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Departamento de Ciencias de la Salud

Efecto del entrenamiento de fuerza y/o una dieta hipocalórica en la síntesis de moléculas que modulan el metabolismo de la glucosa a través de la resistencia a la insulina

TESIS DOCTORAL

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Índice

Declaración

Yo, Cristina Martínez Labari, declaro que la recopilación de trabajos que se presenta en esta tesis se basa en 2 artículos (capítulos 2 y 3) que han sido publicados en revistas internacionales científicas revisadas por pares. Para cumplir con los requisitos estilísticos de una tesis, las publicaciones se han ajustado a un mismo formato a lo largo de todo el presente documento. Sin embargo, estas modificaciones no han alterado el contenido de los artículos publicados. A continuación se citan las principales funciones que he desempeñado y de las que he sido responsable durante todo el trayecto de la presente tesis doctoral.

Capítulos 2 y 3

Fernández-Real JM, Izquierdo M, Ortega F, Gorostiaga E, Gómez-Ambrosi J, Moreno-Navarrete JM, Frühbeck G, **Martínez C**, Idoate F, Salvador J, Forga L, Ricart W, Ibañez J.The relationship of serum osteocalcin concentration to insulin secretion, sensitivity, and disposal with hypocaloric diet and resistance training. J Clin Endocrinol Metab. 2009 Jan;94(1):237-45. doi: 10.1210/jc.2008-0270. Epub 2008 Oct 14.

Fernández-Real JM, Izquierdo M, Moreno-Navarrete JM, Gorostiaga E, Ortega F, **Martínez C,** Idoate F, Ricart W, Ibañez J. Circulating soluble transferrin receptor concentration decreases after exercise-induced improvement of insulin sensitivity in obese individuals. Int J Obes (Lond). 2009 Jul;33(7):768-74. doi: 10.1038/ijo.2009.99. Epub 2009 Jun 2.

El equipo de investigación formado por los profesionales del Centro de Estudios, Investigación y Medicina del Deporte (CEIMD) del Instituto Navarro de Deporte y Juventud (INDJ) del Gobierno de Navarra dirigido por el Dr. Javier Ibáñez y el Dr. Mikel Izquierdo principalmente, y del que yo soy miembro, concibió la idea y diseñó un estudio de investigación sobre el efecto del entrenamiento de fuerza y/o una dieta hipocalórica en un grupo de mujeres obesas. Dentro del proyecto de investigación, del que han salido a parte de los mencionados artículos (presentados en los capítulos 2 y 3 de esta tesis), otras publicaciones se pueden diferenciar diversas partes experimentales. Dentro de la parte específica de entrenamiento de fuerza del proyecto, entre otras funciones,

participé como científica del ejercicio físico y del deporte supervisando el entrenamiento del grupo de las mujeres obesas, sedentarias y sin patologías asociadas en el desarrollo del protocolo de fuerza y ayudé en la cuantificación y recálculo de las cargas además de realizar las valoraciones finales de fuerza (test 1RM), recogida de datos y análisis de datos de las variables. Por otro lado, ya finalizada la investigación, estuve encargada de la recepción de los datos bioquímicos, diseñé la base de datos y realicé los pertinentes análisis estadísticos, tomé parte en la interpretación y valoración de los mismos. Preparé tablas y figuras previas que tras las revisiones se vieron modificadas para su publicación. Por último, realicé los informes reportando información sobre los datos de mayor interés para entregar a los sujetos del estudio y participé en la discusión de los resultados de estos dos trabajos y del resto de publicaciones relacionadas con el proyecto.

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En primer lugar, quisiera darles las gracias a mis padres. Hace relativamente poco tiempo comprendí vuestra forma de hacer las cosas. Me lo habéis dado todo; autonomía para decidir, educación para crecer y sobre todo me habéis enseñado a valorar el sacrifico que cuesta conseguir aquello que me he propuesto en la vida.

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"Quien no sabe lo que busca no entiende lo que encuentra".
Claude Bernard (1813-1878)

Cristina

Resumen

Resumen

La presente tesis doctoral gira en torno a la necesaria búsqueda de información sobre nuevas moléculas moduladoras del metabolismo de la glucosa y su efecto en la mejora de la Resistencia a la Insulina a través de la dieta y el ejercicio físico. Para ello se han llevado a cabo 2 trabajos de investigación basados en el efecto de la dieta hipocalórica individualizada y/o el ejercicio de fuerza progresivo.

Estudio 1 (Capítulo 2): El hueso se comporta como un órgano endocrino al secretar osteocalcina que produce un aumento de la secreción y sensibilidad a la insulina en modelos animales. Esta reciente descripción de la osteocalcina como hormona secretada por el hueso y su impacto en la sensibilidad a la insulina en modelos animales nos proporcionó un marco de estudio para comprobar si la osteocalcina circulante también podría estar asociada con efectos metabólicos en los seres humanos. Por ello, el objetivo de este primer trabajo era evaluar la osteocalcina y su relación con la secreción y sensibilidad a la insulina en tres grupos de varones y mujeres no diabéticos. Presentamos datos sobre 1) un estudio transversal con 149 varones en los que se analizó la sensibilidad a la insulina mediante la técnica Mínimal model; 2) un estudio longitudinal con 26 mujeres distribuidas en un grupo control, un grupo que hizo dieta y otro grupo que realizó dieta y ejercicio de fuerza (pérdida leve de peso del 7,3 y 8,7% respectivamente); y 3) un estudio longitudinal con 20 sujetos que realizaron únicamente dieta hipocalórica (pérdida moderada de peso del 16,8%). En el estudio transversal, se pudo apreciar una mayor concentración de osteocalcina circulante en los varones con IMC < 25kg/m^2 que se asoció con una mayor sensibilidad y secreción de insulina. En el segundo estudio, la pérdida leve de peso producida en los sujetos que hicieron únicamente dieta produjo una ligera mejora en la sensibilidad a la insulina pero sin cambios significativos en la osteocalcina. Sin embargo, el grupo que realizó dieta y ejercicio de fuerza tuvo un efecto estadísticamente significativo sobre la osteocalcina y sensibilidad a la insulina y redujo la grasa visceral sin producir descensos en la masa muscular del muslo. En el tercer estudio, los sujetos con dieta más restrictiva que perdieron un 16,8% de peso corporal presentaban unos niveles de osteocalcina incrementados después de la pérdida de peso, sin mejorar sustancialmente la sensibilidad a la insulina.

Estudio 2 (Capítulo 3): Recientemente se ha encontrado que el receptor de transferrina soluble circulante (sTfR) está asociado negativamente con la sensibilidad insulina. Por ello, nuestro objetivo con este trabajo fue evaluar la concentración de sTfR circulante después de la pérdida de peso inducida por la dieta o por la dieta más el entrenamiento de fuerza en mujeres obesas. La sensibilidad a la insulina se determinó mediante el Modelo de evaluación homeostática (HOMA, Homeostasis Model Assessment). Pudimos comprobar que después de 16 semanas la sensibilidad a la insulina mejoró en ambos grupos de intervención. Sin embargo, únicamente vimos una mejora significativa (disminución) del sTfR en el grupo que hizo dieta y entrenamiento de fuerza. El mantenimiento de la masa muscular y la mejora de fuerza del grupo que realizó el entrenamiento de fuerza sugieren que el ejercicio físico tiene la capacidad de inducir una regulación sobre el sTfR mediada por la mejora de la sensibilidad a la insulina.

A través de estos estudios hemos podido comprobar los beneficios de la dieta y el ejercicio físico de fuerza en la concentración de moléculas que mejoran la sensibilidad a la insulina produciendo de esta forma una reducción del riesgo de padecer patologías crónicas asociadas a la Resistencia a la Insulina.

Capítulo 1

Introducción

Introducción, objetivos y lista de abreviaturas

Introducción

Las cifras de glucemia durante un periodo de 24 horas es, de media, ~90 mg/dl con una máxima concentración que habitualmente no excede de 165 mg/dl tras la ingesta de una comida y no suele disminuir por debajo de 55 mg/dl después de realizar ejercicio o ayuno (60h). Esta relativa estabilidad contrasta con la situación de otros sustratos como el glicerol, el lactato, los ácidos grasos libres (AGL) y los cuerpos cetónicos, cuyas fluctuaciones son superiores (Shrayyef & Gerich, 2010).

Este reducido rango que define la normoglucemia es mantenido a través de la regulación y contrarregulación de un complejo sistema neuro-hormonal: cuando se produce una disminución de la glucosa plasmática de 20 mg/dl (de 90 a 70 mg/dl) se suprime la liberación de insulina y con ello se reduce la absorción de glucosa en ciertas áreas del cerebro (por ejemplo, hipotálamo, donde se localizan los sensores de la glucosa), esto hará que se active el Sistema Nervioso Simpático y se desencadene la activación de hormonas contrarreguladoras (glucagón, catecolaminas, cortisol y GH). Todos estos cambios producen un incremento de la glucosa plasmática y disminuye su utilización hasta restablecer valores estables de glucemia. Por otro lado, un aumento de 10 mg/dl de la glucemia estimulará la producción de insulina y suprimirá la secreción de glucagón para prevenir mayores incrementos de glucosa y mantener unos niveles adecuados (Shrayyef & Gerich, 2010).

La glucosa plasmática proviene tanto de la dieta como del resultado de la depleción de glucógeno hepático (glucogenólisis) o de la formación de glucosa en hígado a partir de otros precursores como el lactato, piruvato, amino ácidos y glicerol (gluconeogénesis) (Shrayyef & Gerich, 2010).

Importancia de la homeostasis de la glucosa

En definitiva, la homeostasis de la glucosa consiste en el mantenimiento de los niveles de glucemia en unos niveles adecuados durante todo el día para mantener el equilibrio del organismo. A pesar de que los AGL son el combustible más utilizado por la mayoría de los órganos, la glucosa es en condiciones fisiológicas estables el principal combustible

metabólico del cerebro. Esto ocurre debido a la baja concentración de otros posibles sustratos (por ejemplo, los cuerpos cetónicos) o por la limitación de poder atravesar las barreras hematoencefálicas (por ejemplo, los AGL). Después de un ayuno prolongado y debido al incremento en su concentración circulante, los cuerpos cetónicos pueden ser utilizados de forma significativa por el cerebro. El cerebro no es capaz de sintetizar glucosa y no puede acumularla en forma de glucógeno que asegure sus necesidades para más de unos pocos minutos. Por ello, el cerebro depende de un continuo abastecimiento de glucosa plasmática.

Una concentración de glucosa plasmática menor de 20 mg/dl respecto de los valores normales provoca una limitación de su transporte de glucosa para la utilización por el cerebro. Una glucemia menor de 55 mg/dl afecta al cerebro, mientras que un descenso más severo y una prolongada hipoglucemia producen convulsiones, daño permanente del cerebro y en ocasiones, la muerte. Por otro lado, un ligero aumento de la concentración de la glucemia, que suele ocurrir en pacientes con intolerancia a la glucosa, incrementa el riego de morbilidad cardiovascular (Shrayyef & Gerich, 2010).

Factores que influyen en la homeostasis de la glucosa

Los factores más importantes en todo momento son las hormonas (insulina, glucagón y catecolaminas), la actividad del Sistema Nervioso Simpático y las concentraciones de otros sustratos (AGL). En un tiempo más prolongado (horas-días), adquieren importancia otras hormonas (cortisol y Hormona del crecimiento (GH)), algunos factores nutricionales (composición de la dieta), la condición física y el ejercicio, acompañados de cambios en la sensibilidad a las hormonas (Gerich, 1993). El cortisol, la GH y las catecolaminas afectan a la homeostasis de la glucosa alterando la sensibilidad a la insulina y la disponibilidad de sustratos alternativos.

Insulina

La insulina es la principal responsable de controlar la captación, utilización y almacenamiento de nutrientes celulares; aumenta la absorción de glucosa de la sangre, principalmente hacia el músculo y el tejido adiposo, en donde promueve su conversión a glucógeno y triglicéridos, respectivamente, inhibiendo al mismo tiempo su degradación. Además, en el hígado inhibe la gluconeogénesis, la glucogenólisis y la cetogénesis, y promueve la síntesis de proteínas principalmente en el músculo (Heesom, Harbeck, Kahn, & Denton, 1997).

El principal regulador de secreción de insulina es la concentración de glucosa plasmática: el aumento de glucemia después de la ingesta de una comida produce un aumento de 3 o 4 veces la concentración de insulina en los siguientes 30-60 minutos, mientras que una disminución de glucosa en plasma por debajo de 50 mg/dl dará lugar a la reducción de un 80-90% en los niveles de insulina en sangre. Un incremento agudo de aminoácidos y, en menor medida, de AGL también aumenta la secreción de insulina (Gerich, 1993; Gerich, 2000; Stumvoll et al., 2000; Woerle et al., 2003)

Con la ingesta de alimentos, los factores intestinales llamados incretinas (por ejemplo, péptido inhibidor gastrointestinal (PIB) y péptido similar al glucagón (GLP-1)) aumentan la secreción de insulina. Es por esto que las concentraciones de insulina en plasma aumentan en mayor medida después de una carga oral de glucosa que después de idénticas concentraciones de glucosa por vía intravenosa (Woerle et al., 2003; Gosmanov et al., 2005).

