaflet with written general dietary recommendations, biweekly menus (table I), and seasonal receipts. An informative website was available for all participants (http://www.glyndiet.org/). In order to obtain the desired weight loss, a 500 kcal restriction in diet was applied to each participant. Total daily energy expenditure for each participant was estimated using the WHO (2001) equations corrected by the physical activity degree.

Ethical committee

The Institutional Review Board of University Hospital of Sant Joan de Reus (Spain) approved the
study protocol on February 2009. The trial was registered in International Standard Randomized Controlled Trial Number Register (ISRCTN54971867).

Measurements

Individual examination visits were scheduled at baseline, after 15 days of intervention, and then monthly until the end of the study. Across the visits, different evaluations and questionnaires were conducted to assess changes on anthropometry and the adherence to the intervention.

Anthropometry and blood pressure

Each examination visit included the evaluation of anthropometry and blood pressure. Body weight and
height were measured using calibrated scales and a wall-mounted stadiometer with subjects wearing light clothes and no shoes by trained staff. Their body mass index was calculated as the weight (kg) divided by the square of the height (m). Waist circumference was measured twice at the midway between the lowest rib and the iliac crest. Body composition was measured by bio-electrical impedance analysis (TANITA TBF-300, Arlington Heights, USA). Blood pressure was measured in the non-dominant arm, using a validated semiautomatic oscillometer (Omron HEM-705CP, Hoofddorp, Netherlands), in duplicate with a five-minute interval between each measurement, and the mean of these values was recorded.

**Dietary assessment**

Dietary intake was estimated at baseline and at the 1st, 3rd and 6th month of intervention by mean of 3-day dietary records including two workdays and a weekend day. Subjects were encouraged to record the food that they eat; otherwise trained dieticians estimated weight using an illustrated book of food portions. Energy and nutrient intake were calculated from Spanish food composition tables. Values of GI for each food were extracted from the International Glycemic Index and Glycemic Load Values using glucose as the reference scale. The dietary glycemic index was calculated according to the equation:

\[
\text{Dietary GI} = \sum \text{GI}_a \times (\text{CHO}_a / \text{CHO}_{a-n})
\]

where GI\(_a\) represents the glycemic index of the food, CHO\(_a\) the available carbohydrate of the food and CHO\(_{a-n}\) represents the total available carbohydrate.

Dietary glycemic load was calculated as follow:

\[
\text{Dietary GL} = \sum \text{GI}_a \times \text{CHO}_a / 100
\]

Satiety was evaluated at baseline and at the end of the study. Participants completed a short subjective questionnaire measuring the rates of hunger, fullness, satiety and desire to eat at breakfast, lunch and dinner using visual analogue scales (VASs). VASs were represented by a 100 mm line that goes to 0 to 10, where 0 represents “extremely hungry” and 10 “I’m hungry as I’ve ever been”. Subjects had to rate their subjective levels of satiety before having each meal and every 30 minutes during four hours after.

**Physical activity**

As dietary intake, physical activity was evaluated 3 times along the intervention using the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire. There was no specific intervention on physical activity during the 6 months of the intervention. Subjects were encouraged to continue with their normal patterns of physical activity.

**Tolerance and side effects**

In each month visit, dietitians assessed any adverse effects occurred by administering a checklist of symptoms including: mouth symptoms; bloating, fullness, or indigestion; altered bowel habit; and any other diet-related symptoms.

