# INSTITUTO POLITÉCNICO NACIONAL

**Escuela Nacional de Medicina y Homeopatía**

**Sección de Estudios de Posgrado e Investigación**

# “Evaluación de la expresión génica en el tejido adiposo en respuesta al tratamiento acupuntural de la obesidad”

TESIS

QUE PARA OBTENER EL GRADO DE

**Doctorado en Ciencias en Biotecnología**

PRESENTA

**M. en C. Jessica M. García Vivas**

Directores de Tesis:

Dra. en C. Laurence A. Marchat

Dr. en C. Carlos Galaviz-Hernández

México DF, 3 de febrero 2015

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En la Ciudad de **México, D.F.** el día 20 del mes de **febrero** del año **2015**, la que suscribe **Jessica María García Vivas** alumna del Programa de **Doctorado en Ciencias en Biotecnología en Red**, con número de registro **B102477**, adscrita a la **Escuela Nacional de Medicina y Homeopatía**, manifiesta que es autora intelectual del presente trabajo de Tesis bajo la dirección de la D. en C. Laurence A. Marchat M y del D. en C. Carlos Galaviz Hernández y cede los derechos del trabajo titulado "**Evaluación de la expresión génica en el tejido adiposo en respuesta al tratamiento acupuntural de la obesidad"**, al Instituto Politécnico Nacional para su difusión, con fines académicos y de investigación.

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*Y DESIGNACIÓN DE DIRECTORES DE TESIS*

México, D.F. a 26 de noviembre del 2010

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|---|---|---|
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Con registro: B 1 0 2 4 7 0

Aspirante de: Doctorado en Ciencias en Biotecnologia en Red

1.- Se designa al aspirante el tema de tesis titulado:
Evaluación de la expresión génica en el tejido adiposo en respuesta al tratamiento acupuntural de la obesidad.

De manera general el tema abarcará los siguientes aspectos:
Determinar los cambios en los niveles séricos de adipocinas en respuesta al tratamiento. Evaluar si hay cambios a nivel transcripcional. Identificar otros genes cuya expresión este modulada por el tratamiento acupuntural

2.- Se designan como Directores de Tesis a los Profesores:
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3.- El trabajo de investigación base para el desarrollo de la tesina será elaborado por el alumno en:
El laboratorio 2 de biomedicina molecular de la ENMyH
que cuenta con los recursos e infraestructura necesarios.

4.- El interesado deberá asistir a los seminarios desarrollados en el área de adscripción del trabajo desde la fecha en que se suscribe la presente hasta la aceptación de la tesis por la Comisión Revisora correspondiente:

INSTITUTO POLITECNICO NACIONAL SECRETARIA DE INVESTIGACION Y POSGRADO * 06 MAY 2011 RECIBIDO DIRECCION DE POSGRADO

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En la Ciudad de Mexico siendo las 15:00 horas del día 3 del mes de febrero del 2015 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de la ENMyH para examinar la tesis titulada:

"Evaluación de la expresión génica en el tejido adiposo en respuesta al tratamiento acupuntural de la obesidad"

Presentada por el alumno:

| García | Vivas | Jessica María |
|---|---|---|
| Apellido paterno | Apellido materno | Nombre(s) |

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aspirante de:

Doctorado en Ciencias en Biotecnología

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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Este trabajo fue realizado en el laboratorio de **Biomedicina Molecular 2** de la Sección de Posgrado e Investigación **(SEPI)** de la Escuela Nacional de Medicina y Homeopatía del Instituto Politécnico Nacional **(ENMyH-IPN)** bajo la dirección de la Dra. en C. Laurence A. Marchat y del Dr. en C. Carlos Galavíz Hernández.

Este proyecto fue apoyado por el Consejo Nacional de Ciencia y Tecnología **(CONACYT)** con el número de proyecto **113148** y por la Secretaría de Investigación y Posgrado del Instituto Politécnico Nacional **(SIP-IPN)** con los números de proyecto **20131116** y **20143948**

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# Índice

# Abreviaturas

| | |
|---|---|
| **5-HT** | Serotonina |
| **ACSL3** | Gen Long chain acyl-CoA synthetase-3(por sus siglas en inglés) |
| **ACTH** | Hormona adrenocorticotropica (del inglés Adrenocorticotropic hormone) |
| **AgrP** | Proteína relacionada con Agouti (del inglés Agouti-related peptide) |
| **AM** | Acupuntura con moxibustion |
| **APM** | Acupuntura profunda con moxibustión |
| **BL 20** | Punto vejiga 20 o Pishu |
| **BL 23** | Punto vejiga 23 o Shenshu |
| **CACNA1** | Gen Calcium channel, voltage-dependent, L type, alpha 1C subunit (por sus siglas en inglés) |
| **CART** | Péptido Regulador de la Transcripción de Anfetamina y Cocaína (del inglés cocaine- and amphetamine-regulated transcript) |
| **CCK** | Colecistocina |
| **CGM** | Catgut embebido con moxibustión |
| **CV 12** | Punto Ren mai 12 o Zhongwan |
| **CV 6** | Punto Ren mai 6 o Qihai |
| **EA** | Electroacupuntura |
| **EAM** | Electroacupuntura con moxibustión |
| **ECV** | Enfermedad cardiovascular |
| **ENSANUT** | Encuesta Nacional de Salud y Nutrición |
| **FDA** | Food and Drug Administration (por sus siglas en inglés) |
| **GABA** | Ácido gamma-aminobutírico (del inglés γ-Aminobutyric acid) |
| **HDL** | Lipoproteína de alta densidad (del inglés High-density lipoprotein ) |

| | |
|---|---|
| **HOMA-IR** | Indice de resistencia a la insulina (del inglés homeostatic model assessment-Insulin resistence) |
| **HPA** | Eje Hipotalámico–pituitario–adrenal |
| **ICC** | Índice cintura cadera |
| **IL-6** | Interleucina 6 |
| **IMC** | Índice de masa corporal |
| **IRS-1** | Receptor de insulina 1(del inglés insulin receptor substrate-1) |
| **JAK** | Del ingles Janus-activated kinases |
| **LH** | Hormona leutinizante |
| **LRa** | Receptor de leptina a |
| **LRb** | Receptor de leptina b |
| **MC3** | Receptor de melanocortina 3 |
| **MC4** | Receptor de melanocortina 4 |
| **MTC** | Medicina Tradicional China |
| **NEUROD1** | Gen de diferenciación neurogénica 1 |
| **NPC1** | Gen Niemann-Pick C1 |
| **NPY** | Neuropéptido Y |
| **OCDE** | Organización para la Cooperación de Desarrollo Económico |
| **OMS** | Organización Mundial de la Salud |
| **PDE8B** | Gen fosfodiesterasa 8B (del ingles Phosphodiesterase-8B) |
| **POMC** | Pro-opiomelanocortina |
| **PPARGC1A** | Gen proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α (por sus siglas en inglés) |
| **PRKAA2** | Gen 5´-AMP-activated protein kinase catalytic subunit α2 (por sus siglas en inglés) |
| **PYY3-36** | Péptido YY3-36 |

| | |
|---|---|
| **SLC30A8** | Gen Solute carrier family 30; zinc transporter, member 8 (por sus siglas en inglés) |
| **SM** | Síndrome metabólico |
| **SNC** | Sistema nervioso central |
| **SP 6** | Punto bazo 6 o Sanyinjiao |
| **ST 25** | Punto estómago 25 o Tianshu |
| **ST 36** | Punto estómago 36 o Zusanli |
| **STAT 5** | Señal de transducción y activador de la transcripción 5 (del inglés Signal Transducer and Activator of Transcription 5) |
| **TNF-α** | Factor de necrosis tumoral α (del inglés tumor necrosis factor α) |
| **UCN3** | Gen urocortina 3 |
| **UCP** | Proteínas desacoplantes (del inglés uncoupling protein) |
| **VLDL** | Liporoteína de muy baja densidad (del inglés very low-density lipoprotein) |
| **WHO** | World Health Organization |
| **α-MSH** | Hormona α melanocito estimulante (por sus siglas en inglés α -melanocyte-stimulating hormone) |

# Resumen

La obesidad favorece el desarrollo de enfermedades degenerativas que son las principales causas de muerte tanto en los países desarrollados como en vías de desarrollo. Se debe a un desequilibrio entre el consumo y gasto energético, lo cual induce un aumento en el tejido adiposo y consecuentemente, una desregulación de las adipocinas que regulan el hambre y saciedad a nivel del sistema nervioso central. El tratamiento de la obesidad incluye generalmente una dieta balanceada y el ejercicio, así como medicamentos y en algunos casos cirugía. Además, las terapias alternativas representan una opción atractiva para un número creciente de pacientes obesos. Particularmente, la acupuntura, una técnica de la Medicina Tradicional China, ha demostrado ser una terapia eficaz para la reducción del peso corporal así como la regulación de parámetros bioquímicos asociados. Por lo que es de nuestro interés investigar los cambios en la expresión génica del tejido adiposo en respuesta al tratamiento acupuntural de la obesidad, con la finalidad de contribuir al entendimiento de las bases moleculares del efecto de esta terapia en el tratamiento de esta enfermedad.

En este estudio, comparamos el efecto de la estimulación de un mismo grupo de puntos corporales con cinco técnicas de acupuntura en mujeres obesas. Los resultados mostraron que todos los tratamientos fueron eficientes para reducir de manera significativa el peso. De manera interesante, el tratamiento con catgut embebido y moxibustión también permitió disminuir los niveles de insulina y HOMA-IR, de tal manera que las mujeres regresaron a un estadio de sensibilidad a la insulina, lo que sugiere que este tratamiento podría reducir el riesgo de diabetes asociado a la obesidad en estas pacientes. Sin embargo, no encontramos cambios en los niveles de adipocinas circulantes (leptina, adiponectina, resistina) y TNF- α en suero en respuesta al tratamiento. Por otro lado,el análisis de la respuesta transcripcional al tratamiento en el tejido adiposo mediante ensayos de microarreglos de DNA mostró que se modula, de manera significativa, la expresión de genes involucrados en el control de la homeostasis, el metabolismo de lípidos, así como la transducción olfatoria y la vía de señalización

del ácido gamma-aminobutírico. En conjunto, estos datos sugieren que los efectos del tratamiento con catgut embebido y moxibustión en la reducción del peso y la resistencia a la insulina están asociados a la regulación de eventos bioquímicos que se alteran en la obesidad.

# Abstract

Obesity promotes degenerative diseases which are the leading causes of death in developed and developing countries. It is due to an imbalance between spending and energy consumption, which induces an increase in adipose tissue and consequently a deregulation of adipokines that regulate hunger and satiety in the central nervous system. The treatment of obesity generally includes a balanced diet and exercise, as well as medication and in some cases surgery. In addition, alternative therapies represent an attractive option for a growing number of obese patients. Particularly, acupuncture, a technique of Traditional Chinese Medicine has proved being effective in reducing body weight and regulating associated biochemical parameters. So it is our interest to investigate changes in gene expression in adipose tissue in response to acupuncture treatment of obesity, in order to contribute to the understanding of molecular basis about the effects of this therapy in the treatment of this disease.

In this study, we compared the effects of the stimulation of the same set of body points by five acupuncture techniques in obese women. The results showed that all treatments were effective to significantly reduce body weight. Interestingly, acupuncture catgut embedding therapy with moxibustion also helped to reduce insulin levels and HOMA-IR, so that women returned to an insulin sensitivity state, suggesting that this treatment could reduce the risk of diabetes associated to obesity in these patients. However, we did not find any changes in circulating adipokines (leptin, adiponectin, resistin) and TNF-alpha levels in response to this treatment. Furthermore, analysis of the transcriptional response to treatment in adipose tissue by DNA microarray assays revealed the significant modulation of genes involved in homeostasis control, lipid metabolism, olfactory transduction, and gamma-aminobutyric acid signaling pathway. Taken altogether, our data suggest that the effects of catgut embedding therapy with moxibustion in weight and insulin resistance reduction are associated with the regulation of biochemical events that are altered in obesity.

# INTRODUCCIÓN

**1. ¿Qué es la obesidad?**

La obesidad se define como una acumulación anormal o excesiva de grasa que puede ser perjudicial para la salud (WHO, 2014); es un desorden metabólico que resulta de un desequilibrio entre la ingesta y el gasto energético. Esta enfermedad crónica se debe principalmente a un consumo excesivo de dietas ricas en grasas y altas en carbohidratos, aunado a la baja o nula actividad física. Su importancia en la última década radica en que se presenta en todos los grupos de edad y estratos socioeconómicos y afecta a la mayoría de los paíse, debido en gran medida, a la creciente urbanización y el desarrollo económico, que han producido cambios en los estilos de vida de la población mundial, con modificaciones en la dieta y la actividad física, según la Organización Mundial de la Salud (OMS). Adicionalmente, en el desarrollo de la obesidad contribuyen factores fisiológicos, psicológicos, socioeconómicos, culturales, metabólicos y genéticos que favorecen el desarrollo de esta enfermedad (Galic *et al.,* 2010; Jéquier, 2002).

De acuerdo a la OMS, la obesidad se diagnostica principalmente a través de la medición del índice de masa corporal (IMC). Ideado por el estadístico belga L. A. J. Quetelet, el IMC es un indicador simple de la relación entre el peso en kilogramos y la talla en metros, de acuerdo a la siguiente fórmula: IMC ($kg/m^2$) = Peso (kg) / Estatura ($m^2$). El IMC permite valorar la adiposidad o volumen de grasa del cuerpo, y de esta manera definir la corpulencia del individuo. Así, se considera que los individuos adultos con un IMC < 18.5 $kg/m^2$ presentan un bajo peso; un IMC entre 18.5 y 24.9 $kg/m^2$ indica un peso normal; si el valor es entre 25 y 29.9 $kg/m^2$, se habla de preobesidad o sobrepeso; y cuando el IMC es superior a 30 $kg/m^2$, entonces el individuo presenta obesidad, con diversos grados hasta llegar a la obesidad mórbida si el IMC es superior a 40 $kg/m^2$ (WHO, 2013).

En las personas obesas, la grasa abdominal, más que otros tipos de grasa, puede provocar inflamación ya que los tejidos se estresan fácilmente y desarrollan una respuesta inflamatoria. Si es una situación a corto plazo, desaparece y todo regresa a la normalidad, pero cuando se queda esa grasa en la cintura, esta inflamación puede resultar crónica y dañar el tejido, lo que puede causar que el cuerpo, como consecuencia de la obesidad, desarrolle resistencia a la insulina y alteraciones a nivel cardiovascular. Por lo que es importante valorar la grasa abdominal en los pacientes obesos.

Una manera práctica de obtener un indicador de la grasa intraabdominal y de la grasa visceral, es determinar la circunferencia de cintura, la cual se mide en el punto medio entre la última costilla y la parte superior de la cresta iliaca. Según la OMS, la circunferencia de cintura debe ser menor a 90 cm en hombres con una estatura menor a 180 cm y menor a 95 cm cuando la estatura es mayor a 180 cm. En el caso de las mujeres, la cintura debe ser menor de 80 cm si miden menos de 170 cm de altura y si su estatura es mayor a 170 cm debe ser menor de 85 cm. Los valores superiores de circunferencia de cintura se asocian a un mayor riesgo de padecer enfermedades cardiovasculares (Per Björntorp, 1987; WHO, 2000).

Otra opción para medir los niveles de grasa intraabdominales es la determinación del índice cintura/cadera (ICC), que relaciona el perímetro de la cintura con el de la cadera en centímetros. La circunferencia de la cadera se mide a nivel de los trocánteres mayores, que en general coinciden con la sínfisis pubiana. La OMS considera que los valores normales del ICC son de 1 para el hombre y de 0,8 para la mujer. Valores superiores del ICC pueden representar un factor de riesgo para el desarrollo de enfermedades cardiovasculares, así como presión arterial elevada y diabetes, entre otras enfermedades relacionadas con la obesidad (Pouliot *et al.,* 1994).

## 2. Enfermedades asociadas a la obesidad

La obesidad, principalmente la obesidad de tipo central o visceral, está estrechamente relacionada con el síndrome metabólico (SM), el cual es un conjunto de múltiples anomalías que, además de la obesidad central, incluye hipertensión arterial, dislipidemias (aumento de triglicéridos en las VLDL, disminución del colesterol tipo HDL) y resistencia a la insulina (glucosa alta en la sangre, hiperinsulinemia, intolerancia a la glucosa). El diagnóstico del SM implica la presencia de al menos tres de los siguientes datos clínico: elevación de la presión arterial, dislipidemia aterogénica, elevación de triglicéridos, disminución de colesterol y de lipoproteínas de alta densidad (HDL-C), hiperglucemia, y obesidad central (Alberti *et al.,* 2009).

Por lo que, la obesidad es un factor de riesgo importante para varias enfermedades degenerativas, incluyendo diabetes tipo 2 (Colditz *et al.,* 1995), enfermedades cardiovasculares e infartos al miocardio (Wilson *et al.,* 2002), infertilidad (Pasquali *et al.,* 2007) y diferentes tipos de cáncer: colon, próstata, endometrio y mama (Vucenik and Stains*,* 2012) que representan las primeras causas de muerte por cáncer en el mundo y en México (Gale, 2008; Wang *et al.,* 2008, Ren and Kelley, 2009; Antuna-Puente *et al.,* 2008).

## 3. Epidemiología de la obesidad en el mundo y en México

La obesidad ha cobrado gran interés en los últimos años debido a su alta incidencia y su alarmante aumento a nivel mundial. La OMS publicó que en el 2008, a nivel mundial existían más de 1.4 mil millones (35%) de adultos mayores de 20 años con sobrepeso, de los cuales arriba de 200 millones de hombres y cerca de 300 millones de mujeres eran obesos. Además se determinó que el 65% de la población mundial vive en países donde el sobrepeso y la obesidad afectan la salud de más personas que la ausencia de alimento (WHO, 2014). Por otra parte, se predice que en el 2020, la obesidad afectará al 30-37% de los hombres y 34-44% de las mujeres en el mundo (Bibbins-Domingo *et al.,* 2007).

La obesidad es una epidemia mundial que afecta tanto a países desarrollados como países en vías de desarrollo. Así, en los Estados Unidos de América, más del 30% de la población padece obesidad (Flegal *et al.,* 2010), mientras que en Europa se presentó entre el 10% y el 30% de la población adulta (Balkau *et al.,* 2010).

En México, la OMS estimó que en el año 2008, el 32.1% de la población padecía obesidad y un 68.3% sobrepeso, ubicando a México en el segundo lugar mundial por debajo de los EUA (WHO, 2014). Además, la Organización para la Cooperación de Desarrollo Económico (OCDE, por sus siglas en inglés) reportó que México era el país más obeso del mundo con un 30% de obesidad en la población y con un 69.5% de sobrepeso y obesidad, en el año 2010. Pero en el 2012, México volvió al segundo lugar, por debajo de EUA a nivel global, aun si la prevalencia de obesidad en mujeres aumentó a un 37.5% contra 34.5% en el 2010 (OECD, 2014). Por otra parte, según la Encuesta Nacional de Salud y Nutrición del 2012, el 73% de las mujeres mexicanas presentan obesidad y sobrepeso (ENSANUT, 2012).

Estos datos muestran que la obesidad se ha convertido en un problema serio de salud en muchos países incluido México (Rivera *et al.,* 2002; Popkin *et al.,* 2012). En el 2008, Stevens *et al,* publicó que el 75% de las muertes en México del 2004, estaban relacionadas con la obesidad, ya sea por complicaciones de la obesidad y/o por enfermedades relacionadas con ella (Stevens *et al.,* 2008). Esto es un dato relevante ya que la OCDE, reportó que por cada 15 kg de peso extra por persona, hay un aumento del 30% de riesgo por muerte prematura (OECD, 2010). Consecuentemente en el informe de la Secretaría de Salud se ha estimado que las pérdidas económicas por muertes prematuras a causa de la obesidad en México alcanzaron $1,931 millones de dólares en el 2008 (Secretaría de Salud, 2010). Asimismo, en el 2010 el gasto en México por estas enfermedades fue de $ 806 millones de dólares y se calcula que este gasto aumentará hasta 1,200 y 1,700 millones de dólares en el 2030 y 2050, respectivamente (Rtveladze *et al.,* 2013)

## 4. Regulación del peso corporal y alteraciones en obesidad

### 4.1. Regulación del hambre y saciedad

La necesidad de obtener energía y almacenarla es fundamental para cualquier ser vivo. El hambre es la primera señal en este proceso; indica que el organismo empieza a sufrir una disminución en sus niveles energéticos y lo lleva a adquirir nuevamente energía y nutrientes esenciales para poder sobrevivivir. Es por esto que la regulación de la ingesta calórica y del gasto energético es un importante evento para la homeostasis del organismo. Es un proceso muy complejo que está regulado por diferentes vías y/o señales redundantes para salvaguardarlo (Berthoud *et al.,* 2002; Berthoud *et al.,* 2008)

La regulación de la homeostasis energética promueva la estabilidad en la cantidad de energía corporal almacenada en forma de grasa. Involucra la participación de señales nerviosas periféricas y distintas moléculas y mediadores con acciones neuroendocrinas que se integran a nivel del sistema nervioso central (SNC) en los clásicamente conocidos centros hipotalámicos del hambre y saciedad, a partir de los cuales se produce una respuesta eferente que controla la cantidad de energía consumida o almacenada a corto, medio o largo plazo.

Conceptualmente, el apetito se relaciona con las señales que inician la ingesta, la satisfacción, con las señales de terminación de la ingesta y la saciedad con las señales que mantienen la inhibición de la ingesta durante el periodo intercomidas, hasta que se detectan nuevamente las señales provocados por el hambre y por lo tanto un nuevo consumo. Por tanto, el hecho de comer se trata de un sistema homeostático de “feed-back” motivado para detectar las señales de hambre, buscar e iniciar la comida, detectar las calorías y nutrientes ingeridos y finalizar la ingesta cuando el aporte energético necesario ha sido cubierto.

El órgano central en la regulación de la homeostasis energética es el SNC. El cerebro recibe señales liberadas del tejido adiposo, del tracto gastrointestinal, páncreas e hígado; estas señales se integran a nivel del núcleo arcuato del hipotálamo, el cual es un centro regulador y productor de factores hormonales que

participan en la regulación del equilibrio energético del organismo y de las funciones apetito-saciedad (Führer *et al.,* 2008). Esta región del cerebro recibe inervación de numerosas áreas, en especial del nucleus tractus solitarius y del área postrema del tallo cerebral, las cuales transmiten numerosas señales neuronales y hormonales del tracto gastrointestinal. Estas señales activan vías efectoras centrales que involucran tanto al sistema nervioso como a una red compleja de proteínas o neuropéptidos, que generan respuesta en el consumo de energía (apetito y saciedad) y así como en el gasto energético (termogénesis). Estas respuestas incluyen la activación neuroendócrina, conducta motora y actividad en el sistema nervioso simpático, las cuales regulan varios procesos fundamentales del metabolismo, incluyendo lipólisis, secreción de insulina y glucagón del páncreas, así como síntesis y liberación de glucosa en el hígado, dando como resultado el control del hambre y la saciedad (Havel *et al.,* 2004).

En la regulación de la ingesta participan numerosos péptidos que son producidos por diferentes órganos o tejidos entre los que destacan: el neuropéptido Y (NPY), MSH α, CART y AgRP proveniente del hipotálamo; la colecistocinina, la grelina y el péptido PYY3-36 producidos por el tracto digestivo; la adiponectina, la leptina, la resistina así como proteínas proinflamatorias como el TNFα, sintetizados principalmente por el tejido adiposo (Erlanson-Albertsson, 2005)

**4.2. Moléculas involucradas en la fisiología del hambre a nivel del hipotálamo**

Los neuropéptidos orexigénicos, que inducen el apetito, de mayor jerarquía son el neuropéptido Y (NPY) y la proteína relacionada con Agouti (AgrP), mientras que los neuropéptidos anorexigénicos, que inhiben el apetito, son la proopiomelanocortina (POMC) y el transcrito regulado por cocaína y anfetamina (CART).