Los procesos metabólicos varían en su sensibilidad a la insulina y en sus características con respecto a la dosis-respuesta. En los niveles basales observados en estado de postabsorción (entre 5-10 mU/ml), la insulina está inhibiendo la glucosa y la liberación de AGL un 30-50% (contrarrestando el efecto del glucagón y el Sistema Nervioso Simpático), mientras que tiene un efecto insignificante sobre la captación de glucosa en los tejidos. La máxima eliminación de la glucosa y la liberación de AGL se observa normalmente con concentraciones de insulina postprandial (aproximadamente de 40-50 mU/ml), mientras que la estimulación máxima en la captación de la glucosa por los tejidos requiere concentraciones de insulina en plasma superior a 300 mU/ml que no se ven en

condiciones fisiológicas normales excepto en individuos con resistencia a la insulina en los que, por supuesto, ese nivel no produce el efecto máximo (Gerich, 1993; Stumvoll et al., 2000; Woerle et al., 2003; Bolli, Gottesman, Cryer, & Gerich, 1984).

En relación con los mecanismos de acción de la insulina, estos comienzan con su interacción con receptores específicos en la superficie de la célula. El receptor de la insulina se extiende por la membrana plasmática y tras la unión con la insulina transmite la señal al interior de la célula, que es ampliada y desencadena una variedad de respuestas intracelulares favoreciendo el almacenamiento de energía e inhibiendo la movilización de reservas de energía.

Resistencia a la insulina

La Resistencia a la Insulina (RI) es una disminución de la respuesta de los tejidos periféricos diana como el músculo, el tejido adiposo y el hígado a una concentración fisiológica de insulina, con lo que la RI se caracteriza por una disminución tanto de la sensibilidad a la insulina de los tejidos como del metabolismo de la glucosa, lípidos y proteínas.

Los factores que contribuyen a la RI son múltiples (Wilcox, 2005), aunque en estos momentos está bien establecido que su alta incidencia es debida en gran parte a la obesidad (Amati et al., 2009), más concretamente a la grasa visceral (Bonora, 2000), y a que el músculo esquelético juega un papel central en su desarrollo (Zierath, Krook, & Wallberg-Henriksson, 2000) . Sin embargo, el mecanismo subyacente en la relación entre la obesidad y la RI del músculo todavía es materia de debate. La teoría predominante se basa en la incapacidad del tejido adiposo para almacenar el exceso de energía, lo que resulta en una salida elevada de AGL desde los depósitos de grasa a otros tejidos como el músculo esquelético (Sethi & Vidal-Puig, 2007). El contenido excesivo de lípidos dentro de este tejido causa alteraciones metabólicas, incluyendo RI.

En definitiva, el músculo esquelético, en virtud de su masa y su elevado índice de transporte de glucosa estimulado por la insulina, representa un tejido muy importante en el desarrollo de la RI (Caro, Dohm, Pories, & Sinha, 1989). Así, la RI del músculo esquelético representa el principal defecto en el mantenimiento de unos niveles

normales de glucemia (Zierath et al., 2000) y a menudo se acompaña de diferentes alteraciones metabólicas y cardiovasculares, como la hipertensión, dislipidemia, diabetes mellitus tipo 2 y aterosclerosis (DeFronzo & Ferrannini, 1991).

Estrategias para mejorar la Resistencia a la Insulina

Cambios en el estilo de vida como la pérdida de peso y el ejercicio físico se reconocen como intervenciones no farmacológicas eficaces con efectos beneficiosos sobre las enfermedades metabólicas y cardiovasculares, incluida la RI (Ryan, 2000; Lapidus et al., 1984; Braith & Stewart, 2006; Palermo, Maggi, Maurizi, Pozzilli, & Buzzetti, 2014).

De hecho y, teniendo en cuenta aspectos relevantes de la alimentación, en los últimos años, varios trabajos de meta-análisis han encontrado que las pautas de la dieta mediterránea tradicional sin restricciones calóricas, produce efectos beneficiosos significativos sobre los factores de riesgo metabólico, entre los que se encuentra la RI (Kastorini et al., 2011; Garcia et al., 2016), consiguiendo resultados notorios en la disminución de la circunferencia de la cintura en personas que siguieron ese patrón alimentario (Alvarez-Perez et al., 2016).

Se ha descrito también que las pérdidas de peso corporal de entre el 5-10% parecen ser suficientes para incidir sobre la RI (Garvey et al., 2016). Principalmente se recomiendan dietas hipocalóricas equilibradas, con un déficit energético de 500-1000 Kcal respecto a la dieta habitual que generen pérdidas de peso en torno al medio kilogramo por semana (Eckel, 2008). Es conveniente que estas dietas además, sean bajas en Ácidos Grasos Saturados (AGS) e Hidratos de Carbono (HC) de alto índice glucémico (IG) (Wolever, 2000; Macdonald, 2016) , en las que el aporte de omegas-3 y de fibra sea suficiente para generar mejoras en la sensibilidad de la insulina (McAuley & Mann, 2006).

El ejercicio físico claramente mejora la sensibilidad a la insulina del músculo pero los mecanismos son poco claros (Sjoberg et al., 2017). Sin embargo sabemos que , el ejercicio físico es capaz de reducir la hiperglucemia característica en la RI por dos vías: una primera vía temprana, que se desarrolla en los 30-60 minutos posteriores al ejercicio, independiente de la insulina, con un alto ritmo de transporte de glucosa al interior de la célula mediado por la translocación del transportador de glucosa GLUT-4 al sarcolema; y

una segunda vía dependiente de la insulina, caracterizada por un aumento de la sensibilidad a la insulina circulante con una mayor absorción de glucosa por el músculo y mayor actividad de la glucógeno sintetasa. La mayor sensibilidad del músculo a la insulina después del ejercicio puede prolongarse hasta unas 48 horas (Beelen, Burke, Gibala, & van Loon, 2010).

Tradicionalmente, ha sido el entrenamiento aeróbico el más recomendado para favorecer la pérdida de peso en pacientes con sobrepeso u obesidad (Ross et al., 2004); sin embargo, desde hace años, se prescribe entrenamiento de fuerza para promover la pérdida de grasa corporal mientras se preserva la masa muscular (Ibanez et al., 2010; Garvey et al., 2016). En este sentido, diferentes estudios han demostrado que, sin una dieta hipocalórica concomitante, el entrenamiento de fuerza por si sólo disminuye significativamente la grasa visceral en varones adultos (Ibanez et al., 2005) y mujeres (Treuth et al., 1995), mejorando la sensibilidad a la insulina (Ibanez et al., 2005). Rice y colaboradores (1999) concluyeron que, la pérdida de peso en varones obesos inducida por el ejercicio independientemente de si era aeróbico o de fuerza, tiene mejores efectos sobre el metabolismo de la glucosa que la dieta sola.

Siendo conscientes de la importancia del metabolismo de la glucosa y la disponibilidad energética para el organismo vemos la necesidad de explorar los mecanismos fisiológicos a través de los cuales una dieta hipocalórica y el ejercicio físico influyen positivamente en la RI. En definitiva, unos de los objetivos prioritarios de los estudios que dieron origen a las publicaciones que se detallan más adelante fue valorar si ciertas moléculas estudiadas en modelos animales se comportan de la misma manera en humanos obesos mejorando la RI.

En este contexto, Lee et al. (2007) han demostrado en modelo animal que el hueso regula el “eje insulina-glucosa” y el metabolismo energético. Esto es un hallazgo fascinante. En concordancia con este nuevo concepto, el hueso se comporta como un órgano endocrino hasta entonces desconocido que secreta osteocalcina. La liberación de osteocalcina provoca un aumento de la secreción de insulina, un descenso de la glucosa sanguínea, un aumento de la sensibilidad de la insulina, el descenso de la grasa visceral, y un incremento del gasto energético. De hecho, en ratones, la falta de osteocalcina se

relacionó con un descenso de la proliferación de células beta y RI, así como con una anormal cantidad de grasa visceral y un aumento del nivel de triglicéridos (TG) en suero.

Esta descripción de la osteocalcina como una hormona derivada del hueso que afecta a la sensibilidad a la insulina en modelos animales nos proporciona un marco teórico para comprobar si la osteocalcina circulante también está asociada con cambios metabólicos en humanos y más concretamente en humanos obesos.

Para examinar si la osteocalcina circulante está asociada con cambios metabólicos en humanos se llevaron a cabo 3 estudios.

1) Un estudio transversal para comprobar si esta hormona está asociada con la sensibilidad a la insulina en 149 varones sin ningún tipo de enfermedad metabólica.

2) Un estudio longitudinal para comprobar si la osteocalcina está relacionada tanto con los cambios inducidos por una dieta hipocalórica diseñada para perder peso corporal de manera suave y progresiva (0.5 kg de peso corporal por semana), como con los cambios inducidos por una intervención de dieta más ejercicio físico (la misma dieta hipocalórica antes mencionada más un programa progresivo de entrenamiento de fuerza de 2 sesiones por semana).

3) Un estudio longitudinal para comprobar si la osteocalcina está relacionada con los cambios inducidos por una dieta hipocalórica diseñada para perder peso corporal de manera más pronunciada (1 kg de peso corporal por semana) en pacientes obesos.

Por otro lado, sabemos que la insulina es una hormona anabólica que estimula la absorción celular de muchos nutrientes incluyendo aminoácidos, cationes y hierro. El hierro es transportado en la sangre por la transferrina y cedido a los tejidos por un proceso mediado por un receptor de membrana específico para la tranferrina, presente en todos los tejidos, denominado receptor de la transferrina (TfR). La insulina es capaz de causar una estimulación rápida y pronunciada en la absorción de hierro por diferentes tipos de células, lo que hace que la TfR se redistribuya moviéndose desde el compartimento de la membrana intracelular hasta la superficie de la célula (Finch, 1994; Davis, Corvera, & Czech, 1986; Yokomori et al., 1991). A causa de la expresión de la TfR, es posible detectar una forma soluble de TfR en suero, la sTfR. La concentración

circulante de sTfR es proporcional a la expresión celular de la TfR asociada a la membrana (Baynes & Cook, 1996) y esto indica la estrecha relación entre el TfR y el sTfR . La concentración de sTfR, obviamente, está directamente relacionada con las exigencias celulares de hierro.

Algunos investigadores han observado que la concentración de sTfR en suero está ligada a la Resistencia a la Insulina (Fernandez-Real et al., 2007) observando una relación inversa entre la sensibilidad a la insulina y la concentración de sTfR. Es decir, cuanto menor era la sensibilidad a la insulina, mayor era la concentración de sTfR.

Ya que la insulina provoca un aumento de la concentración de sTfR en suero en modelos animales (Clairmont & Czech, 1990), se podría sospechar que la hiperinsulinemia puede contribuir a tener concentraciones demasiado altas de sTfR en individuos con una tolerancia a la glucosa alterada.

En concordancia con todas estas observaciones, y con el objetivo de arrojar más luz sobre la relación de la concentración de TfR con la sensibilidad a la insulina, 26 mujeres obesas fueron distribuidas en tres grupos diferentes de manera aleatoria: un grupo control (7 mujeres), grupo dieta hipocalórica (8 participantes) y un grupo dieta más entrenamiento de fuerza (11 mujeres). Con este diseño experimental, el objetivo principal fue evaluar si, la mejora de la sensibilidad a la insulina en los diferentes grupos de intervención, afectaba o no la concentración de sTfR.

Lista de abreviaturas

Abreviatura	Significado
AGL	Ácido grasos libres
AGS	Ácido Graso Saturado
IG	Índice glucémico
HC	Hidratos de Carbono
GH	Hormona del crecimiento
PIB	Péptido inhibidor gastrointestinal
GLP-1	Péptido similar al glucagón
RI	Resistencia a la Insulina
sTfR	Forma soluble receptor de la transferrina
TG	Triglicéridos
TfR	Receptor de la transferrina

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Capítulo 2

The Relationship of Serum Osteocalcin Concentration to Insulin Secretion, Sensitivity, and Disposal with Hypocaloric Diet and Resistance Training

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The Relationship of Serum Osteocalcin Concentration to Insulin Secretion, Sensitivity, and Disposal with Hypocaloric Diet and Resistance Training

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Abstract

Context: Bone has recently been described as exhibiting properties of an endocrine organ by producing osteocalcin that increases insulin sensitivity and secretion in animal models.

Objective and Design: We aimed to evaluate circulating osteocalcin in association with insulin sensitivity and insulin secretion in three different studies in nondiabetic subjects: one cross-sectional study in 149 men (using minimal model), and two longitudinal studies in two independent groups (one formed by 26 women, and the other by 9 men and 11 women), after a mean of 7.3 and 16.8% weight loss, and after a mean of 8.7% weight loss plus regular exercise.

Results: In the cross-sectional study, circulating osteocalcin was associated with insulin sensitivity, mainly in lean subjects, and with insulin secretion (only in lean subjects). A mean of 16.8%, but not 7.3% weight loss, led to significant increases in circulating osteocalcin. However, a mean of 8.7% weight loss plus regular exercise led to the more pronounced effects on the serum osteocalcina concentration, which increased in parallel to reduced visceral fat mass, unchanged thigh muscle mass, and increased leg strength and force. The post intervention serum levels of osteocalcin were associated with both insulin sensitivity (r=0.49;*P*=0.03) and fasting triglycerides (r=0.54; *P*=0.01).The change in visceral fat was the parameter that best predicted the change in serum osteocalcin, once age, body mass index, and insulin sensitivity changes were controlled for (*P*=0.002).

