



## INCLUSION/EXCLUSION CRITERIA

- 18-65 years
- BMI >18,5 kg/m<sup>2</sup> and <40 kg/m<sup>2</sup>
- No diabetics, no cancer
- No pregnant
- European race
- If your work is outside of the range between 7:00 a.m. and 11:00 p.m. In the case that the patients have been shift worker, they will can participate if they leave the shift during 2 years.
- Not take melatonin in the last two weeks, and the two weeks of the study
- Not eat the following drugs:
  - I. Those that affect blood sugar values: a) insulin; b) medications for type 2 diabetes such as Metformin or other oral antidiabetics such as sulfonylureas, meglitinides, or glitazones; c) Corticosteroids / steroids; d) growth hormone
  - II. Those that affect blood clotting: e) anticoagulant drugs, or anticoagulants such as heparin, warfarin (Coumadin) or copylores (Plavix)
  - III. Those that affect the values of melatonin or sleep and circadian rhythms: f) beta-blockers for hypertension (high blood pressure), such as metoprolol (Lopressor), mexiletine, propranolol or verapamil; g) medications for sleep disorders (circadian rhythms) such as lithium, ramelteon, hypnotics (such as Ambien, Sonata or Lunesta) or other stimulants such as Provigil
- Name if you take medicines such as nonsteroidal, anti-inflammatory drugs such as ibuprofen or aspirin
- History (family history questions) to non-patients Garaulet
  - Level of studies: school / institute / university
  - If you are a smoker, how many cigarettes / day

## MEASUREMENTS

### 1. Food timing and dietary record:

- 7 consecutive days dietary food intake (mobile app and foods pictures)

### 2. Light exposure: HOB0 pendant sensor (7 consecutive days included those days of the study).

The day of the study, be sure that during OGTT (Oral Glucose Tolerance Test) in the late condition (1 hour before their habitual bedtime) the light not be more than 10 luxes and that this situation star 30 minutes before the time of the OGTT. In the early condition (4 hour before their habitual bedtime) the light would be more than 500 luxes

### 3. Anthropometric and biochemistry measurements:

- BMI
- Waist and Hip (cm).
- Impedance data: % Fatty Mass, Kg Fatty Weight, Kg Lean Mass, Kg Muscle Mass, Kg Total Water, % Total Water, Kg Bone Mass, Basal Metabolism (KJ), Basal Metabolism (Kcal), Metabolic Age, Visceral Fat Level, Degree of Obesity (%)
- Sex, age
- Menopause (Yes/No) and the date of the last menstruation. The patient should be pipi before the impedance, all of the impedances would be at the same time (19:00-20:00h).

### 4. Questionnaires:

- 1) Demographics and Health Survey: socioeconomic status, habitual alcohol intake, smoking, sleep apnea, insomnia
- 2) Inform consent
- 3) Anthropometric characteristics and OGTT conditions
- 4) International Physical Activity Questionnaire (IPAQ)
- 5) Morningness Eveningness Questionnaire (MEQ)



- 6) Emotional Eating Questionnaire (EEQ)
- 7) Food and Sleep log (food and timing)
- 8) Pittsburgh Sleep Quality Index
- 9) Day of the experiment menu
- 10) Control sheet
- 11) Insomnia Severity Index (ISI)
- 12) Patient Health Questionnaire-9 (PHQ-9)

### 5. Actigraphy

Actigraphy (sensor). For 7 consecutive days to record the activity rhythm the week before the OGTT. It should be the week before the study to know how is melatonin (although if you cannot it could be two weeks before)

### 6. OGTTs

We will administer 75 grams of glucose to the subject after the basal measurement in both conditions. Fisher Scientific; Fisher brand Glucose Tolerance Test Beverage, 75 g (75 Sun-dex); Orange flavored, caffeine free, non-carbonated; 10 oz fluid / 296 ml.

Link: <https://www.fishersci.com/shop/products/fisherbrand-glucose-tolerance-test-beverages-9/401223fb>

Write the time of each extraction

No more than 2 minutes in drink the glucose

8-h fasting

2 measures or conditions:

- 4 h before their habitual bedtime.
- 1 h before their habitual bedtime (should come 1h before of the T0 and keep in the dark). The samples will be just before the glucose T0 and at T30', T60', T 90'y T120' minutes after the glucose.
- Serum insulin and glucose.

### 7. Determinations

- Glucose and insulin in the 5 times (T0, T30', T60', T 90'y T120')
- Melatonin in T0' and T120'

### 8. Subjects report

- 1) Demographics and Health Survey: socioeconomic status, habitual alcohol intake, smoking, sleep apnea, insomnia
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**11. Store aliquots:**

- Glucose:** 30-400  $\mu$ l
- Insulin:** 30-400  $\mu$ l
- Melatonin:** 30-400  $\mu$ l
- Serum:** 400  $\mu$ l
- DNA:** 200  $\mu$ l

200-300  $\mu$ l: 1 for insulin, 1 for melatonin 1for glucose, and the rest for store in the freezer.