UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

DEPARTAMENTO DE MEDICINA

RELACIÓN ENTRE LA EXPRESIÓN DE ADIPOCITOQUINAS EN EL TEJIDO ADIPOSO EPICÁRDICO Y LA ENFERMEDAD CARDIOVASCULAR

MEMORIA

Que presenta para optar al Grado de Doctor

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El Prof. D. José Ramón González Juanatey, Catedrático del Departamento de Medicina de la Universidad de Santiago de Compostela y Jefe de Servicio de Cardiología y Unidad Coronaria del Hospital Clínico Universitario de Santiago, la Dra. Dña. Sonia Eiras Penas, Investigadora del Instituto de Investigaciones Sanitarias (IDIS) del Hospital Clínico Universitario de Santiago, y la Dra. Dña. Lilian Grigorian Shamagian,

CERTIFICAN QUE:

La presente memoria, titulada "Relación entre la expresión de adipocitoquinas en el tejido adiposo epicárdico y la enfermedad cardiovascular", que presenta D. Elvis Teijeira Fernández para optar al Grado de Doctor por la Universidad de Santiago de Compostela, ha sido realizada bajo su dirección en el Servicio de Cardiología y Unidad Coronaria y en el Instituto de Investigaciones Sanitarias del Hospital Clínico Universitario de Santiago, y autorizan su presentación a fin de que pueda ser juzgada por el tribunal correspondiente.

Y para que así conste, firman la presente en Santiago de Compostela, a 7 de octubre de 2010.

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A mi madre.

A mis hermanos.

A Marga.

What we know is a drop,
what we don't know is an ocean.

Isaac Newton

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CAPÍTULO 1

INTRODUCCIÓN GENERAL

En las dos últimas décadas, la tradicional visión del tejido adiposo como un mero depósito energético ha dejado de ser válida, ya que se ha puesto de manifiesto que la grasa corporal es un auténtico órgano endocrino de gran complejidad.[1] Hoy día, sabemos que el tejido adiposo expresa y secreta varias moléculas bioactivas conocidas como adipoquinas o adipocitoquinas, que pueden ejercer efectos a nivel local –ya sea por mecanismos autocrinos o paracrinos– y a nivel sistémico. Además de emitir estas señales eferentes, el tejido adiposo expresa múltiples receptores que le permiten responder a las señales procedentes de diversos ejes hormonales y del sistema nervioso central.[2]

Los tejidos grasos están compuestos no sólo por adipocitos, sino también por una matriz de tejido conjuntivo, células nerviosas, del estroma vascular y del sistema inmune.[3] Aunque los adipocitos son responsables de la secreción de un gran número de hormonas, el estroma del tejido adiposo también posee una capacidad secretora importante.[4]

El hecho de que tanto el exceso como el déficit de determinados depósitos adiposos se asocien al desarrollo de complicaciones metabólicas[4, 5] enfatiza la relevancia del tejido adiposo como órgano endocrino. Por otra parte, la distribución de la grasa corporal influye en el riesgo de desarrollo de enfermedades cardiovasculares y metabólicas. Clásicamente, el patrón de obesidad "central" se asocia de manera más directa a un incremento del riesgo que el patrón de obesidad "periférica". Estas observaciones sugieren que los tejidos adiposos viscerales y subcutáneos, a pesar de su aparente similitud, poseen propiedades muy diferentes,[6] con una implicación distinta en la patogenia de las enfermedades cardiovasculares y metabólicas. En este sentido, la grasa visceral, pero no la subcutánea, se considera uno de los principales componentes del síndrome metabólico y desempeña un papel fundamental en su fisiopatología.[7]

Aunque inicialmente el interés por el tejido adiposo visceral se centró sobre todo en la grasa intraabdominal, en los últimos años el tejido adiposo epicárdico (TAE) ha cobrado protagonismo como productor de adipocitoquinas de gran relevancia fisiopatológica.[8] Los

efectos paracrinos de las adipocitoquinas producidas por el TAE tienen especial interés dada la localización anatómica de este tejido, si bien los efectos autocrinos y endocrinos que pueden ejercer tampoco son desdeñables.[9]

ADIPOQUINAS Y COMPLICACIONES METABÓLICAS DE LA OBESIDAD.

El descubrimiento de las adipoquinas en los últimos años ha supuesto un revulsivo en el conocimiento de la fisiología y fisiopatología del tejido adiposo. A mediados de 1990, se describió por primera vez la expresión del factor de necrosis tumoral alfa (TNF-α) en el tejido adiposo de humanos y roedores obesos,[10, 11] y desde entonces se han ido descubriendo otras proteínas secretadas por el tejido adiposo. Muchas de estas adipoquinas se han implicado en la patogénesis de la inflamación crónica y de la resistencia a la insulina, ambas asociadas a la obesidad.

Se observó también que el tejido adiposo humano contiene un número elevado de macrófagos residentes[12] que, una vez activados, son los principales responsables de la producción de citoquinas proinflamatorias (TNF-α, interleuquina (IL)-6 e IL-1).[13] Es probable que exista una serie de mecanismos que subyacen a la infiltración de macrófagos en el tejido adiposo. Una posibilidad es la producción por parte de los adipocitos de quimioquinas que propiciarían la quimiotaxis de los macrófagos. Además del aumento de la infiltración macrofágica en el tejido adiposo, la obesidad se asocia a cambios en el fenotipo de los macrófagos, que incrementan su actividad proinflamatoria.[14] Los adipocitos expresan niveles bajos de proteína quimiotáctica de monocitos (MCP)-1, y su expresión está incrementada en los sujetos obesos.[15]

Por otra parte, la mayoría de los macrófagos rodean adipocitos necróticos, conformando una estructura histológica típica, lo cual sugiere que los macrófagos infiltran el tejido adiposo como parte de su función *scavenger* como respuesta a la necrosis de los adipocitos.[16] En animales de experimentación se ha demostrado que el número de macrófagos y la presencia de estas estructuras histológicas es mayor en los individuos obesos.[17]

Existen varias causas potenciales de necrosis adipocitaria y de infiltración macrofágica. Por una parte, se ha propuesto que la hipoxia podría ser uno de los fenómenos desencadenantes de la necrosis de los adipocitos,[18] ya que con la obesidad aumenta el tamaño de los adipocitos y disminuye el aporte sanguíneo que reciben. Se ha observado que la inducción de hipoxia *in vivo* conlleva el incremento de la expresión de varias citoquinas inflamatorias.[19] Por otro lado,

también se postula que la acumulación de triglicéridos y el crecimiento de los adipocitos en el tejido adiposo es un proceso benigno que evita la lipotoxicidad en el hígado, músculo esquelético y otros depósitos ectópicos.[20] La inflamación del tejido adiposo ocurriría cuando se limita la expansión adipocitaria, bien sea por alteraciones en el desarrollo de los adipocitos, por disminución de la síntesis de lípidos o por factores de la matriz que impiden la hipertrofia del tejido. Un ejemplo del desarrollo limitado de los adipocitos es la lipodistrofia. Los sujetos que la presentan tienen menos tejido adiposo en localizaciones habituales pero mayor cantidad de grasa ectópica, y desarrollan lipotoxicidad y resistencia a la insulina.[21]

-ADIPONECTINA.