Tanto NPY como AgrP, son potentes orexigénicos liberadas por el mismo grupo de neuronas en la región del núcleo arcuato del hipotálamo (Hahn *et al.,* 1998).

Ambos pueden producir hiperfagia, reducen el gasto energético y promueven el almacenamiento de triglicéridos en el tejido adiposo (Nogueiras *et al.,* 2007)

En especial, NPY se produce tanto central como periféricamente, (Gehlert *et al.,* 1987; Morris, 1989), pero es en el hipotálamo donde participa de manera importante en el balance energético y responde a cambios en el estatus energético. Por ejemplo, sus niveles incrementan en ausencia de alimento y vuelven a sus niveles basales después de alimentarse (Beck *et al.,* 1990*;* Brady *et al.,* 1990; Kalra *et al.,* 1991). En concordancia a su participación en la homeostasis energética y el control de peso, la liberación del NPY está sujeta a regulación de leptina, insulina y cortisol, ya que disminuye cuando se elevan los niveles de leptina e insulina y se incrementa al aumentar la grelina, la hormona de crecimiento y los niveles de glucocorticoides (Cyr *et al.,* 2013)

La proteína AgrP es un potente antagonista de los receptores de melanocortina MC3 y MC4, constituyendo un importante factor del proceso metabólico que regula el comportamiento alimentario y el peso corporal (Ollmann *et al.,* 1997). Sus niveles basales están elevados en los sujetos obesos, como han demostrado algunos estudios en roedores en los que la administración de esta sustancia aumenta notablemente la ingesta y el peso de los animales. Igualmente, los ratones transgénicos que expresan AgrP en cantidades excesivas desarrollan obesidad (Gonçalves *et al.,* 2014). En el humano, se ha descubierto la presencia de un polimorfismo en el gen de AgrP en los individuos genéticamente delgados (Marks *et al.,* 2004).

Por otro lado, POMC es sintetizada en las células corticotrópicas de la glándula pituitaria anterior y del núcleo arcuato del hipotálamo, como precursor de numerosos neuropéptidos. Ejerce su efecto anorexígeno uniéndose a los receptores de melanocortina MC4 (Foster *et al.,* 2003) y por lo tanto inhibiendo la acción la proteína AgrP (Pritchard *et al.,* 2002). Además POMC produce la Hormona α melanocito estimulante (α-MSH, por sus siglas en inglés) que está

sujeta a la regulación de la leptina. El ayuno reduce las concentraciones del RNAm de POMC en el núcleo arcuato, debido a una menor señal de leptina ya que en las neuronas POMC se expresan receptores de leptina (Schwartz *et al.,* 1997; Cheung *et al.,* 1997). El hecho de que la AgrP y la POMC se expresan en el núcleo arcuato, y que dicha expresión está regulada por el ayuno y por la deficiencia de leptina, indica que el papel de los receptores de la melanocortina en el SNC es importante en la regulación del peso corporal, siendo necesario un balance adecuado entre la acción de la α-MSH y de la AgrP para la regulación de la ingesta y del gasto energético. Aunque en la actualidad se considere al NPY como la molécula orexígena más potente, su efecto permanece pocas horas, mientras que el de la AgrP persiste durante más tiempo, aproximadamente una semana tras su administración intracerebroventricular.

La proteína CART se expresa en el núcleo arcuato, en las mismas neuronas que la POMC, y también tiene un marcado efecto anorexígeno (Abbott *et al.,* 2001). En los animales desprovistos de alimento, se observa un importante reducción de la síntesis de este péptido en el núcleo arcuato del hipotálamo (Aja *et al.,* 2002) En los ratones transgénicos *ob/ob*, se encuentra en escasa cantidad pero tras la administración de leptina se normalizan los niveles (Kristensen *et al.,* 1998), lo que demuestra la importancia de ésta en su regulación. El CART actúa sobre el núcleo paraventricular, regulando funciones autonómicas y neuroendocrinas relacionadas con la alimentación pero no modifica el gasto de energía, por lo que su acción parece ser exclusivamente inhibidora del apetito. Todo ello sugiere que el CART se encuentra implicado en el control de la homeostasis de la energía (Asakawa *et al.,* 2001). La leptina y las anfetaminas estimulan la producción de éste péptido por neuronas hipotalámicas que expresan POMC y se reduce la ingesta de alimentos.

### 4.3. Moléculas involucradas en la fisiología del hambre a nivel de otros órganos y tejidos

Como se mencionó anteriormente, la regulación del hambre y saciedad involucra no sólo señales del hipotálamo, sino también señales provenientes de otros órganos, particularmente el tracto digestivo, el páncreas, el hígado y el tejido adiposo.

La grelina que se produce principalmente en el estómago y en menor proporción en el intestino, el riñón, el corazón, el páncreas, el testículo, el hipotálamo, la hipófisis y la placenta, es un importante factor orexígeno (Lazarczyk *et al.,* 2003). Sus niveles plasmáticos son altos en personas sanas antes de las comidas y en situaciones de ayuno o caquexia, pero disminuyen después de comer (Cummings *et al.,* 2001). La grelina tiene acción antileptina y a pesar de ser una proteína sintetizada en el estómago, su existencia y la de sus receptores en el hipotálamo sugiere que a ese nivel está implicado un efecto de regulación de la ingesta a corto y largo plazo. Además de que estimula la liberación de hormona de crecimiento, disminuye la oxidación de grasa y aumenta la ingesta de alimentos, así como la adiposidad (Kojima *et al.,* 2001).

La colecistocina (CCK) es un factor anorexigénico que es producido por el duodeno en respuesta a la entrada del bolo alimentario. A través de su unión a receptores, produce una disminución del grado de llenado gástrico y contribuye a su distensión; además produce sensación de saciedad por medio de la constricción pilórica que inhibe el vaciamiento gástrico. Por otra parte, la ingesta también estimula la liberación de CCK cerebral que ejerce un efecto anorexígeno (Gibbs *et al.,* 1973; Gibbs *et al.,* 1986)

El péptido PYY3-36 que pertenece a la familia de neuropéptidos, es una molécula anorexigénica secretado por las células L del intestino, fundamentalmente en el intestino delgado distal, en respuesta al alimento, además

de páncreas y cerebro (Batterham *et al.,* 2003; Lluís *et al.,* 1989). Los niveles sanguíneos de PYY3-36 permanecen altos entre comidas y se ha observado que el aumento de PYY3-36 disminuye en un 36% el volumen ingerido de forma inmediata y un 33% el volumen total de 24 horas, por lo que es considerado como el principal regulador de la ingesta en periodos de tiempo intermedios (Freddie *et al.,* 1991; Batterhamn *et al.,* 2003)

La insulina producida por el páncreas, también tiene un efecto anorexigénico. Se ha observado que la inactivación de los receptores para insulina en el cerebro se acompaña de un cuadro de hiperfagia, obesidad, hiperinsulinemia e hiperleptinemia y disfunción de LH (hormona leutinizante) (Brüning *et al.,* 2000). Tanto la insulina como la leptina son cruciales para la regulación a largo plazo del equilibrio de la energía. Ambas son liberadas en forma proporcional a la grasa corporal y transportadas hacia el cerebro, en las áreas hipotalámicas donde se encuentran sus respectivos receptores, y modulan la expresión de los neuropéptidos que regulan la conducta alimentaria y el peso corporal, lo cual produce inhibición de la ingesta de alimentos y aumento del gasto de energía, de una manera dependiente de la dosis. Entre sus diferencias se observa que la secreción de la insulina se produce en respuesta a una sola comida, mientras que con la secreción de la leptina esto no ocurre. La insulina parece desempeñar un papel clave, aumentando con un retraso de varias horas la leptinemia.

Acontinuación se describe con más detalle las moléculas producidads por el tejido adiposo que tienen un papel en la fisiología del hambre.

## 5. Tejido adiposo y adipocinas relacionadas con obesidad y respuesta inflamatoria

El tejido adiposo es considerado un órgano dinámico involucrado en muchos procesos fisiológicos y metabólicos. Está formado principalmente por adipocitos, los cuales son células redondas que contienen una vacuola lipídica que representa el 95% del peso celular y desplaza a los demás organelos a la periferia. Los

adipocitos están inmersos en una red de fibras de colágeno junto con otras células como fibroblastos, macrófagos y preadipocitos (Ahima, 2006).

Existen dos tipos de tejido adiposo: blanco y pardo o marrón. El tejido adiposo pardo normalmente se dispone alrededor de los vasos sanguíneos. Su color se debe a la gran cantidad de mitocondrias que poseen las células que lo conforman; estas mitocondrias carecen del aparato celular para transformar la energía liberada por la oxidación de los ácidos grasos en ATP, pero expresan altas cantidades de proteínas desacoplantes (UCP por sus siglas en inglés) que desacoplan la oxidación de los ácidos grasos de la producción de ATP, permitiendo la liberación de la energía en forma de calor o termogénesis.

El tejido adiposo blanco, es el más abundante del organismo humano adulto (aproximadamente 20% del peso corporal). En condiciones normales el 80% del tejido adiposo está localizado en el tejido celular subcutáneo y el 20% restante representa el tejido adiposo visceral, el cual está constituido por adipocitos de un tamaño más reducido, con menor capacidad de almacenamiento, más vascularizado, mayor inervación simpática y gran número de receptores β3-adrenérgicos que facilitan su actividad metabólica.

Anteriormente, el tejido adiposo ha sido considerado como el principal reservorio de lípidos del organismo, principalmente en forma de triglicéridos. Sin embargo, varios estudios han mostrado que es un tejido que expresa y secreta una gran variedad de péptidos activos conocidos como adipocitoquinas o adipocinas, las cuales actúan de forma local (autócrina/parácrina) y sistémica (endócrina). Además expresa numerosos receptores que le permiten responder a señales aferentes de distintos órganos endócrinos y del sistema nervioso central (Elissondo *et al.,* 2008; Kershaw *et al.,* 2004). Particularmente, el tejido adiposo tiene un papel primordial en la homeostasis de varios procesos fisiológicos, entre los cuales se incluyen: la ingesta de alimentos, la regulación del equilibrio

energético, la acción de la insulina y el metabolismo de la glucosa (Frühbeck *et al.,* 2001; Sánchez-Muñoz *et al.,* 2005).

Dentro de las adipocinas sintetizadas a nivel del tejido adiposo y que tienen un papel en la regulación del hambre y saciedad, y por lo tanto en el control del peso corporal, podemos mencionar principalmente a la leptina, adiponectina, resistina y TNF-α.

### 5.1. Leptina

La leptina producida por el tejido adiposo funciona como un sensor que vigila el nivel de los depósitos de energía, es decir de la masa del tejido adiposo. Unida a la forma corta de su receptor (LRa) viaja por torrente sanguíneo y cruza la barrera hematoencefálica para interactuar con la forma larga del receptor de leptina (LRb) en el hipotálamo (Elmquist *et al*., 1999, Pan *et al.,* 2012). Esta señal es recibida e integrada por las neuronas hipotalámicas y una respuesta efectora, que incluye la modulación de los centros de apetito y actividad del sistema nerviosos simpático, regula los dos principales determinantes del equilibrio de energía: ingesta y gasto. El efecto general de la leptina es reducir los depósitos grasos y promover la delgadez mediante un sistema regulador de retroalimentación. (Jéquier , 2002).

Los efectos de la leptina en el hipotálamo son mediados por su unión al receptor de leptina, perteneciente a la familia gp130 de receptores de citocinas, lo cual activa un factor de la transcripción de genes de dos poblaciones de neuronas hipotalámicas. Este proceso disminuye la expresión de los dos neuropéptidos orexigénicos (inductores de la ingesta de alimentos) antes mencionados: el NPY y el AgrP. Por otra parte, aumenta la expresión de los péptidos anorexigénicos (inhibidores de la ingesta de alimentos): α-MSH y CART. El resultado de la supresión de neuropéptidos orexigénicos y la inducción de anorexigénicos contribuye a promover la sensación de saciedad, por lo que la leptina puede considerarse como una adipocina anorexigénica (Oswal *et al.,* 2007).

La secreción de leptina presenta un ritmo circadiano con incremento nocturno, sus concentraciones plasmáticas son bajas cerca del amanecer y empiezan a incrementarse hacia las 3:00 p.m., alcanzando sus valores máximos durante la noche. Estas variaciones no se relacionan con la ingesta de alimentos ni con aumentos en la concentración de insulina circulante inducida por alimentos (Langendonk *et al.,* 1998)

Es notable que la mayoría de los individuos obesos presentan concentraciones plasmáticas de leptina altas que no producen el efecto anorexigénico esperado, lo cual indica que la obesidad puede vincularse con una resistencia a la leptina en estos pacientes, y no con una deficiencia en leptina (Shimizu *et al.,* 2007). Este efecto puede ocurrir al menos en dos distintos niveles: una saturación en el transporte de leptina a través de la barrera hematocefálica y/o anormalidades en el grado de activación del receptor de leptina y/o la transducciòn de la señal. (El-Haschimi *et al.,* 2000).

### 5.2. Adiponectina

La adiponectina es una adipocina producida exclusivamente por el tejido adiposo (Maeda *et al.,* 1996). Se expresa en altas cantidades en individuos sanos y delgados. Particularmente, las concentraciones en plasma oscilan entre 5 y 30 g/mL, por lo que la adiponectina representa aproximadamente el 0.01% del total de las proteínas plasmáticas (Arita *et al.,* 1999) y su concentración plasmática es aproximadamente tres veces superior a la mayoría de las hormonas (Elissondo *et al.,* 2008). Sin embargo, en los pacientes que presentan resistencia a la insulina u obesidad, la expresión de la adiponectina y sus concentraciones en plasma se encuentran disminuidas. Esto sugiere que los individuos con altas concentraciones de adiponectina son menos propensos a desarrollar diabetes tipo 2 que aquellos con concentraciones bajas (Sánchez-Muñoz *et al.,* 2005).

La adiponectina tiene acciones antiaterogénicas, ya que puede inhibir la formación de la placa ateromatosa en casi todas sus etapas, por sus efectos directos sobre el endotelio (antiaterogénicos y antiinflamatorios) (Yamauchi *et al.,* 2003).

Existen reportes que sugieren la existencia de una asociación entre los niveles de adiponectina y la enfermedad cardiaca, ya que altos niveles de adiponectina reducen el riesgo de padecer un infarto agudo al miocardio; otros autores observaron un descenso en los niveles de adiponectina después de un infarto (Pischon *et al.,* 2004; Zoccali *et al.,* 2002; Adamczak *et al.,* 2003; Iwashima *et al.,* 2004). Además, la adiponectina puede promover la oxidación hepática de ácidos grasos, lo cual puede producir un efecto protector contra el desarrollo de hígado graso (Mendez-Sanchez *et al.,* 2005).

Por último, se cree que la adiponectina tiene un efecto antiangiogénico, ya que *in vitro* se observó que inhibe la migración y proliferación de células endoteliales y previene la formación de nuevos vasos sanguíneos; estas acciones están mediadas por la activación de apoptosis en las células endoteliales, vía la activación de las caspasas 3, 8 y 9 (Brakenhielm *et al.,* 2004). Estudios que evalúan niveles de adiponectina en diferentes tipos de cáncer apoyan esta teoría ya que asocian bajos niveles de ésta con mayor riesgo de padecer cáncer de endometrio (Petridou *et al.,* 2003; Dal Maso *et al.,* 2004), de mama (Mantzoros *et al.,* 2004; Chen *et al.,* 2006), de estómago (Ishikawa *et al.,* 2005), de próstata (Goktas *et al.,* 2005) y de colon (Ogunwobi *et al.,* 2006).

En los pacientes obesos, se ha visto que los niveles plasmáticos de adiponectina disminuyen y se correlacionan en forma negativa con los niveles de triglicéridos (Stefan *et al.,* 2005). Disminuye la sensibilidad a la insulina y la oxidación de los ácidos grasos, lo que induce un aumento en los niveles circulantes de ácidos grasos y del contenido de triglicéridos intramiocelulares y hepáticos (Ferré, 2004)

### 5.3. TNF-α

El TNF-α es una proteína de 26 kDa en la membrana celular y sufre un corte que da lugar a su forma soluble de 17 kDa (Moller, 2000). Es una citocina pro-inflamatoria, producida por una variedad de tipos celulares, pero principalmente por macrófagos y linfocitos; también puede ser producida por adipocitos, en menor cantidad, en humanos. Algunos autores mencionan que el TNF-α no es producido directamente por los adipocitos, sino por el efecto sistémico de la leptina u otra adipocina, ya que no siempre está aumentada su concentración en la obesidad (Bastard *et al.,* 2006). Sin embargo, otros autores reportan que disminuye su concentración en sujetos obesos después de la pérdida de peso, mejorando su sensibilidad a la insulina (Kern *et al.,* 2001). Por otra parte, se ha observado aumento en la forma de TNF-α unida a la membrana ( 26 kDa) en los individuos obesos, la cual puede actuar de manera autócrina en los procesos de citotoxicidad e inflamación por interacción celular (Xu *et al.,* 2002; Xu *et al.,* 2002).

En roedores, se cree que el TNF-α juega el papel más importante en la fisiopatología de la resistencia a insulina a través de la fosforilación del sustrato-1 del receptor de insulina (IRS-1, insuline receptor substrate-1) en sus residuos de serina, el cual podría interactuar con la subunidad beta del receptor de insulina y detener su vía de señalización (Hotamisligil *et al.,* 1993; Stephens *et al.,* 1997; Bastard *et al.,* 2006)

### 5.4. Resistina

En ratones, la resistina es producida por los macrófagos y tiene un papel en el desarrollo de la resistencia a la insulina, ya que induce resistencia a la insulina en el hígado y aumenta la producción de glucosa. En humanos, no está del todo claro cuál es su función; sin embargo se ha reportado aumento en las concentraciones séricas de resistina en individuos obesos y con diabetes tipo 2

(Gharibeh *et al.,* 2010; Nagaev *et al.,* 2001; McTernan *et al.,* 2002; Wang *et al.,* 2002)

Existen grandes discrepancias entre sí la resistina en humanos es secretada por el tejido adiposo o por los monocitos y macrófagos, ya que en algunos estudios no se ha encontrado presencia o solamente se ha detectado niveles muy bajos del RNAm de este gen en los adipocitos, mientras que en otros trabajos, se han observado niveles de expresión de la resistina significativos en leucocitos mononucleares y macrófagos, además de los adipocitos (Fain *et al.,* 2003; Patel *et al.,* 2003). Es por esta razón que existe un debate entre el rol funcional de la resistina en el ratón y en el humano.

Normalmente la concentración de resistina en suero está en el rango de 7 - 22 ng/mL en el humano. La resistina desempeña importantes funciones reguladoras aparte de su papel en la resistencia a la insulina y la diabetes en una variedad de procesos biológicos: la aterosclerosis y la enfermedad cardiovascular (ECV), enfermedad de hígado graso no alcohólica (Sheng *et al., 2008)*, enfermedad autoinmune, cáncer, asma, enfermedad inflamatoria del intestino y la enfermedad renal crónica. La evidencia sugiere que la resistina está implicado en procesos patológicos que conducen a enfermedades cardiovasculares incluyendo la inflamación (Filkova *et al.,* 2009), la disfunción endotelial, la trombosis, la angiogénesis y la disfunción de las células del músculo liso (Calabro *et al.,* 2011; Carmeliet, 2005; Chen *et al.,* 2010; Chu *et al.,* 2008; Di Simone *et al.,* 2006). Aunque su mecanismo de acción y participación en vías de señalización en las células blanco aún no queda muy claro.

## 6. Tratamiento de la obesidad

El objetivo del tratamiento de la obesidad es reducir el peso corporal para conseguir una mejoría en las enfermedades asociadas a esta enfermedad. Se considera que una disminución progresiva del peso, con pequeñas pérdidas de peso de 5 -10% del peso inicial, permite que los resultados se mantengan a largo plazo (Daza, 2009).

La OMS hace mención que la dieta saludable además de la actividad física suficiente y regular, son los principales factores de promoción y mantenimiento de una buena salud durante toda la vida, mientras que las dietas malsanas y la inactividad física son dos de los principales factores de riesgo de hipertensión, hiperglucemia, hiperlipidemia, sobrepeso u obesidad y de las principales enfermedades crónicas, como las cardiovasculares, el cáncer o la diabetes. Consecuentemente menciona que a nivel mundial hay 2,7 millones de muertes anuales debido a una ingesta insuficiente de frutas y verduras, y 1,9 millones de muertes anuales a causa de la inactividad física. Por lo tanto, ya que la mejora de la dieta y la promoción de la actividad física representan una oportunidad única para elaborar y aplicar una estrategia eficaz que reduzca sustancialmente la mortalidad y la morbilidad de la obesidad a nivel mundial, la OMS adoptó en mayo de 2004 la Estrategia mundial sobre régimen alimentario, actividad física y salud (WHO 2014)

### 6.1. Modificaciones en la dieta

Generalmente, el tratamiento de la obesidad inicia con cambios en la dieta, buscando reducir el consumo energético a través de una disminución del consumo de alimentos ricos en grasas y carbohidratos. Se recomienda un aporte aproximado de 500 calorías menos que el gasto calórico total previamente estimado, buscando de esta forma una reducción de 0.5 a 1 kg por semana. Las dietas muy bajas en calorías no se recomiendan, ya que se han registrado efectos adversos y de rebote. Una dieta normal debe ser aproximadamente de 1800 kcal. Una vez alcanzado el peso deseado, es necesario instaurar un programa de mantenimiento para que el peso corporal no suba más de 2.5 kg.

Las recomendaciones de la OMS (WHO, 2014), tanto poblacionales como individuales son:

- Lograr un equilibrio calórico y un peso saludables.

- Reducir la ingesta calórica procedente de las grasas, cambiar las grasas saturadas por las insaturadas y eliminar los ácidos grasos trans.
- Aumentar el consumo de frutas, verduras, legumbres, cereales integrales y frutos secos.
- Reducir la ingesta de azúcares libres.
- Reducir el consumo de sal (sodio), cualquiera que sea su fuente, y garantizar que la sal consumida esté yodada.

### 6.2. Modificaciones en la actividad física

Se considera actividad física cualquier movimiento corporal producido por los músculos esqueléticos que exija gasto de energía. Se ha observado que la inactividad física es el cuarto factor de riesgo en lo que respecta a la mortalidad mundial (6% de las muertes registradas en todo el mundo). Además, se estima que la inactividad física es la causa principal de aproximadamente un 21%-25% de los cánceres de mama y de colon, el 27% de los casos de diabetes y aproximadamente el 30% de la carga de cardiopatía isquémica.
Un nivel adecuado de actividad física regular en los adultos:

- reduce el riesgo de hipertensión, cardiopatía coronaria, accidente cerebro-vascular, diabetes, cáncer de mama y de colon, depresión y caídas;
- mejora la salud ósea y funcional, y
- es un determinante clave del gasto energético, y es por tanto fundamental para el equilibrio calórico y el control del peso.

La actividad física no debe confundirse con el ejercicio. Este es una variedad de actividad física planificada, estructurada, repetitiva y realizada con un objetivo relacionado con la mejora o el mantenimiento de uno o más componentes de la aptitud física. La actividad física abarca el ejercicio, pero también otras actividades que entrañan movimiento corporal y se realizan como parte de los momentos de

juego, del trabajo, de formas de transporte activas, de las tareas domésticas y de actividades recreativas.

Aunado a una reducción de la dieta, es importante aumentar el gasto energético mediante la práctica de algún deporte, para promover la utilización de los triglicéridos almacenados en el tejido adiposo grasa como fuente energética, para de esta manera regular el peso corporal. Se considera que por sí sola, la actividad física puede permitir una disminución del 2 a 3% en el IMC, pero es mucha mas eficiente cuando se asocia a una dieta balanceada (WHO, 2014).