Conclusion: Circulating osteocalcin could mediate the role of bone as an endocrine organ in humans. (***J Clin Endocrinol Metab* 94: 237–245, 2009**)

Introduction

Abnormalities of bone metabolism are well known to occur in subjects with obesity and type 2 diabetes. Even increased adiposity in children is a risk factor for fracture (Goulding, Jones, Taylor, Williams, & Manning, 2001). Patients with type 2 diabetes are prone to fracture, although their bone density may not be particularly low (Schwartz et al., 2001). The rate of bone turnover is decreased in patients with type 2 diabetes, as reflected by diminished expression of biomarkers of bone resorption and formation, including osteocalcin, an osteoblast-specific protein (Gerdhem, Isaksson, Akesson, & Obrant, 2005). In fact, several studies have previously demonstrated that serum osteocalcin was reduced in patients with type 2 diabetes (Bouillon et al., 1995; Pietschmann, Schernthaner, & Woloszczuk, 1988; Pedrazzoni et al., 1989; Rico, Hernandez, Cabranes, & Gomez-Castresana, 1989).

Lee *et al.* (2007) have recently demonstrated in mice that bone regulates the insulin/glucose axis and energy metabolism. This is a fascinating new concept according to which the bone behaves as an endocrine organ by secreting osteocalcin, which leads to increased insulin secretion, lower blood glucose, increased insulin sensitivity, decreased visceral fat, and increased energy expenditure. In fact, mice lacking osteocalcina displayed decreased β-cell proliferation and insulin resistance, an abnormal amount of visceral fat, and increased serum triglyceride levels (Lee et al., 2007). Similar information in humans is lacking.

This recent description of osteocalcin as a bone-derived hormone impacting on insulin sensitivity in animal models provided us a framework to test whether circulating osteocalcin could also be associated with metabolic effects in humans. In fact, there are few studies that have evaluated circulating osteocalcin in relation to insulin sensitivity in humans.

Subjects and Methods

Cross- sectional study

A total of 149 consecutive men [mean age, 50.2 ± 11.7 yr; range, 30–68 yr; mean body mass index (BMI) 27.6 ± 3.5 kg/m^2 were recruited in an ongoing study dealing with insulin sensitivity in northern Spain. Subjects were randomly located from a census, and they were invited to participate. Participation rate was 71%. Inclusion criteria were: 1) BMI <40 kg/m^2; 2) absence of systemic disease; and 3) absence of infection within the previous month. None of the control subjects were taking medications or had evidence of metabolic diseases other than obesity. Liver disease and thyroid dysfunction were specifically excluded by biochemical work-up. All subjects had fasting plasma glucose below 7.0 mM and were taking no medications. Type 2 diabetes was ruled out by an oral glucose tolerance test according to criteria from the American Diabetes Association. Insulin sensitivity was measured using the frequently sampled iv glucose tolerance test with minimal model analysis. Insulin secretion was calculated as the insulin area during the first 10 min of the frequently sampled iv glucose tolerance test. This test also provides the insulin disposition index, a parameter emerging from the model, which represents the ability of the pancreatic islets to compensate for insulin resistance.

In brief, the experimental protocol started between 0800 and 0830 h after an overnight fast. A needle was inserted into an antecubital vein, and patency was maintained with a slow saline drip. Basal blood samples were drawn at -30, -10, and -5 min, after which glucose (300 mg/kg body weight) was injected over 1 min starting at time 0, and insulin (Actrapid, 0.03 U/kg; Novo Nordisk, Bagsvaerd, Denmark) was administered at time 20 min. Additional samples were obtained from a contralateral antecubital vein up to 180 min.

Effects of slight weight loss with or without regular physical activity

Sedentary, nonsmoking, obese (BMI, 30-40 kg/m^2) women, aged 40-60 yr, were recruited through an advertisement in a local newspaper. Before inclusion in the study, all candidates were thoroughly screened by an extensive medical history, resting and maximal exercise electrocardiograms, and blood pressure measurements. Cardiovascular, neuromuscular, arthritic, pulmonary, or other debilitating diseases as determined via one or all of the screening tools were reasons for exclusion from the study. None of the subjects received any medication. All subjects were carefully informed about the possible risks and benefits of the project and then provided written consent forms before participating in the study. This project was approved by the ethical committee of the regional health department.

Participants were randomized to three groups: a control group (C; n = 7); a diet group (D; n = 8) with a caloric restriction of 500 kcal/d; and a diet and resistance training group (D+RT; n = 11) with the same caloric restriction as group D and a 16-wk supervised RT program of two sessions per week. During the 16 wk of the study, the subjects maintained their customary recreational physical activities (*e.g.* walking). The baseline characteristics of the subjects are presented in Table 1.

Table1. Baseline and follow-up characteristics of the slight weight loss study

	Control (n= 7) age 51.5 ± 7.2 yr			Diet (n= 8) age 51.6 ± 6.6 yr			Diet + exercise (n= 11) age 47.7 ± 6.5 yr			
	Pre	**Post**	***P***	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***	***P*a** (age , 0.36)
IBM (kg/m^{-2})	34.58 ± 3.90	34.38 ± 3.55	**0.52**	34.11 ± 3.89	31.62 ± 3.92	**0.009**	34.27 ± 2.78	31.33 ± 2.00	**<0.0001**	0.96
Weight	88.68 ± 12.74	88.15 ± 11.74	**0.506**	87.22 ± 18.01	80.81 ± 16.64	**0.012**	88.08 ± 12.63	80.36 ± 9.11	**<0.0001**	0.98
WHR	0.91 ± 0.01	0.89 ± 0.02	**0.26**	0.93 ± 0.02	0.91 ± 0.03	**0.08**	0.92 ± 0.03	0.88 ± 0.03	**0.026**	0.126
Thigh muscle area	46,667.6 ± 7,736	46,465.5 ± 7,167.4	**0.74**	48,966.4 ± 10,883.2	47,226.8 ± 11,609	**0.039**	46,867.6 ± 9,021.3	46,470.2 ± 8,839.2	**0.87**	0.87
Thigh fat area	88,332 ± 19,339	89,127.2 ± 19,090.9	**0.59**	87,641.9 ± 21,716.5	72,697.6 ± 18,365.1	**0.004**	10,4923.7 ± 16,539.6	87,479.1 ± 14,200.8	**<0.0001**	0.11
Visceral AT	3,370.78 ± 1,228.42	3,329.26 ± 1,187.23	**0.65**	3,243.75 ± 1,085.33	2,557.51 ± 1,171.16	**0.007**	3,211.30 ± 1,232.09	2,528.43 ± 1,039.89	**0.0001**	0.96
Total AT	16,833.4 ± 4,183.2	16,623.5 ± 3,785.1	**0.53**	16,973.7 ± 4,787.1	13,333.2 ± 4,840.8	**0.001**	18,205 ± 3,337.9	13,810.7 ± 2,201.8	**<0.0001**	0.73
1RM arms	34.28 ± 6.56	34.81 ± 6.38	**0.20**	30.31 ± 5.25	29.21 ± 5.86	**0.175**	32.95 ± 6.96	43.63 ± 7.61	**<0.0001**	0.47
1RM legs	187.28 ± 30.47	188.71 ± 31.25	**0.17**	182.87 ± 39.80	205.37 ± 67.07	**0.09**	175.00 ± 33.68	274.54 ± 64.00	**<0.0001**	0.75
HOMA	4.00 ± 1.54	3.01 ± 1.50	**0.08**	3.93 ± 2.61	2.92 ± 2.20	**0.066**	3.19 ± 1.53	2.13 ± 1.07	**0.029**	0.605
Adiponectin	13.17 ± 3.24	12.67 ± 2.19	**0.44**	12.33 ± 4.85	12.68 ± 4.24	**0.63**	14.15 ± 4.55	12.90 ± 3.55	**0.106**	0.66
Log sTNFR2	0.76 ±.03	0.75 ± 0.05	**0.97**	0.74 ± 0.10	0.69 ± 0.05	**0.15**	0.73 ± 0.15	0.75 ± 0.11	**0.65**	0.98
Log osteocalcin	0.24 ± 0.35	0.07 ± 0.56	**0.12**	0.32 ± 0.31	0.31 ± 0.43	**0.87**	0.02 ± 0.39	0.34 ± 0.19	**0.006**	0.63

Values are mean ± SD. AT, Adipose tissue; 1RM, one repetition maximum; sTNFR2, serum TNF receptor 2; WHR, waist-to-hip ratio. [a] ANOVA *P* for baseline characteristics among groups.

Diet

Diet was designed, in both D and D+RT groups, to reduce 500 kcal/d according to a previous evaluation of the habitual physical activity of each subject by accelerometry (TriTrac-R3D System, Software Version 2.04; Reining International, Madison, WI). This diet was designed to elicit a 0.5-kg weight loss per week. The C group was asked to maintain body weight. Throughout the 16-wk intervention period, body weight was recorded every 2 wk in both D and D+RT groups. Each subject of the intervention groups participated in a series of 1-h seminars (every 2 wk) wherein the dietitian taught proper food selection and preparation, eating behavior, control of portion sizes, and modification of binge eating and other adverse habits. The average compliance with the diet classes and the exercise sessions was above 95%.

RT program

The strength training program was a combination of heavy resistance and "explosive" strength training. The subjects were asked to report to the training facility two times per week for 16 wk to perform dynamic resistance exercise for 45–60 min per session. A minimum of 2 d elapsed between two consecutive training sessions. Each training session included two exercises for the leg extensor muscles (bilateral leg press and bilateral knee extension exercises), one exercise for the arm extensor muscle (the bench press), and four to five exercises for the main muscle groups of the body. Only resistance machines (Technogym, Gambettola, Italy) were used throughout the training period. In all the individual exercise sessions performed, one of the researchers was present to direct and assist each subject toward performing the appropriate work rates and loads. Lower and upper body maximal strength was assessed at wk 0 and 16 by using one repetition-maximum actions.

Magnetic resonance (MR)

The volumes of visceral and abdominal sc adipose tissue were measured by MR.MR imaging was performed with a 1Tmagnet (Magnetom Impact Expert; Siemens Corporation, New York, NY) using body coil. The subjects were examined in a supine position with both arms positioned parallel along the lateral sides of the body. The following procedures, in chronological order, were carried out: upper part of the body, subject repositioning; and lower part acquisition. We obtained a spoiled T1 weighted gradient-echo sequence with repetition time (TR) = 127 msec and echo time (TE) = 6 msec. Each half body volume was scanned using two stacks, each containing 10 contiguous 10-mm-thick slices. Each stack was acquired in 20 sec, and interleaved slice order was used. Afield of view of 500 mm was used, and all the stacks were acquired with breath holding. Depending on the height of the person, this resulted in a total of 31–40 axial images per person. The total investigation time was about 5 min.

MR imaging of both thighs was then obtained. T1-weighted sequence was used with a repetition time (TR) of 645 msec and a spin echo time (TE) of 20 msec. The field of view was 500 x 500 mm, and the matrix was 512 x 192. The slices were 10 mm thick, with no gap between the slices. The thighs were scanned using two stacks, each containing 15 contiguous 10-mm-thick slices; the scan was performed axially from articular boundary of lowest external femoral condyle. The images were retrieved from the scanner according to a DICOM (Digital Imaging and Communications in Medicine) protocol. The acquired axial MR images were transferred to an external personal computer running Windows XP. The level of each abdominal image was labeled using sagittal scout images, referred to discal level. We used a specially designed image analysis software (SliceOmatic 4.3, Tomovision Inc., Montreal, Canada) for quantitative analysis of the images.

Effects of moderate weight loss

To evaluate further the effect of moderate weight loss on circulating osteocalcin after weight loss, 20 Caucasian obese volunteers (9 males, 11 females; age range, 21 to 66 yr) attending the Endocrinology Department at the University Clinic of Navarra were recruited. Patients underwent a clinical assessment including medical history, physical examination, body composition analysis, and comorbidity evaluation, as well as nutritional interviews performed by a multidisciplinary consultation team. All subjects were nonsmokers. Patients with signs of infection were excluded. Obese patients were not receiving statins or any antidiabetic medication.

Type 2 diabetes mellitus was defined following the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus based on both fasting plasma glucose concentrations and plasma glucose 2 h after an oral glucose tolerance test.

Diet

Weight loss was achieved by prescription of a diet providing a daily energy deficit of 500-1000 kcal/d as calculated from the determination of the resting energy expenditure through indirect calorimetry (Vmax29; SensorMedics Corporation, Yorba Linda, CA) and multiplication by 1.4 as indicated for sedentary individuals to obtain the patient's total energy expenditure (Gomez-Ambrosi et al., 2007). This hypocaloric regime allows a safe and steady weight loss of 0.5-1.0 kg/wk when strictly followed and supplied 30, 54, and 16% of energy requirements in the form of fat, carbohydrates, and protein, respectively.