La adiponectina, también conocida como Acrp30, adipoQ, apM1 o GBP28, consta de 244 aminoácidos y es el principal producto proteico de los adipocitos, donde se produce en su mayor parte.[22] La hormona circula en plasma en concentraciones elevadas, de 3 a 30 μg/mL, representando el 0,01% de las proteínas plasmáticas totales.[23] Fue identificada simultáneamente por varios grupos independientes en ratones y en humanos hace poco más de una década, y el gen que la codifica (AMP1) se localiza en el cromosoma 3q27.[22, 24]

La secuencia proteica de la adiponectina consta de cuatro dominios (Figura 1-1). El primero es un péptido señal situado en la zona amino-terminal, que permite la secreción de la hormona al exterior de los adipocitos. El segundo es una región de 28 aminoácidos con variabilidad entre especies. El tercero es un dominio colágeno constituido por 22 tripletes glicina-X-tirosina. Y por último, presenta un dominio globular en la región carboxi-terminal. La secuencia de la hormona se asemeja a la de otras moléculas conocidas, como C1q y los colágenos VIII y X. Por otra parte, mientras que los monómeros de adiponectina, de unos 30 kDa, no se encuentran en plasma, sí se han encontrado trímeros y hexámeros de bajo peso molecular, que predominan en el espacio intracelular, y formas oligoméricas de alto peso molecular que son predominantes en plasma. Se ha observado que las diversas formas pueden poseer distintos efectos de

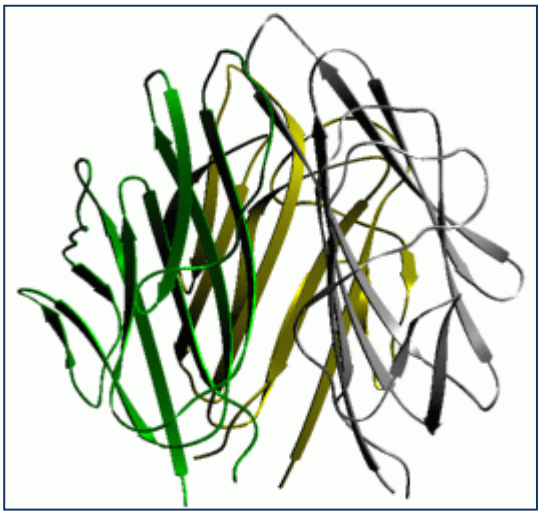

FIGURA 1-1. Estructura de la adiponectina.

señalización en el aparato cardiovascular.[25, 26] Asimismo, en plasma también circulan fragmentos globulares pequeños resultantes de la proteólisis de adiponectina, que son farmacológicamente activos y contribuyen a la regulación del peso corporal y de la oxidación de ácidos grasos en ratones.[27]

La insulina disminuye los niveles de expresión de adiponectina tanto en ratones como en humanos,[28] mientras que las tiazolidinedionas, potentes agonistas de los receptores activados por el proliferador de peroxisomas (PPAR)-γ, aumentan su expresión.[28, 29] La mayoría del resto de factores que presentan un impacto significativo en la regulación de adiponectina, como las citoquinas (IL-6 y TNF-α), la hormona del crecimiento, las catecolaminas, los glucocorticoides, la prolactina y los andrógenos, ejercen efectos inhibitorios.[30]

En la Tabla 1-1 se enumeran los principales efectos fisiológicos de la adiponectina. A pesar de que la hormona se produce principalmente en el tejido adiposo,[23] los individuos obesos presentan niveles plasmáticos menores, lo cual puede deberse a la supresión de la transcripción de adiponectina en estos sujetos y al efecto inhibitorio causado por las citoquinas inflamatorias. También se observan concentraciones plasmáticas menores de la hormona en varones respecto a mujeres, pero no variaciones horarias, lo cual sugiere que la regulación de su secreción se realiza más bien a largo plazo.[31]

La adiponectina se correlaciona negativamente con el índice de masa corporal (IMC), los niveles plasmáticos de triglicéridos y la glucemia en ayunas; y positivamente con la edad y los niveles de colesterol HDL.[35] Se postula que el paradójico aumento de adiponectina con la edad pueda estar en relación con una cierta "resistencia a la adiponectina".

TABLA 1-1. Resumen de los efectos fisiológicos de la adiponectina.[27, 32-34]

- Adhesión de monocitos al endotelio.
- Producción de citoquinas por los macrófagos.
- Fagocitosis.
- Inhibición de la acumulación de LDLc acetiladas.
- Aumento de la sensibilidad a la insulina.
- Estimulación de la beta-oxidación lipídica.
- Disminución de la transformación de macrófagos a células espumosas.
- Inhibición de la expresión del receptor "scavenger" de macrófagos (MSR).
- Inhibición dosis-dependiente de la unión del ligando a MSR.

Receptores de adiponectina.

Se han clonado dos formas de receptores de adiponectina, conocidas como adipoR1 y adipoR2. AdipoR1 es un receptor de alta afinidad por el dominio C-terminal globular, con muy baja afinidad por la molécula completa de adiponectina, y se expresa de forma predominante en el músculo esquelético y cardiaco. Por el contrario, adipoR2 presenta afinidad intermedia por ambas formas de adiponectina y es más abundante en el hígado.[36]

Los resultados de los experimentos de sobreexpresión y bloqueo de adipoR1 y adipoR2 sugieren que estos receptores median el aumento de la actividad de los ligandos de monofosfato de adenosina (AMP) y PPAR-γ, así como la oxidación de los ácidos grasos y la captación de glucosa.[36] Los receptores de adiponectina se expresan en las células beta pancreáticas,[37] los macrófagos y las lesiones ateroscleróticas.[38] En la célula beta, la expresión del receptor de adiponectina aumenta con la exposición al oleato, y el tratamiento con el dominio globular de adiponectina induce la expresión de lipoproteina-lipasa.[37]

La adiponectina y las enfermedades metabólicas y cardiovasculares: estudios experimentales.

-Adiponectina y sensibilidad a la insulina.

La activación de la quinasa de AMP (AMPK) por la unión de adiponectina a los receptores adipoR1 y adipoR2 induce la expresión de PPAR-α y aumenta la expresión genética de los enzimas implicados en la betaoxidación de los ácidos grasos y en la captación de glucosa.[36] La adiponectina disminuye la producción hepática de glucosa mediante la inhibición de los enzimas de la gluconeogénesis y contribuye así a la reducción de la glucemia en animales diabéticos y no diabéticos.[39] La administración de adiponectina mejora la sensibilidad a la insulina en ratones deficitarios *(knockout)* de adiponectina sometidos a una dieta hiperlipídica.[34] Por otra parte, aunque las hormonas tiroideas y la hormona del crecimiento también alteran la sensibilidad a la glucosa, sólo la insulina y los glucocorticoides suprimen la expresión de adiponectina en los adipocitos.[40]

-Adiponectina, angiogénesis y función endotelial.

