# FAT AND CARBOHYDRATES IN THE DIET: ITS METABOLIC CONTRIBUTION TO OBESITY IN CHILEAN WOMEN

ERIK O. DÍAZ, GABRIELA SALAZAR, RICARDO UAUY

Energy and Metabolism and Stable Isotopes Laboratory, Instituto de Nutricion y Tecnologia de los Alimentos (INTA), Avenida J.P. Alessandri 5540, Santiago, Chile

***Abstract***

*It has been found that children and adults in the Chilean population are getting obese in a rapidly raising proportion. There is a cohort of children less than six years old, which are regularly controlled by the Ministry of Health. From this information and studies carried out at INTA, it is known that the prevalence is raising continuously. Unfortunately, this can not be ascertained in adults where the nutritional situation is assessed only in small groups, which are not representative of the general population. The problem with adults is that the healthy population does not attend to the medical clinics unless they are already ill. The studies conducted in Chilean adults have found that >40% of low socio-economic status (SES) women are suffering from obesity. A intriguing aspect in our situation is that although sedentarism is frequent in adult women (as a possible cause of positive energy balance), their intake is based on a high proportion of carbohydrates (CHO) but not much fat (50-70 g on average). It may be suggested that the excess CHO can be converted into fat through denovo lipogenesis but this process is less important as cause of obesity in humans.*

*A more plausible cause of this problem is likely to be related to the diet. The oxidation hierarchy of macronutrients shows that whenever CHO and fat are available, the former will be firstly oxidised. This way, fat can be spared even when eaten in small amounts, accumulating in the mid-long term. Another important dietary aspect is provided by its fatty acids composition that according to animal studies, seems to modulate fat oxidation. In addition to these, glycemic effects of CHO eaten in combination with the same meal can further potentiate fat storage.*

*This proposal aims to test the dietary effects mentioned above by using indirect calorimetry in tandem with stable isotopes methodologies in a group of normal weight and obese women.*

## 1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Chile has been through the epidemiology transition and its population's lifestyle is now quite comparable to developed countries. Within the many changes, diet and physical activity patterns (found almost invariably associated to low SES groups in many societies). Obesity is highly prevalent, reaching >40% in middle age women from low SES populations. The diet has been mainly based on CHO (>60% of energy intake) based on foods such as pasta, bread and rice. The amount of fat on average is no more than 25-27% of the energy intake. Other countries where obesity is equally prevalent but with a higher dietary fat content seek these two characteristics as ideal.

Dietary components must be seen not only from the energy balance perspective but also from the composition of the dietary macronutrients, especially CHO and fat. It is now known that different CHO vary in their glycemic effects. The last FAO expert meeting on this nutrient has addressed this topic. They strongly recommended assessing the glycemic index (GI) of the usual foods and meals since they vary depending on the combination of local foods and preparation. Another important issue in this regard is the composition of fatty acids in the diet. It is known that their oxidation rates varies according to chain length, number of double bonds and isomerism. For example, linoleic acid has a high rate of oxidation compared to saturated fatty acids with the same chain length and isomerism. Within unsaturated fatty acids, the n-3 family is readily oxidised compared with the n-6 family. The same applies to fat mobilization. Literature on this topic comes mainly from animal studies and only few from humans. Certainly more human research is required. The available information suggests that the type of CHO and fatty acids in the diet can independently influence the metabolic route of the fuel mixture. Furthermore, and more importantly, there is another effect of high GI meals eaten in combination with fat where both can potentiate the lipogenic effect of the other through insulin activity. High levels of insulin will have an anabolic effect on fatty acids (theoretically more on saturated and long chain fatty acids).

Given a positive energy balance, obesity can certainly develop this way. This situation can be particularly valid for Chilean women with an apparently normal fat intake. In Chile, there is no national nutrition survey or a follow-up of the changes in the fatty acid composition of the usual diet, much less is known about the glycemic effect of meals. Effective dietary recommendations can be foreseen from this study to prevent obesity and its associated comorbidities.

Similar studies are conducted to assess the effect of the changes in the fatty acid w3/w6 ratio of the diet and its effects on the expression of some genes related to energy metabolism [1]. Much more has been done in the relation of dietary fatty acids and insulin resistance. Variations in serum, liver and muscle arachidonic acid levels are found to be associated to lipogenesis and insulin action, implying the role of this fatty acid in the energy substrate utilization [2,3,4]. Palmitic acid and other saturated fats have been shown to promote insulin resistance in rats [5,6]. Linoleic acid and other essential fatty acids are protective and facilitate the glucose clearance by the cells [7,8]. The mobilization of fatty acids from the adipose tissue and visceral mass has also been found to depend on the type of fatty acid stored. The longer the chain length and the unsaturation degree, the easiest the mobilization and oxidation of them in the body [9,10,11,12,13,14].