In this study, body weight was measured with a digital scale to the nearest 0.1 kg, height was measured to the nearest 0.1 cm with a Holtain stadiometer (Holtain Ltd., Crymych, UK), and body fat was estimated by air-displacement-plethysmography (Bod-Pod; Life Measurements, Concord, CA). Data for estimation of body fat by this plethysmographic method has been reported to agree closely with the traditional gold standard hydrodensitometry (underwater weighing) (Ginde et al., 2005).

The experimental design was approved, from an ethical and scientific standpoint, by the Hospital's Ethical Committees from all participant institutions in the three different studies, and volunteers gave their informed consent to participate in all the studies.

Analytical determinations

In all studies, blood samples were collected after an overnight fast in the morning to avoid potential confounding influences due to hormonal rhythmicity. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Intraassay and interassay coefficients of variation (CVs) were less than 4% for all these tests.

Measurements of serum adiponectin and plasma osteocalcin were centralized in a single laboratory. Osteocalcin was measured by an Enzyme Amplified Sensitivity Immunoassay (EASIA) kit (DRG Instruments GmbH, Marburg, Germany). Sensitivity of the method, the detection limit, defined as the apparent concentration two SD values above the average OD at zero binding, was 0.4 ng/ml, and the intra- and interassay CVs were less than 10%. Serum adiponectin levels were measured by a commercially available ELISA kit (Linco Research, St. Charles, MO). The intra- and interassay CVs were less than 8%. The lowest level of adiponectin that can be detected by this assay is 0.78 ng/ml. There was no cross-reactivity with other cytokines or hormones.

In the cross-sectional study, serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Brea, CA). Serum insulin levels were measured in duplicate by monoclonal immunoradiometric assay [IRMA or enzyme-amplified sensitivity immunoassay (EASIA), Medgenix Diagnostics, Fleunes, Belgium]. Intraassay and interassay CVs were similar to those previously reported (Gubern et al., 2006; Fernandez-Real et al., 2006).

In the study of the effects of slight weight loss with or without regular physical activity, resting blood samples were drawn at wk 0 and 16. The subjects reported to the laboratory and sat quietly for 10–15 min before giving a blood sample. Basal glycemia was analyzed using an enzymatic hexokinase method (Roche Diagnostics, Mannheim,

Germany). Serum insulin levels were measured in duplicate by monoclonal immunoradiometric assay (INSI-CTK Irma; DiaSorin, Madrid, Spain). Intraassay and interassay CVs were less than 5%. To estimate insulin resistance, the homeostatic model assessment (HOMA) index was calculated as fasting insulin concentration (µU/ml) x fasting glucose concentration (mmol/liter)/22.5.

In the study of the effects of moderate weight loss, plasma glucose was analyzed by an automated analyzer (Roche/Hitachi Modular P800, Basel, Switzerland) as previously described (Gomez-Ambrosi et al., 2006). Insulin was measured by means of an enzyme-amplified chemiluminescence assay (Immulite, Diagnostic Products Corp., Los Angeles, CA). An indirect measure of insulin sensitivity was calculated from the fasting plasma glucose and insulin concentrations by using the quantitative insulin sensitivity check index (Katz et al., 2000; Gomez-Ambrosi et al., 2002).

Statistical analysis

Pearson's correlation was used to evaluate the associations among continuous variables. Those parameters that did not follow a normal distribution were log-transformed. Comparisons of quantitative variables among groups were made using ANOVA. Multiple regression models were used to assess the influence of osteocalcin on insulin sensitivity, taking into account potential factors associated with this variable such as BMI and waist-to-hip ratio. The models were built in a customized way by means of the enter method, which takes into account the simultaneous influence of all variables; this is a procedure for variable selection in which all variables in a block are entered in a single step. We chose this conservative method given the relatively low number of subjects studied. Furthermore, regression diagnostics were checked by using the inverse normal plot of the residuals and plots of the residuals against the fitted values. Influence analyses were also performed by means of Cook's D. Moreover, the problems of colinearity were solved by centering some of the variables. The statistical package used was Stata v.8 (StataCorp, College Station, Texas). Levels of statistical significance were set at $P < 0.05$.

Results

Cross-sectional study

We evaluated 149 men, aged 50.2 ± 11.7 yr, with mean BMI 27.6 ± 3.5 kg/m^2, a median insulin sensitivity of 2.35*10^{-4}*min^{-1}*mU/liter (interquartile range, 1.23–3.2), and a median insulin secretion of 359.1 mU/liter*min^{-1} (interquartile range, 186.6–511.1). Median circulating osteocalcina was 6.1 (interquartile range, 3.5-8.1) ng/ml. Osteocalcin was positively linked to insulin sensitivity among these 149 otherwise healthy men (r = 0.23; *P* = 0.006; Fig. 1). The statistical power of this association was 81% (α = 0.05, β = 0.20).

Interestingly, the association appeared stronger in lean subjects (BMI <25 kg/m^2) (Fig. 1, *enclosed legend*), although the comparison between slopes did not reach statistical significance (*P* = 0.3). Among lean subjects, osteocalcin was the most significant factor impacting on insulin sensitivity (45% of its variance), even after accounting for the effects of age, BMI, and waist diameter in a multiple linear regression analysis. Among lean subjects, we also observed a positive association between circulating osteocalcin and insulin secretion (r = 0.41; *P* = 0.03) and the insulin disposition index (r = 0.43; *P* = 0.02). Circulating adiponectin was available in 137 of these subjects and showed a positive association with osteocalcin (r = 0.19; *P* = 0.02).

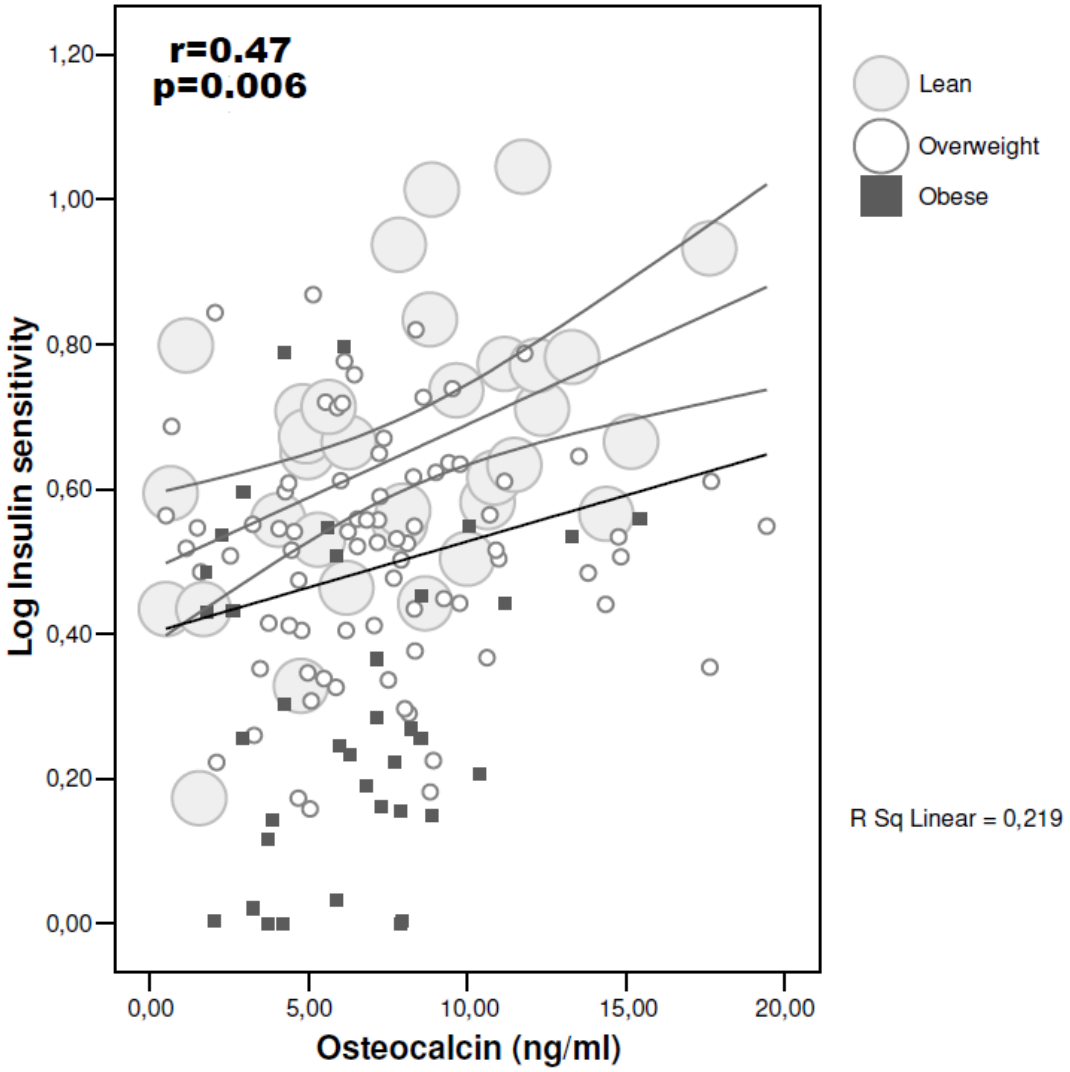

Figure 1. Linear relationship between circulating osteocalcin and insulin sensitivity in 149 men of the cross-sectional study (single line and correlation coefficient) and in lean men (95% confidence interval for the mean and correlation coefficient in the *upper left corner*). Lean subjects are defined as having a BMI less than 25 kg/m^2; overweight as BMI at least 25 but less than 30 kg/m^2; and obese as BMI greater than 30 kg/m^2. We used the nontransformed value of osteocalcin because the Kurtosis and skewness values were closer to 0 than the log-transformed value in this population.

Effects of weight loss on circulating osteocalcin

Given this cross-sectional association, we also aimed to evaluate the effects of weight loss and physical exercise on circulating osteocalcin. Twenty-six obese women were randomized to follow a structured RT program and a hypocaloric diet (D+RT; n=11), compared with only a hypocaloric diet (D; n = 8) and a control group (C; n = 7), in which no action was taken. Baseline characteristics were similar in the three groups (Table 1). Serum osteocalcin was negatively associated with insulin resistance (HOMA value, r = - 0.43; *P* = 0.03) and positively with circulating adiponectin (r = 0.45; *P* = 0.02). Baseline osteocalcin was not significantly associated with total fat (r = - 0.27; *P* = 0.18), visceral fat (r = - 0.24; *P* = 0.2), or fasting triglycerides (r = 0.01; *P* = 0.9).

After 16 wk, no significant changes were observed in the different parameters evaluated in the control group (Table 1). In the diet group, a 7.3% weight loss was accompanied by reduced total and visceral fat mass and thigh muscle mass. Insulin sensitivity tended to improve. No significant changes in serum osteocalcina concentrations were observed. In the diet plus RT group, despite the fact that weight loss was of similar magnitude (- 8.7%), osteocalcin increased significantly (Fig. 2). The statistical power of this change in serum osteocalcin was 96% (α = 0.05, β = 0.20). This was observed in parallel with reduced visceral fat mass, unchanged thigh muscle mass, and increased leg strength and force (Fig. 3). In all subjects as a whole (n = 26), the change in circulating osteocalcin was significantly associated with the change in visceral fat (r = - 0.59; *P* = 0.001). However, in the subgroup analysis, this relationship was not significant in the control group (r = 0.25; *P* = 0.6) or in the diet group (r = - 0.40; *P* = 0.3).