La adiponectina ejerce efectos antiinflamatorios uniéndose específicamente a los colágenos tipos I, III y V del espacio subendotelial de las arterias lesionadas.[41] Los ratones con déficit de

adiponectina presentan alteraciones en la revascularización tras la isquemia, mientras que la sobreexpresión de la hormona la facilita.[42]

En animales de experimentación se ha demostrado también que el suplemento de adiponectina estimula la angiogénesis. La adiponectina estimula la migración de las células endoteliales y previene la apoptosis *in vitro*.[43, 44] Los ratones deficitarios de adiponectina presentan un número reducido de células endoteliales progenitoras circulantes en condiciones de isquemia. Por otra parte, la incubación de células mononucleares humanas de sangre periférica con adiponectina induce el aumento del número de progenitores circulantes de células endoteliales, y la adiponectina favorece su quimiotaxis y la diferenciación hacia estructuras reticulares.[45]

En línea con estos hallazgos, se ha observado que la adiponectina estimula la producción de óxido nítrico en las células endoteliales a través de la fosforilación de la óxido nítrico sintasa endotelial (eNOS) tanto por mecanismos dependientes como por mecanismos independientes del enzima AMPK.[42] Los ratones con déficit de adiponectina sometidos a una dieta hipersódica presentan mayor presión arterial, una tasa elevada de infarto cerebral tras el daño por isquemia y reperfusión, menor expresión de eNOS en la aorta y menor activación de eNOS en el tejido cerebral isquémico.[46, 47] Por tanto, el eje regulador adiponectina-eNOS protege contra el desarrollo de insuficiencia vascular y disfunción endotelial.

-Adiponectina y aterosclerosis.

La administración de adiponectina a ratones deficitarios de apolipoproteína E reduce el tamaño de la lesión aterosclerótica y la expresión del receptor *scavenger* clase A, de TNF-α y de la molécula de adhesión vascular celular tipo 1 (VCAM-1).[48] Por otra parte, la adiponectina reduce la expresión de VCAM-1 inducida por TNF-α en las células endoteliales, suprimiendo la activación del factor nuclear (NF)-κB.[49] En humanos, también suprime la expresión de receptores *scavenger* clase A y de este modo inhibe la transformación de los macrófagos a células espumosas, reduce la producción de TNF-α estimulada por lipopolisacáridos[32] y aumenta la producción de IL-10 por parte de los macrófagos.[50] Además, facilita la eliminación de células apoptóticas por los macrófagos y modula los procesos inflamatorios.[51] Así, la adiponectina posee propiedades antiaterogénicas y podría ser beneficiosa para el tratamiento y la prevención de enfermedades ateroscleróticas.

-Adiponectina y daño miocárdico por isquemia y reperfusión.

Recientemente, se ha comprobado que los ratones deficitarios de adiponectina desarrollan infartos de miocardio más extensos tras la inducción de daño por isquemia y reperfusión. En estos animales de experimentación, el daño producido por la isquemia y la reperfusión genera un incremento de la actividad apoptótica del miocardio y de la expresión de TNF-α. Sin embargo, la administración de adiponectina revierte dicho efecto, y de ese modo disminuye el tamaño del infarto y mejora la función cardiaca.[52, 53]

La protección que confiere la adiponectina contra el daño por isquemia y reperfusión está ligada a la inhibición del exceso de estrés oxidativo y nítrico inducido por peroxinitritos.[52] La adiponectina suprime el exceso de producción de especies reactivas de oxígeno en varios tipos celulares, incluidas las células endoteliales.[54] La reducción de especies reactivas de oxígeno y de nitrógeno puede contribuir a la acción protectora de la hormona en el infarto de miocardio. Por otra parte, se ha observado que el aumento de la producción de adiponectina debido a la restricción calórica ofrece al miocardio cierta resistencia al daño por isquemia y reperfusión.[55] Estos hallazgos sugieren que el tratamiento con adiponectina podría tener utilidad clínica en el manejo de pacientes con infarto agudo de miocardio.

El daño miocárdico por isquemia y reperfusión produce un descenso transitorio de la concentración de adiponectina. Además, la administración de adiponectina a ratones deficitarios da lugar a su acumulación en el miocardio de los animales con daño por isquemia y reperfusión, pero no en los que no son sometidos a ese daño.[56] Probablemente, la adiponectina se acumula en tejidos sometidos a isquemia y ejerce allí una función cardioprotectora. Se ha observado que adiponectina se expresa en cardiomiocitos,[57] y que los niveles locales de la hormona se incrementan en modelos experimentales de daño miocárdico[56] y disminuyen en la miocardiopatía dilatada.[58] Es posible que la adiponectina producida localmente ejerza una función cardioprotectora, aunque esta hipótesis no ha sido confirmada hasta el momento.

En cardiomiocitos cultivados sometidos a hipoxia y reoxigenación, la adiponectina suprime la apoptosis promoviendo la señalización por AMPK. La adiponectina también inhibe la producción de TNF-α inducida por lipopolisacáridos, una acción antiinflamatoria mediada en parte por su capacidad de activar la síntesis de prostaglandina E2 dependiente de ciclooxigenasa (COX)-2. La expresión de COX-2 inducida por la adiponectina disminuye al

bloquear el enzima esfingosina quinasa-1, y el tratamiento con antagonistas del receptor esfingosina-1-fosfato también disminuye la expresión de COX-2 en respuesta a adiponectina.[53] En línea con estas observaciones *in vitro*, el miocardio de los ratones deficitarios de adiponectina muestra una disminución de la inducción de COX-2 tras el daño por isquemia y reperfusión respecto al de los ratones con fenotipo salvaje. Además, la inhibición de COX-2 revierte parcialmente las acciones inhibitorias de la adiponectina sobre el tamaño del infarto y la producción de TNF-α.[53]

En conclusión, la adiponectina protege el corazón de la isquemia aguda mediante dos mecanismos independientes: los efectos antiapoptóticos mediados por AMPK y los efectos antiinflamatorios mediados por COX-2.

-Adiponectina e hipertrofia miocárdica.

Estudios experimentales han demostrado que la adiponectina previene el desarrollo de hipertrofia ventricular izquierda. Los ratones con déficit de adiponectina presentan hipertrofia ventricular concéntrica severa y una mayor mortalidad tras la sobrecarga de presión causada por la constricción aórtica, respecto a los ratones con fenotipo salvaje.[59] De manera inversa, la adiponectina atenúa la hipertrofia cardiaca tras la sobrecarga de presión o la administración de angiotensinógeno II.[60, 61]

En cardiomiocitos cultivados, la adiponectina estimula la fosforilación de AMPK y suprime la activación de las proteín-quinasas reguladas por señales extracelulares (ERKs) y la hipertrofia inducidas por los receptores alfaadrenérgicos o la endotelina-1.[60] El efecto inhibitorio de la adiponectina sobre la hipertrofia ventricular está mediado por la activación de la señalización de AMPK. Las interacciones de adiponectina con los receptores adipoR1 y adipoR2 inducen la activación de AMPK y de p38, y facilitan la captación de glucosa y la betaoxidación de los ácidos grasos,[36] como se comentó previamente. Ambos receptores se encuentran en el miocardio y median la activación de AMPK por la adiponectina en modelos de hipertrofia *in vitro*.[62] En conjunto, estos hallazgos sugieren que el eje de señalización adiponectina-AMPK en cardiomiocitos limita el remodelado cardiaco patológico. La adiponectina estimula la actividad de los PPAR-α, y esta estimulación disminuye al administrar un inhibidor de AMPK. Por tanto, la adiponectina inhibe la fibrosis cardiaca por lo menos en parte a través de la activación de los PPAR-α dependiente de AMPK.[61]

-Adiponectina y disfunción ventricular.