### 6.3. Modificaciones conductivo-conductuales

El tratamiento de la obesidad, basado en cambios en la dieta y la actividad física, en muchos casos, también debe ser asociado a un programa de modificación conductivo-conductual, para obtener resultados positivos. Muchos pacientes obesos enfrentan problemas relacionados con el estrés, frustraciones, depresión, necesidades y temores en diversos eventos de su vida, encontrando un refugio en la comida como método de compensación. En otros casos, son los hábitos alimenticios familiares que son la causa de la obesidad (Bravo *et al.,* 2011). Por lo cual, es necesario implementar técnicas de auto monitoreo de la ingesta, de la actividad física y del manejo del estrés, así como fomentar en los pacientes a actividades más saludables, para lograr modificar el estilo de vida de los pacientes obesos (Cuevas *et al.,* 2005).

### 6.4. Tratamiento farmacológico

La obesidad también puede ser controlada mediante un tratamiento farmacológico, siempre y cuando esté asociado al enfoque básico con modificaciones en la dieta, actividad física y cambios en el estilo de vida. En general, el tratamiento farmacológico solo se recomienda a pacientes con un IMC mayor a 30 $kg/m^2$ (o mayor a 27 $kg/m^2$ si presenta comorbilidades asociadas) que no logren una reducción de peso significativa con los cambios antes mencionados. Los medicamentos utilizados pretenden producir una pérdida de peso y grasa corporal

a través de tres principales mecanismos: disminución de la absorción intestinal de las grasas, disminución de la ingesta y incremento del gasto energético. Dentro de los medicamentos actualmente aprobados por la FDA (Food and Drug Administration, de los Estados Unidos de América), podemos mencionar el orlistat (Xenical de Roche, Alli de GSK), el lorcaserín (conocido por Belviq) y la fentermina combinada con topiramato (Qsymia).

El orlistat es un inhibidor de las lipasas pancreáticas y gástricas que descomponen los triglicéridos en ácidos grasos absorbibles en el lumen del tracto gastrointestinal, de tal manera que los triglicéridos de la dieta se excretan sin digerir. Puede contribuir a disminuir el riesgo cardiovascular, los niveles de proteína C reactiva, la lipemia postprandial y los valores de citoquinas pro inflamatorias, además de que favorece el aumento de la adiponectina (Rubio *et al.,* 2007). Sin embargo, se han descrito efectos secundarios relacionados principalmente con desórdenes gastrointestinales, como son la producción de heces grasosas o con mala consistencia debido a la presencia de las grasas, el incremento de la flatulencia y la incontinencia fecal; además afecta la absorción de vitaminas liposolubles. Por lo que el orlistat debe ser utilizado sólo por personas que tienen serios problemas de obesidad y bajo supervisión médica.

El lorcaserín es un agonista del receptor de serotonina del subtipo 5-HT2c en el hipotálamo. Activa la síntesis de POMC, produciendo como resultado una señal de saciedad que promueve la pérdida de peso. Los efectos adversos más frecuentes incluyen cefalea, mareos, fatiga, náuseas, boca seca, y constipación. También se ha reportado casos de priapismo, así como un riesgo de trastornos psiquiátricos (excitación, alucinaciones) y cognitivos (atención). Además, no se recomienda su uso en personas que consumen otros medicamentos con efectos serotoninérgicos, debido a que las interacciones de drogas a nivel del receptor de serotonina pueden generar el llamado síndrome serotoninérgico caracterizado por alteraciones del estado mental, sistema motor autónomo y trastornos musculares (Thomsen *et al.,* 2008; Meltzer *et al.,* 2013)

El Qsymia es una combinación de fentermina y topiramato, en una fórmula de liberación prolongada. La fentermina es un supresor del apetito y estimulante del tipo anfetamina. El topiramato se usa tratar convulsiones en las personas con epilepsia y prevenir migrañas. Además, el topiramato es un antagonista de dos moléculas orexigénicas, el neuropéptido Y y la anandamida, y un agonista de los receptores centrales de la leptina y insulina. La acción del topiramato en el SNC, especialmente en el hipotálamo, induce anorexia y pérdida de peso, por reducción de la ingesta (Caricilli *et al.,* 2012). Sin embargo, el tratamiento con Qsymia produce parestesias, mareos, alteraciones del gusto, insomnio, constipación y xerostomía, así como problemas cardiovasculares (alteraciones en la frecuencia cardiaca y presión arterial) del sistema nervioso central (trastornos del humor, sueño, y trastornos cognitivos), oftalmológicos, entre otros.

### 6.5. Tratamiento quirúrgico

Finalmente, los pacientes obesos que cumplen con los siguientes criterios: edad entre 18 y 60 años, IMC > 40 kg/m$^2$ (o > 35 kg/m$^2$ con comorbilidades, obesidad mórbida desde más de 5 años, fracaso del tratamiento médico, sin contraindicaciones psiquiátricas, entre otros, pueden ser considerados aptos una intervención quirúrgica (Rubio *et al.,* 2007).

La cirugía bariátrica consiste en producir cambios en la fisiología del organismo para conseguir pérdidas de peso mantenidas y duraderas en el tiempo. Las técnicas y los procedimientos quirúrgicos son técnicamente complejos y no están exentos de complicaciones a corto ni medio plazo.

Los procedimientos restrictivos pretenden limitar la cantidad de comida que se ingiere. Por ejemplo, el procedimiento de la banda gástrica ajustable por laparoscopia consiste en la colocación de una banda alrededor de la parte superior del estómago creando restricción a la comida ingerida. En general, la pérdida de peso no supera el 40% de pérdida de exceso de peso en 5 años. Por otra parte, el procedimiento de la manga gástrica consiste en el corte longitudinal

del estómago por vía laparoscópica, para quitar la parte más ancha del estómago, haciendo el estómago más pequeño y suprimiendo la producción de grelina, lo que favorece la saciedad temprana y permite perder hasta el 50% del exceso de peso en 5 años.

El procedimiento conocido como Bypass gástrico es una combinación de procedimientos restrictivos y de mal-absorción. Es el procedimiento quirúrgico que se realiza con más frecuencia en los Estados Unidos y alrededor del mundo. Este procedimiento comprende el corte del estómago para crear una pequeña bolsa gástrica, el corte del intestino delgado y la reconstrucción del estómago mediante la unión al intestino. El Bypass Gástrico permite que los pacientes pierdan hasta un 75% de su peso inicial en un periodo de 5 años. Además, se controlan de manera eficiente los problemas relacionados con la obesidad.

Todos los métodos antes mencionados pueden ayudar los pacientes obesos a perder peso. Sin embargo, los resultados dependen en gran medida de la voluntad de cada individuo; los fármacos anti-obesidad pueden producir efectos secundarios graves y a menudo no muestran beneficios a largo plazo; además los procedimientos de cirugía bariátrica presentan varios riesgos para la salud. Por otra parte, el tratamiento de la obesidad y las comorbilidades asociadas, representa un gasto económico enorme para el sector salud de todos los países. Por lo que es importante investigar terapias alternativas que sean económicas, eficientes y menos invasivas, como lo es la medicina tradicional china (MTC), particularmente la acupuntura.

# ANTECEDENTES

## 1. Medicina Tradicional China y obesidad

La medicina tradicional china (MTC) es un sistema médico muy completo y bien estructurado cuyas bases teóricas tienen más de 2000 años de existencia. Ha permanecido vigente en China y ha traspasado fronteras llegando a obtener un reconocimiento a nivel mundial, de tal manera que en la actualidad ha sido incorporada e integrada con los modelos médico-científicos contemporáneos.

El modelo de la MTC esta basado en la existencia de una "fuerza energética" llamada "qi" que circula en el cuerpo a través de "canales" o "meridianos". Establece que la salud depende del buen funcionamiento y armonía de un sistema conformado por "órganos", "sustancias vitales" (energía, sangre y líquidos) y "canales", el cual permite la libre circulación de la "energía". Cuando se rompe este equilibrio y el flujo de la "energía" que garantizan una adecuada producción de sustancias vitales y su distribución a todo el organismo, sobreviene la enfermedad (Lacey *et al.,* 2003). En la MTC, el diagnóstico particular incluye a la integración de "síndromes" basándose en manifestaciones clínicas, la evaluación especializada del pulso radial y en la observación de la lengua. Además se fundamenta en diversas características y funciones específicas de los diferentes "sistemas orgánicos" (órganos), las cuales no corresponden estrictamente a las descritas en la medicina moderna aunque siempre es posible definir e integrar los dos sistemas médicos (Revisión en García-Cardona *et al.,* 2011).

La MTC utiliza diversas técnicas los entre las cuales destacan:

- Herbolaria: preparaciones a base de hierbas para estimular y regular las funciones del organismo.
- Tui-Na: técnicas de masaje chino, con pleno conocimiento de los canales energéticos acupunturales para lograr un equilibrio energético corporal.
- Qi-Gong: práctica de movimientos corporales en coordinación con la respiración para controlar y regular el flujo de energía por el organismo.

- Moxibustión: es la aplicación de calor en puntos de acupuntura por medio de conos o puros de moxa, su materia prima principal son hojas de *Artemisa vulgaris*
- Acupuntura: que involucra la utilización de agujas insertadas en distintos puntos del organismo. Se puede dividir en acupuntura manual, electroacupuntura que consiste en la estimulación eléctrica constante y periódica en la superficie corporal o sobre las agujas ya previamente insertadas; además puede haber estimulación con láser, tachuelas, acupresión, inserción de catgut, que tiene como ventaja la producción de un estímulo más intenso y prolongado en cuanto a intensidad y frecuencia y la auriculoterapia que es una variante de la acupuntura en la cual se aplican estímulos en puntos de la oreja para la prevención y tratamiento de diversas enfermedades. Particularmente, la acupuntura es ampliamente usado para el manejo de obesidad.

**2. Bases moleculares de la acupuntura**

En la MTC, la acupuntura consiste en estimular puntos anatómicos específicos mediante la inserción de agujas finas, con la finalidad de producir una señal mecánica de transducción que tenga un efecto prolongado en el organismo para restablecer la libre circulación del “qi” y por lo tanto la salud (Man, 1971). Hasta la fecha, no se conoce con certeza cómo actúa la acupuntura a nivel molecular, sin embargo existen varios trabajos enfocados a descifrar los mecanismos moleculares que pudieran explicar los efectos de la acupuntura en diversas enfermedades.

La inserción de la agujas en el músculo estimulan las fibras nerviosas sensitivas (Kagitani *et al.,* 2005), lo que esto causa la liberación de varios neuropéptidos, por ejemplo sustancia P, péptido relacionado con el gen de la calcitonina, entre otros, en el área. Consecuentemente, aumenta la microcirculación (Holmang *et al.,* 2002; Jansen *et al.,* 1989; Sato *et al.,* 2000). Mediante el sistema simpático, la estimulación acupuntural llega a la médula espinal y puede modular los órganos

que corresponden a la misma zona de inervación (Sato *et al.,* 1997; Springer-Verlag *et al.,* 2009). La señal también es transferida al cerebro, el cual libera diferentes sustancias (opioides), entre las cuales destaca la beta-endorfina producida por el hipotálamo, las cuales modulan la expresión de otras moléculas que inducen cambios funcionales al nivel del órgano blanco. En base a estos datos, una hipótesis es que la acupuntura modula la actividad del sistema nerviosos simpático y afecta el eje hipotalámico–pituitario–adrenal (HPA) a mediante la modulación de la secreción de la hormona adrenocorticotropica (ACTH) y cortisol (Ahsin *et al.,* 2009; Harbach *et al.,* 2007).

El restablecimiento del "qi" se obtiene a través de breves movimientos de rotación de las agujas insertadas en los puntos corporales. Se ha reportado que la interacción de las agujas con el tejido conectivo intersticial, las fibras elásticas y el colágeno, podría inducir una respuesta caracterizada por cambios en la matriz extracelular, rearreglo del citoesqueleto intracelular y contracción celular, generación de señales de transducción autocrinas y paracrinas que modifiquen la matriz extracelular circundante para amplificar la respuesta terapéutica (Langevin *et al.,* 2001). El tejido conectivo intersticial también tiene un rol importante en la neuromodulación asociada a la acupuntura, ya que los cambios que sufre promueven la estimulación de fibras nerviosas aferentes, las cuales transmiten la señal al sistema nervioso central para inducir la liberación de opioides como la β-endorfina, la encefalina y la dinorfina (Han *et al.,* 2004; Kawakita *et al.,* 2006).

Otra línea de investigación está enfocada en la modulación de proteínas específicas, como lo es el factor de transcripción STAT 5 (del inglés signal transducer and activator of transcription 5, el cual es activado a través de la vía JAK (del ingles Janus-activated kinases)/STAT mediada por citocinas. Así, se ha reportado que la estimulación del punto acupuntural Zusanli (ST36) esta asociada a un aumento en la expresión y actividad de unión al DNA de STAT5 en células mononucleares de sangre periférica. De acuerdo a los autores, estos resultados confirman que la acupuntura produce una respuesta molecular en el organismo; particularmente sugieren la participación de la vía de transducción de señales

mediada por citocinas y JAK/STAT en los efectos inmunomoduladores de la acupuntura. (Liu *et al.,* 2006).

### 3. Tratamiento acupuntural de la obesidad

El término chino para obesidad utilizado es *fei pang* 肥胖 que en general se puede traducir como “gordura, grasa, grasa fértil, gordura o grasa generada con facilidad”. La MTC describe a la obesidad como una enfermedad compleja que involucra principalmente a los “sistemas orgánicos” o energéticos del bazo, hígado y riñón, que al alterarse modifican el metabolismo de los líquidos corporales, los cuales producen productos patológicos llamados “humedad” y “flema”, que de acuerdo con la MTC se “acumulan” y “estancan” en diferentes partes del cuerpo en forma de grasa. Esta “flema-grasa” se convierte en un producto capaz de generar o detonar una gran diversidad de procesos patológicos que afectan al organismo en múltiples sistemas según el Su Wen del Huangdi Neijing (Clásico interior del emperador amarillo) en la primera parte: Su wen (Preguntas básicas) (Flaws, 2002; García-Cardona *et al.,* 2011) Por lo tanto la obesidad se relaciona principalmente con el concepto “acumulación de flema” y la “flema” tiene su origen principalmente en la disfunción de un sistema orgánico-energético llamado *Pi* 脾 Bazo (Sionneau, 2000)

En los últimos años, la acupuntura ha empezado a ser considerada como una alternativa terapéutica atractiva y eficiente para el tratamiento de la obesidad en los países occidentales. Por lo que OMS promueve la realización de estudios científicos de los tratamientos acupunturales con la finalidad de validar esta medicina, mejorar su aceptación por la medicina moderna, y así extender su uso como una opción terapéutica simple, barata y efectiva (WHO, 1996).

Varios reportes clínicos y científicos han demostrado la eficacia de la acupuntura en el control de la obesidad, no sólo para reducir el IMC, sino también para regular diferentes parámetros bioquímicos, como son el perfil lipídico que incluye

triglicéridos, colesterol y LDL, además de lipoproteínas (lipoproteina A, apolipoproteina A y apolipoproteina B), hormonas (leptina, grelina, colecistokinina, adiponectina), neurotransmisores (beta-endorfina), el metabolismo de la glucosa (insulina, peptido-c, glucosa), así como marcadores de inflamación e inmunológicos (TNF-α, IgG, IgA, IgM, y IgE) . La electroacupuntura es la técnica más utilizada en estos estudios, seguramente por ser la técnica en la que se pueden regular la estimulación por las agujas a través de los estímulos eléctricos usados durante el tratamiento. Sin embargo, también existen reportes acerca de los efectos de la acupuntura manual, catgut embebido, acupuntura auricular, entre otras. Estos datos se describen en los Capítulos 1 y 2 con más detalles.

# JUSTIFICACIÓN

La obesidad es un problema de salud pública mundial que favorece el desarrollo de problemas cardiovasculares, diabetes y cáncer, que representan las primeras causas de muerte en el mundo. Generalmente, el tratamiento de esta enfermedad consiste en cambios en la dieta y aumento de la actividad física, en ocasiones asociados a un medicamento o una cirugía. Sin embargo, los resultados dependen en gran medida de la voluntad del paciente; además, los fármacos que se utilizan pueden producir efectos secundarios graves y a menudo no muestran beneficios a largo plazo.

La acupuntura, una técnica de la MTC, representa una terapia alternativa atractiva para el tratamiento de la obesidad ya que es económica, eficiente, menos invasiva, y sin efectos secundarios. Particularmente, se ha reportado que la disminución del peso corporal esta asociada a una modulación de los parámetros bioquímicos y algunas adipocinas en pacientes.

Por lo que es de nuestro interés investigar los cambios de la expresión génica en el tejido adiposo en respuesta al tratamiento acupuntural de la obesidad, con la finalidad de contribuir al entendimiento de las bases moleculares del efecto de esta terapia en el tratamiento de la obesidad.

# OBJETIVOS

**Objetivo general**

Determinar los cambios de expresión génica en el tejido adiposo que están asociados al tratamiento acupuntural de la obesidad.

**Objetivos particulares**

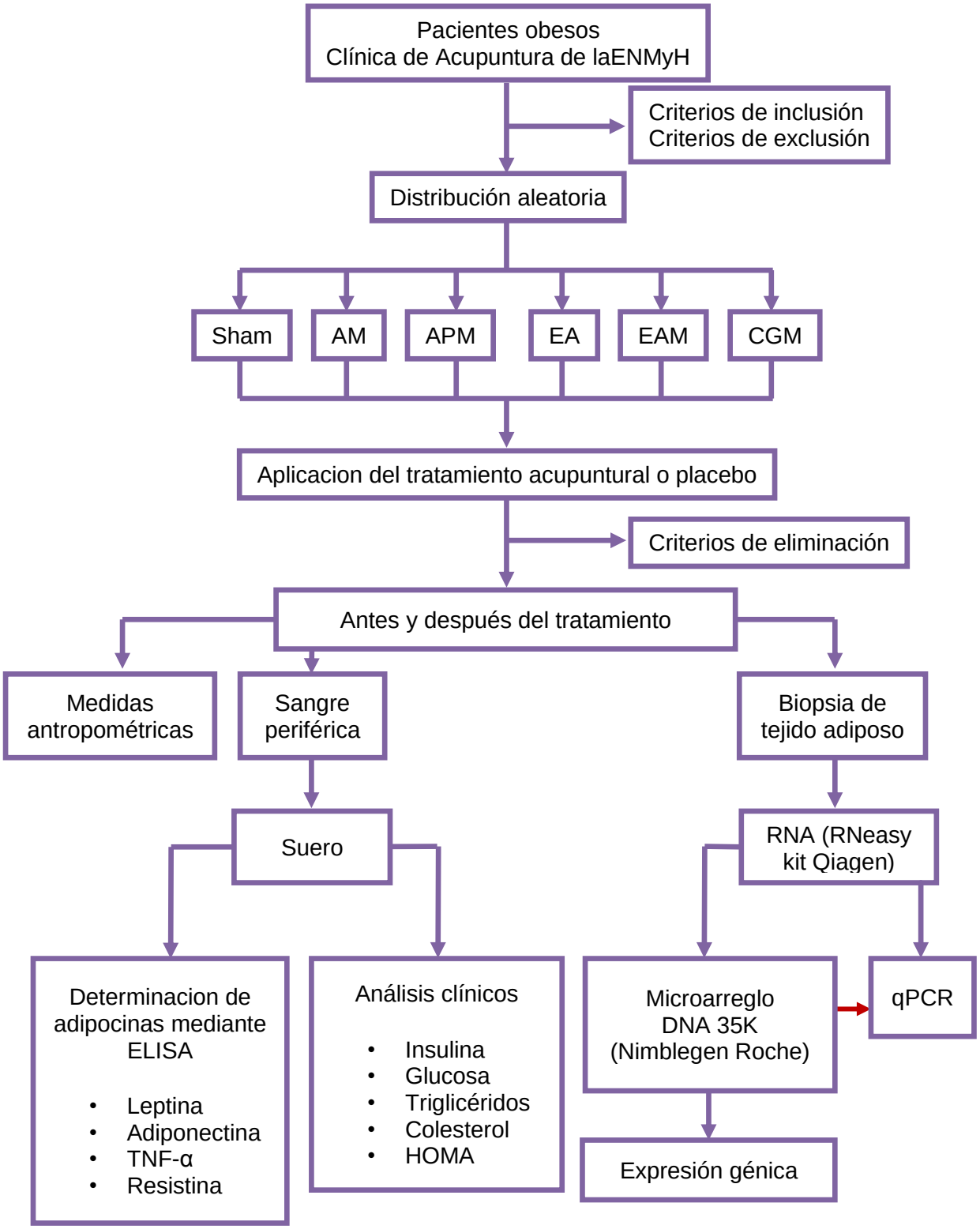
1) Comparar la eficacia de diferentes tratamientos acupunturales en los parámetros antropométricos y clínicos de pacientes obesos.

2) Determinar el efecto del tratamiento acupuntural más eficiente sobre las concentraciones de adipocinas (leptina, adiponectina, TNF-α y resistina) en suero.

3) Identificar genes cuya expresión sea modulada por el tratamiento acupuntural seleccionado en el tejido adiposo de pacientes obesos.

4) Determinar las vías metabólicas y bioquímicas que se modifican en respuesta al tratamiento acupuntural seleccionado en pacientes obesos.

# ESTRATEGIA EXPERIMENTAL



# Capítulo 1

## Effects of Acupuncture on Obesity and Adipokines Involved in Body Weight Control.

**García-Vivas Jessica**, González-González Roberto, García-Cardona Ma. Del Carmen, López-Camarillo César and Marchat Laurence A. Effects of Acupuncture on Obesity and Adipokines Involved in Body Weight Control. Homeopathy & Ayurvedic Medicine 2013, 2:3 http://dx.doi.org/10.4172/2167-1206.1000129. Review article.

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Review Article Open Access

# Effects of Acupuncture on Obesity and Adipokines Involved in Body Weight Control

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**Abstract**

Obesity is a worldwide disease that results from a deregulation of energy balance and changes in adipokines and other molecules with metabolic relevance. Pharmacological treatments for obesity are often associated to drug adverse effects. Among alternative and complementary therapeutic methods for obesity treatment, Traditional Chinese Medicine and particularly acupuncture that have been practiced for thousands of years in China, have been increasingly used for the efficient control of body weight without producing negative side effects and weight regain. Several works have suggested that the effects of acupuncture may be related to hypothalamus stimulation, which may regulate the production of some proteins involved in food intake and energy expenditure balance. In this review, we present the main results of English publications obtained from PubMed database as well as data from works published in original Chinese language. These reports describe the clinical effectiveness of acupuncture as a treatment for obesity. They also provide evidence about the regulation of the principal adipokines related to obesity, namely leptin and adiponectin, as well as other relevant biochemical molecules. Although further well-designed and controlled studies are required, this knowledge contributes to gain some insight in the mechanisms underlying the effect of acupuncture for obesity treatment.