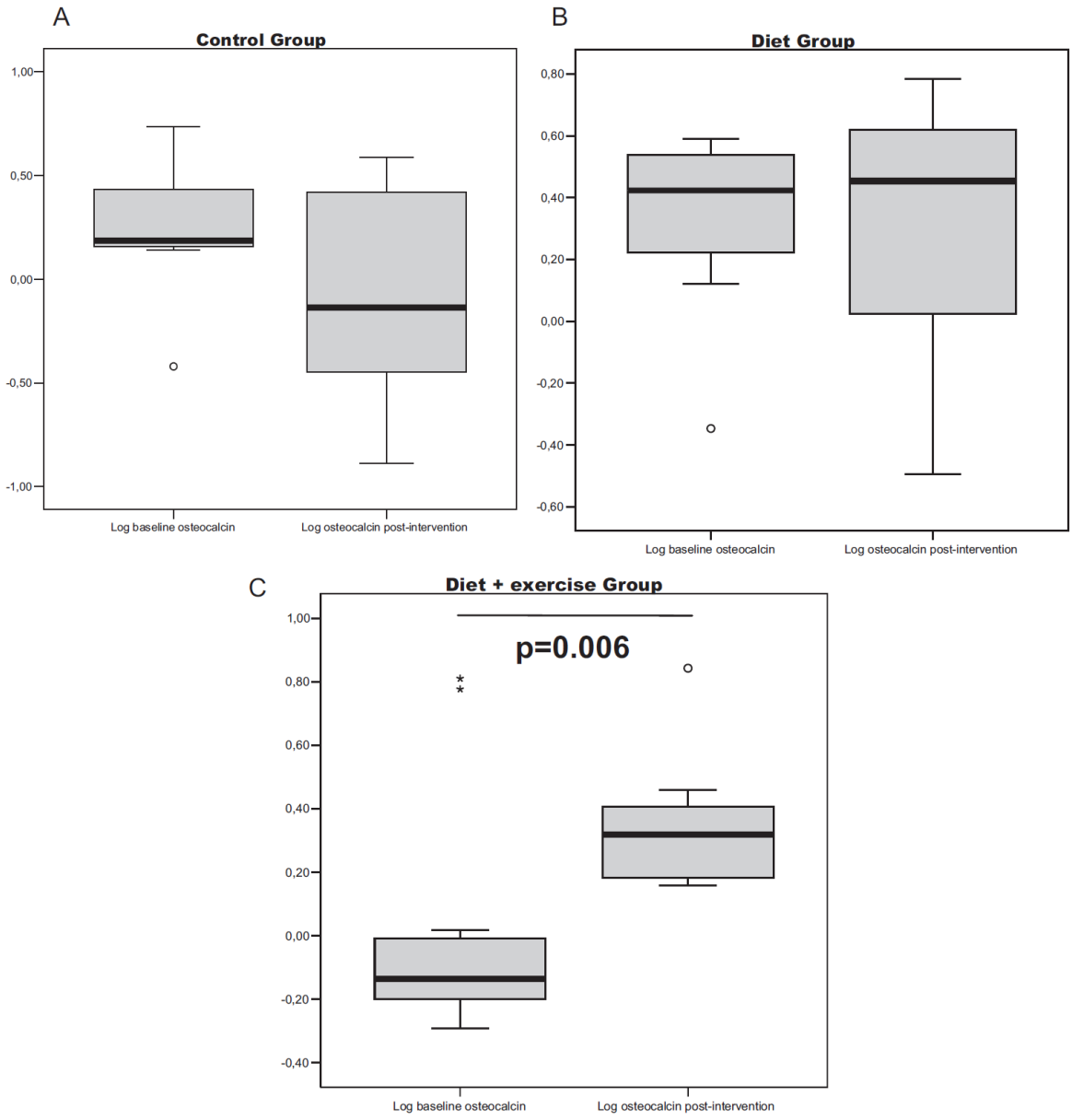

Figure 2. Changes in circulating osteocalcin in obese women (n = 26) enrolled in the slight weight loss intervention. A, Control subjects, in whom no action was taken (n = 7); B, diet-induced weight loss (n = 8); C, weight loss induced by diet plus regular exercise (n = 11). *Edges of gray box* indicate 25th and 75th percentiles. *Horizontal line in middle of box* depicts median. *Whiskers* indicate minimum/maximum values.

In the intervention groups (both D and D+RT, n = 19) the post intervention serum levels of osteocalcin were associated with both insulin resistance (r = - 0.49; *P* = 0.03) and fasting triglycerides (Fig. 3D). However, we did not observe significant relationships between the change in circulating osteocalcina and the change in fasting triglycerides. In all subjects as a whole (n = 26), the change in visceral fat was the single parameter that best predicted the change in serum osteocalcin, once age, BMI, and insulin sensitivity changes were controlled for (*P* = 0.002) (Table 2). When the change in leg muscle strength was introduced in the model, both variables contributed to 30% of the variance in changing serum osteocalcin (Table 2).

Table 2. Multiple linear regression analysis with the change in circulating osteocalcin as dependent variable in the slight weight loss study

Change in osteocalcin	β	*P* value	β	*P* value
Age	-0.17	0.34	-0.009	0.85
Change in BMI	-0.12	0.57	-0.19	0.87
Change in insulin sensitivity	-0.10	0.58	-0.12	0.94
Change in visceral fat	-0.52	0.002	-0.46	0.005
Change leg muscle strenght			0.51	0.002
Ajusted R^2	0.24		0.30	

We then questioned whether the magnitude of weight loss was insufficient to impact on circulating osteocalcin concentration. To this end, we studied subjects that underwent a more prolonged period of treatment, achieving a mean weight loss of -16.8%. The characteristics of these subjects are shown in Table 3. Baseline osteocalcin was not significantly associated with insulin sensitivity (r = 0.25, *P* = 0.3) and tended to be negatively associated with total fat mass (r = - 0.33, *P* = 0.1). In these subjects, mean osteocalcin was increased after weight loss (Fig. 4).

The statistical power of this change in serum osteocalcin was 78% ($\alpha = 0.05$, $\beta = 0.20$). However, we found no associations between the change in serum osteocalcin and changing insulin resistance, circulating adiponectin, or triglycerides (r values between - 0.32 and 0.26, $P > 0.1$). Interestingly, among men, the decrease in waist diameter tended to be associated with the increase in osteocalcin but this was not statistically significant (r = - 0.51, $P = 0.1$, n = 9).

Table 3. Effect of moderate weight loss in obese patients after a dietary intervention

	Before weight loss	**After weight loss**	
n	**20**	**20**	***P***
Age (yr)	43.4 ± 9.4	44.1 ± 9.2	
Body weight (kg)	109 ± 7	91 ± 4	< 0.001
BMI (kg/m2)	38.0 ± 2.0	32.0 ± 2.1	< 0.0001
Body fat (%)	45.9 ± 2.0	38.6 ± 2.1	< 0.0001
Waist circumference (cm)	115 ± 4	103 ± 3	
WHR	0.95 ± 0.02	0.94 ± 0.02	0.175
Glucose (mmol/liter)	5.6 ± 0.2	5,1 ± 0,1	0.043
Insulin (mU/ml)	21.4 ± 5.2	11.8 ± 1.7	0.112
QUICKI	0.312 ± 0.009	0.341 ± 0.012	0.031
Leptin (ng/ml)	39.2 ± 12.1	25.4 ± 10.2	0.044

To convert glucose to milligrams per deciliter, divide by 0.05551. WHR, Waist-to hip ratio.

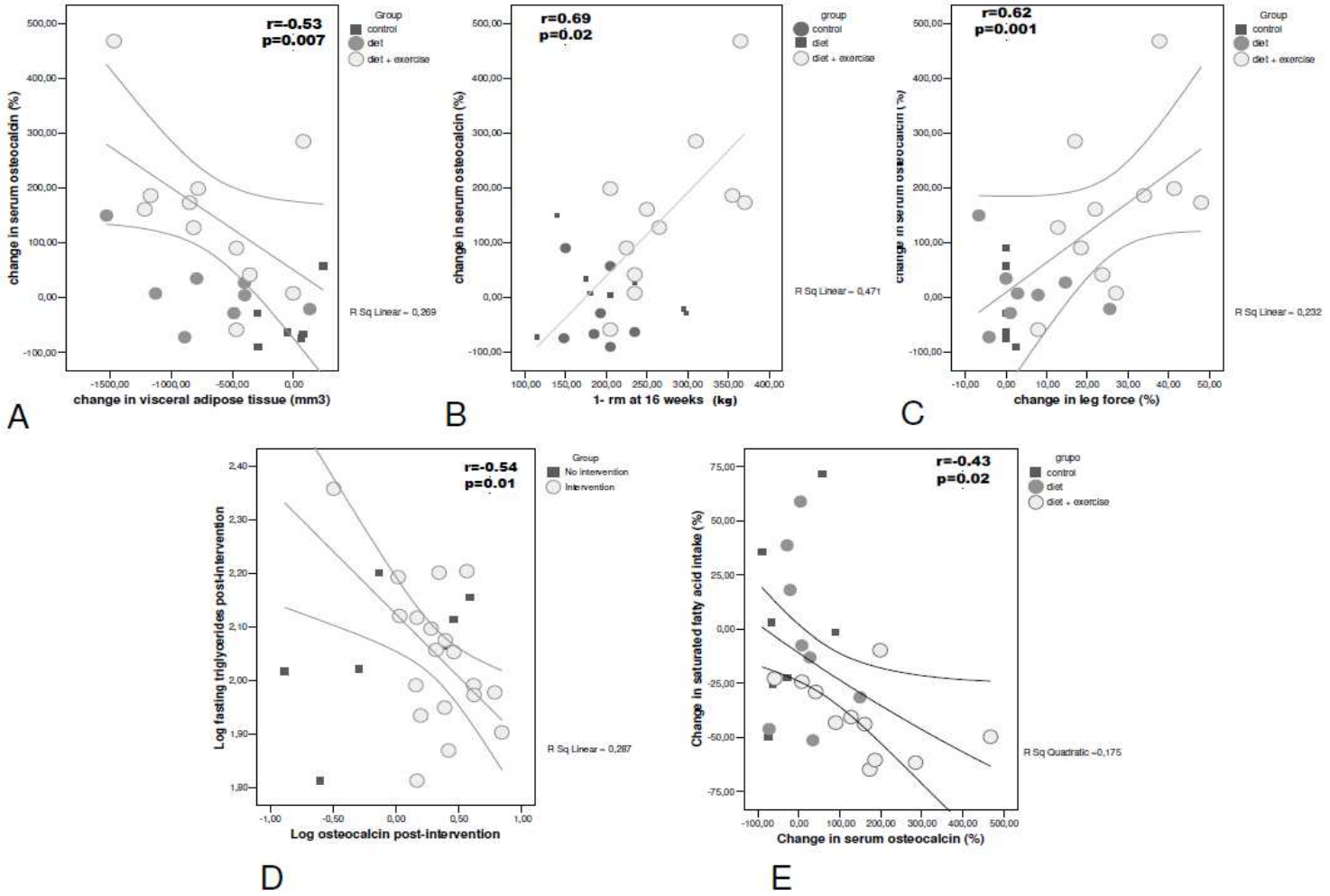

Figure 3. Factors associated with changing osteocalcin in obese women (n =26) enrolled in the slight weight loss intervention. Variables associated with the change in serum osteocalcin: A, change in visceral adipose tissue; B, absolute leg force (1-rm); and C, changes in leg force. D, Log osteocalcin was associated with fasting triglycerides only after weight loss in the intervention group as a whole: diet only and diet + RT groups together. E, Relationship between the change in saturated fatty acid intake and change in osteocalcin after weight loss. The coefficients shown only represent *open circles* in all panels.

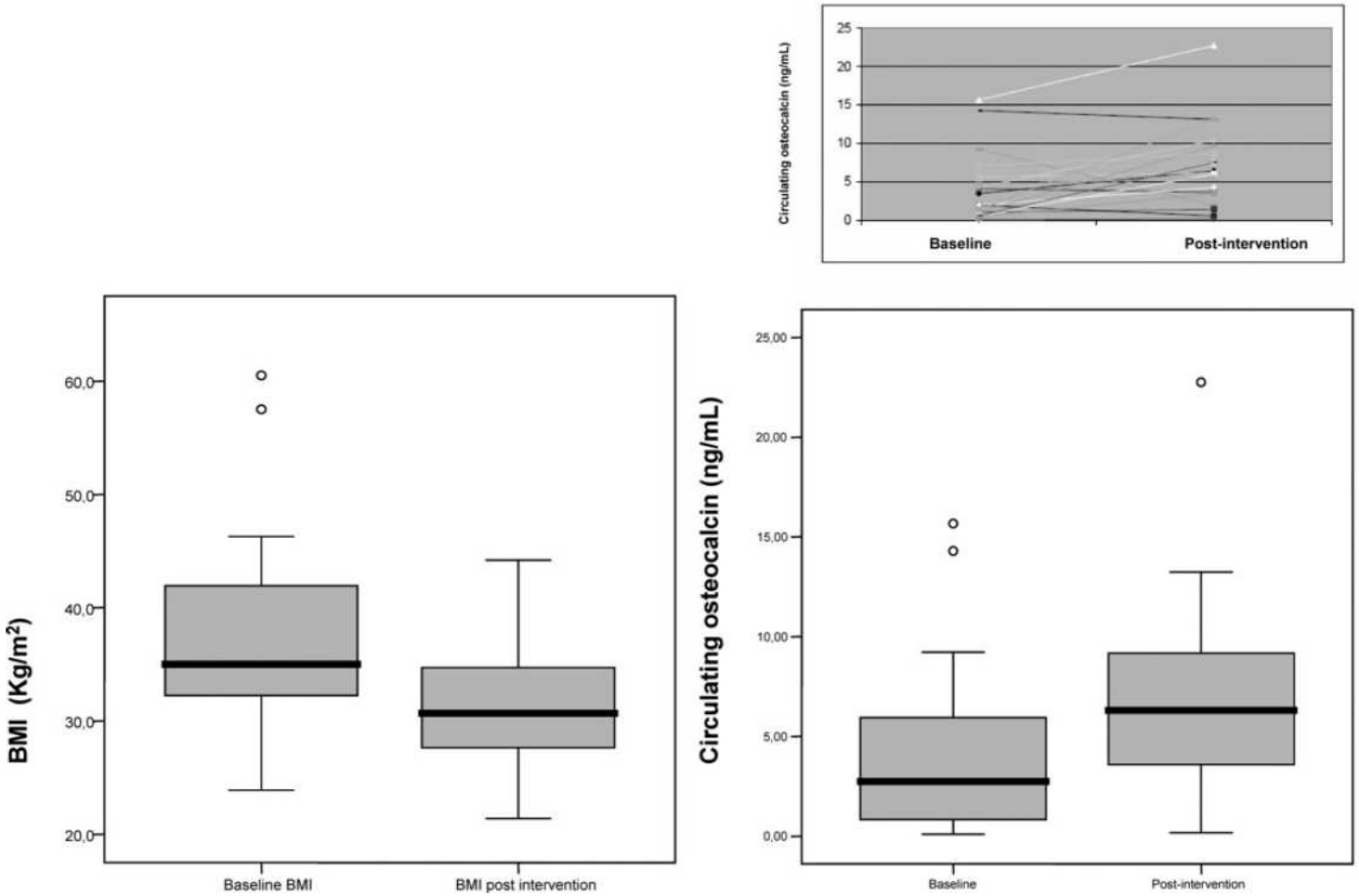

Figure 4. Obese men and women (n = 20) enrolled in the moderate weight loss intervention. Changes in BMI (*left panel*) and osteocalcin (*right panels*). *Edges of gray box* indicate 25th and 75th percentiles. *Horizontal line in middle of box* depicts median. *Whiskers* indicate minimum/maximum values.