Studies performed in Chile have shown that obesity is highly prevalent on low SES >40 years old women [15]. We have recently finished a study to describe the conditioning factors of obesity in this group of women [16]. The variables measured were 24-hour dietary recall and food frequency questionnaires, physical activity (heart rate monitoring and doubly-labelled water [DLW]), energy expenditure (DLW) and resting metabolic rate under fasting conditions. The hypothesis was that there were metabolic and behavioural differences between obese and normal weight women that can help to explain their nutritional condition. The study compared a group of 21 obese and 21 normal women. The main findings were that the two groups of women are equally sedentary, there were no alterations in their resting metabolism, total energy expenditure by DLW is not finished yet but, based on the rest of the data, no differences can be expected. Composition of the diet differed but not when corrected by kilogram of body weight. Energy intake was similar to the estimated energy requirements in both groups. The study allowed to obtain a valuable information on the number of meals per/day, food composition of the main meals, and frequency of foods eaten at the different meal times. The total amount of fat eaten ranged on average from 50 g in normal weight and 75 g in obese women. There were no differences in the proportions of energy obtained from CHO, fat and protein which was 60, 25 and 15% respectively. Considering that the metabolic variables in this study have been obtained during fasting, the next step within this CRP will be to assess the possible metabolic differences under feeding conditions.

### 1.1 Detailed research objectives

This study aims to assess:

a) The fatty acid composition of the test meals and fasting plasma tryglicerides.

b) The energy and macronutrient content of the usual diet by measuring duplicate samples of one day meals.

c) The substrate oxidation (or storage) of dietary CHO and fat in the usual meals by the combined use of $^{13}C$ substrates and indirect calorimetry.

d) The glycemic response (glycemic index, GI) to the most common foods for breakfast.

e) The energy expenditure during resting metabolic rate (RMR) and free-living conditions (sub-sample by DLW) to assess the energy expenditure, physical activity pattern and expenditure, and body composition.

f) The usual dietary intake assessed by food recall and food frequency questionnaires.

g) The agreement between energy expenditure and intake from DLW measurements as a validation of the food intake questionnaires.

h) Additional objective: To set-up a labelling system for $^{13}C$ starch.

### 1.2 Hypotheses

a) The ratio of saturated fatty acid/polyunsaturated fatty acid (SFA/PUFA) in the diet is inversely associated to the total and exogenous CHO oxidation.

b) The plasma SFA/PUFA will be higher in obese compared to normal women.

c) The previous responses will be further strengthened by a high glycemic index meal.

## 2. METHODS

This project is a pilot study aimed to gather information about the dietary and metabolic conditioning factors of obesity in a group of Chilean women. A challenge meal will be used to study the effects on macronutrient's oxidation. Unfortunately, there is no adequate data on the possible response of these women to the feeding regime to be used in the sample size calculations. Variability in the plasma fatty acid profile in Chilean adults (unpublished) allowed to calculate a sample of 17 subjects per group.

### 2.1. Sample

Considering possible losses, 20 healthy, middle age (30-50 years old) women, will be divided in two nutritional categories: normal weight defined as BMI 20-25 kg/m2 and obese 30-40 kg/m2. Exclusion criteria will be the presence of any chronic disease such as diabetes, hypertension, hyperlipemia and thyroid disorders. The sample will be obtained from a poor urban population in the East Side of the city. For each obese woman a corresponding normal weight control will be studied. The variables to be used for this matching will be age, smoking habits, occupation (housewife, manual work, office work) and menopause (or oestrogen therapy), if present.

### 2.1. Methodology

The study is divided in five stages:

a) **At home:** Initial contact and first selection will be applied at the house level based on the exclusion criteria.

b) **At INTA:** A medical examination will be performed to the pre-selected women to further exclude any unidentified medical condition. On the same occasion, a glucose tolerance test will be performed using 75g of oral glucose and two blood samples to measure glucose and insulin levels (0 and 2 hours after the dose).

c) **At INTA:** Selected women will be invited to INTA to measure RMR and DLW dose (in a sub-sample n=5).

d) **At home:** To apply the dietary intake questionnaires and fit the heart rate monitors on women. To provide information and appointment for the next stage.

e) **At INTA:** Test meal, blood samples (fasting and post meal determinations of glucose levels for glycemic index and fasting plasma fatty acids profile). Total body calorimetry during 12 hours. Breath test for $^{13}C$ analysis.

### 2.3. Procedures

a) Determinations of plasma glucose, insulin and fatty acid profile will be performed at INTA and under fasting conditions. For the calculation of the glycemic index, plasma glucose will be assessed from the antecubital vein every fifteen minutes during two hours following the test meal. Calculations will be according to the last FAO Expert Committee on CHO [17]. The ratio of SFA/PUFA will be calculated in the fasting plasma samples, composition of fatty acids of plasma triglycerides will be assessed and compared with food intake data knowing that their composition is a good sample of the present fat intake composition.

b) Resting metabolic rate will be assessed in fasting conditions and it will be used to calculate the activity (TEE/RMR) ratio. The energy content of the test meal will be equivalent to 1/3 of the measured RMR.

c) Free-living energy expenditure by DLW will be obtained by the multipoint method according to Coward, *et al.*[18]. Measurements will be performed in our own laboratory at a minimal cost.