**Keywords:** Acupuncture; Adiponectin; Body weight control; Inflammation; Leptin; Obesity; Traditional chinese medicine

**Abbreviations:** ACOX: Acyl-Coa Oxidase; AGRP: Agouti-Related Peptide; α-MSH: α-Melanocyte-Stimulating Hormone; BE: Beta Endorphin; BMI: Body Mass Index; CART: Amphetamine-Related Transcript; CNS: Central Nervous System; CSF: Cerebrospinal Fluid; DIO rat: Diet-Induced Obese Rat; EA: Electro Acupuncture; FFA: Free Fatty Acid; HDL: High Density Lipoprotein; Hsp: Heat Shock Protein; LDL: Low Density Lipoprotein; MCP-1: Monocyte ChemoAttractant Protein-1; NPY: Neuropeptide Y; NRF 1: Nuclear Respiratory Factor 1; PGC-1α: Peroxisome Proliferator-Actived Receptor Coactivator-1α; POMC: Pro-Opiomelacortin; PPAR γ: Peroxisome Proliferator-Activated Receptor γ; SIRT1: Skeletal Sirtuin 1 Protein; TCM: Traditional Chinese Medicine; TNF α: Tumor Necrosis Factor α; WAT: White Adipose Tissue

## Introduction

Obesity is a global public health problem that presents the characteristics of a pandemic due to its rapidly increasing incidence [1-3]. Notably obesity promotes hyperglycemia, hyperinsulinemia and hyperleptinemia, as well as glucose intolerance and insulin resistance [4] that are part of the metabolic syndrome [3,5,6]. It also represents a risk for degenerative illnesses, including coronary diseases, heart attack, infertility, erectile dysfunction, arthropathies, neuropathies, as well as colon, prostate, endometrial and breast cancer [7]. These obesity-related diseases cause an excessive economic cost and represent the main causes of death worldwide. Together with a balanced diet and physical exercise, anti-obesity drugs and surgery processes can help patients to lose weight. However, they usually produce adverse effects and weight regain is very common if patients do not strictly follow nutritional recommendations and come back to their sedentary life style. Therefore, there is an increasing interest in alternative and complementary therapeutic methods for obesity treatment. Among these, Traditional Chinese Medicine (TCM) and particularly acupuncture that have been practiced for thousands of years in China, represent a suitable therapeutic approach for individuals with obesity, without producing negative side effects and weight regain. This review describes the main metabolic pathways involved in body weight regulation, with anemphasis in leptin and adiponectin adipokines that are essential for the control of food intake and energy expenditure balance. It also describes the clinical effectiveness of acupuncture treatment for obesity and provides evidence about the effects on these adipokines and other relevant biochemical parameters, from extensive analyses of English articles published in PubMed database, as well as works published in original Chinese language in acupuncture and TCM journals.

## Obesity

Obesity is defined as an abnormal increase of fatty acids storage in an expanded adipose tissue mass [3] and accumulation of ectopic fat, which is associated to an increased number and size of adipocytes as a result of passive overconsumption of high-fat and carbohydrates-rich diets, and low physical activity. This energy imbalance is due to numerous physiological, psychological, socioeconomic, cultural, emotional, metabolic and genetic factors, whose complex roles are not fully understood yet [8,9]. At cellular level, obesity is also related

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with activation of immunocompetent cells, such as macrophages, which produce a low-grade chronic inflammation characterized by abnormal cytokine production, increased acute-phase reactants and other mediators, and activation of a network of inflammatory signaling pathways [6].

Involuntary control of energy homeostasis is thought to be crucial to maintain the equilibrium between energy intake and expenditure. In general, body weight is regulated by the central nervous system (CNS), mainly hypothalamus, which controls hunger and satiety in response to a complex network of signals from endocrine tissues, including pancreas (insulin), adipose tissue (leptin, adiponectin) and stomach (ghrelin) [10]. These peripheral signals provide information about the ingested food to the brain, which responds via integrated neuropeptide pathways that are related to energy homeostasis, including neuroendocrine activation from the pituitary gland, motor behavior, and autonomic activity, which is a fundamental metabolic process, including lipolysis, insulin and glucagon secretion from pancreas, and glucose synthesis from liver [11].

Adipose tissue is not only a passive reservoir of energy [12], it also plays a role in energy homeostasis by regulation of whole body Free Fatty Acid (FFA) homeostasis. It stores FFAs in the form of triglycerides through their esterification to glycerol in periods of calorie abundance, and releases them back to the circulation in times of energy shortage [3]. In response to nutrient, neural and hormonal signals, adipose tissue also secretes bioactive peptides, called adipokines, which act at local (autocrine/paracrine) and systemic (endocrine) levels to control feeding, thermogenesis, immunity and neuroendocrine function [13]. In addition to these efferent signals, adipose tissue expresses receptors to respond to afferent signals from hormone systems and CNS [14]. It also has a role in insulin resistance and cardiovascular complications [6].

The predominant type of adipose tissue commonly called "fat" is the White Adipose Tissue (WAT) located in subcutaneous region and around viscera [13]. It consists of adipocytes surrounded by vascularized and innervated loose connective tissue with macrophages, fibroblasts, adipocytes precursors and other cells. Notably, WAT is responsible for the synthesis of adipokines for body weight control: leptin, adiponectin, acylation stimulating protein and resist in are produced by adipocytes, while TNFα and IL-6 are synthesized by macrophages [15-17].

## Main Adipokines Involved in Body Weight Control

### Leptin

The best known adipokine that promotes weight loss is leptin that acts on the hypothalamus (arcuate nucleus), suppressing food intake and stimulating energy expenditure through increased thermogenesis [8,18]. The regulatory feedback includes the following steps: 1) a sensor (leptin production by adipose cells) monitors the level of energy stores (size of adipose tissue mass); 2) hypothalamic centers receive and integrate the leptin signal through leptin receptors; 3) effectors systems (mainly sympathetic nervous system) control energy intake and energy expenditure [8]. Leptin released from WAT enters into the circulating system, binds to a short form of leptin receptor (LRa) and crosses the blood-brain-barrier [19,20]. In the hypothalamus, leptin binds to the long form of leptin receptor (LRb) [21] in two different groups of neurons. One population synthesizes and releases two orexigenic neuropeptides: the neuropeptide Y (NPY) and the agouti-related peptide (AGRP); the other produces two anorexigenic proteins: the anorexigenic peptide α-melanocyte-stimulating hormone (α-MSH), which derived from pro-opiomelacortin (POMC) and the cocaine and amphetamine-related transcript (CART) [22].

Leptin concentration in plasma and mRNA expression level in adipose tissue are related to obesity severity, which means that an increase of fat mass is associated with an increase of leptin, making it an indicator of total fat mass [23,24]. Most obese subjects have a high plasma leptin concentration and present leptin resistance, which may contribute to deregulation of body weight control. This might result from: 1) a limitation of blood-brain-barrier [25,26], in which leptin receptor (LRa) that transports leptin into the cerebrospinal fluid (CSF) is saturated [19]; 2) mutation of LRb leptin receptor in hypothalamus [27] or 3) inhibition of leptin signaling mechanism in hypothalamus, like STAT3 pathway [28]. Therefore leptin can be successfully used as an anti-obesity tool only in the small number of obese patients with leptin deficiency.

### Adiponectin

Exclusively produced by mature adipocytes [29], adiponectin is an abundant plasma protein [30] that exists as low-molecular-weight trimers to high-molecular-weight dodecamers [31]. It is an anti-inflammatory adipokine that regulates energy balance and peripheral lipid metabolism [32,33], stimulating fatty acid oxidation and glucose untaken in skeletal muscles and adipose tissue, both dependent on AMPK signals [34-36]. Moreover, its binding to adiponectin receptors (AdipoR1 and AdipoR2), which colocalize with leptin receptor in hypothalamus, regulates energy expenditure through the same signaling pathway than leptin [37]. Adiponectin has anti-diabetic and anti-atherogenic roles, which are negatively correlated with obesity and insulin sensitivity markers, like waist-hip ratio, insulin resistance, dyslipidemia, diabetes and cardiovascular disease [38]. Therefore a low plasma adiponectin level is considerate as an independent risk factor for type II diabetes [6,24,39,40].

## Traditional Chinese Medicine and Acupuncture for the Treatment of Obesity

### Definition

Traditional Chinese Medicine (TCM) is a complete medical system that has been used to diagnose, treat, and prevent illnesses for more than 2,000 years. TCM is based on the belief in yin and yang (defined as opposing energies): when they are in balance, there is health; when they are out of balance, it means illness [41]. According to TCM, life force or "Qi" circulates longitudinally throughout the body within 12 energy pathways named "energetic channels" or "meridians" [42] that connect with what Western medicine defined as organs or tissues. Health depends on the proper function and harmony of a system consisting of "organs", "vital substances" (energy, blood, fluids) and "energetic channels". When balance is disrupted, vital substances production and distribution throughout the body is altered and disease occurs [43]. Stimulation of specific points in the "channels", called "acupoints", using different methods described in Table 1, can normalize Qi to restore health [42].

### Molecular mechanisms of acupuncture

Recent experimental studies have tried to investigate the molecular mechanisms underlying the effects of acupuncture, one of the most used methods of TCM.Using functional magnetic resonance imaging, Napadow et al. showed that limbic system is central for the acupunctural effect (acupuncture, electroacupuncture or tactile pressure), although

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| Methods | Short Describtion |
|---|---|
| Needling or traditional acupuncture | Fine stainless-steel needles are inserted through the skin to stimulate acupoints manually |
| Electroacupuncture | The needles receive a electrical stimulation to improve the stimulation of acupoints |
| Auricular acupuncture | Application of needle at acupoints in the ear |
| Staplepuncture or tacks | A short acupuncture needle is taped into specific body points for extended periods with pressure |
| Acupressure | Massage or pressing at acupoints |
| Moxibustion | Application of heat, by burning a pellet or cigar of combustible material (*Artemisa vulgaris*) on or near the point to stimulate |
| Cupping | Use of cups from different materials to create negative pressure on skin surface to increase blood circulation and acupoint stimulation |
| Transcutaneous electrical nerve stimulation | Application of electrode pads to skin surface, which transmit a mild current into the acupoints |
| Catgut embedding | Introduction of catgut (a type of cord made of natural fiber from sheep or goat intestine wall) on acupoints to enhance the stimulation for 10-15 days |
| Chinese Herb | Utilization of plant, animal, human, and mineral products usually obtained by decoction |
| Laser stimulation | Use of low output light power (2.5-5 mW) beam to stimulate body or ear acupoints |

**Table 1:** The main methods in TCM.

different pathways and neurobiological responses are activated depending on stimulated points [44]. In contrast, there was no response in limbic system when no-points were stimulated as control [45]. These molecular mechanisms may explain the effects of acupuncture in obesity control since limbic system includes the hypothalamus region that regulates food intake and energy expenditure balance. Other studies have revealed that endogenous opioid peptides(enkephalin, beta-endorphin, endomorphin, dynorphin) in CNS mediate the analgesic effect of Electro acupuncture [46]. However, the exact mechanisms underlying the beneficial effects of acupuncture in many pathologies, including obesity, remain largely unknown.

## Obesity in TCM

The Chinese term for obesity is "fei pang" (肥胖) which means "fat, grease easily generated". TCM describes obesity as a complex condition involving energy systems of spleen, liver and kidney. The alteration of their energy creates an imbalance in body fluids metabolism, which generates pathological products called "moisture" or "humidity" and "phlegm" that accumulate in different parts of the body as fat. This phlegm-fat turn constitutes a pathological product that triggers a wide variety of disease processes, affecting multiple body systems. TCM also considers that poor eating habits and sedentary lifestyles cause obesity, and recognizes that heredity and congenitally determined constitution are important. According to the characteristics of each patient, obesity can correspond to distinct deficiency syndromes (Table 2) and distinct points should be stimulated. The individual diagnostic also includes clinical manifestations, the radial pulse, and a specialized evaluation of the tongue [43].

## Experimental evidence of the effects of acupuncture on adipokines regulating body weight

An exhaustive search in PubMed database allows the release of numerous reports about the use of acupuncture for obesity treatment. Because one of the coauthor of the present review is a specialist in acupuncture medicine and has Chinese language knowledge, we were able to analyze both English and Chinese language publications, which is somewhat difficult for most scientists. In addition, thanks to his personal access to works published in acupuncture and Traditional Chinese Medicine journals in original Chinese language, we also have the opportunity to review clinical and experimental reports published by acupuncture experts from China, where acupuncture has been practiced for thousands of years.

The majority of publication related to acupuncture and obesity, usually analyzed the effects on body mass and anthropometric data, but little is known about the regulation of biochemical parameters and adipokines, mainly leptin and adiponectin that are relevant for body weight control. Here we describe several results of the main reports related to Electro Acupuncture (EA) that is the most commonly used method for obesity treatments; we also include some interesting data about catgut embedding and other acupuncture protocols. These reports were selected because they used control group (placebo) and present experimental data related to leptin and adiponectin (and other molecules) that might contribute to explain the molecular mechanisms of the beneficial effects of acupuncture.

## Electro acupuncture

Electro acupuncture is the most frequently used acupuncture technique to control body weight, because the stimulation is constant and easily measurable in Hertz (Hz). Therefore, the parameters of EA can be precisely characterized, which allows reproducible results.

In 2005, you and Hung showed that 100 Hz EA (bilateral Zusanli (ST36) and Sanyinjiao (SP6) for 30 min during 14 days) significantly inhibits weight gain in Wistar diet-induced obese (DIO) rats, with a decrease in triglycerides and an increase in HDL. Leptin and insulin levels were significantly increased in control group, while they remained unchanged in EA group [47]. In human, a similar reduction in body weight, lipid profile (triglycerides, total cholesterol and LDL), as well as in waist and hip circumference, was observed when patients were treated with EA for six weeks (bilateral Tianshu (ST25), bilateral Weidao (GB28), Zhongwan (CV12), Shuifen (CV9), Guanyuan (CV4), Sanyinjiao (SP6), as well as Quchi (LI11) and Fenlong (ST40) for obese patients with higher energy, or Qihai (CV6) and Yinlingquan (SP9) for patients with lower energy, using 30-40 Hz and dense-disperse wave), followed by a six weeks period without any treatment for six weeks and another six weeks period with a low-calorie diet for. These results demonstrated that EA exhibits long-term effects on body homeostasis in obese patients [48]. Body weight and serum leptin reduction (($p<0.000$)in response to EA (ear points Sanjiao (Hungry) and Shenmen (Stomach), and body points Hegu (LI4), Quchi (LI11), Tianshu (ST25), Zusanli (ST36), Neiting (ST44), Taichong (LV3) and Qihai (CV6), once daily, for 30 minutes, during 20 days) were also associated with an increase in serum beta endorphin (BE) levels ($p<0.05$). Authors hypothesized that the effect of EA in modulating serum BE level could enhance lipolytic activity, which may induce weight loss by mobilizing energy stores [49]. A randomized, sham-controlled preliminary trial confirmed that the significant reduction of body weight and body mass index (BMI) in obese women treated with EA (Hegu (LI4), Shenmen (HT7), Zusanli (ST36), Neiting

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| Syndrome | Recommended points (occurrence frequency in %) | |
|---|---|---|
| Spleen and obstruction of humidity deficiency | • YinlingquanSP9 (25)<br>• PishuBL20 (25)<br>• ZusanliST36 (87)<br>• ZhongwanCV12 (45) | • NeiguanPC6 (13)<br>• TianshuST25 (28)<br>• Qihai CV6 (39) |
| Heat and obstruction of humidity and phlegm in the stomach | • TianshuST25(92)<br>• FenglongST40 (53)<br>• NeitingST44 (33)<br>• QuchiLI11 (36) | • ZhongwanCV12 (45)<br>• ShuidaoCV28 (30)<br>• PishuBL20 (19) |
| Liver energy stagnation and phlegm | • TaichongLV3 (62)<br>• ZhigouTH6 (18)<br>• QimenLV14 (10)<br>• NeiguanPC6 (31) | • HeguLI4 (68)<br>• ZhongwanCV12 (80)<br>• ShanzhongCV17 (6) |
| Energy deficiency and blood stagnation | • ZhongwanCV12 (45)<br>• SanyinjiaoSP6 (60)<br>• ZusanliST36 (88)<br>• Guilai ST29 (8) | • TaichongLV3 (28)<br>• HeguLI4 (31)<br>• GeshuBL17 (16)<br>• TianshuST25 (80) |
| Obstruction and turbidity center by phlegm | • ZhongwanCV12 (45)<br>• TianshuST25 (88)<br>• QihaiCV6 (40)<br>• ZusanliST36 (88) | • YinlingquanSP9 (20)<br>• TaichongLV3 (28)<br>• NeiguanPC6 (14)<br>• PishuBL20 (20) |
| Yang deficiency of spleen and kidney | • ShenshuBL23 (57)<br>• PishuBL20 (100)<br>• Tianshu ST 25 (100) | |

**Table 2:** Recommended points used for obesity treatment according to the different syndromes in TCM.

(ST44), and Sanyinjiao (SP6) bilaterally, two sessions of 20 minutes/week for five weeks) was related to decreased levels of leptin. They also observed reduced insulin levels, and increased levels of ghrelin and cholecystokinin [50]. Lou et al. also reported that EA can significantly reduce leptin levels and increase adiponectin serum levels in obese [51]. Finally, study of Fan et al. evidenced that EA at lateral Housanli and Neiting (ST44) with 2-15 Hz, 4mA for 49 days, was more effective than the anti-obesity drug, sibutramine, to reduce body mass through the regulation of adiponectin and insulin levels in obese rats [52].

To confirm that EA, and not the stress produced by the manipulation, was responsible for body weight reduction and adipokines modulation, Kim et al. compared three groups of rats: AL (fed *ad libitum* without any treatment), Holder (fed *ad libitum* with daily holder restraint) and EA (fed *ad libitum* with daily holder restraint and 100 Hz EA stimulation) groups. After the four-week experimental period, they evidenced that food intake and body weight reduction in EA group was associated to increased serum leptin levels, as previously reported. Interestingly, the level of stress hormones, such as epinephrine and norepinephrine, and corticosterone, was increased in Holder group, but not in EA group. Altogether, these results suggested that the effect of EA on body weight was through increasing leptin, but was not due to the stress caused by the daily holder restraint [53]. Taken altogether, these studies indicated that EA might help to control obesity owing to its beneficial effects on hormones that participate in pathways regulating body weight, namely leptin and adiponectin, among others. An interesting study showed that the significant reduction in food intake and body weight in DIO rats treated with 2 Hz EA (Zusanli (ST36) and Sanyinjiao (SP6) with intensity increasing stepwise from 0.5-1-1.5 mA daily for 30 minutes), was associated with increased levels of α-MSH peptide and POMC mRNA in hypothalamus, and an elevated α-MSH concentration in CSF. These data suggested that the mechanism by which EA controls body weight in rat involves α-MSH that has an anorexigenic effect [54]. In another study, in addition to the effect on appetite and α-MSH, the application of 2 Hz EA (four weeks, three sessions/week) in DIO rats also induced increase in anorexigenic CART peptide, and a decrease in orexigenic peptide NPY in hypothalamus. The modulation of these neuropeptides could explain the reduction of food intake and body weight in rat. Notably, 2 Hz EA treatment induced a more important reduction in food intake, body weight and ghrelin levels, that 100 Hz EA; while 100 Hz EA was more efficient to reduce cholesterol and triglycerides, and increase plasma leptin [55].

Some of the studies described above showed that EA not only modulates leptin and adiponectin, but also affects insulin levels. This was confirmed in works published by Cabioğlu and colleagues in which EA application (ear points Sanjiao (Hungry) and Shenmen (Stomach), and body points Hegu (LI4), Quchi (LI11), Tianshu (ST25), Zusanli (ST36), Neiting (ST44) and Taichong (LV3), once daily, for 30 minutes, during 20 days) produced a 4.8% weight reduction and a significant decrease ($p<0.05$) in total cholesterol, triglyceride and LDL in obese women [56], as well as a significant decrease in lipoprotein A and apolipoprotein B ($p<0.05$), indicating that EA therapy might be a useful approach for both losing weight and reducing risk factors for associated cardiovascular diseases [57]. Interestingly, the effects of EA also included an increase in serum insulin and c-peptide levels ($p<0.001$) and a decrease in glucose levels ($p<0.01$), suggesting that EA can help to control serum glucose levels through regulation of serum insulin and c-peptide levels [58]. These results are consistent with those of Lin et al. which reported that EA treatment with 15 Hz at bilateral Zusanli (ST36) was able to significantly reduce HOMA index from 7.29 ± 3.0 to 3.3 ± 1.1 in rats with insulin resistance induced by prednisolone, while HOMA index was 5.1 ± 1 in control. Plasma levels of FFAs were significantly decreased after 60 minutes of prednisolone injection (until 16 ± 20% in EA group, compared with 72 ± 31% in control). Therefore, the positive effect of EA on insulin resistance could be by lowering plasma FFAs levels [59]. Another study also demonstrated that the decrease in FFAs levels in response to 3 Hz EA (Zusanli (ST36) and Guanyuan (CV4), five sessions/week, eight weeks), was associated to up-regulation of skeletal Sirtuin 1 (SIRT1) protein expression, peroxisome proliferator-actived receptor γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF 1) and acyl-CoA oxidase (ACOX). Authors concluded that low-frequency EA improves insulin sensibility in obese diabetic mice probably through activation of SIRT 1/PGC-1α in skeletal muscle [60].

Obesity is closely associated with a chronic inflammation and a few studies have described the anti-inflammatory actions of acupuncture in the treatment of obesity. For example, results from Yu et al. evidenced that strong EA (20 Hz, 5 V at Zusanli (ST36) and Sanyinjiao (SP6) daily for 14 days) was more effective to regulate body weight, as well as triglycerides, cholesterol, HDL and LDL, than weak EA (20 Hz, 2.5 V) in obese rats. Interestingly, RT-PCR assays using RNA obtained from epididymis adipose tissue revealed a significant reduction of transcripts corresponding to that monocyte chemo attractant protein-1 (MCP-1) and TNFα, both pro inflammatory molecules. Then the modulation of inflammation could contribute to the effects of EA in obese patients [61].

## Catgut embedding

Currently, many clinical studies are evaluating the effect of catgut implantation in obese patients and animal models. This method consists in the protein magnetization line with catgut embedding apparatus implanted in the corresponding acupuncture points to produce a sustained and effective stimulation for about 15 days. All the studies clearly demonstrate the efficiency of catgut embedding to regulate body weight; they also evidence changes in several markers.

For example, in the study of Gao et al., rats exhibited a reduced weight compared to control group, an increase in PPARγ-mRNA and

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a reduction in total cholesterol, LDL, hepatic lipoprotein lipase and triglycerides levels in response to two sessions of catgut implantation at points Housanli (posterior ST36 Zusanli), Tianshu (ST25) and Pishu (BL20) [62]. Another report demonstrated that catgut embedding had a better anti-obesity action than manual acupuncture. Interestingly, change in body weight was associated with a significant reduction of serum leptin, triglycerides and cholesterol levels, as well as a significant increase in hypothalamus leptin after four weeks of treatment in obese rats ($p<0.05$) [63]. In addition to modify lipid profile, catgut implantation in abdomen points during 90 days produced a decrease in glucose levels and fasting insulin in patients with metabolic syndrome, which indicates that catgut embedding method could be useful in patient with metabolic syndrome, probably through insulin reduction [64]. In a recent report, Yan et al. also reported those 90 days-treatment (one catgut application every two weeks) caused a significant reduction of insulin and leptin resistance, as well as a diminution in total cholesterol, triglycerides and LDL levels, while HDL increased significantly. Interestingly, after one year, patients did not present insulin resistance anymore. Moreover, leptin receptor mRNA expression, leptin and insulin levels were significantly increased in hypothalamus of obese rats with catgut application, which suggested that catgut embedded can modify gene expression [65].

## Other acupuncture techniques for the treatment of obesity

As described in Table 1, TCM and acupuncture includes a large number of methods. Although EA and catgut embedding seem to be the more efficient for obesity treatment, the other techniques have been also shown to reduce body weight and regulate adipokines.