El papel de la adiponectina en el desarrollo de insuficiencia cardiaca se ha estudiado utilizando un modelo de ratón con infarto de miocardio. Respecto a los ratones con fenotipo salvaje, los ratones deficitarios de adiponectina desarrollan mayor dilatación del ventrículo izquierdo, hipertrofia y disfunción contráctil tras el infarto.[63] La alteración de la función ventricular izquierda se acompaña de hipertrofia miocitaria, aumento de la apoptosis, fibrosis intersticial y reducción de la densidad capilar en los bordes de la zona infartada.

Además, la administración de adiponectina a ratones con fenotipo salvaje disminuye la dilatación ventricular izquierda y mejora la función ventricular, asociada a un incremento de la densidad capilar y una disminución de la hipertrofia, la apoptosis y la fibrosis intersticial tras el infarto. Los ratones con déficit de adiponectina presentan también un mayor grado de insuficiencia cardiaca tras la sobrecarga de presión, respecto a los ratones de fenotipo salvaje.[59]

En conclusión, la adiponectina parece proteger el corazón frente al remodelado patológico crónico, si bien la interpretación de los datos epidemiológicos resulta compleja, como se verá más adelante.

La adiponectinemia y las enfermedades metabólicas y cardiovasculares: estudios epidemiológicos.

-Adiponectina y diabetes mellitus tipo 2.

La hipoadiponectinemia se asocia al síndrome metabólico y al desarrollo de diabetes mellitus tipo 2,[64, 65] y la concentración de adiponectina se correlaciona de forma directa con la sensibilidad a la insulina.[66] Los individuos con diabetes mellitus tipo 2 establecida presentan menores concentraciones plasmáticas de adiponectina que los controles no diabéticos,[67] y lo mismo ocurre con los pacientes con síndrome metabólico respecto a los controles.[68]

-Adiponectina y cardiopatía isquémica.

Varios estudios muestran que los niveles de adiponectina son menores en los pacientes con manifestaciones clínicas de enfermedad arterial coronaria.[69, 70] Sin embargo, la asociación entre la adiponectinemia y la enfermedad arterial coronaria todavía es controvertida, ya que algunos estudios prospectivos no encontraron asociación significativa[71, 72] o tan sólo una débil asociación entre los niveles bajos de adiponectina y la enfermedad coronaria.[73] La disparidad en los resultados puede deberse a diferencias poblacionales, y por tanto el papel de la

hipoadiponectinemia como factor independiente asociado a la enfermedad arterial coronaria todavía no está claro.

Por otra parte, los pacientes que padecen síndrome coronario agudo presentan menores concentraciones plasmáticas de adiponectina.[35] La hipoadiponectinemia se asocia además a un mayor riesgo de infarto agudo de miocardio en varones, independientemente de los niveles de proteína C reactiva o del estatus glucémico.[74] Incluso se llegó a proponer como punto de corte de riesgo de síndrome coronario agudo una concentracion de adiponectina inferior a 5,5 μg/mL.[75]

También se ha observado que un rápido descenso del nivel de la hormona tras el infarto agudo de miocardio[76] así como niveles persistentemente bajos predicen el desarrollo de eventos cardiacos en varones con infarto agudo de miocardio.[77] Finalmente, resulta muy interesante que los niveles plasmáticos de adiponectina medidos tras el intervencionismo coronario pueden servir como predictor independiente de la mejoría de la función cardiaca durante el seguimiento.[78]

-Adiponectina e hipertensión arterial.

Los resultados de los estudios diseñados para comprobar la relación entre la adiponectina plasmática y la hipertensión arterial no son homogéneos, si bien algunos sí han demostrado que existe asociación entre la hipoadiponectinemia y la hipertensión arterial.[79, 80] Por otra parte, se observó que algunos fármacos antihipertensivos ejercen efectos paralelos sobre la concentración plasmática de adiponectina, la presión arterial y la sensibilidad a la insulina.[81]

-Adiponectina e hipertrofia ventricular izquierda.

La hipertrofia ventricular izquierda se relaciona en parte con la obesidad, y algunos estudios clínicos han investigado la posible implicación de los niveles de adiponectina en su patogenia. La hipoadiponectinemia se asocia a la progresión de la hipertrofia ventricular izquierda, que se acompaña con frecuencia de disfunción diastólica,[82] y se ha demostrado también que los niveles de adiponectina se correlacionan negativamente con la masa ventricular izquierda.[83] Sin embargo, puesto que la hipoadiponectinemia puede incrementar el riesgo de hipertensión arterial,[79] su contribución al desarrollo de hipertrofia ventricular podría deberse, por lo menos en parte, a la modulación de la presión sanguínea.

-Adiponectina e insuficiencia cardiaca.

Varios estudios prospectivos han analizado la asociación entre los niveles de adiponectina y la insuficiencia cardiaca. La hiperadiponectinemia se asocia a una mayor mortalidad y severidad de la enfermedad en pacientes con insuficiencia cardiaca crónica, incluida la miocardiopatía dilatada.[84-86] Los pacientes con caquexia presentan niveles elevados de adiponectina respecto a los pacientes no caquécticos,[87] y un IMC bajo asociado a niveles elevados de adiponectina puede estar relacionado con el aumento de la mortalidad tras el inicio del cuadro de insuficiencia cardiaca.[88] Por el contrario, en pacientes asintomáticos, los niveles plasmáticos de adiponectina parecen carecer de utilidad como predictores pronósticos de insuficiencia cardiaca.[89]

En pacientes con manifestaciones clínicas de insuficiencia cardiaca, los niveles de adiponectina son elevados y persiste la controversia acerca de la utilidad de la hormona como predictor pronóstico en esta situación. Teniendo en cuenta que la adiponectina ejerce efectos beneficiosos en el remodelado cardiaco patológico, los niveles paradójicos asociados a insuficiencia cardiaca podrían explicarse por la existencia de cierto fenómeno de "resistencia a la adiponectina",[90] comentada previamente en relación a los niveles más elevados de la hormona en individuos de edad avanzada.

-Adiponectina y arteriopatía periférica.

Los niveles de adiponectina se correlacionan positivamente con el índice tobillo-brazo, con la distancia máxima de marcha y con la distancia de claudicación.[91] Sin embargo, en pacientes con arteriopatía periférica sometidos a cirugía de revascularización, la hipoadiponectinemia se asocia a un aumento del riesgo de muerte.[92] Por tanto, en estadios tempranos, los niveles de adiponectina plasmática se correlacionan negativamente con la severidad de la arteriopatía periférica, pero no ocurre lo mismo en pacientes con enfermedad avanzada.