Discussion

Summarizing the associations with insulin sensitivity, we found that fasting osteocalcin was associated with insulin sensitivity cross-sectionally in 149 men and in 26 obese women with a wide range of BMI, but not in 20 obese men and women with a low BMI range. The change in circulating osteocalcin was significantly associated with the change in insulin sensitivity in the slight weight loss group (both D and D+RT groups, r = - 0.50; *P* = 0.02; n = 19) but not in the moderate weight loss group.

The main findings of this study are: 1) the association between circulating osteocalcin and insulin sensitivity; 2) the association between osteocalcin and insulin secretion and insulin disposition index among lean men; 3) the observation that slight diet-induced weight loss *per se* did not lead to significant changes in serum osteocalcin concentration; 4) a weight loss of similar magnitude plus regular physical activity resulted in increased circulating osteocalcin; 5) the increase in serum osteocalcin concentration was associated with changes in visceral fat mass and, importantly, with changes in leg muscle strength; and 6) moderate weight loss also resulted in increased osteocalcin but without relationship to insulin sensitivity or fasting triglycerides.

In parallel with the findings described in experimental animals (Lee et al., 2007), we found that the baseline circulating osteocalcin concentration was associated with insulin sensitivity and secretion and circulating adiponectin (lean and obese men and women). After slight weight loss, osteocalcin correlated with fasting triglycerides in obese women.

Osteocalcin knockout mice showed increased visceral fat (Lee et al., 2007). In our study, serum osteocalcin significantly increased in parallel to reduced visceral fat mass after diet and regular exercise in obese women .In the slight weight loss study, baseline osteocalcina was not significantly different among groups (Table 1). By chance, mean values of osteocalcin were higher in the D+RT group compared with the other groups. This difference was not statistically significant, even if we compared this group to the remaining subjects (*P* = 0.3). In fact, baseline mean log osteocalcina in the D+RT group was very similar to that present in the control group after follow-up.

It could be argued that the change in insulin resistance and fat mass was, to some extent, similar in the D group and the D+RT group (the change in insulin resistance in the D group did not reach statistical significance). However, the most striking differences between these groups were the change in leg force, which was strongly related with the change in serum osteocalcin in univariant and multivariate analysis. Thigh muscle mass was unchanged after diet plus regular exercise in association with increased leg strength and force. In contrast, in the D group, thigh muscle mass was significantly decreased after 16wk (Table 1). In a previous study, as little as a 5%weight loss plus regular exercise also led to increased osteocalcin (Hinton, Rector, & Thomas, 2006).

There is a considerable body of evidence gathered from studies over the past half century indicating that regular physical activity reduces the risk of cardiovascular disease. Regular physical activity is particularly beneficial to individuals with insulin-resistant conditions, such as obesity, type 2 diabetes, and the metabolic syndrome (Gill & Malkova, 2006). Although the postexercise increase in muscle insulin sensitivity has been characterized in considerable detail, the basic mechanisms underlying this phenomenon remain a mystery (Holloszy, 2005). Like exercise, stimulation of muscles to contract *in situ* results in an increase in insulin sensitivity (Cartee & Holloszy, 1990). In contrast, stimulation of muscles immersed in Krebs-Henseleit-bicarbonate buffer to contract *in vitro* does not result in enhanced insulin sensitivity (Holloszy, 2005; Cartee & Holloszy, 1990; Gao, Gulve, & Holloszy, 1994). The explanation for this finding was that an as-yet-unidentified serum protein must be present during contractile activity in order for the increase in insulin sensitivity to occur (Gao et al., 1994). The mechanism responsible for the permissive effect of serum has not yet been elucidated. Also, like contractile activity, the effects of exercise, hypoxia, and 5-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (AICAR, a pharmacological activator of AMPK) on insulin sensitivity require the presence of serum during the treatment period (Fisher, Gao, Han, Holloszy, & Nolte, 2002).

We propose that osteocalcin represents this missing link in the exercise-induced improvement in insulin sensitivity. Exercise is thought to act on the skeleton through muscle pull, producing strains on the skeleton that are perceived by bone cells. We observed that a change in leg force was associated with a change in serum osteocalcin concentration (Fig. 3C). Exercise may stimulate increased secretion of osteocalcin by

bone that positively impacts on insulin secretion and insulin sensitivity. We further propose that diet-induced weight loss and exercise lead to changes in insulin sensitivity by different mechanisms. Although prolonged dieting induced changes in circulating osteocalcin, the magnitudes of these changes were not associated with the metabolic profile.

Moderate, but not slight, weight loss led to significantly increased circulating osteocalcin levels, possibly indicating only increased bone turnover. This supports previous findings in which osteocalcin increased after diet-induced weight loss (Viapiana et al., 2007; Bowen, Noakes, & Clifton, 2004). As previously suggested, the overall increase in bone turnover may be unfavorable for maintaining bone mass after diet induced weight loss (Viapiana et al., 2007). A study of the ratio of undercarboxylated osteocalcin to total osteocalcin after diet and after exercise might provide the clue for the study of their association with insulin sensitivity.

Not all reports on the effects of weight loss or exercise on circulating osteocalcin levels are concordant. Villareal *et al.* (2006) reported no significant changes in osteocalcin levels with weight loss due to caloric restriction. However, no obese subjects were included in this study (Villareal et al., 2006). Interestingly, these authors found that exercise was associated with preservation of bone mineral density that could be mediated through exercise-induced bone loading (Villareal et al., 2006). We here suggest that bone loading could elicit increased osteocalcin production. On the other hand, weight gain also led to increased osteocalcin in patients with anorexia nervosa, possibly indicating, again, increased bone remodeling (Ricci et al., 1998).

Several studies have previously demonstrated that serum osteocalcina was reduced in patients with type 2 diabetes (Bouillon et al., 1995; Pietschmann et al., 1988; Pedrazzoni et al., 1989; Rico et al., 1989). To our knowledge, this would be the first study evaluating osteocalcina in association with insulin sensitivity in humans, and the first study showing exercise-induced changes in circulating osteocalcina in association with insulin sensitivity, visceral fat mass, and muscle strength. However, the lack of data on undercarboxylated osteocalcin is a limitation of this study. Lee *et al.* (2007) reported that undercarboxylated osteocalcin was the active form of osteocalcin in rodent models.

In summary, our findings suggest that osteocalcin might be an active regulator of insulin sensitivity by bone.

Acknowledgments

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Capítulo 3

Circulating soluble transferrin receptor concentration decreases after exercise-induced improvement of insulin sensitivity in obese individuals

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Circulating soluble transferrin receptor concentration decreases after exercise-induced improvement of insulin sensitivity in obese individuals

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Abstract

Background: Circulating soluble transferrin receptor (sTfR) has been recently found to be associated negatively with insulin sensitivity.

Objective: To evaluate circulating sTfR concentration after changing insulin sensitivity in obese individuals.

Design: Circulating sTfR concentration was evaluated after diet-induced weight loss in obese women (diet (D) group, n=8); after diet-induced weight loss plus resistance training (D+RT group, n=11); and after follow-up without weight loss (control (C) group, n=7).

Results: After 16 weeks, insulin sensitivity (HOMA (Homeostasis Model Assessment) value) significantly improved in parallel to weight loss (- 7.3%) and reduced total fat mass (evaluated using magnetic resonance imaging) in the D group. Thigh muscle mass decreased significantly (P=0.03). Serum sTfR concentration did not change significantly. In the D+RT group, weight loss (- 8.7%) and improvement of insulin sensitivity were of similar magnitude. Thigh muscle mass was preserved (P=0.8). Serum sTfR concentration decreased significantly (P=0.001). Interestingly, higher the thigh muscle

volume after weight loss, higher the decrease in circulating sTfR concentration. We also found that higher the increases in leg force at week 16, higher the decrease in circulating sTfR concentration in all individuals as a whole. No significant changes were observed in insulin sensitivity, sTfR concentration or thigh muscle mass in the C group.

Conclusion: These findings suggest a long-term regulation of serum sTfR concentration by exercise-induced improvement of insulin sensitivity in obese individuals.

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Keywords: weight loss; iron; transferrin receptor; insulin sensitivity

Introduction

Insulin is an anabolic hormone that stimulates the cellular uptake of many nutrients, including amino acids, cations and iron. Iron circulates in a form bound to transferrin and is taken up from the blood by a high-affinity specific transferrin receptor (TfR) (Finch, 1994; Davis, Corvera, & Czech, 1986; Yokomori et al., 1991). Insulin is known to cause a rapid and marked stimulation of iron uptake by different cell types, redistributing TfRs from an intracellular membrane compartment to the cell surface (Finch, 1994; Davis et al., 1986; Yokomori et al., 1991). Iron uptake by insulin parallels its effects on glucose transport (Tanner & Lienhard, 1989).

As a result of the externalization of TfR during the endocytic cycle, a soluble form of TfR (sTfR) can be detected in serum. Circulating sTfR concentrations are proportional to cellular expression of the membrane-associated TfR (Baynes & Cook, 1996). Serum sTfR concentration is closely related to cellular iron demands.

In an earlier paper, we provided evidence according to which serum sTfR concentration was linked to insulin resistance (Fernandez-Real et al., 2007). We observed that the lower the insulin sensitivity, the higher the sTfR concentration. Furthermore, insulin sensitivity, independently of age, obesity and iron status, contributed independently to the variance of circulating sTfR concentration (Fernandez-Real et al., 2007). As insulin injection increases serum sTfR concentration in animal models (Clairmont & Czech, 1990), hyperinsulinaemia may contribute to the inappropriately high sTfR concentration detected in individuals with altered glucose tolerance.

Serum sTfR concentrations seemed to be not acutely regulated by insulin, were given the unaltered serum sTfR levels during an oral glucose tolerance test (Fernandez-Real et al., 2007). Recently, resistance training (RT) has been shown to improve insulin sensitivity in individuals with type 2 diabetes (Ibanez et al., 2005). Taking into account these observations, 26 obese women were randomized to follow a structured RT program and a hypocaloric diet (n=11), compared with hypocaloric diet alone (n=8) and a group of individuals in whom no action was implemented (n=7). We aimed to

evaluate whether the improvement of insulin sensitivity affects serum sTfR concentration.

Subjects and methods

A total of 26 sedentary, non-smoking, obese (body mass index, 30-40 kg m^{-2}) women, aged 40-60 years, participated in this study. Before inclusion in the study, all candidates were thoroughly screened. None of the participants received any medication, and blood disorders, including anemia, were excluded in all participants. This project was approved by the ethical committee of the Regional Health Department. Participants were randomized to into three groups: a Control group (C; n=7); a diet group (D; n=8) with a caloric restriction of 500 kcal per day; and a diet and resistance training group (D+RT; n=11) with the same caloric restriction as group D and a 16-week supervised resistance training program of 2 sessions per week. During the 16 weeks of the study, the participants maintained their customary recreational physical activities (for example, walking). The baseline characteristics of the participants are presented in Table 1. Three women in the control group, three in the D group and four in the D+RT group were post-menopausal. In the remaining women, the participants were studied in the follicular phase (days 3-9 of the menstrual cycle).

Diet

Diet was designed in both D and D+RT groups to reduce 500 kcal per day, according to an earlier evaluation of the habitual physical activity of each participant by an accelerometer (TriTrac-R3D System, Software Version 2.04; Madison, WI, USA). This diet was designed to elicit a 0.5 kg weight loss per week. The C group was asked to maintain body weight. Throughout the 16-week intervention period, the body weight was recorded every 2 weeks in both D and D+RT groups. Each participant of the intervention groups participated in a series of 1-h seminars (every 2 weeks), wherein the dietician taught proper food selection and preparation, eating behavior, control of portion sizes, and modification of binge eating and other adverse habits. The average compliance with the diet classes and the exercise sessions was above 95%.

Resistance training program

The strength training program was a combination of heavy resistance and 'explosive' strength training. The participants were asked to report to the training facility twice per week for 16 weeks to perform dynamic resistance exercises, for 45-60 min per session. A minimum of 2 days elapsed between two consecutive training sessions. Each training session included two exercises for the leg extensor muscles (bilateral leg press and bilateral knee extension exercises), one exercise for the arm extensor muscle (the bench press) and four to five exercises for the main muscle groups of the body. Only resistance machines (Technogym, Gambettola, Italy) were used throughout the training period. In all the individual exercise sessions performed, one of the researchers was present to direct and assist each participant toward performing the appropriate work rates and loads. Lower and upper body maximal strength was assessed at weeks 0 and 16 by using 1-RM (repetition maximum) actions.