One of the most interesting studies about the effects of acupuncture on adipokines, was performed in a diabetic and obese rat model, stimulating Housanli (ST 36) and Yishuand Neiting (ST 44) points. After four weeks of treatment, the authors reported significative changes in the level of several adipokines in circulating blood, namely adiponectin, as well as resistin and TNFα. This indicated that acupuncture can modulate/regulate various biochemical pathways, including metabolism and inflammation, producing different physiological effects that contribute to the control of obesity. These effects were similar to those observed in the group treated with glibenclamide. However, acupuncture treatment did not produce the secondary effects associated with glibenclamide [66]. Auricular acupressure combined with a low-calorie diet was able to produce a significant reduction in plasma leptin levels (18.57%, $p<0.01$) and body fat mass (4%, $p<0.05$) in obese patients compared to control group after six weeks treatment [67]. Ippoliti et al. reported that hypocaloric traditional Chinese and western diets produced the same significant reduction in body weight, BMI and waist circumference and leptin levels, without affecting TNFα nor ghrelin levels. Notably, when traditional Chinese diet was associated to auricular acupuncture (Hunger, Shenmen, Liver, Kidney, Lung, Stomach and Mouth points), patients reported a higher reduction of hunger feeling compared with sham group. Unfortunately, the authors did not describe any

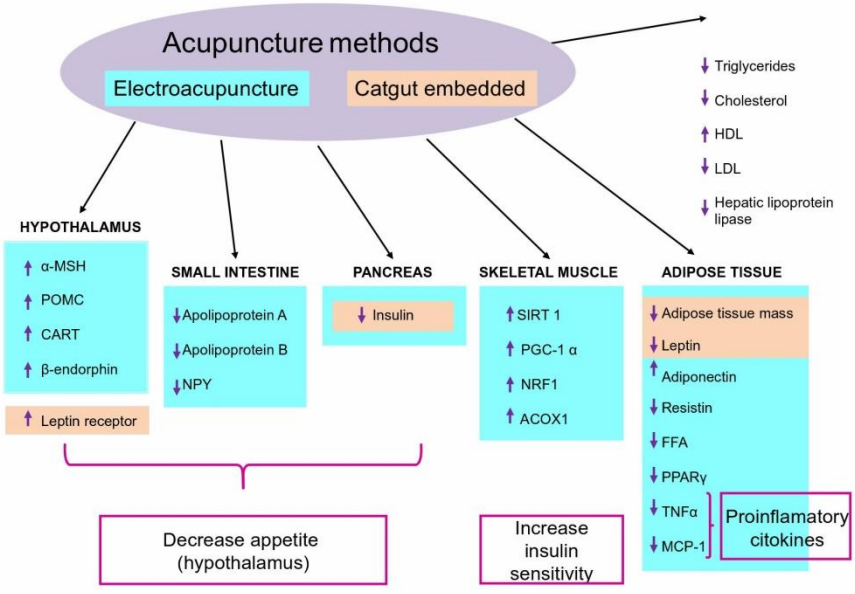


**Figure 1: Schematic representation of how the most efficient acupuncture methods for obesity treatment affect the expression of specifc molecules in different tissues and organs.** Because of they have effects on various systems, acupuncture methods (blue: electroacupuncture; green: catgut embedded) can reduce appetite, increase insulin sensitivity, reduce proinflamatorycitokines and regulate lipid metabolism. α-MSH: α-Melanocyte-stimulating hormone; POMC: Pro-opiomelanocortin ; CART: cocaine- and amphetamine-regulated transcript; SIRT 1: Sirtuin 1; PGC-1 α: Peroxisome proliferator-activated receptor gamma coactivator 1-α; NRF1: Nuclear respiratory factor 1; ACOX 1: Peroxisomal acyl-coenzyme A oxidase 1; HDL: High density lipoprotein; LDL: Low density lipoprotein; FFA: Free fatty acids; PPAR γ : peroxisome proliferator-activated receptor γ; TNF- α: Tumor necrosis factor α; MCP-1: Monocyte Chemoattractant Protein-1.

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results about leptin, TNFα and ghrelin concentrations [68]. Finally, Rahsepar et al. performed a randomized clinical trial to compare the effects of acupuncture *versus* auricular acupuncture in obese patients. Subjects received treatment in combination with low-calorie diet for six weeks (first period) followed by a low-calorie diet alone in the next six weeks (second period). The authors observed that both treatments significantly reduced anthropometric parameters and anti-Heat shock proteins (Hsp) antibodies in the first period. Interestingly, only patients treated with acupuncture showed anthropometric and lipid profile (excepted for HDL) changes in the second period, demonstrating the more sustained effects of acupuncture in comparison with auricular acupuncture. The authors concluded that although both auricular and acupuncture are effective to control body weight, dyslipidemia and immune system, acupuncture induced the most significant changes [69].

## Conclusion

Obesity is not only a nutrition disease that results from an imbalance between energy intakes *versus* calorie consumption; it is a complex metabolic illness that involves disequilibrium in various systems summarized in the psycho-neuro-endocrine-immune axis. As a global effect, the adipocyte far from being an allied cell becomes the enemy of the obese individual, which sometimes can lead to death. Emerging experimental evidence described above shows that acupuncture has multi-faceted effects in obese patients (Figure 1). Consistent with the clinical effects on body weight, acupuncture protocols are able to modify serum levels of leptin and adiponectin adipokines, as well as insulin, α-MSH, POMC, CART, SIRT 1, PPARγ, TNFα and MCP-1, among others. As a result, there is a modulation of various biochemical pathways, including metabolism, inflammation, sympathetic activity and defective insulin signaling pathways, unlike anti-obesity drugs usually limit their action to a specific pathway of body weight control. This clearly shows that acupuncture and its related techniques, in combination with the understanding of etiology, physiology and syndromatic differentiation of TCM offer an attractive alternative therapy for the treatment of obesity. These therapeutic methods do not only improve the state of the psycho-neuro-endocrine-immune axis mentioned above, they also contribute to increase the relationship between the different systems involved in body weight regulation, so that the adipocyte becomes again a friendly cell and not a time bomb. However, additional studies are required to fully understand the molecular basis acupuncture treatment for obesity. Because of the complexity of its physiopathology, obesity treatment requires the participation of a multidisciplinary team, which means experts in acupuncture working with researchers, to be able to correlate the effect of distinct acupuncture procedures on weight control with modifications of molecular mechanisms and factors involved in energy balance. Such studies will help to determine new strategies for more effective and safer control of this worldwide pandemic illness.

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# Capítulo 2

## Acupoint catgut embedding therapy with moxibustion reduces the risk of diabetes in obese women.

**Jessica M. Garcia-Vivas**, Carlos Galaviz-Hernandez, Flavia Becerril-Chavez, Francisco Lozano-Rodriguez, Absalom Zamorano-Carrillo, Cesar Lopez-Camarillo and Laurence A. Marchat. Journal of Research in Medical Sciences. 2014, 2014 Jul;19(7):610-6.

ORIGINAL ARTICLE

# Acupoint catgut embedding therapy with moxibustion reduces the risk of diabetes in obese women

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**Background:** Obesity is a major health problem worldwide for which conventional therapy efficacy is limited. Traditional Chinese medicine, particularly body acupoint stimulation, provides an alternative, effective, and safe therapy for this medical challenge. The present study was designed to compare the effects of distinct methods to stimulate the same set of acupoints, on anthropometric and biochemical parameters in obese women. **Materials and Methods:** Ninety-nine obese women were randomly assigned to six groups of treatment: Acupuncture with moxibustion, long needle acupuncture with moxibustion, electroacupuncture (EA), EA with moxibustion, embedded catgut with moxibustion (CGM) and sham acupuncture as control. Obesity-related parameters, including body weight, body mass index (BMI), waist and hip circumferences, waist/hip ratio, biochemical parameters (triglycerides, cholesterol, glucose, insulin) and homeostasis model of assessment - insulin resistance (HOMA-IR) index, were determined before and after each treatment. **Results:** Body weight and BMI were significantly reduced in response to all treatments. Interestingly, acupoint catgut embedding therapy combined with moxibustion was the only treatment that produced a significant reduction in body weight (3.1 ± 0.2 kg, $P < 0.001$), BMI (1.3 ± 0.1 kg/m$^2$, $P < 0.001$), insulin (3.5 ± 0.8 mcU/ml, $P < 0.1$) and HOMA-IR (1.4 ± 0.2 units, $P < 0.01$) in comparison with sham group. Furthermore, this treatment was able to bring back obese women to a state of insulin sensitivity, indicating that acupoint catgut embedding therapy combined with moxibustion could be useful as a complementary therapy to reduce the risk of diabetes associated to obesity in women. **Conclusion:** Overall, our results confirmed the effectiveness of acupoints stimulation to assist in the control of body weight in women. They also highlighted the more favorable effects of embedded catgut-moxibustion combination that may be due to the extended and consistent stimulation of acupoints.

**Key words:** Acupuncture therapy, complementary therapies, insulin resistance, moxibustion, obesity

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## INTRODUCTION

Obesity is a worldwide public health problem. In the United States, more than 30% of the adult population is obese;[1] in Europe similarly, adult obesity incidence is between 10% and 30%.[2,3] Due to the coexistence of malnutrition problems with an overconsumption of energy-dense diets and low physical activity, obesity has also become a serious disease concern in some developing countries, including Mexico.[4,5] Obesity is related to multiple metabolic abnormalities of the metabolic syndrome, which represent risk factors for cardiovascular diseases that are the leading causes of death worldwide.[6,7] Therefore, it is fundamental to implement health strategies to control obesity and its associated comorbidities.

Treatment of obesity requires a balanced diet and physical exercise. Pharmacological therapy and bariatric surgery represent an option in selected cases. In the last decade, many people have turned to complementary and alternative medicine to aid with weight loss. The traditional Chinese medicine (TCM) offers a complete medical system that has been used to diagnose, treat, and prevent illnesses for >2,000 years. One of the most commonly used therapies of TCM is acupuncture, which consists in stimulating specific points on the body (acupoints), by inserting thin metal needles into superficial structures such as skin, subcutaneous tissue, or muscles, in order to remove blockages in the flow of vital energy or life force called "qi" that circulates throughout the body through a system of pathways called channels.[8] Acupoint stimulation can also be performed by moxibustion that involves burning mugwort (moxa) near the skin,[9] or catgut embedding in which an implanted surgical chromic thread extend the stimulation for 15-18 days.[10]

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Several reports showed the beneficial effects of acupoint stimulation for obesity control. Auricular acupressure (i.e., stimulation of acupoints in or behind the ear) with a low-calorie diet produced a significant reduction of body fat mass and leptin levels in obese patients.[11] Weight loss in obese women treated with electroacupuncture (EA) (i.e., stimulation of acupoints by a small electric current) has been associated with changes in lipid profile, lipoproteins, leptin, insulin, glucose, neurotransmitters, as well as in inflammatory and immunological markers.[12-18] Acupoint catgut embedding therapy also produced a decrease in lipids, and a significant reduction of insulin and leptin resistance. Moreover, insulin sensitivity remained for a 1-year period, demonstrating the sustained effects of this method.[19,20] All these studies stimulate distinct sets of acupoints, and the number and duration of the sessions vary, which makes difficult to compare the efficiency of the different treatments. Here, we aimed to compare the effects of the stimulation of the same set of acupoints by distinct methods, namely, acupuncture, EA, moxibustion and catgut embedding, in obese women. Specifically, we evaluated changes in anthropometric and biochemical parameters related to obesity in response to each treatment.

## MATERIALS AND METHODS

### Subjects

The current study was designed as a randomized, placebo-controlled, clinical study. Patients were recruited at the Acupuncture Clinic of the National School of Medicine and Homeopathy of the National Polytechnic Institute in Mexico City, Mexico, according to the following eligibility criteria: Women between the ages of 18 and 45 with body mass index (BMI) ≥25 kg/m$^2$, without a known metabolic syndrome. Smokers or alcoholic participants, pregnant or breast-feeding women, and patients with previous acupuncture or drug treatment for obesity in the last 6 months, were excluded. Patients who failed to attend at least one session were eliminated. The Ethics Committee of the National School of Medicine and Homeopathy of the National Polytechnic Institute approved the protocol. The research was conducted in accordance with the last update of Helsinki declaration. All selected participants signed the informed consent.

### Treatments

Women ($n$ = 138) that were selected according to eligibility and exclusion criteria, were randomly assigned into six groups of treatment following an adaptive biased-coin randomization method, namely urn randomization: Acupuncture with moxibustion (AM), long needle acupuncture with moxibustion (LNAM), EA, EA with moxibustion (EAM), catgut embedding with moxibustion (CGM) and sham acupuncture (placebo) as control. The study was single blind, i. e. participants did not know if they were assigned to a treatment group or the placebo group. Acupuncture points were selected to cover the main syndromes related to obesity according to TCM as follows: CV6 (Qihai), CV12 (Zhongwan), and bilateral ST25 (Tianshu), ST36 (Zusanli) and SP6 (Sanyinjiao) for all groups, as well as BL20 (Pishu) and BL23 (Shenshu) for moxibustion stimulation. For the AM group, sterile stainless-steel acupuncture needles (Natural) (1.5 cun length at ST36 and SP6 acupoints; 3 cun length at CV6, CV12 and ST25 acupoints) were inserted to a depth of approximately 1-2 cm after skin sterilization. For LNAM protocol, 1.5 cun length needles were used to stimulate ST36 and SP6 acupoints, while 6 cun length needles were inserted at CV6, CV12 and ST25 acupoints to a depth of approximately 10-12 cm. BL20 and BL23 points were also stimulated with moxibustion in both AM and LNAM groups. For EA and EAM protocols, 3 cun acupuncture needles (at CV6, CV12 and ST25 acupoints), and 1.5 cun needles (at ST36 and SP6 acupoints) were connected to an acupuncture stimulator (KWD-808 li, GreatWall Brand) using dispersed-dense wave at 4 Hz high density frequency. The main advantage of EA is that the insertion of needles does not require extreme precision because the current delivered through the needle stimulates a larger area than the needle itself. In addition, BL20 and BL23 points were stimulated with moxibustion in the EAM group. For CGM protocol, a hypodermic needle (21 G × 32 mm) was used to introduce a chromic catgut strand 00 (Catgut acupuncture kit, Shuangyi) at CV6, CV12, ST25, ST36 and SP6 acupoints. The catgut thread was later completely absorbed by the body (about 18-21 days). BL20 and BL23 points were also stimulated with moxibustion. Finally, sham acupuncture involved the use of stainless-steel needles covered with a plastic film and a cap to avoid needle insertion, as previously described by Takakura and Yajima.[21] Subjects from AM, LNAM, EA, EAM and sham groups received two treatments per week for a total of 6-week (acupuncture for 20 min with or without moxibustion for 5 min in each session). For CGM group, catgut was implanted each 3-week, for a total of 6-week, whereas moxibustion was applied twice a week. Patients were asked to keep their usual lifestyle, including their diet and physical activity. These recommendations were reinforced at each session all over the protocol.

### Clinical and biochemical evaluation

A standard health questionnaire with a complete medical history was administered by a physician at the beginning of the protocol; age and anthropometric values were also assessed. Patients' body weight (kg) was measured in their underwear to the nearest 0.1 kg using a calibrated balance scale. Height (m) was measured to the nearest centimeter using a rigid stadiometer. BMI was calculated and expressed in kilogram per meter square following World Health Organization recommendations. Waist circumference (cm)

was measured midway between the lateral lower rib margin and the iliac crest, to the nearest 0.1 cm; hip circumference (cm) was measured at the level of major trochanters through the pubic symphysis; the waist-hip ratio (WHR) index was also determined. A venous blood sample was taken from the left cubital vein at 07:00 to 08:00 am after an overnight fast in order to determine total cholesterol, triglycerides glucose, and insulin concentrations using standard protocols. Normal values for cholesterol and triglycerides were defined as <150 mg/dl and <200 mg/dl, respectively. Normal values for glucose and insulin were defined as 80-110 mg/dl and 2-20 mcU/ml, respectively. Insulin resistance was determined by the homeostasis model of assessment - insulin resistance (HOMA-IR), using the following formula: HOMA-IR = (fasting insulin [mcU/ml] × fasting glucose [mg/dl])/405. A HOMA-IR index of 3.8 was chosen as the cut-off point to define insulin resistance.[22] At the end of the 6-week trial, anthropometric and biochemical data were obtained again for each patient.

### Statistical analyses

Prism 5 (GraphPad Software, Inc.) was used to conduct statistical analyses. Descriptive characteristics of the variables (weight, BMI, waist circumference, hip circumference, WHR, triglycerides, cholesterol, glucose, insulin and HOMA-IR index) were expressed as mean ± standard error of the mean. The paired Student's *t*-test was used to compare initial and final data in each group. ANOVA-Tukey's multiple comparison test was used to compare mean age, baseline anthropometric and biochemical data, as well as mean changes of each parameter between groups. The level of significance was set as $P \leq 0.05$.

## RESULTS

### Tolerance and general effects

All treatments were very well-tolerated, and none of the obese women suspended the therapy due to adverse side-effects. Most patients reported a reduction of appetite and anxiety; some of them also reported an increase in energy. Unfortunately, these data were not rigorously documented by the physician.

### Effects on anthropometric data

A total of 200 women that complied with all the eligibility criteria described above were enrolled into the study, but 62 were excluded. During the treatment period, several women were eliminated because they failed to attend at least one session and only 99 women completed the protocol [Figure 1]. Mean age was 35.9 ± 7.8 years, with the following distribution: 18-20 years, 3%; 21-25 years: 10%; 26-30 years, 10%; 31-35 years, 17%; 36-40 years, 17% and 41-45 years, 43%. Body weight range was 66.6-138.5 kg (87.06 ± 15.9 kg). Most patients (62/99) were classified as obesity I (30 < BMI ≤ 34.9 kg/m$^2$); the others presented obesity II (16/99: 35 < BMI ≤ 39.9 kg/m$^2$) and III (18/99; BMI ≥ 40 kg/m$^2$), and only three patients were overweight (25 < BMI ≤ 29.9 kg/m$^2$).

The cohort available for final statistical analysis included 22 patients in AM, 10 patients in LNAM, 10 patients in EA, 20 patients in EAM, 25 patients in CGM, 12 patients in sham group. Mean age and baseline anthropometric data are described in Table 1. Mean age, body weight, BMI, waist and hip circumferences, as well as WHR, were not significantly different among groups according to ANOVA test (data not shown). Mean BMI was between 33.4 ± 1.3 kg/m$^2$ and 38.6 ± 2.6 kg/m$^2$, and mean WHR was higher than 0.83 in all groups, confirming that women presented obesity I and II, with a cardiovascular risk.

At the end of the study, body weight and BMI were significantly reduced in all groups ($P < 0.001$), although final BMI values still corresponded to obesity I and II [Table 1]. A comparative analysis between groups revealed that body weight was significantly reduced in response to all treatments in comparison with the sham group, according to ANOVA-Tukey's test. Consequently, BMI was also significantly reduced in all groups when compared with sham group. In addition, body weight reduction was higher in LNAM group when compared with EAM group ($P < 0.01$); BMI reduction was higher in LNAM when compared with EAM ($P < 0.001$) and CGM ($P < 0.05$) groups, whereas BMI reduction was higher in EA group in comparison with EAM group ($P < 0.01$) [Figure 2]. In contrast, no changes were observed in waist circumference,

**Table 1: Initial and final values of anthropometric parameters in the different groups of treatment**

| Group | Age (years) | Weight (kg) | | BMI (kg/m$^2$) | | Waist circumference (cm) | | Hip circumference (cm) | | WHR | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Sham | 39.5±1.4 | 87.5±3.0 | 86.3±2.9*** | 35.7±1.2 | 35.2±1.2*** | 105.8±1.7 | 100.6±1.5 | 117.8±3.9 | 112.1±4.3 | 0.90±0.0 | 0.90±0.0 |
| AM | 38.6±1.0 | 88.6±2.4 | 86.0±2.4*** | 36.0±0.8 | 35.0±0.8*** | 110.1±3.3 | 105.3±3.1 | 121.0±2.6 | 117.4±2.5 | 0.90±0.0 | 0.89±0.0 |
| LNAM | 38.2±1.1 | 88.95±3.4 | 84.21±3.5*** | 33.4±1.3 | 31.41±1.3*** | 99.6±3.8 | 96.1±3.7 | 119±3.7 | 117±3.6 | 0.83±0.0 | 0.81±0.0 |
| EA | 36.4±1.3 | 91.8±7.8 | 87.7±7.7*** | 38.6±2.6 | 36.8±2.6*** | 107±4.7 | 103±4.2 | 119±6.3 | 117.6±6.4 | 0.90±0.0 | 0.88±0.0 |
| EAM | 39.2±1.2 | 86.7±3.4 | 83.9±3.5*** | 35.6±1.0 | 34.4±1.0*** | 100.3±5.4 | 97.5±5.5 | 119.3±6.9 | 116.7±6.8 | 0.84±0.0 | 0.83±0.0 |
| CGM | 35.7±1.8 | 84.9±2.5 | 82.3±2.5*** | 33.5±0.7 | 32.1±0.7*** | 104.0±2.0 | 100.4±1.8 | 117.3±2.1 | 113.4±2.0 | 0.88±0.0 | 0.88±0.0 |

Values are presented as mean ± SEM. ***$P < 0.001$ final versus initial in each group (paired Student's *t*-test). AM = Acupuncture with moxibustion; LNAM = Long needle acupuncture with moxibustion; EA = Electroacupuncture; EAM = Electroacupuncture with moxibustion; CGM = Catgut embedding with moxibustion; BMI = Body mass index; WHR = Waist/hip ratio; SEM = Standard error of the mean

hip circumference, or WHR in patients from all groups at the end of the protocol [Table 1].

### Effects on biochemical parameters

Mean baseline biochemical data are described in Table 2. Concentrations of triglycerides, cholesterol, glucose and insulin age, as well as HOMA-IR, were not significantly different among groups according to ANOVA test (data not shown). Patients in all groups have high triglycerides values, while cholesterol concentrations were in normal range (except in EA group). Glucose and insulin concentrations were in normal range, indicating that subjects were not diabetic. However, HOMA-IR values were equal or higher than 3.8 in sham, AM, LNAM, EA and CGM groups, showing that these obese women had insulin resistance at the beginning of the protocol.