Intervenciones terapéuticas que aumentan los niveles de adiponectina.

-Modificaciones del estilo de vida.

Diversas intervenciones terapéuticas han demostrado incrementar los niveles de adiponectina. Por una parte, la pérdida de peso prolongada puede llegar a normalizar los niveles de la hormona.[67] La combinación de una dieta hipocalórica y de actividad física moderada conlleva una pérdida de peso significativa e incrementa los niveles de adiponectina plasmática,

especialmente en los sujetos diabéticos.[93] Durante el adelgazamiento, la isoforma de alto peso molecular de adiponectina aumenta significativamente, mientras que los niveles de trímeros y hexámeros disminuyen.[44]

Por otra parte, en pacientes con enfermedad arterial coronaria, se ha observado que los niveles plasmáticos de adiponectina se asocian negativamente con el tabaquismo.[94] En adipocitos murinos cultivados se ha demostrado también que la nicotina y el peróxido de hidrógeno reducen la expresión de ARN y la secreción de adiponectina de forma dosis-dependiente.[95]

-Bloqueo del sistema renina-angiotensina-aldosterona.

Los fármacos que bloquean el sistema renina-angiotensina-aldosterona aumentan significativamente los niveles de adiponectina sin modificar el grado de adiposidad. Se ha observado que el tratamiento con losartán o con ramiprilo aumenta los niveles de adiponectina y mejora la sensibilidad a la insulina.[96, 97]

-Agonistas de PPAR-α.

Los agonistas de los PPAR-α mejoran la sensibilidad a la insulina y reducen la adiposidad en animales de experimentación.[98] El receptor adipoR2 se induce tanto por PPAR-α como por PPAR-γ. El tratamiento con fenofibrato aumenta la sensibilidad a la insulina en pacientes con hipertrigliceridemia primaria,[99] y también se observó que incrementa los niveles de adiponectina sin ejercer influencia sobre el peso corporal.[100]

-Agonistas de PPAR-γ.

Las tiazolidinedionas actúan como agonistas PPAR-γ e inducen la expresión y la secreción de adiponectina en humanos y roedores *in vivo* e *in vitro* sin afectar el peso corporal.[29] Tras el tratamiento con tiazolidinedionas, los niveles de adiponectina se incrementan de manera uniforme en diabéticos, así como en controles obesos y con normopeso.[101] Por el contrario, en un estudio realizado en pacientes diabéticos obesos, se observó que el tratamiento con metformina no modifica los niveles plasmáticos de adiponectina, aun consiguiendo un control glucémico similar.[102]

-Estatinas y betabloqueantes.

Los efectos de las estatinas sobre la sensibilidad a la insulina son controvertidos, ya que los resultados de los diferentes estudios al respecto llegan a conclusiones dispares.[103, 104]

Por otra parte, el aumento de la actividad el sistema nervioso simpático disminuye los niveles plasmáticos de adiponectina, y los agonistas betaadrenérgicos y los análogos de AMP inhiben su expresión genética.[105] Por el contrario, los betabloqueantes de nueva generación aumentan los niveles plasmáticos de adiponectina y mejoran la sensibilidad a la insulina.[106]

-LEPTINA.

La leptina es una proteína de 167 aminoácidos codificada por el gen *OB* (locus 7q 31.3) y secretada sobre todo por los adipocitos. Sus niveles plasmáticos se incrementan en la obesidad y se correlacionan con la proporción de grasa corporal total.[107]

Los ratones con mutación del gen *OB* presentan obesidad severa, pero el déficit de leptina es muy raro en humanos. En adultos, dicho déficit produce diversas alteraciones como hiperfagia y obesidad, hiporrespuesta de células T, hiperinsulinemia, resistencia a la insulina, hiperlipidemia, disfunción inmunológica y alteraciones neuroendocrinas.[108]

Los efectos de la leptina son mediados por receptores localizados principalmente en células del sistema nervioso central, pero también en otros tipos celulares, como los adipocitos y las células endoteliales. El receptor de leptina pertenece a la familia de receptores de citoquinas y está relacionado con la vía del trasductor de señal y activador de la transcripción-3 (STAT-3), entre otras. STAT-3 es esencial en la regulación de la ingesta, la neoglucogénesis hepática y la secreción de gonadotropina,[109] pero no influye en el control del metabolismo del tejido adiposo por parte de la leptina.[110]

Existen diversos factores que estimulan la liberación de leptina, como TNF-α y otras citoquinas proinflamatorias, insulina, glucosa, estrógenos, y probablemente a nivel local angiotensina II y endotelina. La presencia de receptores de leptina en varios órganos sugiere que la hormona participa en múltiples procesos fisiológicos: en el crecimiento, el control metabólico, la regulación inmunológica, la regulación de la sensibilidad a la insulina[111] o la reproducción.[107] Además, se han descrito otras acciones de la leptina, como la proliferación y diferenciación de monocitos, y la liberación de TNF-α y de IL-6 por los monocitos.

El estudio WOSCOPS[112] mostró que la leptina es un factor de riesgo de aterosclerosis independiente de la edad, la presión arterial sistólica, los niveles plasmáticos de lípidos, el

IMC y los niveles de proteína C reactiva (PCR). También se encontró asociación entre los niveles plasmáticos elevados de leptina y el ictus,[113] la calcificación de las arterias coronarias y el infarto agudo de miocardio.[114] No obstante, en un estudio prospectivo y metaanálisis reciente, solamente se observó una asociación moderada y no estadísticamente significativa entre los niveles de leptina y el desarrollo de enfermedad arterial coronaria, dependiente en parte del IMC.[115]

El tratamiento con leptina recombinante humana revierte la hiperfagia, la obesidad, el hipogonadismo y las alteraciones inmunológicas asociadas al déficit de leptina,[108] y también es un tratamiento prometedor en el manejo de las complicaciones de la lipodistrofia.[116] Sin embargo, la utilización de la leptina en el tratamiento de la obesidad típica y de sus complicaciones metabólicas no ha sido exitosa, probablemente por algún mecanismo de resistencia a la hormona.[117]

Por tanto, es muy probable que la leptina desempeñe un papel crucial en muchos procesos fisiológicos y que actúe como un marcador de la cantidad de grasa corporal, pero su implicación directa en la patología cardiovascular todavía no se ha demostrado de manera consistente.

-FACTOR DE NECROSIS TUMORAL ALFA (TNF-α).