Magnetic resonance

The volumes of visceral and abdominal subcutaneous adipose tissue were measured by magnetic resonance. Magnetic resonance imaging was carried out with a 1T magnet (Magnetom Impact Expert, Siemens) using body coil. The participants were examined in a supine position with both arms positioned parallel along the lateral sides of the body. The procedure was carried out on the upper part of the body, followed by lower part acquisition after repositioning of the participant. We obtained a spoiled T1-weighted gradient-echo sequence with repetition time of 127 ms and echo time of 6 ms. Each half-body volume was scanned using two stacks, each containing 10 contiguous 10mm thick slices. Each stack was acquired in 20 s and interleaved slice order was used. An FOV (field of view) of 500 mm was used, and all the stacks were acquired with breath holding. Depending on the height of the person, this resulted in a total of 31-40 axial images per person. The total investigation time was about 5 min.

Magnetic resonance imaging of the both thighs was then obtained. T1-weighted sequence was used with a repetition time of 645 ms and a spin echo time of 20 ms. The field of view was 500 x 500 mm and the matrix was 512 x 192. The slices were 10 mm thick, with no gap between the slices. The thighs were scanned using two stacks,

each containing 15 contiguous 10 mm thick slices; the scan was performed axially from articular boundary of lowest external femoral condyle. The images were retrieved from the scanner according to a DICOM (Digital Imaging and Communications in Medicine) protocol. The acquired axial magnetic resonance images were transferred to an external personal computer running Windows XP. The level of each abdominal image was labeled using sagittal scout images, referred to as the distal level. We used a specially designed image analysis software (SliceOmatic 4.3, Tomovision Inc, Montreal, Canada) for quantitative analysis of the images.

The experimental design was approved, from an ethical and scientific standpoint, by the Hospital Ethics Committee and volunteers gave their informed consent to participate in the study.

Analytical determinations

In all studies, blood samples were collected after an overnight fast in the morning to avoid potential confounding influences due to hormonal rhythmicity. Total serum triglycerides were measured through the reaction of glycerol-phosphate oxidase and peroxidase. Intra-assay and interassay coefficients of variation were <4%.

Serum sTfR was measured using a double monoclonal sandwich enzyme immunoassay (ELISA) (Biovendor, Palacheho tr., Brno, Czech Republic). Intra- and inter-assay coefficients of variation were <4.5%.

Serum adiponectin levels were measured by a commercially available enzyme-linked immunoassay, ELISA kit (LINCO Research, Missouri, USA). The intra- and inter-assay coefficients of variation were <8%. The lowest level of adiponectin that can be detected by this assay is 0.78 ng ml^{-1}. No significant cross-reactivity with other cytokine or hormone molecules was detected.

Resting blood samples were drawn at weeks 0 and 16. The participants reported to the laboratory and sat quietly for 10-15 min before giving a blood sample. Basal glycemia was analyzed using an enzymatic hexokinase method (Roche Diagnostics, Mannheim, Germany). Serum insulin levels were measured in duplicates by monoclonal immunoradiometric assay (INSI-CTK Irma, DiaSorin, Madrid, Spain). Intra-assay and

inter-assay coefficients of variation were >5%. To estimate insulin resistance, the HOMA (Homeostasis Model Assessment) index was calculated as fasting insulin concentration (μU ml^{-1}) x fasting glucose concentration (mmol l^{-1})/22.5.

Statistical analysis

Descriptive results of continuous variables are expressed as mean (s.d.). Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test and then variables were given a base-10 log transformation, if necessary. These parameters (HOMA value, triglycerides, sTfR) were analyzed on a log scale and tested for significance on that scale. The anti-log-transformed values of the means (geometric mean) are reported in Table 1. The relation between variables was tested using Pearson's test (with log-transformed values) and multiple linear regression analysis. We used paired and unpaired t-tests and ANOVA (analysis of variance) test with Bonferroni's post hoc analysis for comparisons of quantitative variables. The statistical analyses were carried out using the program SPSS (v.12.0 for Windows; Chicago, IL, USA).

Table 1. Antropometrical and biochemical characteristics of study subjects

	Control			**Diet**			**Diet + exercise**			***P*[a]**
n	**7**			**8**			**11**			
Age	**51.5 ± 7.2**			**51.6 ± 6.6**			**47.7 ± 6.5**			0.36
	Pre	**Post**	***P***	**Pre**	**Post**	***P***	**Pre**	**Post**	***P***	-
IBM (kg m^{-2})	34.58 ± 3.90	34.38 ± 3.55	**0.52**	34.11 ± 3.89	31.62 ± 3.92	**0.009**	34.27 ± 2.78	31.33 ± 2.00	**<0.0001**	0.96
Weight (kg)	88.68 ± 12.74	88.15 ± 11.74	**0.506**	87.22 ± 18.01	80.81 ± 16.64	**0.012**	88.08 ± 12.63	80.36 ± 9.11	**<0.0001**	0.98
WHR: Wais-to-hip-ratio	0.91 ± 0.01	0.89 ± 0.02	**0.26**	0.93 ± 0.02	0.91 ± 0.03	**0.08**	0.92 ± 0.03	0.88 ± 0.03	**0.026**	0.126
Thigh muscle volumen (cm^3)	46667 ± 7736	46465 ± 7167	**0.74**	48966 ± 10883	47226 ± 11609	**0.039**	46867 ± 9021	46470 ± 8839	**0.87**	0.87
Thigh fat volumen (cm^3)	88332 ± 19339	89127± 19090	**0.59**	87641 ± 21716	72697 ± 18365	**0.004**	104923 ± 16539	87479 ± 14200	**<0.0001**	0.11
Visceral adipose tissue (cm^3)	33708 ± 1228	3329 ± 1187	**0.65**	3243 ± 1085	2557 ± 1171	**0.007**	3211 ± 1232	2528 ± 1039	**0.0001**	0.96
Total adipose tissue (cm^3)	16833 ± 4183	16623 ± 3785	**0.53**	16973 ± 4787	13333 ± 4840	**0.001**	18205 ± 3337	13810 ± 2201	**<0.0001**	0.73
Arm force (1-RM, kg)	34.28 ± 6.56	34.81 ± 6.38	**0.20**	30.31 ± 5.25	29.21 ± 5.86	**0.175**	32.95 ± 6.96	43.63 ± 7.61	**<0.0001**	0.47
Leg force (1-RM, kg)	187.28 ± 30.47	188.71 ± 31.25	**0.17**	182.87 ± 39.80	205.37 ± 67.07	**0.09**	175.00 ± 33.68	274.54 ± 64.00	**<0.0001**	0.75
HOMA	4.00 ± 1.54	3.01 ± 1.50	**0.08**	3.93 ± 2.61	2.92 ± 2.20	**0.066**	3.19 ± 1.53	2.13 ± 1.07	**0.029**	0.605
Fasting Triglycerides (mg per 100 ml)	127 ± 31.4	117.4 ± 30.6	**0.15**	131.3 ± 44.3	140.2 ± 45.8	**0.38**	109.1 ± 29.7	99.4 ± 22.2	**0.26**	0.35
Adiponectin	13.1 ± 3.2	12.6 ± 2.1	**0.44**	12.3 ± 4.8	12.6 ± 4.2	**0.63**	14.1 ± 4.5	12.9 ± 3.5	**0.106**	0.66
Soluble transferrin receptor (ng ml^{-1})	1.56 ± 0.3	1.49 ± 0.3	**0.39**	1.56 ± 0.4	1.46 ± 0.3	**0.23**	1.51 ± 0.5	1.27 ± 0.4	**0.001**	0.72

Abbreviations: BMI, body mass index, HOMA, Homeostasis Model Assessment; 1-RM, one-repetition maximum. [a] ANOVA *P* for baseline characteristics among groups.

Results

Baseline characteristics were similar in the three groups (ANOVA *P*, Table 1). Circulating sTfR concentration correlated positively with HOMA value in all participants as a whole (r = 0.36, *P* = 0.04).

Diet led to weight loss (- 7.3%) and reduced total fat mass, and thigh muscle and adipose mass in the D group. Insulin sensitivity (HOMA value) tended to improve. Serum sTfR concentration did not change significantly (Figure 1). In the D+RT group, weight loss (- 8.7%) and improvement of insulin sensitivity were of similar magnitude. In contrast to the D group, thigh muscle mass was preserved, and leg strength and force increased significantly (Figure 2). In this D+RT group, serum sTfR concentration decreased significantly (Figure 1).

No significant changes were observed in the different parameters evaluated (Table 1) in the C group, although HOMA value tended to decrease, possibly in the context of observation.

Interestingly, higher the thigh muscle volume, higher the decrease in circulating sTfR (Figure 2). Similarly, higher the change in leg force at weeks 8 and 16 (Figure 2) and higher the absolute value of arm force (panel d, Figure 2), higher the decrease in circulating sTfR in all participants as a whole. There was no significant relationship between sTfR concentration and adiponectin or adiponectin changes.

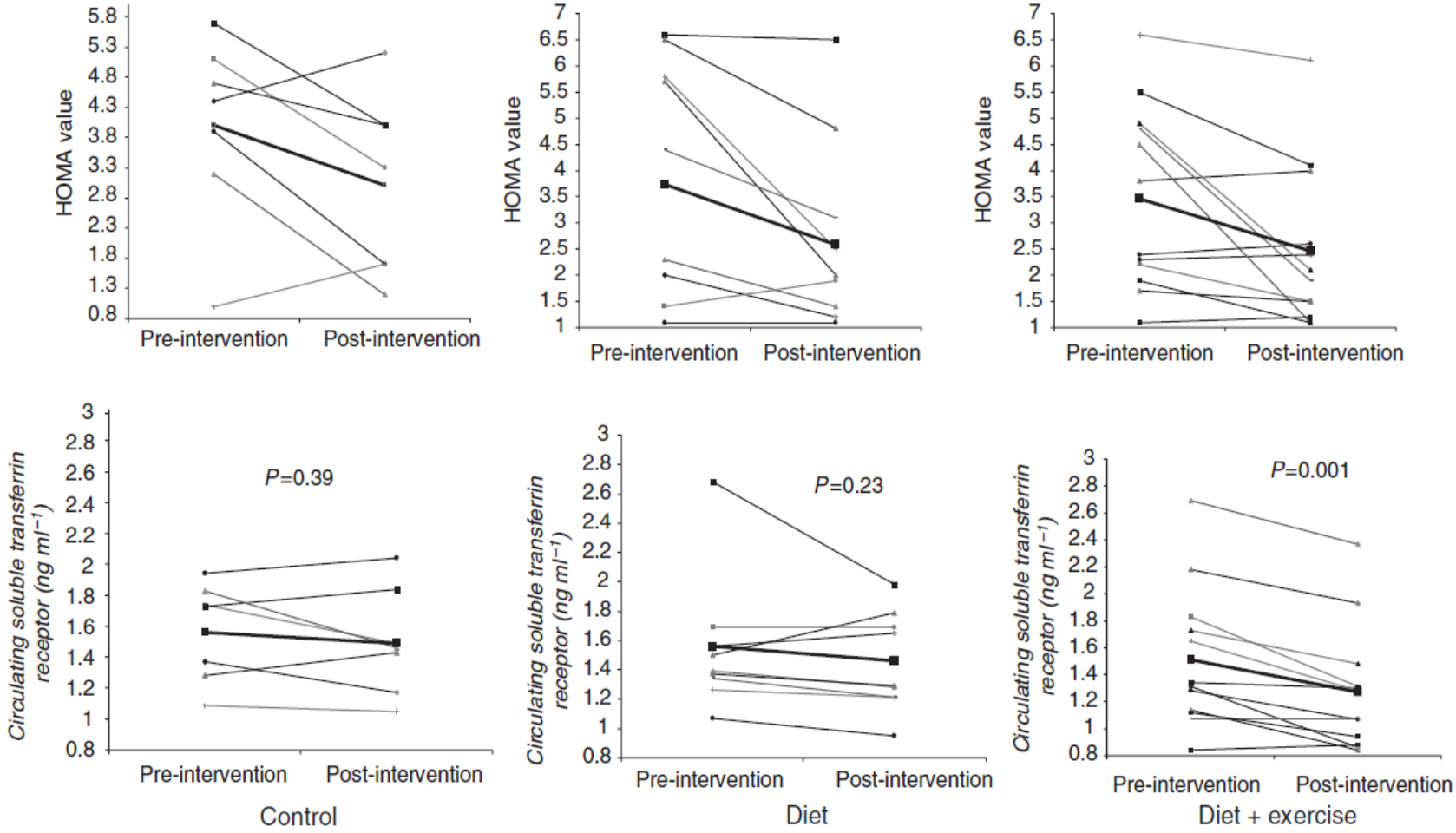

Figure 1. Homeostasis Model Assessment (HOMA) value (upper panels) and serum soluble transferrin receptor (sTfR) concentration (lower panels) before and after each intervention period.