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**Table I**

Quantitative example of a daily menu for the three arms of dietary intervention

<table>
<thead>
<tr>
<th></th>
<th>Low-GI diet</th>
<th>High-GI diet</th>
<th>Low-Fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td>Skimmed milk, whole-grain cereals or whole-grain bread with olive oil and nuts</td>
<td>Skimmed milk, breakfast cereals with chocolate and fruit</td>
<td>Low-fat milk, white bread sandwich with white cheese and fruit</td>
</tr>
<tr>
<td><strong>Mid-morning snack</strong></td>
<td>Whole-grain sandwich with white cheese and olive oil</td>
<td>White bread sandwich with ham and olive oil</td>
<td>Low-fat yogurt with glucose and white toast</td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td>Stewed lentils with vegetables, baked sole with salad, fruit and whole-grain bread</td>
<td>Green salad, white pasta with Bolognese sauce, fruit and white bread</td>
<td>Mashed potato, grilled turkey with artichokes, fruit and white bread</td>
</tr>
<tr>
<td><strong>Afternoon snack</strong></td>
<td>Low-fat yogurt, fruit, whole-grain bread with olive oil</td>
<td>Full-fat yogurt, fruit and Rich Tea biscuits</td>
<td>Low-fat yogurt with breakfast cereals with chocolate, glucose, fruits</td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td>Salad with goat cheese, omelette with vegetables, fruit and whole-grain bread</td>
<td>Rice salad, grilled salmon with vegetables, fruit and white bread</td>
<td>Vegetable soup, scrambled eggs with mushrooms, fruit and white bread</td>
</tr>
</tbody>
</table>

GI: Glycemic index.
Biological samples collection and store

Blood and urine samples were collected at baseline and at the end of the study. Aliquots of EDTA plasma, citrate plasma, buffy coat and serum were kept frozen (-80°C) for further determinations of satiety markers, inflammatory cytokines and other metabolic risk markers. Specific RNA tubs were also collected and kept frozen at -20°C for further analysis of mRNA expression (Applied Biosystems, Life Technologies, UK). At the same time, platelets, erythrocytes and mononuclear cells were isolated from EDTA plasma tubes and preserved for further analysis. Simultaneously, complete blood cell count, fasting plasma glucose, glycosylated haemoglobin, lipid profile, urea and creatinine concentrations, transaminases and coagulation tests were determined in a centralized laboratory using routine analysis methods. The 24-hour urine samples were collected, the volume of the sample was quantified and aliquots of 2 ml were kept frozen at -80°C.

Additionally, adipose tissue samples were obtained in a subgroup of subjects at baseline and at the end of the study. Subcutaneous adipose tissue samples were removed by incisional biopsy on the right side of the abdomen under local anaesthesia. The adipose tissue samples were immediately frozen in liquid nitrogen for a better preservation and were conserved at -80°C.

Evaluation of postprandial response

At baseline, a study test breakfast was served to all subjects according with dietary characteristics of the intervention group assigned. After 2 hours, a blood extraction was performed to collect blood samples for further biochemical analysis. Ratings of satiety were evaluated during a 4-h postprandial period using VASs.

Statistical analysis

Sample size was estimated considering the weight loss as the primary outcome. Based in previous studies,5,19 sample size estimated was 33 subjects for Low-GI and High-GI groups and 25 subjects for low-fat group, with an alpha error of 5% and 90% of power. Expecting a 15% of dropouts, we decided to include 40 subjects for each one of the intervention groups to compensate the possible losses.

All analyses will be based on an intention-to-treat approach. Differences between the final and baseline visits for continuous measures will be expressed as means and standard deviation. Variables that did not fit a normal distribution (Kolmogorov-Smirnov test) will be treated in its logarithmic form. The primary analysis will be done by analysis of variance of repeated measures with change in BMI between final visit and baseline visit as the dependent variable and intervention group as the independent variable. We will also conduct post-hoc comparisons within groups to observe the individual effects of the interventions. In addition, we will assess the predictive capacity of inflammatory cytokines over the body weight loss through multiple linear regression models and the changes of these cytokines throw the 6 months are effected by the intervention after adjusting for potential confounders. Level of significance was set at P < 0.05. All analyses will be performed with the newest version of the statistical software package SPSS for windows.