TNF-α es una citoquina proinflamatoria inicialmente descrita como un factor inducido por endotoxinas, con capacidad de necrosis tumoral. Se trata de una proteína transmembrana de 26 kDa, con un fragmento biológicamente activo de 157 aminoácidos y 17 kDa que ejerce sus efectos mediante su unión a receptores tipo I y II.[118] En el tejido adiposo, TNF-α se expresa en los adipocitos y células del estroma vascular,[119] pero sobre todo en los macrófagos del estroma. Los adipocitos expresan ambos tipos de receptores de TNF-α, ya sea ligados a la membrana o bien en forma soluble.[118]

Los ratones con déficit de TNF-α o de sus receptores no presentan resistencia a la insulina inducida por la obesidad.[120] Los niveles plasmáticos de TNF-α, así como sus niveles de expresión genética en el tejido adiposo, están elevados en situaciones de resistencia a la insulina.[121] Por otra parte, la infusión de TNF-α inhibe la captación de glucosa inducida por la insulina en sujetos sanos.[122] Sin embargo, los intentos de neutralizar los efectos de TNF-α para mejorar la resistencia a la insulina en humanos no han resultado satisfactorios, aunque algunos estudios han mostrado una ligera mejoría con la inhibición de esta citoquina.[123, 124] Los

limitados efectos de TNF-α sobre la resistencia a la insulina podrían explicarse por sus acciones paracrinas, aunque los mecanismos implicados en la sobreexpresión de TNF-α asociada a la obesidad y las señales moleculares que subyacen a las alteraciones metabólicas relacionadas con TNF-α todavía se desconocen.

Recientemente, se ha estudiado también el papel de la respuesta inflamatoria en la fisiopatología de la cardiopatía isquémica. TNF-α estimula la expresión de moléculas de adhesión en las células endoteliales, por lo que podría contribuir al inicio del proceso aterosclerótico.[125] TNF-α se ha asociado a un aumento del riesgo de reinfarto y de muerte cardiovascular tras un primer infarto agudo de miocardio.[126] Los niveles de esta citoquina se correlacionan con el índice tobillo-brazo, que se utiliza para predecir la severidad de la enfermedad arterial periférica,[127] y también con la carga de aterosclerosis observada mediante ecocardiografía carotídea en varones sanos de mediana edad.[128]

Sin embargo, otros investigadores han sugerido que los niveles de receptor de TNF (TNFR) pueden ser un mejor marcador de la carga ateroscleróstica que el propio TNF-α, e incluso se ha observado que son los niveles de TNFR, pero no los de TNF-α, los que se asocian con la aterosclerosis carotídea en individuos menores de 70 años.[129] También se ha estudiado la implicación de TNF-α en la fisiopatología de la insuficiencia cardiaca congestiva, pero el ensayo ATTACH no ha demostrado la utilidad de inhibidores de TNF-α en el tratamiento de esta entidad.[130]

Por otra parte, TNF-α y adiponectina están estrechamente relacionadas. La adiponectina inhibe la adhesión de monocitos y la expresión de moléculas de adhesión inducidas por TNF-α[69] y disminuye la secreción de TNF-α por los macrófagos,[32, 49] mientras que TNF-α tiene la capacidad de reducir la expresión de adiponectina en los adipocitos mediante la supresión de la actividad de su promotor.[29]

-PROTEÍNA QUIMIOTÁCTICA DE MONOCITOS TIPO 1 (MCP-1).

MCP-1 consta de 76 aminoácidos y es una de las principales proteínas que intervienen en los procesos de quimiotaxis y reclutamiento de macrófagos. Se secreta sobre todo por los propios macrófagos, las células endoteliales y los adipocitos,[131] y los individuos obesos presentan mayores niveles plasmáticos y sobreexpresión de la citoquina en el tejido adiposo.[15] Los ratones con déficit de MCP-1 o de sus receptores presentan una disminución de la infiltración de macrófagos en el tejido adiposo y una mejoría de la función metabólica.[12, 131] Sin embargo,

este hallazgo no fue confirmado en un estudio más reciente,[132] lo cual sugiere que puedan existir otras moléculas que también desempeñen un papel relevante en el reclutamiento de macrófagos en el tejido adiposo, como la proteína inflamatoria de macrófagos tipo 1[133] o la osteopontina.[134] MCP-1 contribuye a la resistencia a la insulina, la esteatosis hepática[131] y la aterogénesis.[135]

-INTERLEUQUINA (IL)-6.

IL-6 es una citoquina de 185 aminoácidos con efectos pro y antiinflamatorios en el aparato cardiovascular. Se produce tanto en células del sistema inmune como en células endoteliales, miocitos vasculares, miocitos isquémicos y adipocitos.[107] El tejido adiposo es el responsable de la producción de un 30% de la IL-6 circulante.[119]

IL-6 ejerce diversos efectos fisiológicos, que incluyen la estimulación de la diferenciación de las células B, la activación de las células T, la activación de los macrófagos y de las células *natural killers* (NK), y la estimulación de los hepatocitos para producir reactantes de fase aguda.[136] IL-6 contribuye a la producción de PCR,[137] que en pacientes con enfermedad coronaria se asocia a la presencia de lesiones coronarias complejas.[138] Por otra parte, IL-6 sirve como mediador de los efectos biológicos de la PCR.

La expresión de IL-6 es mayor en TAE que en TAS en pacientes con enfermedad coronaria,[8] lo cual sugiere que IL-6 podría ejercer importantes efectos a nivel local. Al igual que otras citoquinas proinflamatorias, IL-6 es un factor inflamatorio clave en la patogenia de la enfermedad vascular aterosclerótica,[139] ya que desempeña un papel importante en la desestabilización y rotura de la placa aterosclerótica en las arterias coronarias.[107]

Estudios epidemiológicos han demostrado que los niveles elevados de IL-6 y de PCR se asocian a un incremento del riesgo de mortalidad en pacientes ancianos.[140] Los niveles séricos elevados de IL-6, junto con los de otras citoquinas, también se asocian a un peor pronóstico en los pacientes hospitalizados por angina inestable; asimismo, los pacientes con mayores complicaciones durante la evolución hospitalaria de su cuadro clínico presentaban niveles más elevados de IL-6.[141]

IL-6 también desempeña probablemente un papel notorio en la fisiopatología de la insuficiencia cardiaca, la miocarditis y la hipertrofia miocárdica, y se relaciona con la severidad de la disfunción ventricular izquierda y con la activación del sistema renina-angiotensina-aldosterona.[136]

-IL-10.

IL-10 es una citoquina que consta de dos monómeros de 160 aminoácidos cada uno. Posee potentes efectos antiaaterogénicos y su función principal es limitar la respuesta inflamatoria.[142] En pacientes con angina inestable, se observó que aquellos que desarrollaban eventos cardiovasculares durante un seguimiento de tres meses presentaban menores niveles basales de IL-10,[143] y que los niveles elevados de IL-10 también se asocian a un mejor pronóstico en los pacientes con síndrome coronario agudo.[144]

Sin embargo, el efecto benificioso de los niveles altos de IL-10 se limita a los pacientes que presentan concentraciones elevadas de PCR, en los que además IL-10 se asocia con una mejor vasorreactividad endotelial.[145] Estos datos apoyan el concepto de que el balance entre citoquinas pro y antiinflamatorias en un determinante principal de la inestabilidad de la placa y del pronóstico de los pacientes con síndrome coronario agudo.

-INHIBIDOR DEL ACTIVADOR DEL PLASMINÓGENO TIPO 1 (PAI-1).