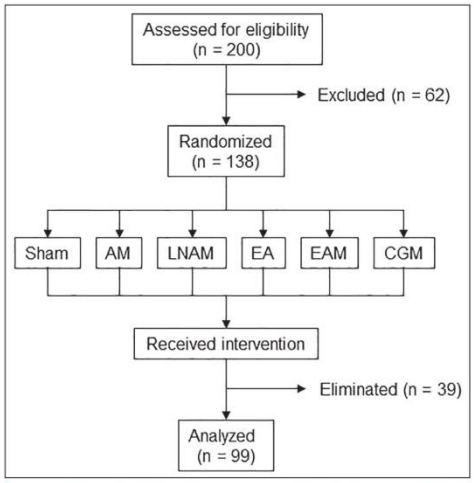


**Figure 1:** Study design. Participants were recruited at the Acupuncture Clinic of the National School of Medicine and Homeopathy of the National Polytechnic Institute in Mexico City, Mexico. Eligibility criteria were: Women between the ages of 18-45, with body mass index ≥25 kg/m$^2$, without a known metabolic syndrome. Smokers or alcoholic women, pregnant or breast feeding women, and women with previous acupuncture or drug treatment for obesity in the last 6 months, were excluded. The study was designed as a randomized, placebo-controlled and single blind study

At the end of the study, we observed a significant reduction of triglycerides in EA, EAM ($P < 0.05$) and CGM ($P < 0.01$) groups [Table 2]. But these changes were not significant in comparison with sham and other groups, when data were compared using ANOVA test (data not shown). In contrast, cholesterol was not affected [Table 2]. Additionally, AM, EAM, and CGM treatments led to a reduction of glucose ($P < 0.01$), but there was no significant difference with sham group according to ANOVA test (data not shown). Interestingly, insulin was reduced in response to CGM treatment ($P < 0.01$), while HOMA-IR was reduced in EA ($P < 0.01$) and CGM ($P < 0.001$) groups [Table 2]. Multiple comparison analysis revealed that changes in triglycerides and glucose were not significant in comparison with sham and other groups, when data were compared using ANOVA test (data not shown). In contrast, the reduction of insulin ($P < 0.05$) and HOMA-IR ($P < 0.01$) observed in

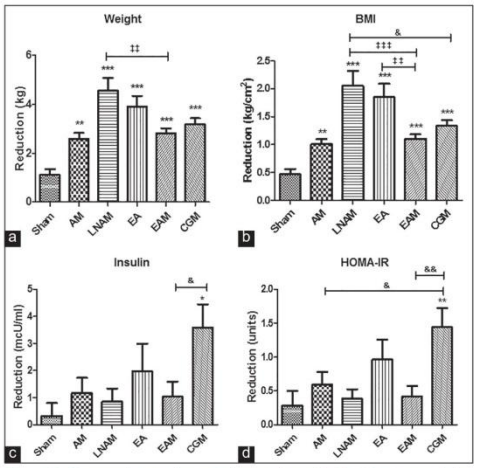


**Figure 2:** Comparative analyses of changes in weight (a), body mass index (b), insulin (c) and homeostasis model of assessment - insulin resistance (d) between groups of treatment. Data were compared using ANOVA-Tukey's multiple comparison test. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ versus sham. $^{\&\&}P < 0.01$, $^{\&}P < 0.05$ versus catgut embedding with moxibustion. $^{\ddagger\ddagger\ddagger}P < 0.001$, $^{\ddagger\ddagger}P < 0.01$ versus electroacupuncture with moxibustion

**Table 2: Initial and final values of biochemical parameters in the different groups of treatment**

| Group | Triglycerides (mg/dl) | | Cholesterol (mg/dl) | | Glucose (mg/dl) | | Insulin (mcU/ml) | | HOMA-IR | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Sham | 187.7±18.7 | 186.6±13.7 | 186.1±12.3 | 189.4±11.5 | 92.5±3.2 | 89.2±2.0 | 16.3±1.5 | 16.0±1.2 | 3.8±0.4 | 3.5±0.3 |
| AM | 177.6±12.8 | 174.8±10.6 | 184.2±8.0 | 189.5±7.3 | 95.1±1.2 | 91.5±1.2** | 16.8±1.1 | 15.2±1.0* | 4.0±0.3 | 3.5±0.2 |
| LNAM | 181.8±47.3 | 148.1±24.9 | 185.4±33.2 | 183.8±25.9 | 93.2±7.0 | 89.6±6.3 | 19.6±3.8 | 18.0±3.2 | 4.6±1.1 | 4.0±0.9 |
| EA | 184.1±49.2 | 146.1±50.0* | 254.8±22.8 | 237.7±13.5 | 100.8±7.5 | 100.6±4.5 | 19.4±2.3 | 14.3±1.3 | 4.7±2.0 | 4.2±1.8** |
| EAM | 186.8±15.1 | 168.5±9.9* | 193.6±8.9 | 195.0±10.0 | 91.9±1.3 | 86.9±1.4** | 15.2±1.1 | 14.2±1.0 | 3.4±0.2 | 3.0±0.2 |
| CGM | 173.9±16.4 | 143.2±11.1* | 194.9±8.2 | 191.0±7.8 | 99.9±3.5 | 89.6±1.8** | 16.1±1.3 | 13.2±1.2** | 4.2±0.4 | 2.9±0.3*** |

Values are presented as mean ± SEM. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ final versus initial in each group (paired Student's *t*-test). SEM = Standard error of the mean; HOMA-IR = Homeostasis model of assessment - insulin resistance; AM = acupuncture with moxibustion; LNAM = Long needle acupuncture with moxibustion; EA = Electroacupuncture; EAM = Electroacupuncture with moxibustion; CGM = Catgut embedding with moxibustion

CGM group was significant in comparison with sham group (ANOVA-Tukey's test) [Figure 2].

## DISCUSSION

In the last decade, there is an increasing interest for alternative medicine, such as the TCM, to help for obesity control, which represents a major public health problem worldwide. Notably, different acupuncture related methods involving the stimulation of body acupoints, have been reported as safe and efficient therapies to reduce body weight and associated parameters. The results of the present study confirm that 6-week treatments based on the stimulation of the same set of acupoints by AM, LNAM, EA, EAM, and embedded catgut with moxibustion (CGM) were able to significantly reduce body weight and BMI in obese women, as it has been previously reported by different groups. Moreover, our data validate that CV6, CV12, ST25, ST36, and SP6, as well as BL20 and BL23 acupuncture points, are relevant for regulating anthropometric parameters, whatever the method used for stimulation, probably because these acupoints cover most syndromes that are related to obesity according to TCM. Tang *et al*. also reported a decrease in body weight and BMI by stimulating almost the same set of acupoints through EA and catgut embedding therapy.[23] In addition, no adverse effects of acupuncture treatments were seen in the present study.

The fact that body weight and BMI were slightly reduced in sham group suggests a placebo effect, since patients claimed they did not modify their diet and physical activity. Importantly, these slight changes were not associated with changes in biochemical parameters in this control group. In contrast, in most groups of treatment, body weight and BMI reduction was associated with a significant modulation of biochemical parameters, mainly triglycerides, glucose, and insulin, but not cholesterol. Particularly, triglyceride reduction observed in patients from EA and EAM groups is in agreement with other reports that showed the effect of strong voltage (5-20 V) 30-40 Hz and dense-disperse wave EA to efficiently reduce triglycerides.[12,17,24] We did not find any reports about the modulation of biochemical parameters by EA associated with moxibustion, probably because these methods are not commonly used together. However, our results evidence that their combination could be an interesting complementary strategy to control body weight.

Interestingly, acupoint catgut embedding therapy with moxibustion was the only treatment that produced a significant reduction of triglycerides as previously reported,[20,25,26] with a decrease of glucose, insulin and HOMA-IR index. All treatments involved the stimulation of the same set of acupoints (CV6, CV12, ST25, ST36, and SP6, as well as BL20 and BL23), during the same number of weeks, which strongly suggests that the better effects observed in response to acupoint catgut embedding therapy with moxibustion were directly related to the method used to stimulate these acupoints. Interestingly, the reduction of HOMA-IR index from 4.2 ± 0.4 to normal values (2.9 ± 0.3) shows that obese women who were insulin resistant had become insulin sensitive at the end of the protocol. In contrast, HOMA-IR index was also significantly reduced in EA group, but women remained insulin resistant. Chen *et al*. also reported that embedded catgut allowed control of body weight, insulin and glucose levels, as well as insulin resistance in obese patients.[27] In obese rats, Yan *et al*. reported that catgut embedding allows control of insulin resistance. Moreover, results evidenced a decrease in both serum and hypothalamic levels of leptin, an adipokine that suppresses appetite and promotes energy metabolism.[20] Therefore, we propose that acupoint catgut embedding therapy with moxibustion may be used as a complementary treatment to reduce the risk of diabetes associated to obesity in women.

Although acupoint catgut embedding therapy can be used alone to control obesity, it seems that its combination with another TCM method enhances the effects. Indeed, Tang *et al*. reported that EA with catgut embedding produced a higher reduction in body weight, BMI, and WH index in comparison with EA alone.[23] Similarly, Shi *et al*. showed that EA together with catgut embedding and acupuncture-cupping significantly reduced body weight, BMI, and WHR in comparison with EA alone.[28] In the present work, we also demonstrate the better efficiency of catgut embedding with moxibustion for the control of anthropometric and biochemical parameters, as well as insulin resistance in obese women.

The better effects of acupoint catgut embedding therapy may be related to the persistent stimulation produced by the suture at the acupoints. It has been reported that the combined effects of proteolytic enzymes and macrophage action against the absorbable surgical thread may improve and extend the acupoint stimulation during 18-21 days as a consequence of the mild irritation in subcutaneous tissue.[20] It has also been shown that catgut embedding in specific acupoints can activate the satiety center and inhibit the hunger center through regulation of norepinephrine, dopamine, 5-hydroxytryptamine (serotonin) and 5-hydroxyindoleacetic acid in the feeding center in rats; this may contribute to enhance the effects observed on anthropometric and biochemical parameters.[29]

## CONCLUSION

Our comparative analysis confirmed the efficiency of acupoint stimulation to significantly reduce body weight,

BMI, and several biochemical parameters in obese women, without producing adverse side-effects. Most importantly, our results highlighted that acupoint catgut embedding therapy with moxibustion was also able to reverse insulin resistance, which indicates that acupoint catgut embedding therapy may represent an interesting health strategy to control obesity and its associated comorbidities. However, this study has some limitations, such as the small sample size of each group of treatment, the short duration of the treatment (only 6-week), the use of acupoints without considering the TCM syndromes of each patient, the lack of a systematic control of dietary and physical activity records, and the absence of an appropriate follow-up of possible body weight rebound. Further studies including larger sample sizes, the stimulation of acupoints according to TCM syndromes of each patients, and a longer protocol are needed to demonstrate that acupoint catgut embedding therapy with moxibustion is able to control body weight and insulin resistance in obese women.

## AUTHORS' CONTRIBUTIONS

JMGV collected and processed anthropometric and biochemical data, performed statistical analyses, participated in manuscript preparation. CGH provided assistance in design of the study, data processing and manuscript preparation. FBC provided assistance in recruitment and treatment of patients, data collection and manuscript preparation. FLR provided assistance in the design of the study, analyzed data, and participated in manuscript preparation. AZC provided assistance for data collection and statistical analyses, and participated in manuscript preparation. CLC provided assistance in the design of the study, and participated in manuscript preparation. LAM carried out the design of the study, coordinated the study and wrote the manuscript. All authors have read and approved the content of the final version. In addition, they all agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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# Capítulo 3

**Transcriptomic profiling of adipose tissue in obese women in response to moxibustion-ACET.**

Jessica M. Garcia-Vivas, Carlos Galaviz-Hernandez, Jorge Fernandez-Retana, Abraham Pedroza-Torres, Carlos Pérez-Plasencia, Cesar Lopez-Camarillo, Laurence A. Marchat. Obesity (en preparación)

**Transcriptomic profiling of adipose tissue in obese women in response to ACET-moxibustion.**

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**Keywords:** alternative medecine; adipokines; adipose tissue; gene expression; obesity.

**Running title:** Acupuncture and gene expression in fat.

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**“What is already known about this subject”**

- Obesity is associated to an increased fat mass.
- Adipokines involved in body weight control are deregulated in obesity.
- Acupuncture catgut embedding therapy (ACET) with moxibustion was able to reduce body weight and revert insulin resistance in obese women.

**“What this study adds”**

- ACET-moxibustion does not modify circulating adipokines levels.
- The effects of ACET-moxibustion are associated to the regulation of biochemical events that are altered in obesity.
- Modulated genes can explain the positive effects of ACET-moxibustion in obesity.

## ABSTRACT

**Objective:** Obesity is a chronic and mild systemic inflammatory condition that is characterized by an increased fat mass and deregulation of adipokines mainly involved in the regulation of homeostasis, feeding, thermogenesis, inflammation and insulin resistance. Complementary and alternative medicine represent an effective therapeutic option to aid with weight loss. We reported that acupuncture catgut embedding therapy (ACET) with moxibustion was able to reduce body weight and revert insulin resistance in obese women. Here, we aimed to evidence changes in adipokines and gene expression in adipose tissue that could explain the effects of ACET-moxibustion.

**Design and methods:** Obese women were treated with ACET-moxibustion or sham acupuncture as control. Circulating leptin, adiponectin, TNF-α and resistin were quantified by ELISA. Gene expression in adipose tissue was determined by cDNA microarray assays and assessed by qRT-PCR.

**Results:** ACET with moxibustion did not modify circulating adipokines levels, however correlations with anthropometric and biochemical parameters were affected. Interestingly, transcriptional changes in adipose tissue revealed the modulation of genes participating in homeostasis control, lipid metabolism, olfactory transduction, and gamma-aminobutyric acid signaling pathway.

**Conclusions:** The effects of ACET-moxibustion on body weight and insulin resistance are associated with the regulation of biochemical events that are altered in obesity.

## INTRODUCTION

Obesity is a major public health challenge, affecting 35% of the world population. It is an important risk factor for diabetes and cardiovascular diseases that are leading causes of death worldwide (1). It is a chronic and mild systemic inflammatory condition that is characterized by a disequilibrium between energy intake and expenditure, resulting in an increased fat mass (2). Adipose tissue is composed of different cell types that secrete pro- and anti-inflammatory adipokines, which control multiple physiological and pathological processes. Leptin regulates the production of orexigenic and anorexigenic neuropeptides in hypothalamus to suppress appetite and promote energy expenditure. It also modulates inflammatory response, increasing the expression of pro-inflammatory cytokines, including TNF-α produced by adipocytes and adipose tissue-resident macrophages (3). TNF-α affects insulin signaling by inhibition of insulin receptor tyrosine kinase activity. It also alters adipocyte differentiation, lipid metabolism, and adiponectin secretion, indirectly promoting the development of insulin resistance (4). Adiponectin increases fatty acid oxidation and glucose uptake in muscles and inhibits gluconeogenesis in the liver, improving peripheral insulin sensitivity. Adiponectin also has anti-inflammatory effects inhibiting TNF-α secretion (5). Resistin, predominantly expressed by macrophages, is associated to insulin resistance and TNF-α secretion (6), indicating that it may have an indirect effect on insulin resistance through exacerbating inflammation.

The control of obesity requires a multidisciplinary approach, which includes a balanced diet, physical activity, pharmaceutical regimen and bariatric surgery in selected cases. Complementary and alternative medicine represents an attractive option for weight control. Several reports described the beneficial effects of the Traditional Chinese Medicine, particularly the stimulation of specific body points (acupoints) by acupuncture and related methods, to reduce body weight, and body mass index (BMI) through the regulation of lipids, lipoproteins, hormones, adipokines, neurotransmitters, glucose metabolism, and inflammatory markers (7, 8). A recent review evidenced that acupuncture catgut embedding therapy (ACET),

which involves embedded surgical chromic catgut sutures into the subcutaneous tissue, has the best clinical efficacy in obesity (9). However, little is known about its effects on adipokines synthesis and gene expression in fat. Previously, we showed that ACET with moxibustion was efficient to reduce BMI, and revert insulin resistance, which could help to control diabetes risk in obese women (10). In order to gain insights about the molecular events underlying these effects, here we evaluated changes in circulating adipokines and transcriptome profile of adipose tissue in response to ACET-moxibustion. Results revealed the modulation of genes participating in biochemical pathways that are altered in obesity, which could contribute to the effects of ACET-moxibustion on body weight and insulin resistance in obese women.

## METHODS AND PROCEDURES

### Subjects and treatments

Women included in the study participated in a weight reduction program by different acupuncture methods, including ACET-moxibustion (10). Briefly, overweight/obese women between the ages of 18-45, were recruited at the Acupuncture Clinic of the National School of Medicine and Homeopathy of the National Polytechnic Institute (ENMH-IPN), Mexico City, Mexico. Pregnant or breast feeding women, smoking and alcoholic women, subjects with a known metabolic disease, and previous acupuncture or drug anti-obesity treatment were excluded. For ACET-moxibustion (25 women), a chromic catgut strand 00 was introduced at CV6, CV12, ST25, ST36 and SP6 acupoints; BL20 and BL23 points were stimulated with moxibustion. Catgut was implanted each three weeks; moxibustion was applied twice a week for 5 minutes, for a total of six weeks. Sham treatment (12 women) was performed as described. All participants were asked to keep their usual lifestyle, including diet and physical activity. The research was according to the Helsinki declaration and the Ethics Committee of the ENMH-IPN approved the protocol. Informed consent was signed by all participants (10).

## Biological samples

At the beginning and end of the protocol, a venous blood sample was taken from the left cubital vein after an overnight fast, using a vacutainer blood collection tube without any anticoagulant. Serums were obtained by centrifugation at 3000 rpm for 15 min at 4°C and stored at -20°C. Abdominal subcutaneous adipose tissue (SAT) biopsies were also obtained. After local disinfection with alcohol and under anesthesia with lidocaine containing 2% epinephrine, a small incision (<1 cm) was performed in abdomen with a sterile scalpel. A SAT sample was taken with sterile forceps and kept in Allprotect Tissue Reagent (Qiagen) at -70°C.

## Adipokines determination

Circulating adipokines were determined by Enzyme-Linked ImmunoSorbent Assays (ELISA) using the Human Leptin ELISA kit (Millipore), Human Adiponectin ELISA kit (Millipore), Human Resistin ELISA kit (Millipore) and HumanTNF-α HD ELISA kit (Quantikine). Adipokines concentrations were expressed as mean ± standard error of the mean (SEM). Normal values for leptin, adiponectin and TNF-α were defined as 1-15 ng/ml, 5-20 µg/ml and < 0.7 pg/ml, respectively (11). A resistin concentration >10 ng/ml was considered as high (12).

## Statistical analysis

Initial and final data in each group were compared by the paired Student´s t-test . Correlations between adipokines in each group, between adipokines and anthropometric and biochemical parameters previously reported (10) were evaluated by Pearson analysis. Significance was set as $p \leq 0.05$.

**Microarray assay**

Twelve SAT biopsies were obtained from six randomly selected women, before and after treatment with ACET-moxibustion. The biopsies were homogenized by freezing in liquid nitrogen and manual crushing. Total RNA was extracted using the RNeasy Mini Kit (Qiagen). RNA purity and concentration were determined in a NanoDrop 1000 spectrophotometer (ThermoScientific). RNA integrity was assessed by electrophoresis. dsRNA was obtained from 50 ng RNA using the Complete Whole Transcriptome Amplification Kit (WTA2, Sigma) following manufacturer instructions. Products were purified using the QiAquick PCR purification kit (Qiagen), labeled with Cy3 using NimbleGen One-Color DNA Labeling Kit (RocheNimbleGen) and quantified by spectrophotometry. Labeled cDNA samples were individually hybridized with a NimbleGen microarray 12x135K for 18 h at 42°C according to manufacturer recommendations. This array represents ~45,035 genes (including controls) which were captured by three probes per gene. After hybridization, the array was washed using NimbleGen Wash Buffer kit (Roche) and scanned using NimbleGen MS 200 Microarray Scanner (Roche).

**Data extraction and analysis**

Data were extracted from scanned images using NimbleScan 2.6 software and aligned with microarray archive to perform array normalization using the quantile normalization method (13). Expression value of each gene was obtained using normalized expression values for the corresponding individual probes through the Robust Multichip-Array procedure and data were analyzed based on the RMA processed expression values (14). All files were exported to Microsoft Excel and processed using the ANAIS software (Analysis of NimbleGen Arrays Interface Suite, http://anais.versailles.inra.fr) . For each gene, fold change (FC) was calculated by log2 transformation of the ratio between normalized signal intensity of final and initial sample, from data of all patients. Data of genes with FC≥1.5

were exported to the Genesis application server (15) to identify significantly modulated genes across patients (FC≥1.5, adjusted p≤0.05 in One-Way ANOVA). In addition, heat map was constructed to show the hierarchical clustering of selected modulated genes (p≤0.01 and FC≥1.5) across samples.

**Bioinformatics analysis**

Significantly modulated genes were functionally clustered according to DAVID v6.7 (http://david.abcc.ncifcrf.gov/) and biological pathways were identified through KEGG database. Selected genes were analyzed by GeneCards® (www.genecards.org/) and STRING databases to retrieve direct (physical) and indirect (functional) associations among corresponding proteins.

**qRT-PCR**

Quantitative RT-PCR assay was performed for several for several genes that were selected based on the following criteria: their constant up- or downregulation in the six patients, their high fold change value and their participation in relevant pathways. The beta-actin was used as housekeeping gene. Specific oligonucleotides for selected modulated genes were designed by Prime 3 (http://primer3.ut.ee) and IDT tools (www.idtdna.com/pages/scitools) softwares (Table 1). Total RNA obtained from SAT biopsies was reverse transcribed using SuperScript III First-Strand Synthesis System (Invitrogen). Briefly, RNA (5 µg) was incubated with 50 µM oligo$(dT)_{20}$ at 65°C for 5 min. Then, cDNA Synthesis Mix was added and incubated for 50 min at 50°C followed by 5 min at 85°C. Finally, RNase H (1 µL) was added and the reaction was incubated for 20 min at 37°C. For each gene, cDNA (100 ng) was mixed with primer pairs (5 pg/µl) and qRT-PCR was performed using 5 µl LightCycler® FastStart DNA Master SYBR Green I (Roche) and the Ligth Cycler 480 DNA Real-time PCR system (Roche). Relative mRNA expression of each gene was determined by after normalization to beta-actin levels

using the ΔΔCT method.

## RESULTS

### ACET-moxibustion affects correlations between circulating adipokines and anthropometric/biochemical parameters

A total of 37 obese women were randomly assigned into ACET-moxibustion and sham groups. Mean age, body weight, BMI, waist and hip circumferences, as well as waist/hip ratio, were not significantly different among groups (10). As expected for obese people, circulating levels of leptin were high, while adiponectin levels were low. Resistin levels appeared high, while TNF-α levels were in the normal range. At the end of the protocol, we did not observe any significant changes in leptin, TNF-α and resistin levels in both groups; adiponectin was slightly reduced in CGM group (Table 2).

We previously reported that moxibustion-ACET was able to significantly reduce weight, BMI, triglycerides, glucose, insulin, and HOMA-IR index in obese women (10). Here, we observe that leptin was positively correlated with BMI ($p<0.05$), insulin ($p<0.01$) and HOMA-IR ($p<0.05$); adiponectin was negatively correlated with triglycerides and resistin ($p<0.05$), while TNF-α was positively correlated with triglycerides ($p<0.05$), at the beginning of the protocol. After moxibustion-ACET, leptin was negatively correlated with insulin, HOMA-IR and adiponectin ($p<0.05$); adiponectin remained negatively correlated with triglycerides ($p<0.01$) and resistin ($p<0.05$), while TNF-α was positively correlated with body weight ($p<0.05$) and BMI ($p<0.01$). We also observed a negative correlation between leptin and adiponectin (Figura 1).

### ACET-moxibustion modifies transcriptional profile in SAT

To identify genes that could contribute to anthropometric and biochemical changes

in response to ACET-moxibustion, we analyzed the global transcriptional response of SAT by cDNA microarrays in six women as described in Methods. Of the 45,035 genes contained in the chip, only 8,025 genes (17.84%) were expressed in SAT and 1,791 genes (3.97%) were modulated after intervention. Of these, 1,548 (86.43 %) were significantly upregulated and 243 (13.57%) were down-regulated (Full lists of up- and down-modulated genes are in Supplementary Table 1). Heat map clearly evidenced the hierarchical clustering of selected modulated genes ($p \leq 0.01$ and $FC \geq 1.5$) across samples, which illustrates the transcriptional response to ACET with moxibustion in SAT.

**Biological processes associated with differentially expressed genes in response to ACET-moxibustion**

To identify the biological response to ACET-moxibustion in SAT, significantly modulated genes was classified according to Gene Ontology categories by DAVID Bioinformatic Database. Biological functions include among others, biological regulation, multicellular organismal process and regulation of metabolic process. According to cellular component analysis, modulated genes are classified as intrinsic to membrane, integral to membrane and plasma membrane, among others. Finally, molecular functions correspond mainly to ion binding, DNA binding and transcription factor activity, among others (**Figure 3**). KEGG pathway classification revealed that 22 genes participate in cytokine-cytokine receptor interaction, 18 genes correspond to calcium signaling and 15 to jak-stat signaling. Other affected pathways are related to cardiac function (dilated cardiomyopathy, hypertrophic cardiomyopathy, cardiac muscle contraction and arrhythmogenic right ventricular), among others (**Table 3**). Interestingly, functional clustering revealed that modulated genes are involved in processes that are altered in obesity, such as homeostasis control (regulation of response to nutrients, homeostatic process, insulin secretion) and lipid metabolism (regulation of fatty acid metabolic process, lipase activity, regulation of lipase activity, diacylglycerol binding, phospholipase C activity and adipocytokine signaling pathway). Another representative functional

theme is olfactory transduction, with the modulation of 44 G protein-coupled receptors (GPCR), rhodosin-like superfamily genes, including 27 olfactory receptor (OR) genes, as well as genes participating in the regulation of adenylate cyclase activity in protein-G signaling and related to cAMP metabolic process. Other modulated genes belong to the gamma-aminobutyric acid signaling pathway (**Table 4**).