Discussion

Diet is the main modifiable factor for preventing and treating obesity and its associated comorbidities. It is therefore imperative to understand the exactly role of the different nutritional strategies on health, and to know what are the mechanisms that might explain such effects towards the design more effective therapeutic and preventive strategies. In opposition to the traditional dietary advices which postulated energy reduction mainly at the expense of fat for the obesity treatment, new nutritional strategies have been addresses not only through the change in the proportion of essential elements, but also the quality thereof. Over the past decade, a growing body of research has linked low GI/GL diets to weight loss. The majority of the studies found a trend in favor of low GI/GL diets, however there are several inconsistencies and no log-term studies, with large differences in dietary GI/GL interventions have been conducted. These discrepancies could be partially explained by the methodology of GI estimation of the diets through the International Glycemic Index and Glycemic Load Values.8 The majority of these values are from studies conducted in Australia or North-America where the foods or their composition may differ from that consumed in the rest of the world. In our specific case, there are few Spanish products with GI values in the international tables. The estimation of the GI of the GLYNDIET interventions must be evaluated with caution.
Fig. 3.—Flowchart of the study. SAT: Subcutaneous Adipose Tissue.

Table II

Baseline characteristics of study subjects by intervention group

<table>
<thead>
<tr>
<th></th>
<th>Low-GI (n = 41)</th>
<th>High-GI (n = 41)</th>
<th>Low-Fat (n = 40)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/Women (n)</td>
<td>8/33</td>
<td>7/34</td>
<td>9/31</td>
<td>0.828</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43 ± 7</td>
<td>44 ± 8</td>
<td>44 ± 8</td>
<td>0.529</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.7 ± 9.6</td>
<td>82.8 ± 9.8</td>
<td>83.5 ± 10.6</td>
<td>0.913</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.2 ± 2.1</td>
<td>30.8 ± 2.2</td>
<td>30.8 ± 2.2</td>
<td>0.602</td>
</tr>
<tr>
<td>Waist circumference(cm)</td>
<td>101.8 ± 7.7</td>
<td>100.4 ± 8.7</td>
<td>103.1 ± 6.9</td>
<td>0.295</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>128.0 ± 17.1</td>
<td>128.5 ± 15.1</td>
<td>131.3 ± 13.9</td>
<td>0.592</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.2 ± 10.8</td>
<td>81.2 ± 9.6</td>
<td>82.8 ± 9.1</td>
<td>0.489</td>
</tr>
<tr>
<td>Current Smoker n (%)</td>
<td>8 (20)</td>
<td>5 (12)</td>
<td>5 (13)</td>
<td>0.573</td>
</tr>
</tbody>
</table>

Data are given as mean (SD) or number (%) unless otherwise indicated. P values of the difference between intervention group (ANOVA for the continuous variables and a 2 test for categorical variables).
Conclusions

The GLYNDIET study has been designed to determine if there is a greater effectiveness of a carbohydrate restricted diet with low-GI compared to an isocaloric diet with carbohydrates high GI or low-fat diet on weight loss in middle long-term. This study will address the different molecular mechanisms that could explain the potential beneficial effect of low-GI carbohydrates on health from different perspectives: the control of satiety (visual analogue scales and biomarkers), modulation of systemic inflammation and the expression of markers of inflammation in adipose tissue, and modulation of the composition and/or activity of various cell populations (lymphocytes, erythrocytes, platelets) for their involvement in inflammatory processes of oxidation and coagulation. Therefore, the results obtained in this study will help establish new nutritional basis for the prevention and/or treatment of obesity and its comorbidities.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We thank all the participants of the GLYNDIET study for their enthusiastic collaboration and the GLYNDIET personnel, Núria Aguilera López, Andrés Díaz-López and Marta Guasch-Ferré, for excellent assistance. CIBERobn is an initiative of ISCIII, Spain. We also acknowledge the grants from Institut d’Investigació Sanitaria Pere Virgili (PV11059S). Institut d’Investigació Sanitaria Pere Virgili has no involvement in the study design, data analysis. We also acknowledge the grants from Institut d’Investigació Sanitaria Pere Virgili (PV11059S) and Fondo de Investigación Sanitaria (PI120153).

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