PAI-1 es una proteína de 379 aminoácidos que inhibe tanto el activador del plasminógeno tisular como el de tipo urokinasa mediante la inhibición de la proteasa serina. Esta inhibición de la fibrinólisis puede contribuir al estado protrombótico.[146] La expresión genética de PAI-1 está regulada por el factor de crecimiento tumoral ß (TGF-ß), que se combina con SMAD fosforilado y se une al promotor de PAI-1.[147] Aunque no exclusivamente, PAI-1 se expresa también en la fracción estromal del tejido adiposo,[148, 149] y sus niveles son mayores en sujetos con complicaciones metabólicas de la obesidad.

Estudios recientes sobre PAI-1 sugieren que se trata de un elemento activo en la patogénesis de la cardiopatía isquémica, ya que tanto los niveles elevados de PAI-1 circulante como el incremento de su actividad se asocian a un aumento de la enfermedad coronaria.[150, 151] Los niveles de PAI-1 son mayores en los sujetos con hiperinsulinemia[152] o diabetes mellitus tipo 2,[153] y probablemente se relacionan también con la oxidación de LDL[154] y con la resistencia a la insulina[155, 156]. El tratamiento hipolipemiante, tanto las estatinas como los fibratos, modula los niveles plasmáticos de PAI-1.[157]

Por tanto, el papel de PAI-1 parece ir más allá de sus efectos sobre la activación del plasminógeno. Probablemente, el desarrollo de antagonistas específicos de PAI-1 abrirá un nuevo campo de investigación que permita aclarar el papel real de PAI-1 en el desarrollo de la aterosclerosis y de la resistencia a la insulina.

-OTRAS ADIPOQUINAS.

La resistina es un péptido de 114 aminoácidos descubierto originalmente como resultado del estudio de la expresión genética del tejido adiposo de ratones sometidos a tratamiento con tiazolidindionas. Los niveles de resistina disminuyen con el tratamiento con tiazolidindionas y son superiores en los ratones con resistencia a la insulina. El tratamiento con anticuerpos anti-resistina mejora la sensibilidad a la insulina y el transporte de glucosa en los adipocitos murinos.[158]

Sin embargo, el papel de la resistina en humanos todavía no se ha esclarecido. Varios estudios que examinaron los niveles plasmáticos de la hormona y sus niveles de expresión en el tejido adiposo no consiguieron demostrar una relación clara con la resistencia a la insulina.[159, 160] Tampoco se conoce todavía su implicación exacta en la fisiopatología de las enfermedades cardiovasculares, aunque algunas observaciones sugieren que los niveles elevados de resistina pueden asociarse a la cardiopatía isquémica y a la insuficiencia cardiaca.[161, 162]

La visfatina es una proteína de 491 aminoácidos que se expresa en múltiples tipos celulares y tejidos, y que se identificó inicialmente como una proteína involucrada en la maduración de las células B.[163] La visfatina se expresa de forma predominante en las células no macrofágicas del estroma adiposo.[164] Se encontró una correlación positiva entre el IMC y la expresión genética de visfatina en el tejido adiposo visceral, así como una correlación negativa entre el IMC y la expresión genética de visfatina en el tejido adiposo subcutáneo,[164, 165] lo cual sugiere que la regulación de esta adipoquina en ambos tejidos es diferente. Algunas observaciones apuntan también a que la visfatina podría estar implicada en el proceso de desestabilización de la placa aterosclerótica.[166]

EL TEJIDO ADIPOSO EPICÁRDICO (TAE).

ANATOMÍA DEL TAE.

El TAE se encuentra entre el epicardio y el pericardio visceral, y es el auténtico depósito graso del corazón. Tanto el TAE como el tejido adiposo abdominal se originan como grasa parda en el mesodermo esplacnopleural durante la embriogénesis, aunque en el adulto constituyen grasa blanca bien diferenciada.[167, 168] Por el contrario, el tejido adiposo mediastínico o paracardiaco, situado en la superficie externa del pericardio parietal, se origina a partir del mesénquima torácico primitivo.[169] El TAE está presente en humanos, en grandes mamíferos y en algunos animales de laboratorio como cobayas y conejos, pero no en roedores pequeños como ratas y ratones, lo cual ha supuesto una cierta limitación para su estudio experimental.

A pesar de tratarse de un componente importante del corazón, los estudios descriptivos sobre el TAE son escasos y no comenzaron hasta mediados del siglo XX, con los trabajos de Reiner.[170] El TAE se localiza fundamentalmente en los surcos interventriculares y auriculoventricular, rodeando los principales vasos epicárdicos, y en menor medida adyacente a la pared libre de aurículas y orejuelas. Cuando está presente en gran cantidad, puede incluso llegar a extenderse por gran parte de la superficie epicárdica ventricular (Figura 1-2). La ausencia de una fascia que lo separe anatómicamente del miocardio y el hecho de que compartan la misma vascularización[9] sugiere la existencia de una estrecha interrelación funcional entre ambos tejidos. La masa de TAE no se asocia a la cantidad de grasa corporal total, pero sí a la de grasa visceral. Por otra parte, el TAE se correlaciona directamente con la masa miocárdica ventricular, y supone el 20% de la masa ventricular total.[171, 172] Puesto que ambos ventrículos poseen la misma cantidad de TAE pero no de miocardio, la

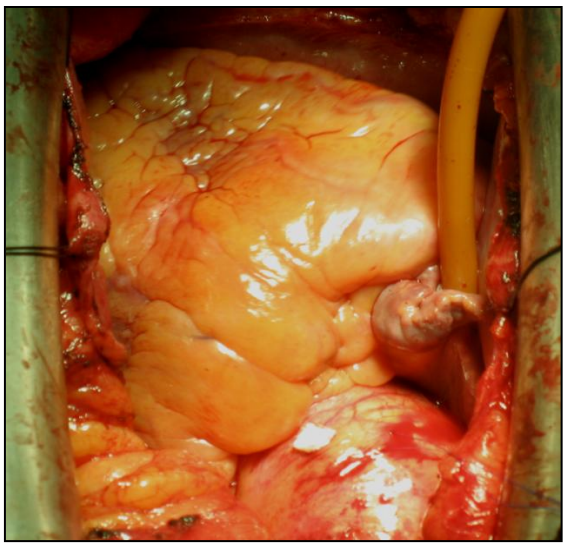

FIGURA 1-2. Tejido adiposo epicárdico, en un paciente sometido a intervención quirúrgica con circulación extracorpórea.

ratio TAE/miocardio es unas tres veces mayor en el ventrículo derecho. Esta ratio se mantiene constante en los sujetos con hipertrofia ventricular, ya que se observa en ellos un incremento notable del TAE, paralelo al del tamaño del ventrículo.[172, 173]

Aunque a raíz de investigaciones en animales de laboratorio se afirmaba inicialmente que el TAE no se deplecciona con el adelgazamiento,[9] estudios recientes demostraron la reducción del TAE en pacientes obesos sometidos a ejercicio físico controlado[174] o a dieta intensiva,[175] si bien la pérdida ponderal de tejido adiposo era muy superior en el depósito visceral que en el epicárdico.[176]

FISIOLOGÍA DEL TAE.