## DISCUSSION

We demonstrate for the first time -until we know- that ACET-moxibustion, a chinese traditional treatment, is useful to reduce body weight and insulin resistance (10) through changes on gene expression in adipose tissue of obese women. Surprisingly, these effects are not directly associated with changes in circulating adipokines, neither in adipokine gene expression in SAT. On the contrary, Chen et al. (2007) (16) showed that ACET is associated with reduced TNF-α levels. This could be due to differences in acupoint selection and stimulation conditions. Indeed, we observed changes in correlations between several adipokines and anthropometric and biochemical parameters, which suggests that ACET with moxibustion modulates, at least partially, physiological and pathological processes related to energy intake and expenditure in SAT.

Obesity is characterized by an excess of adipose tissue and a marked adipocyte dysfunction. White adipose tissue is not only an energy reserve in the form of triglycerides; it is also an active organ for whole body energy homeostasis. Therefore, to gain insights into the molecular mechanisms underlying the effects of ACET-moxibustion on anthropometric and biochemical parameters, we examined the gene expression profile in SAT of randomly selected women. Clinical data of these women were essentially the same as in the overall CGM group (data not shown). ACET-moxibustion significantly affected the expression of a limited number of genes (3.97%) in SAT. Similarly, 2.5% and 4.09% of genes were modulated in SAT after a 10-weeks diet (17) and a 6-month exercise intervention (18), respectively. However, our protocol has a shorter duration (only six weeks).

The higher impact of ACET-moxibustion on gene expression may be related to the persistent stimulation produced by the suture at acupoints. The combined effects of proteolytic enzymes and macrophage action against the absorbable surgical thread causes a mild irritation in SAT, which may improve and extend acupoint stimulation (19). In addition, we cannot discard that a long-term ACET-moxibustion could lead to even more marked effects.

A relevant finding of our study is that ACET-moxibustion is associated with the regulation of biochemical pathways that are altered in obesity. Thus, several modulated genes participate in homeostasis control, including response to nutrient levels, homeostatic processes and insulin secretion. NPC1 (Niemann-Pick C1) controls intracellular cholesterol homeostasis by regulating the transport of lipoprotein-derived lipids (cholesterol and fatty acids) from late endosomes/lysosomes to other cellular compartments (20). Genetic studies revealed a relation between defects in NPC1 gene and obesity; in addition, NPC1 mRNA levels are increased in fat depots in obese individuals, while they are reduced during weight loss (21). Similarly, NPC1 expression was reduced in SAT of obese women after body weight reduction following ACET-moxibustion, which confirmed the metabolic function of NPC1 in adipose tissue. GHRL (Ghrelin) is involved in the hypothalamic regulation of energy homeostasis, stimulating orexigenic NPY and AgRP neuronal activity (22). It is predominantly synthesized in the stomach, but ghrelin mRNA and peptide have been detected in almost all tissues, including SAT. Circulating and tissue levels of ghrelin drop in diet-induced obesity (23), which is consistent with our results. In addition, recent evidence highlights an important role of ghrelin in glucose homeostasis, inhibiting insulin release from the pancreas and promoting hyperglycemia (24). Accordingly, the reduction of ghrelin in SAT in response to ACET-moxibustion is associated to a reduction of both insulin and glucose levels (10). Peroxisome proliferator-activated receptor alpha (PPARA) regulates numerous genes of energy metabolism. A recent work revealed that PPARA mRNA levels were decreased by both genetic and HFD-induced obesity in white adipose tissue, while fasting increased them.

PPARA activation promotes both adipocyte differentiation and fatty acids oxidation, contributing to the enhancement of whole-body oxygen consumption and suppression of adipocyte hypertrophy (25). Moreover, PPARA activation prevents inflammation in white adipose tissue and enhances the action of adiponectin by increasing adiponectin and adiponectin receptors, which results in the amelioration of obesity-induced insulin resistance (26). Here we found that PPARA was downregulated in SAT, suggesting that it probably does not contribute to the improvement of insulin sensibility after ACET-moxibustion. In fact, this event could be more related to the overexpression of PPARGC1A (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), CACNA1C (Calcium channel, voltage-dependent, L type, alpha 1C subunit), SLC30A8 (Solute carrier family 30; zinc transporter, member 8), NEUROD1 (Neurogenic differentiation 1), and PDE8B (Phosphodiesterase-8B), together with the downregulation of UCN3 (Urocortin 3). CACNA1 is activated in the first phase of insulin secretion (27). NEUROD1 controls insulin expression and β-cell survival (28). SLC30A8 is important for insulin secretion, the regulation of hepatic insulin clearance and the delivery of insulin to peripheral target tissues (29). Diminished PDEB8 potentiates biphasic insulin response to glucose (30). UCN3 is induced in pancreas under excessive caloric conditions and acts locally to increase insulin production, which may contribute to reduced insulin sensitivity and harmful metabolic consequences (31). Moreover, PPARGC1A expression is reduced in adipose tissue in insulin resistance (32). Therefore, the modulation of CACNA1C, SLC30A8, NEUROD1, PDE8B, PPARGC1A, and UCN3, could contribute to the control of insulin resistance by ACET-moxibustion in obese women.

Another interesting event that is affected in response to ACET-moxibustion is lipid metabolism, though the overexpression of PPARGC1A, PRKAA2 (5´-AMP-activated protein kinase catalytic subunit α2), and ACSL3 (Long chain acyl-CoA synthetase-3). A decreased expression of PRKAA2 has been associated with increased body weight and fat mass in high-fat diet mice, as well as impaired insulin sensitivity (33). Similarly, a reduced PPARGC1A expression in adipose

tissue has been associated with obesity, fat mass accumulation and insulin resistance (34). In liver tissue, the activation of ACSL3 leads to a reduction of cholesterol and triglycerides (35). Accordingly with these reports, the upregulation of PRKAA2, PPARGC1A and ACSL3 after ACET-moxibustion, could regulate the perturbed fatty acid metabolism, which is an underlying contributor to the development of obesity. This could also help in the control of insulin resistance in these patients (10). ACET-moxibustion was also associated to activation of phospholipases c (PLCB4, PLCXD3, PLCZ1 and PLCB1) that hydrolyze phospholipids into fatty acids and other lipophilic compounds, playing an essential role in signal transduction pathways through the release of diacyl glycerol (DAG) and the second messenger inositol triphosphate. DAG can also be phosphorylated to form the lipid second messenger phosphatidic acid (PA). Notably, disturbances in DAG/PA balance may impair cellular metabolism, including insulin sensitivity (36). Although the direct role of diacylglycerol kinase (DGK) isoforms in the development of insulin sensitivity and diabetes is not well known, it seems that DGKd contributes to hyperglycemia-induced peripheral insulin resistance (37). Therefore, it is possible that the activation of DGK in response to ACET-moxibustion could contribute to the control of insulin resistance through the inhibition of DAG accumulation. Interestingly, the activation of GPCR by ligand binding stimulates adenylate cyclase and intercellular levels of cAMP rise. Interestingly, alterations in adipocyte adenylate cyclase regulation inhibit lipolysis, promoting obesity (38). Congruently, body weight reduction after ACET-moxibustion is associated with the stimulation of non-odorant GPCR, such as CHRM5 and CHRM2 (Muscarinic acetylcholine receptors $M_5$ and $M_2$), HTR7 (5-$HT_7$ receptor) and TSHR (thyroid-stimulating hormone receptor). HTR7 and TSHR are expressed in various tissues, including fat. In periphery, 5-HTRs are important for serotonin functions in glucose and lipid metabolism. On the other hand, a deregulated expression of TSHR in adipose tissue is associated with obesity, as a result of an increased adipogenesis (39). Therefore, we hypothesize that the stimulation of non-odorant GPCRs may promote lipolysis, contributing to body weight reduction in response to ACET-moxibustion.

On the other hand, the transcriptional response to ACET-moxibustion evidences the activation of the olfatory transduction pathway with the overexpression of odorant GPCRs that are represented by 27 olfactory receptors (OR). Although most components of olfactory-like chemosensory signaling are expressed in the olfactory epithelium, they are also present in non-chemosensory tissues, although their physiological functions remain almost unknown (40). Recently, the expression of several OR in SAT and muscles has been related to the progression of obesity (41). In contrast, here we observed that OR activation in SAT is associated with body weight reduction in response to ACET-moxibustion. Further analyses are required to understand the relevance of OR in obesity.

Finally, our results evidenced the activation of the gamma-aminobutyric acid signaling pathway mediated by the upregulation of five subunits of GABR (Gamma-aminobutyric acid receptor subunit) whose endogenous ligand is γ-aminobutyric acid (GABA). GABR are expressed in neuronal and non-neuronal tissues, included SAT (42). A recent study revealed that oral administration of GABA inhibited the high fat diet-induced obesity and improved glucose intolerance and insulin sensitivity, even after the establishment of obesity and diabetes in mice. Furthermore, it reduced adipocyte hypertrophy and adipose tissue mass (43). Therefore, the activation of peripheral GABA receptors by ACET-moxibustion, such as GABA does, could contribute to signaling pathways activation that result in body weight and insulin resistance reduction.

Taken altogether, our results indicate that the effects of ACET-moxibustion treatment on body weight and insulin resistance in obese women do not involve changes in adipokines expression. Importantly, transcriptome profiling of SAT revealed the modulation of genes involved in biochemical events that are altered in obesity, thus leading to the improvement of anthropometric and biochemical parameters in obese women. However, further experiments are required to elucidate the exact molecular mechanisms of ACET-moxibustion for obesity control.

## CONFLICTS OF INTEREST STATEMENTS

The authors declare no conflicts of interest.

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**Author contributions:** LAM, CGH and CLC conceived the study. Data was collected by JMGV, JFR and CPP, analyzed by JMGV, JFR, CPP, and CGH, and interpreted by MGV, JFR, CPP, CGH, CLC, APT and LAM. Literature searches were performed by JGMV, CGH, CLC, and LAM. Figures were generated by JMGV, JFR, CPC, CLC, APT and LAM. JMGV, CGH and LAM were involved in writing the article and all authors have read and approved the submitted version.

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## FIGURE LEGENDS

**Figure 1. Correlations between adipokines circulating levels and anthropometric/biochemical parameters.** (A-C) Correlation between leptin and BMI (A), insulin (B) and HOMA-IR index (C). (D-F) Correlation between adiponectin and triglycerides (D), leptin (E) and resistin (F); (G-I), Correlation between TNF-α and triglycerides (G), body weight (H) and BMI (I). The curves in the graphs illustrate the correlation at the beginning (closed circles) and at the end (open circles) of the protocol. r, Pearson's correlation coefficient; p, p value.

**Figure 2. Transcriptional response to ACET-moxibustion in SAT. Gene expression in SAT.** A) Heat map showing the hierarchical clustering of selected modulated genes ($p \leq 0.01$ and $FC \geq 1.5$) across samples of six randomly selected obese women.

**Figure 3. Classification of modulated genes according to Gene Ontology categories by DAVID.** A) Biological functions. B) Cellular components. C) Molecular functions.

## TABLE LEGENDS

**Table 1.** Genes and primer sequences used in qPCR assays.

**Table 2.** Changes in adipokines levels in response to ACET-moxibustion of obese women.

**Table 3.** KEGG pathways associated to genes modulated in response to ACET-moxibustion treatment.

**Table 4.** Functional clustering of genes modulated in response to ACET-moxibustion.

## SUPPORTING INFORMATION

**Table S1.** Full list of up- and down-regulated genes (FC > 1.5; $p < 0.05$) in subcutaneous adipose tissue of obese women after ACET-moxibustion treatment.

**Figure 1 .** Correlations between adipokines circulating levels and anthropometric/biochemical parameters.

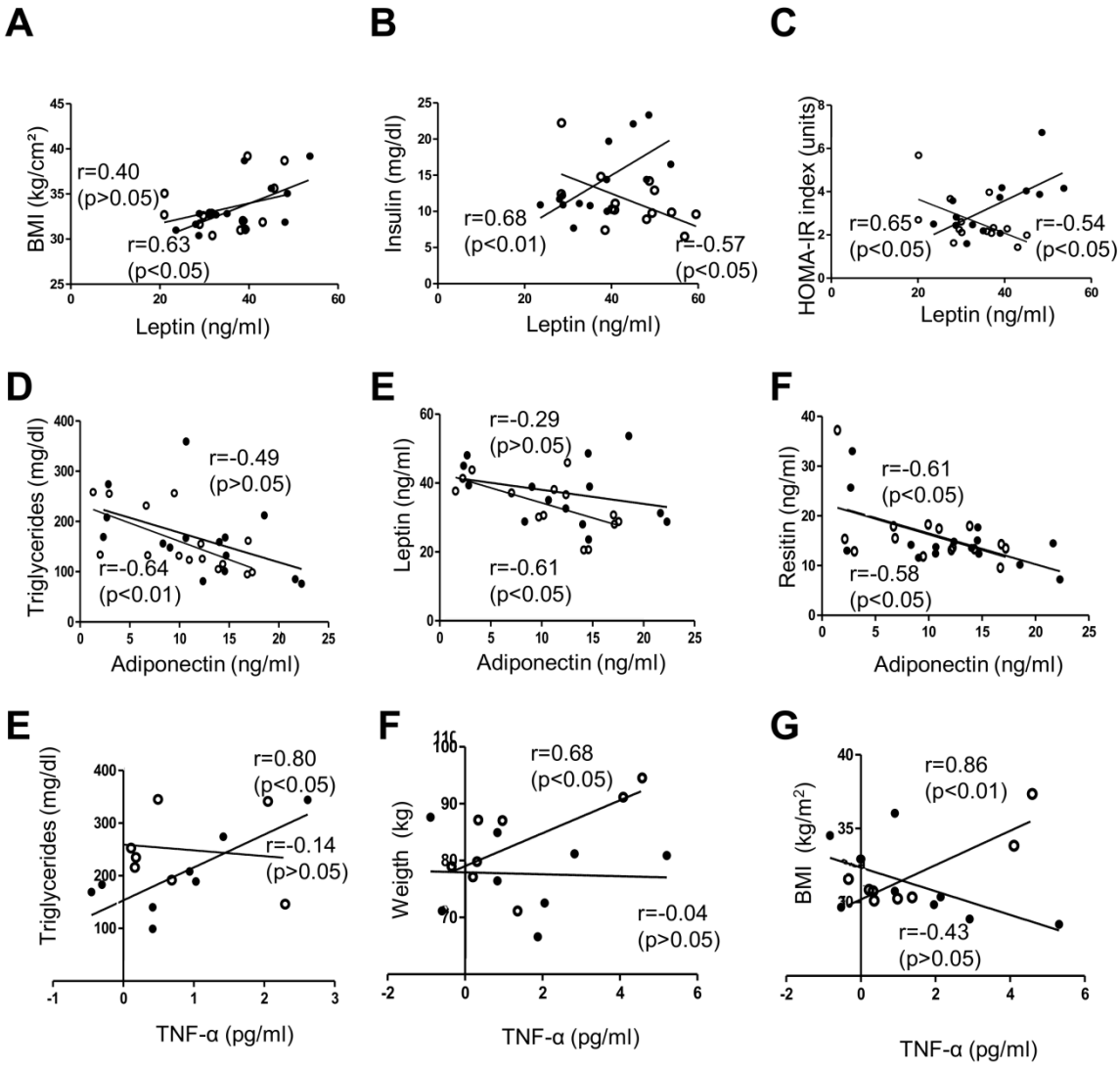

**Figure 2.** Transcriptional response to ACET-moxibustion in SAT. Gene expression in SAT. Heat map showing the hierarchical clustering of selected modulated genes (p≤0.01 and FC≥1.5) across samples of six randomly selected obese women

A

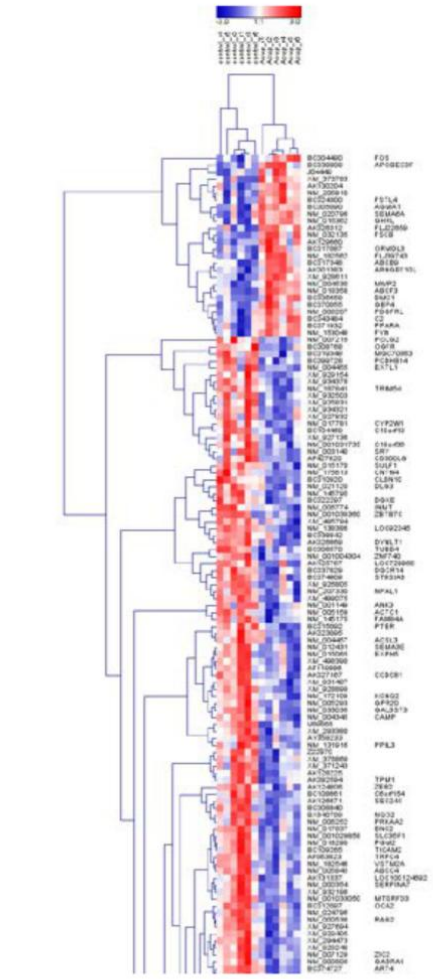

**Figure 3.** Classification of modulated genes according to Gene Ontology categories by DAVID.

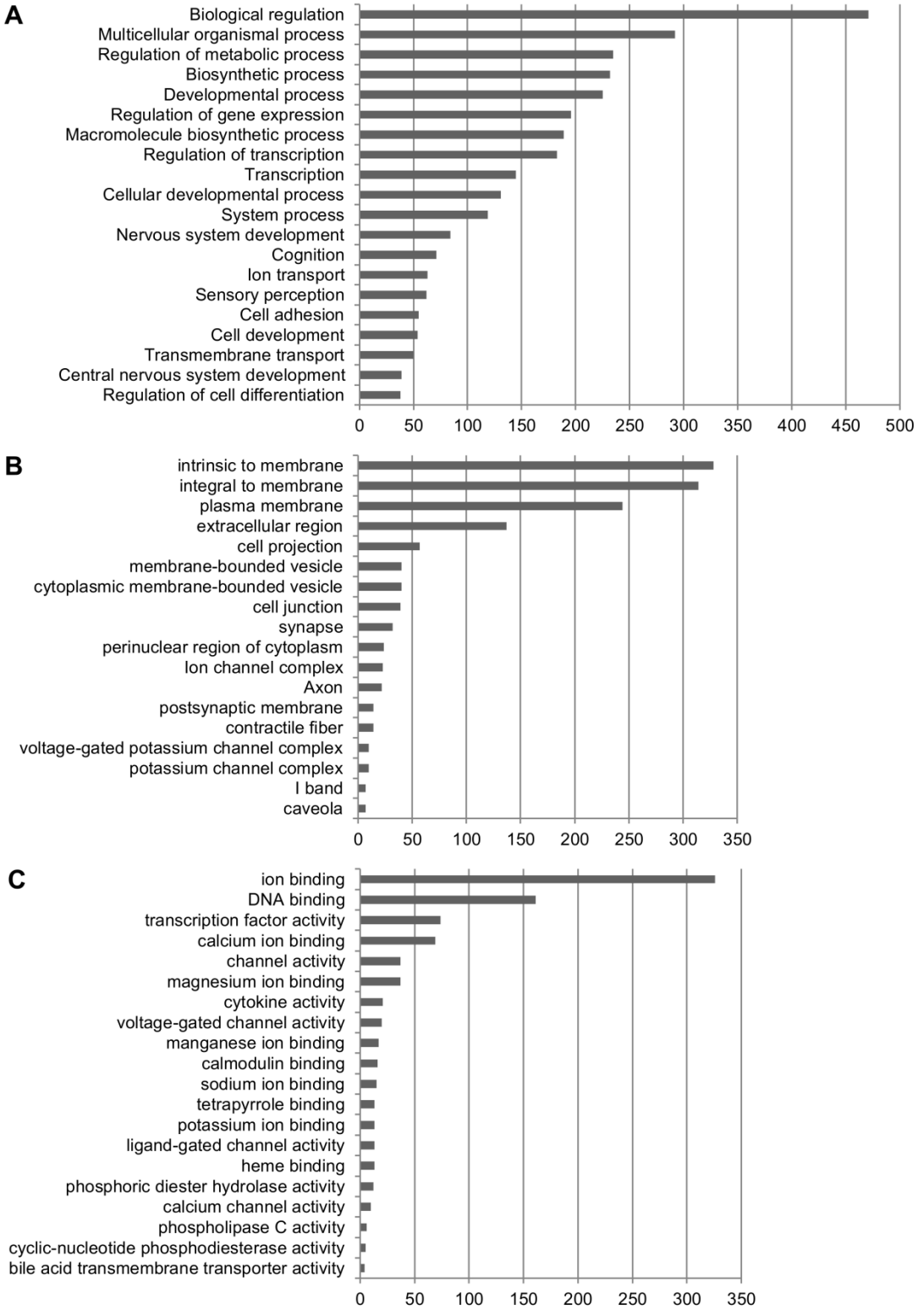

**Table 1.** Changes in adipokines levels in response to ACET-moxibustion of obese women

| Group | Leptin (ng/ml) | | Adiponectin (µg/ml) | | Resistin (ng/ml) | | TNF-α (pg/ml) | |
|---|---|---|---|---|---|---|---|---|
| | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Sham | 34.3 ± 4.8 | 31.4 ± 3.8 | 2.7 ± 0.3 | 2.9 ± 0.2 | 13.4 ± 1.4 | 13.9 ± 1.8 | 0.7 ± 0.3 | 1.4 ± 1.8 |
| CGM | 37.2 ± 2.4 | 33.8 ± 2.1 | 2.8 ± 0.2 | 2.4 ± 0.1* | 15.2 ± 1.6 | 16.1 ± 1.6 | 0.9 ± 0.2 | 0.9 ± 0.3 |

Values are presented as mean ± SEM.

* $p < 0.05$ final *vs* initial in each group (paired Student´s t-test

**Table 2.** Genes and primer sequences used in qPCR assays.

| Gene | FC | P-value* | Primer | Tm (°C) |
|---|---|---|---|---|
| PGM2 | 2.5 | 0.00074 | F: 5'-TCCAGAAGGCAGCGGTCTA | 57 |
| | | | R: 5'-TCCCAAACTCCATTCGGGC | |
| GABRA1 | 1.99 | 0.00578 | F. 5'-ATGATGGAGCTCGAGGCAAA | 57 |
| | | | R: 5'- AGCTCTGAATTGTGCTGGGT | |
| OR6K6 | 2.49 | 0.00075 | F: 5'-GAGCTTGGCCTGCACAGATA | 57.5 |
| | | | R: 5'- TCAGCTGAGTGCATTCCCAG | |
| FSCB | -1.5 | 0.00086 | F: 5'- TGAGGAAACCGTGGTACCTG | 57 |
| | | | R: 5'- GGTCCAGTATCCTGTACGGC | |
| MMP2 | -2.1 | 0.00192 | F: 5'- GTTTCCGCTGCATCCAGACT | 57.5 |
| | | | R: 5'- GGTCCTGGCAATCCCTTTGTA | |
| PDGFRL | -1.5 | 0.00397 | F: 5'- GCTACCCTGCGTATCTGGAC | 58 |
| | | | R: 5'- ATTCACCTGTGTCTGCCGAG | |

F, forward primer; R, reverse primer. FC, fold change from microarray assay.

* p-value from microarray assay.