d) Heart rate monitoring will be assessed in two non-consecutive occasions, recording the time and duration of the major activities. Sleeping time will be specifically recorded since the heart rate during this period is used as the unit to calculate the multiples of activity throughout the day.

e) Dietary intake will be assessed by questionnaires and by chemical analysis of CHO, fat, protein and energy content in most frequent meals.

f) Dietary food quotient will be obtained as suggested by Black and Prentice, *et al.* [19]. This data will be related to the respiratory quotient (RQ) obtained from the calorimeter and the composition of the diet.

g) Total CHO and Fat oxidation and storage will be obtained from the whole body indirect calorimetry according to the formulae given by Elia, *et al.* [20].

h) Thermic effect of food will be calculated as the area under the curve compared to the pre-meal energy expenditure values and the glycemic index of the meal.

i) Given the high costs of CHO and fat isotopes labelled with $^{13}C$, it is proposed to develop our own label by growing wheat under a $^{13}CO_2$ atmosphere as described by Harding, *et al.* [21]. Arrangements are being done to know all the details how to make enriched wheat to be used as part of the CHO eaten within the test meal (wheat is the main source of CHO in our diet). On a separate occasion, exogenous oxidation of $^{13}C$ labelled palmitic acid will be assessed (in this regard, the preferred substrates to test our hypothesis would have been oleic or linoleic acid but this is impossible because of their high cost).

## 3. DETAILED SCHEDULE OF THE RESEARCH PROPOSED

### 3.1. First and second year

#### *3.1.1. Testing of new methodologies*

Duration 3-4 months. It will be necessary to test and standardise some of the methods proposed such as the glycemic index and wheat enrichment with $^{13}C$. Reproducibility of these tests needs to be assured before the start of the project.

#### *3.1.2. Contacts and selection of the study group*

Duration 3-4 months (simultaneous). Key persons are already available in the community. They participate as our contacts and help in the subject's recruitment in conjunction with our field personnel.

#### *3.1.3. Data collection*

Duration 12-14 months. Two persons will be recruited per month with the aim of ending their data collection before other subjects are recruited. This is necessary because there are many variables involved and it is difficult to find the control person to match the obese subject. At the same time some of the laboratory analysis will be conducted in parallel.

#### *3.1.4. Laboratory measurements and data analysis*

Duration 3-4 months. Continuation of the analysis of biological samples. Statistical analysis of the data and drafting of the papers.

#### *3.1.5. Report writing. papers and other dissemination of results*

Duration 3-5 months. To finish writing the papers and reports. Meetings with Scientific Societies, Public (Ministry of Health and Education) and Consumers Associations.

### 3.2. Facilities at INTA, University of Chile

a) Built infrastructure: 10.000 m2. Laboratory of stable isotopes analysis and a calorimetry facility room of 11 m3, including an equipped metabolic kitchen (total surface: 120 m2).

b) Mass spectrometers: 1) Hydra, continuous flow mass spectrometer for liquid and gas analyses of C, H. O. N stable isotopes. 2) Tracer mass and a Roboprep unit for the analysis of gas and solid samples. All of them from Europa Scientific, Crewe, UK.

c) Radio immune analysis equipment

d) HPLC and gas chromatography equipment (Hewlett Packard)

e) Calorimeter room (near completion) and a portable calorimeter (Sensor Medics 2900)

f) Body composition techniques: DEXA (Lunar), underwater weighing and total body water by deuterium analysis

g) Freezers, refrigerators

h) Library and a scientific staff specialised in several areas of nutrition and basic sciences

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TABLE I. MEASUREMENTS AND METHODS

| MEASUREMENT | TECHNIQUE | DETAILS/INSTRUMENT |
|---|---|---|
| **Plasma glucose** | Glucose tolerance test (GTT)<br><br>Glycemic index of test meal | 75 g glucose, blood samples at 0 and 2 hours<br><br>Compared to glucose |
| **Plasma insulin** | RIA | Simultaneous with GTT |
| **Plasma fatty acids** | HPLC | Fatty acid composition of plasma tryglicerides |
| **Resting metabolic rate** | Short term indirect calorimetry | Sensor Medics 2900 |
| **Free-living energy expenditure** | Doubly labelled water<br><br>$D_2O$ (0.05g/kg) and $^{18}O$<br><br>(1.74g/kg of 10% $H_2{}^{18}O$) | Continuous flow mass spectrometer, Hydra, Europa Scientific. Urine collection for fourteen days. |
| **Physical activity** | 24-h heart rate monitoring | Polar Vantage |
| **Dietary intake** | 24-h recall and food frequency questionnaire<br><br>Gas chromatography<br><br>Chemical composition of the food samples | Previous and present dietary intake<br><br>Fatty acid composition of test meals<br><br>Macronutrient's dietary composition. Food quotient |
| **Total CHO and fat oxidation** | Whole body calorimetry<br><br>CHO and lipid oxidation and storage<br><br>Thermic effect of food (TEF) | Instrumentation due to be ready by May 1999. |
| **Exogenous CHO oxidation** | Wheat labelled with $^{13}C$ | Oxidation of $^{13}C$ labelled starch |