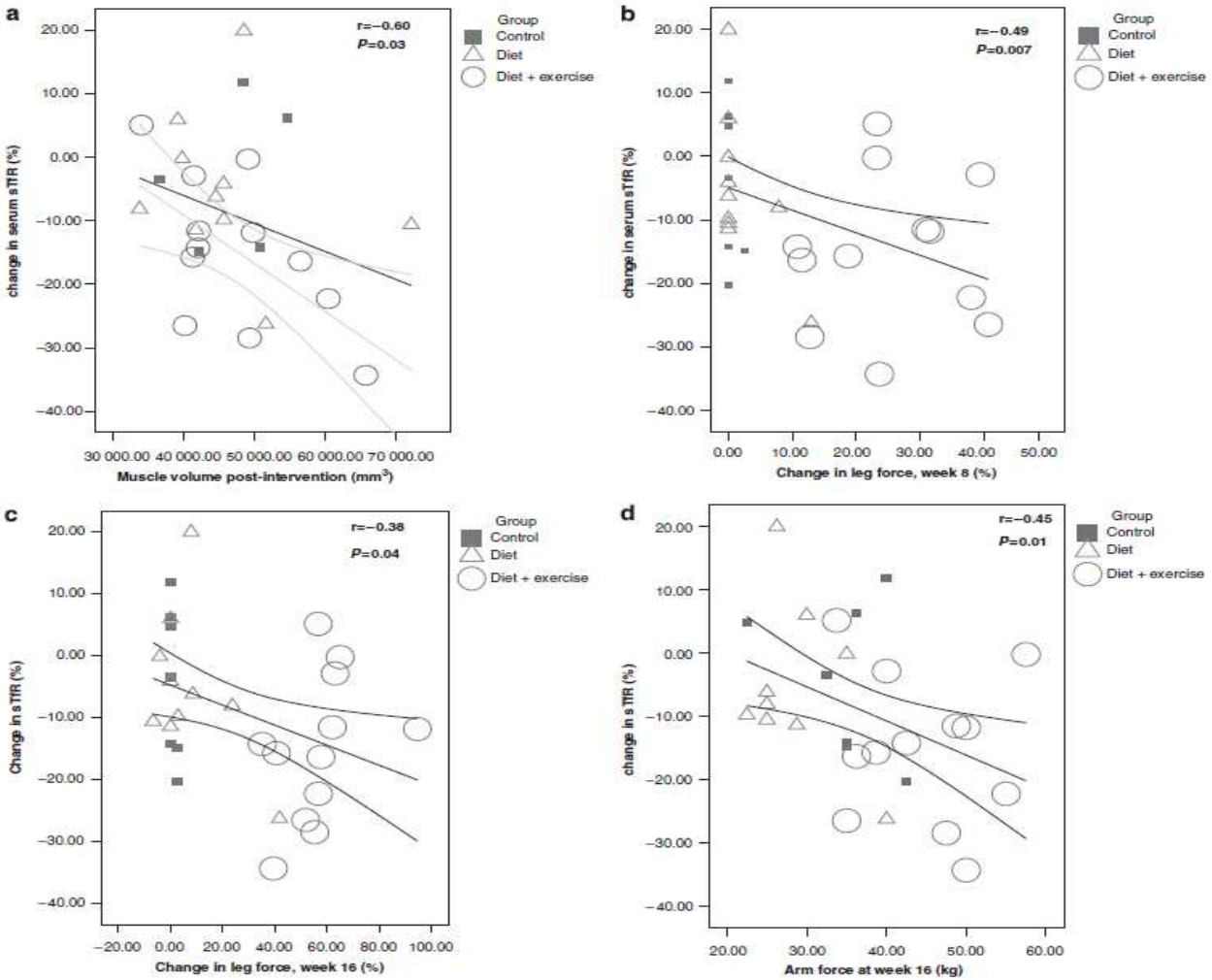

Figure 2. Changes in circulating soluble transferrin receptor (sTfR) according to thigh muscle volume (a), the change in leg force at weeks 8 and 16 (b and c), and the absolute value of arm force (d).

Discussion

The main unprecedented findings in this manuscript are: (1) circulating sTfR decreased significantly after improvement of insulin sensitivity in middle-aged women; (2) this decrease was observed only in the D+RT group despite similar weight loss and improvement of insulin sensitivity than in the D group; (3) the decrease of sTfR was correlated with muscle volume, and leg and arm force (Figure 2). In this sense, the preservation of the muscle volume in the D+RT group compared with decreased muscle volume in the D group was remarkable. The leg and arm force improved only in the D+RT group in which sTfR decreased significantly. Figure 1 discloses the relatively stable values of sTfR in the control and the D groups (except for one participant in this latter group), and the uniform decrease of sTfR in almost all participants in the D+RT group.

The reason for these disparate effects of improved insulin sensitivity induced by diet vs diet plus exercise on circulating sTfR concentrations is currently unknown. However, the maximal effects of insulin and muscular contraction on glucose transport are known to be additive in mammalian skeletal muscle, strongly suggesting that these stimuli act through separate pathways. Contracting myofibers need to obtain glucose very rapidly to cope with the energy demands that increase dramatically when contraction is initiated.

Coderre *et al.* (1995) reported the isolation of distinct insulin- and exercise-sensitive GLUT4 intracellular pools.

Interestingly, the transferrin receptor defines two distinct contraction and insulin-responsive GLUT4 vesicle populations in *in vitro* studies on skeletal muscles (Lemieux, Han, Dombrowski, Bonen, & Marette, 2000). Insulin did not stimulate transferrin receptor recruitment from the GLUT4-containing intracellular fraction to the plasma membrane in skeletal muscle. In contrast, muscular contraction stimulated the recruitment of the transferrin receptor from the same GLUT4-containing intracellular fraction to the plasma membrane (Lemieux et al., 2000). The sTfR is a soluble truncated monomer of tissue receptor, lacking its first 100 amino acids, which circulates in the form of a complex of transferrin and its receptor.

The sTfR is produced by proteolysis, mediated by a membrane-associated serine protease that occurs mostly at the surface of exosomes within the multivesicular intracellular body before exocytosis. The bulk of sTfR measured in serum is proportional to the mass of cellular TfR (Baynes & Cook, 1996). It is thus possible that the exercise-induced stimulation of TfR translocation and recycling endosomes fulfill the dual functions of providing both glucose and iron to contracting myofibers (Lemieux et al., 2000), leading to decreasing serum sTfR concentration in this context. Insulin (improved insulin action by diet alone) failed to induce TfR translocation or caused only a marginal redistribution of the receptor in skeletal muscle (Lemieux et al., 2000).

The activation of TfR recycling in contracted muscle may be important to maintain the levels and activities of iron containing proteins involved in the respiratory capacity of muscle mitochondria. In fact, skeletal muscle represents about 40% of body mass and contains 10-15% of body iron, which is mainly located in myoglobin. Skeletal muscle plays a functional role in oxygen storage, transport and use, and iron is a key component of myoglobin and heme groups of cytochromes.

We are not aware of any study evaluating sTfR in parallel to insulin sensitivity. The concentration of sTfR increased immediately after exercise, as found in several studies (Schumacher, Schmid, Konig, & Berg, 2002; Rocker et al., 2002; Nikolaidis, Michailidis, & Mougios, 2003; Deruisseau et al., 2004; Malczewska, Stupnicki, Blach, & Turek-Lepa, 2004; Robach et al., 2006; Duca et al., 2006; Di, Stel, Banfi, Gonano, & Cauci, 2008). The study of the long-term concentration of serum sTfR was carried out in a group of elite rugby players during a competitive season (Banfi, Del, Mauri, Corsi, & Melegati, 2006). The sTfR concentration increased during the competition period and decreased at the end of the season.

We did not find changes in circulating adiponectin. Plasma adiponectin has been measured in response to acute exercise bouts as well as moderate to long-term training programs in a variety of populations, as recently reviewed (Berggren, Hulver, & Houmard, 2005). Neither an acute bout of stationary cycling nor a 3-week exercise intervention in patients with type 2 diabetes, nor a 6-m exercise intervention led to significant changes in plasma adiponectin (Berggren et al., 2005).

There is a considerable body of evidence gathered from studies over the past half a century indicating that regular physical activity reduces the risk of cardiovascular disease (Gill & Malkova, 2006). Regular physical activity is particularly beneficial in individuals with insulin-resistant conditions, such as obesity, type 2 diabetes and the metabolic syndrome (Holloszy, 2005). Although the post-exercise increase in muscle insulin sensitivity has been characterized in considerable detail, the basic mechanisms underlying this phenomenon remain a mystery (Holloszy, 2005; Cartee & Holloszy, 1990).

In summary, our findings hint at a long-term regulation of serum sTfR concentration by insulin sensitivity. These findings need to be considered when evaluating iron status in obesity (Lecube et al., 2006; Lecube, Hernandez, Pelegri, & Simo, 2008).

Conflict of interest

The authors declare no conflict of interest.

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Capítulo 4

Principales resultados, conclusiones y perspectivas futuras

Estudio 1: The Relationship of Serum Osteocalcin Concentration to Insulin Secretion, Sensitivity, and Disposal with Hypocaloric Diet and Resistance Training

Objetivo: evaluar la osteocalcina circulante y su asociación con la sensibilidad y secreción de insulina en tres estudios diferentes en sujetos no diabéticos.

Principales resultados:

1) Hay una asociación clara entre la osteocalcina y la sensibilidad a la insulina en los 149 varones del estudio transversal y en las 19 mujeres obesas de los dos grupos de intervención (grupo dieta y dieta más ejercicio de fuerza) del primer estudio longitudinal pero no en los 20 sujetos que disminuyeron un 16,8% de peso corporal en el tercer estudio.

2) La pérdida leve de peso a través de una dieta hipocalórica por sí misma no induce a cambios significativos en la concentración de osteocalcina sérica a pesar de mostrar una ligera mejora (no significativa) de la sensibilidad a la insulina; sin embargo,

3) Una pérdida de peso similar realizando, además de la dieta, ejercicio de fuerza se traduce en un aumento de la osteocalcina circulante acompañado por una mejora significativa en el índice HOMA.

4) El aumento en la concentración sérica de osteocalcina se asoció de forma positiva con cambios en la grasa visceral y, sobre todo, con los cambios en la fuerza muscular de las piernas en las mujeres que realizaron trabajo de fuerza.

Conclusiones:

1) Este sería el primer estudio que evalúa la osteocalcina en asociación con la sensibilidad a la insulina en humanos, y el primer estudio que muestra los cambios inducidos por el ejercicio físico en la osteocalcina circulante en asociación con la sensibilidad a la insulina, la grasa visceral, masa y fuerza muscular. Se confirma la relación observada entre la osteocalcina circulante y la sensibilidad a la insulina en humanos de forma similar a lo observado en los modelos animales.

2) Nuestros resultados sugieren que la osteocalcina, sintetizada por el hueso, podría ser un regulador activo de sensibilidad a la insulina en humanos y que el ejercicio físico tiene un papel fundamental en ello.

Perspectivas futuras:

1) La falta de datos sobre la osteocalcina descarboxilada es una limitación de este estudio. Tal y como reportaba el equipo de trabajo de Lee et al. (2007), la osteocalcina descarboxilada fue la forma activa de la osteocalcina en modelos animales por lo que nos queda saber qué ocurre con esa molécula en los seres humanos.

2) Sería interesante valorar las correlaciones entre osteocalcina y los nutrientes de la dieta (Vit. K, Vit. D, Ca, etc.) para la prevención y/o valoración clínica de algunas enfermedades (arteriosclerosis, osteoporosis, etc.).

Bilbiografía

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Estudio 2: Circulating soluble transferrin receptor concentration decreases after exercise-induced improvement of insulin sensitivity in obese individuals

Objetivo: evaluar la concentración sTfR circulante después de los cambios en la sensibilidad a la insulina en individuos obesos.

Principales resultados:

1) El sTfR circulante disminuyó significativamente después de la mejora de la sensibilidad a la insulina en mujeres obesas de mediana edad.

2) Este detrimento en la sTfR fue observado únicamente en el grupo que hizo dieta y ejercicio de fuerza a pesar de que el grupo que siguió la misma dieta hipocalórica sufre una bajada de peso similar y una ligera (que no significativa) mejora de la sensibilidad de la insulina.

3) El descenso del sTfR correlacionó con el volumen muscular y la fuerza de brazos y piernas. En este sentido, se considera indispensable la conservación de la masa muscular que presenta el grupo de intervención que llevó a cabo dieta y entrenamiento de fuerza comparada con la disminución observada en el grupo dieta.

Conclusiones:

1) Nuestros hallazgos parecen indicar que existe una regulación de la concentración de sTfR en suero a través de la sensibilidad a la insulina mejorada después de hacer dieta hipocalórica y entrenamiento de fuerza.

2) El metabolismo del hierro, alterado en personas con obesidad, también es susceptible a mejoras en la RI producidas por la realización de ejercicio de fuerza y dieta hipocalórica. Sería interesante tener en cuenta el sTfR cuando se valora el hierro en personas con obesidad.

Perspectivas futuras:

1) Es necesario estudiar si se generan correlaciones, y de qué tipo, entre las moléculas estudiadas en este trabajo y otras influyentes en la RI para comprender mejor cómo se comporta el organismo en su conjunto.

2) Podríamos valorar qué ocurre con esta molécula cuando únicamente se hace ejercicio (sin dieta), bien sea de tipo aeróbico o anaeróbico para concluir si hay un tipo de esfuerzo que tenga mejor respuesta y poder concretar en qué medida se debe realizar para generar mejoras en las personas con RI.