**Table 3.** Main KEGG pathways associated to genes modulated in response to ACET-moxibustion.

| Pathway | Count | Gene symbol |
|---|---|---|
| Cytokine-cytokine receptor interaction | 22 | EGFR, CSF2, IFNA21, IL8, IL26, FASLG, EDA2R, IL21, CCL28, TNFSF18, CXCL10, CCL22, IL12RB1, CXCL16, IFNA5, IFNA4, CCR10, BMPR1B, IFNA16, CSF2RA, IL1A, IL2 |
| Calcium signaling pathway | 18 | EGFR, PHKA2, PLCZ1, ADCY1, SLC8A1, ERBB4, TRHR, GRM5, CHRM5, P2RX7, PLCB4, CHRM2, PDE1C, HTR7, PLN, RYR2, PLCB1, CACNA1C |
| JAK-STAT signaling pathway | 15 | PIK3CG, CSF2, IFNA21, IL26, IL21, SPRY3, CBLB, STAT4, IL12RB1, IFNA5, IFNA4, IFNA16, CSF2RA, IL13RA2, IL2 |
| Dilated cardiomyopathy | 14 | ACTC1, ADCY1, SLC8A1, ITGA2, CACNG2, CACNB4, ITGA4, CACNG1, TPM1, ITGA8, PLN, SGCD, RYR2, CACNA1C |
| Hypertrophic cardiomyopathy (HCM) | 13 | ACTC1, SLC8A1, ITGA2, CACNB4, ITGA4, CACNG2, CACNG1, TPM1, ITGA8, SGCD, RYR2, PRKAA2, CACNA1C |
| Cardiac muscle contraction | 11 | SLC8A1, ACTC1, COX7B2, COX6A2, RYR2, ATP1A4, CACNG2, CACNB4, CACNA1C, CACNG1, TPM1 |
| Toll-like receptor signaling pathway | 11 | PIK3CG, FOS, IFNA21, IRF5, IL8, IFNA5, IFNA4, TICAM2, IFNA16, TRAF3, CXCL10 |
| Arrhythmogenic right ventricular cardiomyopathy (ARVC) | 10 | SLC8A1, ITGA8, RYR2, SGCD, ITGA2, CACNG2, CACNB4, ITGA4, CACNA1C, CACNG1 |
| Autoimmune thyroid disease | 9 | IFNA21, IFNA5, IFNA4, CTLA4, FASLG, TSHB, IFNA16, TSHR, IL2 |
| Regulation of autophagy | 6 | IFNA21, IFNA5, IFNA4, PRKAA2, IFNA16, ATG3 |

**Table 4.** Functional clustering of genes modulated in response to ACET-moxibustion.

| Process | Up-regulated genes | | | Down-regulated genes | | |
|---|---|---|---|---|---|---|
| | Gene | FC | p-value | Gene | FC | p-value |
| Homeostasis control | | | | | | |
| Regulation of response to nutrient levels | | | | PPARA | -2.0015 | 0.008 |
| | | | | NPC1 | -1.5039 | 0.025 |
| | | | | GHRL | -1.9307 | 0.006 |
| Homeostatic process | ABCA12 | 2.6279 | 0.013 | HP | -2.2237 | 0.036 |
| | PLP1 | 2.5647 | 0.006 | GHRL | -1.9307 | 0.006 |
| | SCN1A | 2.5509 | 0.028 | PDIA6 | -1.7786 | 0.045 |
| | IL2 | 2.5231 | 0.028 | P2RX7 | -1.7461 | 0.049 |
| | POU3F2 | 2.5146 | 0.026 | TP53 | -1.5103 | 0.043 |
| | CACNA1C | 2.4969 | 0.030 | ATP1A4 | -1.5047 | 0.016 |
| | PTH | 2.3444 | 0.029 | NPC1 | -1.5039 | 0.025 |
| | ANGPTL3 | 2.3394 | 0.047 | | | |
| | ITGA2 | 2.1532 | 0.034 | | | |
| | PMCH | 2.1347 | 0.035 | | | |
| | IL1A | 2.0485 | 0.030 | | | |
| | SLC30A8 | 2.0001 | 0.016 | | | |
| | CACNG2 | 1.9995 | 0.014 | | | |
| | SLC39A4 | 1.9502 | 0.017 | | | |
| | CACNB4 | 1.8853 | 0.009 | | | |
| | PLN | 1.8627 | 0.003 | | | |
| | RHOT1 | 1.8479 | 0.024 | | | |
| | TRHR | 1.8339 | 0.023 | | | |
| | NEUROD1 | 1.8294 | 0.039 | | | |
| | GRIK2 | 1.8203 | 0.028 | | | |
| | KCNMA1 | 1.7724 | 0.041 | | | |
| | ERBB4 | 1.6961 | 0.043 | | | |
| | CCL28 | 1.6894 | 0.009 | | | |
| | TERF1 | 1.6482 | 0.039 | | | |
| | RYR2 | 1.6042 | 0.035 | | | |
| | SLC8A1 | 1.5777 | 0.013 | | | |
| | PPARGC1A | 1.5709 | 0.044 | | | |
| | CCR10 | 1.5654 | 0.018 | | | |
| | HEPH | 1.5574 | 0.041 | | | |
| | AFAP1L2 | 1.5086 | 0.005 | | | |
| | IDUA | 1.5064 | 0.015 | | | |
| Insulin secretion | CACNA1C | 2.4969 | 0.030 | | | |
| | SLC30A8 | 2.0001 | 0.016 | | | |
| | NEUROD1 | 1.8294 | 0.039 | | | |
| Regulation of insulin secretion | NEUROD1 | 1.8294 | 0.039 | UCN3 | -1.9012 | 0.015 |
| | PDE8B | 1.6389 | 0.045 | GHRL | -1.9307 | 0.006 |
| Lipid metabolism | | | | | | |
| Regulation of fatty acid metabolic process | PRKAA2 | 1.5598 | 0.005 | PPARA | -2.0015 | 0.008 |
| | PPARGC1A | 1.5709 | 0.044 | | | |
| Adipocytokine signaling pathway | ACSL3 | 1.8389 | 0.006 | PPARA | -2.0015 | 0.008 |
| | PPARGC1A | 1.5709 | 0.044 | | | |
| | PRKAA2 | 1.5598 | 0.005 | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Lipase activity | PLCB4 | 2.4279 | 0.008 | DAGLB | -1.8641 | 0.038 |
| | PLCXD3 | 2.1739 | 0.008 | PLCXD1 | -1.7525 | 0.030 |
| | CHRM5 | 2.1718 | 0.042 | | | |
| | PLCZ1 | 1.9952 | 0.028 | | | |
| | PLCB1 | 1.6242 | 0.015 | | | |
| | LGALS13 | 1.5253 | 0.013 | | | |
| Regulation of lipase activity | ANGPTL3 | 2.3394 | 0.047 | EGFR | -1.5324 | 0.036 |
| | AGTR2 | 2.2125 | 0.019 | | | |
| | HOMER1 | 2.0338 | 0.030 | | | |
| | GRM5 | 1.9525 | 0.025 | | | |
| | CHRM2 | 1.6464 | 0.044 | | | |
| | ARHGAP6 | 1.5366 | 0.009 | | | |
| | P2RY12 | 1.5317 | 0.040 | | | |
| Diacylglycerol binding | DGKE | 3.0056 | 0.002 | STAC3 | -1.7593 | 0.020 |
| | MYO9A | 2.3040 | 0.030 | | | |
| | DGKB | 1.9123 | 0.019 | | | |
| | VAV2 | 1.7820 | 0.026 | | | |
| | RGNEF | 1.6475 | 0.016 | | | |
| | RASGRP3 | 1.6386 | 0.047 | | | |
| | RASGRP1 | 1.6227 | 0.032 | | | |
| Phospholipase C activity | PLCB4 | 2.4279 | 0.008 | PLCXD1 | -1.7525 | 0.030 |
| | PLCXD3 | 2.1739 | 0.008 | | | |
| | CHRM5 | 2.1718 | 0.042 | | | |
| | PLCZ1 | 1.9952 | 0.028 | | | |
| | PLCB1 | 1.6242 | 0.015 | | | |
| Regulation of adenylate cyclase activity involved in G-protein signaling | PTH | 2.3444 | 0.029 | UCN3 | -1.9012 | 0.015 |
| | TSHR | 1.7731 | 0.042 | ADCY1 | -1.7892 | 0.017 |
| | HTR7 | 1.7389 | 0.046 | | | |
| Regulation of cAMP metabolic process | PTH | 2.3444 | 0.029 | ADCY1 | -1.7892 | 0.017 |
| | CHRM5 | 2.1718 | 0.042 | UCN3 | -1.9012 | 0.015 |
| | TSHR | 1.7731 | 0.042 | GNAZ | -2.0385 | 0.024 |
| | HTR7 | 1.7389 | 0.046 | | | |
| **Olfatory transduction** | | | | | | |
| GPCR, rhodopsin-like superfamily | OR6K6 | 2.4944 | 0.0007 | HTR1D | -2.0998 | 0.021 |
| | OR5T2 | 2.3620 | 0.018 | GPR77 | -2.0616 | 0.037 |
| | OR5T3 | 2.2416 | 0.012 | | | |
| | AGTR2 | 2.2125 | 0.019 | | | |
| | GPR88 | 2.1778 | 0.044 | | | |
| | CHRM5 | 2.1718 | 0.042 | | | |
| | OR6C74 | 2.1435 | 0.017 | | | |
| | OR12D3 | 2.0752 | 0.017 | | | |
| | OR5I1 | 2.0748 | 0.021 | | | |
| | GPR22 | 2.0399 | 0.017 | | | |
| | TAS2R42 | 2.0330 | 0.037 | | | |
| | OR1L1 | 1.9609 | 0.009 | | | |
| | GPR174 | 1.9376 | 0.022 | | | |
| | OXGR1 | 1.9053 | 0.009 | | | |
| | OR51A2 | 1.8859 | 0.046 | | | |
| | OR4K13 | 1.8816 | 0.014 | | | |
| | TRHR | 1.8339 | 0.023 | | | |
| | OR4C12 | 1.8333 | 0.037 | | | |
| | GPR25 | 1.8218 | 0.007 | | | |
| | OR13G1 | 1.8053 | 0.025 | | | |
| | OR51T1 | 1.7916 | 0.039 | | | |

| | | | |
|---|---|---|---|
| | TSHR | 1.7731 | 0.042 |
| | OR4A15 | 1.7646 | 0.011 |
| | HTR7 | 1.7389 | 0.046 |
| | OR1A2 | 1.7246 | 0.026 |
| | OR5B3 | 1.7128 | 0.028 |
| | OR13C4 | 1.7057 | 0.038 |
| | OR10A7 | 1.7021 | 0.018 |
| | OR52E4 | 1.6952 | 0.046 |
| | OR2C3 | 1.6918 | 0.008 |
| | OR52A1 | 1.6898 | 0.032 |
| | OR5K3 | 1.6820 | 0.029 |
| | OR5B17 | 1.6620 | 0.027 |
| | CHRM2 | 1.6464 | 0.044 |
| | OR4K17 | 1.6165 | 0.029 |
| | CCR10 | 1.5654 | 0.018 |
| | OR5AK2 | 1.5620 | 0.034 |
| | GPR20 | 1.5452 | 0.003 |
| | OR2AK2 | 1.5450 | 0.036 |
| | OR51F2 | 1.5346 | 0.007 |
| | NPFFR2 | 1.5337 | 0.046 |
| | P2RY12 | 1.5317 | 0.04 |
| **Gamma-aminobutyric acid signaling pathway** | | | |
| | GABRG1 | 2.1056 | 0.040 |
| | GABRA1 | 1.9936 | 0.005 |
| | CACNB4 | 1.8853 | 0.009 |
| | GABRG2 | 1.8330 | 0.029 |
| | GABRA2 | 1.8249 | 0.034 |

# DISCUSION

Debido a la epidemia de obesidad que afecta a todas las poblaciones, en la última década se ha incrementado el uso de la medicina alternativa en el manejo del control de peso. Así, la Medicina Tradicional China, en especial la acupuntura, ha demostrado ser una terapia segura y eficaz para reducir parámetros antropométricos y bioquímicos en pacientes obesos. Sin embargo, la mayoría de los reportes al respecto son estudios clínicos, sin enfoque molecular; algunos carecen de controles adecuados; además, están publicados en idioma chino, lo que dificulta su difusión en la comunidad científica. Por lo que no propusimos realizar un estudio controlado para validar el uso de la acupuntura en el tratamiento de la obesidad e investigar los cambios en la expresión génica en el tejido adiposo, con la finalidad de contribuir al entendimiento de las bases moleculares del efecto de esta terapia en el tratamiento de la obesidad.

Los resultados obtenidos en nuestro estudio al comparar un tratamiento de seis semanas con diferentes técnicas acupunturales (acupuntura con moxibustion (AM), acupuntura profunda con moxibustión (APM), electroacupuntura (EA), electroacupuntura con moxibustión (EAM) y el catgut embebido con moxibustión (CGM) estimulando el mismo grupo de puntos: Qihai (CV 6), Zhongwan (CV 12), bilateral Tianshu (ST 25), Zusanli (ST 36), Sanyinjiao (SP 6), así como Pishu (BL 20) y Shenshu (BL 23) para moxibustión, confirmaron que todos los métodos son eficaces para reducir el peso corporal y el IMC. La selección de estos puntos acupunturales se realizó de acuerdo a la diferenciación sindromática de la MTC, para el tratamiento de la obesidad. Coinciden con el tratamiento previamente reportado por Tang *et al.* en el 2009, quien usó casi los mismos puntos acupunturales. En este trabajo, el autor reporta una mayor reducción de peso y del IMC cuando se utiliza EA en combinación con catgut embebido comparado con EA sola, además de mejorar el sueño, disminuir ansiedad y depresión. Además, se observó una reducción significativa de los parámetros bioquímicos como en los

niveles de triglicéridos en los grupos de EA, EAM y CGM, en glucosa de los grupos de AM, EAM y CGM y en los niveles de insulina en los grupos de AM y CGM. Estos resultados coinciden con lo reportado por otros autores usando varias técnicas, como la EA (Yu *et al*. 2011; Cabioğlu *et al*., 2005; Abdi *et al*., 2012) y el catgut embebido (Yan *et al*. 2012; Wang *et al*., 2009; Gao *et al*., 2011).

De manera interesante, el tratamiento con catgut embebido y moxibustión que implementamos en este estudio, también permitió una reducción del índice de HOMA-IR, de tal manera que las pacientes obesas regresaron a un estado de sensibilidad a la insulina. Este efecto adicional del tratamiento con catgut embebido había sido previamente descrito (Chen *et al* 2007; Yan *et al.,* 2012), y se ha postulado que podría deberse a la permanencia de la sutura de catgut en el tejido subcutáneo durante aproximadamente 18-21 días, lo cual contribuye a activar enzimas proteolíticas y macrófagos (Yan *et al*. 2012). De esta manera, se genera un estímulo constante de la matriz celular e intersticial con la liberación de neurotransmisores en respuesta a la acupuntura (Langevin *et al.,* 2001; Han *et al.,* 2004; Kawakita *et al.,* 2006). Debido a sus efectos sobre los niveles de glucosa, insulina y el índice de HOMA-IR, el tratamiento de catgut embebido con moxibustión podría ser muy útil para controlar la resistencia a la insulina de la pacientes obesas, y por lo tanto el estado pre-diabético asociado a la obesidad.

Considerando que el tratamiento de catgut embebido con moxibustión es el que permitió obtener los mejores resultados en los parámetros antropométricos y bioquímicos en las pacientes obesas, se decidió proceder al análisis de los efectos de este tratamiento acupuntural en el tejido adiposo, mediante la determinación de los niveles circulantes de adipocinas y los cambios en la expresión genética. De manera inesperada, no se observaron cambios significativos en los niveles de adipocinas como leptina, resistina y TNF-a en sangre en el grupo de CG+M. El único cambio significativo fue la reducción de los niveles circulantes de adiponectina probablemente por la activación de citocinas proinflamatorias secretadas en respuesta a la irritación causada por el fragmento de catgut en el

tejido subcutáneo (Yan *et al*. 2012). Hasta donde sabemos, sólo existe un reporte en que se menciona un incremento en los niveles de adiponectina en respuesta al tratamiento con catgut embebido; sin embargo esta combinado con herbolaria china y el tratamiento no es para obesidad, sino para pacientes con síndrome de ovario poliquístico (Tao *et al.,* 2008).

El análisis de la respuesta transcripcional al tratamiento con catgut embebido y moxibustión en el tejido adiposo, tampoco evidenció cambios en los niveles de RNAm de estas adipocinas. Sin embargo, afectó de manera significativa la expresión de 1791 genes, un número similar a lo previamente por Dahlman *et al*. (2005) y Rönh *et al*. (2014) que usaron tratamientos más largos, de 10 semanas y 6 meses, respectivamente (Dahlman *et al*., 2005; Rönn *et al.,* 2014). Particularmente, en nuestro estudio demostramos por primera vez que los efectos del tratamiento con catgut embebido y moxibustión para reducir el peso corporal y la resistencia a la insulina, están asociados a cambios en la expresión genética en el tejido adiposo en mujeres obesas. Los genes modulados participan en vías bioquímicas que se alteran en la obesidad, como son el control de la homeostasis (respuesta response a los niveles de nutrientes, procesos homeostáticos y secreción de insulina) y el metabolismo de lípidos, así como la transducción olfatoria y la vía de señalización del ácido gamma-aminobutírico.

La sobreexpresión del gen NPC1 (Niemann-Pick C1), involucrado en el transporte intracelular de los lípidos (Garver *et al.,* 2000) y la disminución de la grelina que participa en la regulación hipotalámica del hambre/saciedad (Nakazato *et al.,* 2001) son consistentes con los cambios fenotípicos observados en las pacientes. El aumento en la expresión de los genes CACNA1C (Calcium channel, voltage-dependent, L type, alpha 1C subunit) (Gromada *et al.* 1999), SLC30A8 (Solute carrier family 30; zinc transporter, member 8) Tamaki *et al.,* 2013), NEUROD1 (Neurogenic differentiation 1) (Huang *et al.,* 2002), PDE8B (Phosphodiesterase-8B) (Dov *et al.,* 2008), y PPARGC1A (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Hammarstedt *et al.,* 2003), así como la

disminución de la expresión de UCN3 (Urocortin 3) (Li *et al.,* 2007) podrían explicar el control de la resistencia a la insulina que se observa al terminar el tratamiento de catgut embebido con moxibustión. La sobreexpresión de PPARGC1A, PRKAA2 (5´-AMP-activated protein kinase catalytic subunit α2) (Viollet *et al.,* 2003; Chen *et al.,* 2012) y ACSL3 (Long chain acyl-CoA synthetase-3) (Cao *et al.,* 2010), la activación de las fosfolipasas c (PLCB4, PLCXD3, PLCZ1 and PLCB1) (Montell *et al.,* 2001), así como la estimulación de los receptores GPCRs "no-odorantes", CHRM5 y CHRM2 (Muscarinic acetylcholine receptors $M_5$ and $M_2$), HTR7 (5-$HT_7$ receptor) y TSHR (thyroid-stimulating hormone receptor), que funcionan como receptores de neurotransmisores con la serotonina (Lu *et al.,* 2012), podrían en conjunto promover la lipólisis y regular el metabolismo lipídico, contribuyendo de esta manera a la disminución del peso corporal.

También se sobreexpresan los GPCRs de odorantes que son representados por 27 receptores olfatorios (OR). La expresión de ciertos OR en tejido adiposo ha sido relacionada con la progresión de la obesidad (Choi *et al.,* 2013), por lo que es necesario hacer experimentos adicionales para entender como la relevancia de su activación en la reducción del peso corporal en respuesta al tratamiento de catgut embebido con moxibustión.

Finalmente, es posible que la activación de la vía de señalización del GABA mediada por la activación de los GABR (Tian *et al.,* 2011) contribuye a la activación de vías de señalización que resulten en la reducción del peso corporal y la resistencia a la insulina.

Con todos estos resultados, podemos concluir que el tratamiento basado en la estimulación de los puntos acupunturales Qihai (CV6), Zhongwan (CV12), bilateral Tianshu (ST25), Zusanli (ST36), Sanyinjiao (SP6) mediante catgut embebido, así como Pishu (BL20) y Shenshu (BL 23) para moxibustión, es eficaz para reducir el peso corporal, el IMC, los niveles de triglicéridos, glucosa, así como la resistencia

a la insulina, en pacientes obesas. De manera interesante, estos efectos están asociados a la modulación de genes que favorecen al control del hambre, el metabolismo de lípidos y a la regulación correcta de liberación de la insulina.

# CONCLUSIONES GENERALES

1) Todos los tratamientos acupunturales utilizados en este trabajo para estimular el mismo grupo de acupuntos, fueron eficientes para reducir peso e IMC en pacientes obesas.

2) Tambien fueron capaces de modular los parámetros bioquímicos. Particularmente, EA, EAM y CGM redujeron los niveles de triglicéridos; AM, EAM y CGM fueron eficaces para reducir los niveles de glucosa; AM y CGM disminuyeron los niveles de insulina, mientras que EA y CGM redujeron los niveles de HOMA-IR.

3) De manera interesante, CGM fue la técnica más eficiente para reducir los valores de insulina y HOMA-IR, de tal amenra que las pacientes obesas regresaron a un estadio de sensibilidad a la insulina.

4) Sin embargo, no se observó cambios significativos en los niveles de leptina, TNF-α y resistina en suero; sólo se disminuyó significativamente la adiponectina.

5) El análisis de la respuesta transcripcional al tratamiento con CGM en el tejido adiposo mediante ensayos de microarreglos de DNA mostró que se modula, de manera significativa, la expresión de 1791 genes en el tejido adiposo; 1548 genes fueron sobreexpresados y 243 fueron subexpresados.

6) Los genes moduados participan en procesos que están alterados en la obesidad, como son el control de la homeostasis, el metabolismo de lípidos, así como la transducción olfatoria y la vía de señalización del ácido gamma-aminobutírico.

# PERSPECTIVAS

a. Medir niveles en sangre de otros reguladores del hambre/saciedad, como son citocinas (IL-6), grelina, CCK, entre otras, en respuesta al tratamiento con ACET-moxibustión en pacientes obesos.

b. Aumentar la duración del tratamiento para determinar si se obtienen mejores efectos en los parámetros antropométricos y bioquímicos, así como en la modulación de genes en el tejido adiposo.

c. Evaluar nuevamente los parámetros antropométricos y bioquímicos de los pacientes que fueron tratados con ACET-moxibustion, cada mes durante tres meses, para determinar si existe el efecto de rebote.

d. Evaluar si existe un efecto sinérgico al combinarse el tratamiento acupuntural con una dieta baja en calorías.

e. Realizar el estudio en un modelo animal para poder analizar en varios tejidos (sangre, tejido adiposo, hígado, músculo, páncreas, hipotálamo, entre otros), la expresión de los productos de los genes modulados.

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# PRODUCTIVIDAD DURANTE EL DESARROLLO DEL DOCTORADO

**Participación en congresos:**

**VII Encuentro Nacional de Biotecnología del Instituto Politécnico Nacional** del 11 al 13 de Octubre del 2010 Ciudad de Mazatlán, Sinaloa, México.

**7° Congreso Internacional de Ingeniería Bioquímica, 10° Congreso Nacional de Ingeniería Bioquímica, 18° Jornadas de Biomedicina y Biotecnología Molecular** del 28, 29 y 30 de marzo del 2012 Ixtapa, Zihuatanejo.

**XXX Congreso Nacional de Bioquímica** del 2 al 8 de Noviembre del 2014 Guadalajara, Jalisco.

**Participación en artículos:**

**DNA methylation of leptin and adiponectin promoters in children is reduced by the combined presence of obesity and insulin resistance.**

García-Cardona MC, Huang F, García-Vivas JM, López-Camarillo C, Del Río Navarro BE, Navarro Olivos E, Hong-Chong E, Bolaños-Jiménez F,Marchat LA.Int J Obes (Lond). 2014 Nov;38(11):1457-65. doi: 10.1038/ijo.2014.30. Epub 2014 Feb 19.

**Leptin Levels and Nutritional Status of Indigenous Tepehuán and Mestizo Subjects in Durango, Mexico.** Delgadillo Guzmán, D., Marchat Marchau, L. A., Reyes, J. L., Loera Castañeda, V., Sosa Macías, M., García Vivas, J., & Asseff, I. L. (2014). *Disease Markers*, *2014*, 974503. doi:10.1155/2014/974503