A pesar del creciente interés por el TAE, su fisiología todavía no se conoce por completo. Puesto que su situación anatómica resulta desfavorable para la mecánica cardiaca, se planteó que debía de desempeñar algún papel más allá de servir como depósito energético. Por un lado, el TAE parece ejercer una función protectora; y por otro, se asocia a un mayor riesgo de enfermedades cardiovasculares y metabólicas.

En condiciones fisiológicas, el TAE podría servir como sistema tampón de ácidos grasos libres (AGLs), como depósito energético local en situaciones de alta demanda,[177] y también actuar como grasa parda contra la hipotermia.[178] Los adipocitos del TAE presentan una tasa superior de incorporación y secreción de AGLs. En este sentido, aunque no se estudió la lipolisis ni la lipogénesis directamente en el TAE humano, en cobayas se observaron niveles muy superiores a los de otros tejidos grasos, por lo que se postula que el TAE capta y almacena AGLs para proteger a los cardiomiocitos de la exposición a concentraciones excesivas,[177] que dificultarían el ciclo contráctil y favorecerían el desarrollo de alteraciones de la repolarización y de arritmias cardiacas.[103] El TAE también libera AGLs en situaciones de déficit energético[168, 179] como ocurre en la isquemia miocárdica.

Por otro lado, en los últimos años se ha demostrado que el TAE produce una amplia variedad de moléculas bioactivas. De hecho, se trata de una auténtica fuente de adipoquinas proinflamatorias y proaterogénicas, como MCP-1, TNF-α, IL-1beta, IL-6, receptor soluble de IL-6, resistina,[180] visfatina, omentina, leptina, PAI-1 y angiotensinógeno;[8] pero también de adipoquinas antiinflamatorias y antiaterogénicas, como IL-10, adiponectina[181] y adrenomedulina.[182] No obstante, en la actualidad todavía se desconocen los factores que

pueden modificar el equilibrio entre las moléculas pro y antiinflamatorias sintetizadas por el TAE.

Los adipocitos del TAE son de menor tamaño que los de otros depósitos grasos viscerales.[183] Es posible que existan diferencias en el patrón de expresión y secreción de adipoquinas según el tamaño del adipocito, tal como se ha observado que ocurre en adipocitos procedentes del TAS.[184]

EL TAE Y LAS ENFERMEDADES CARDIOVASCULARES Y METABÓLICAS.

Diversos estudios transversales han vinculado recientemente la cantidad de TAE determinada por distintos métodos de imagen con la patología cardiovascular y metabólica. Si bien la tomografía computarizada (TC) y la resonancia magnética (RM) ofrecen mediciones más completas[185] que la ecocardiografía, la correlación con los datos obtenidos mediante las distintas técnicas de imagen es muy satisfactoria,[171] por lo que la ecocardiografía resulta ser un método sencillo y fiable (Figura 1-3). Se ha propuesto la medición de TAE a nivel de la pared libre del ventrículo derecho como un método adecuado y reproducible.[186] En esta localización, el grosor de la capa adiposa es mayor y resulta más fácil la medición en los planos paraesternales longitudinal y transversal. Varios grupos definieron distintos puntos de corte de grosor del TAE medido por ecocardiograma asociados a mayor riesgo de patología cardiovascular y metabólica [186].

La cantidad de TAE se correlaciona positivamente con la edad, el peso, el IMC, el perímetro de la cintura, la ratio cintura-cadera, la presión arterial, la glucemia basal, la insulina en ayunas y los niveles de triglicéridos y de colesterol LDL; y negativamente con los niveles de colesterol HDL y de adiponectina. Los sujetos con mayor masa de TAE presentan además mayor rigidez arterial carotídea, mayor índice de grosor íntima-media carotídeo,

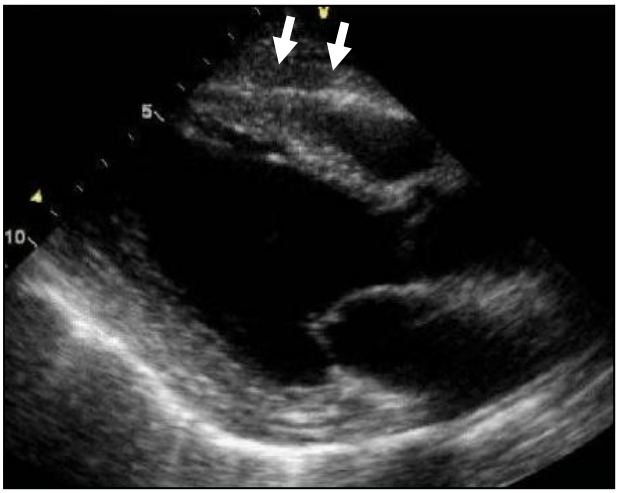

FIGURA 1-3. Ecocardiograma que muestra tejido adiposo epicárdico (flechas) adyacente al ventrículo derecho, en un plano paraesternal longitudinal.

mayor calcificación coronaria y peores parámetros diastólicos.[187-190] El TAE se asocia también a todos los componentes del síndrome metabólico, la resistencia a la insulina, la DM tipo 2 y la aterosclerosis coronaria.[191, 192]

Se ha observado que los volúmenes de TAE superiores a 75 ml predicen la presencia de enfermedad arterial coronaria con sensibilidad y especificidad en torno al 70%.[193] Otro estudio reveló la asociación entre volúmenes excesivamente elevados de TAE (superiores a 300 ml) y la presencia de aterosclerosis coronaria, niveles plasmáticos bajos de adiponectina y de colesterol HDL, y elevados de TNF-α y de PCR ultrasensible.[194] Por otra parte, el volumen de TAE se asocia no sólo a ateromatosis coronaria, sino también a la estabilidad de las placas de ateroma,[195] y el grosor del TAE se ha relacionado con la presencia de disfunción microvascular.[196]

Recientemente, se han publicado varios estudios basados en la cohorte de Framingham que pretendían esclarecer el papel de la grasa pericárdica, definida como aquella que se encuentra en el interior del saco pericárdico. Observaron que la cantidad de tejido adiposo pericárdico medido por TC se correlaciona con múltiples medidas de adiposidad y factores de riesgo cardiovascular, pero el tejido adiposo abdominal presenta una correlación más fuerte con la mayoría de factores de riesgo metabólico. De todas formas, tanto el tejido adiposo pericárdico como el intratorácico se asocian con calcificación vascular, lo cual sugiere que estos tejidos adiposos pueden ejercer efectos tóxicos locales en la vasculatura.[197] El tejido adiposo pericárdico se asocia especialmente a calcificación arterial coronaria.[198] Por el contrario, este tejido, al contrario que la grasa intratorácica, se asocia a la enfermedad cardiovascular de manera independiente respecto a medidas tradicionales de obesidad, pero no al ajustar por factores de riesgo clásicos.[199]

También se encontró asociación entre el tejido adiposo pericárdico –y también la grasa intratorácica, la visceral, el IMC y el perímetro de la cintura– y la masa del ventrículo izquierdo y de la aurícula izquierda, pero no con la del ventrículo derecho. No obstante, en el análisis multivariado sólo persistía la asociación entre el tejido adiposo pericárdico y el tamaño de la aurícula izquierda. Así, los autores sugerían que los efectos sistémicos de la obesidad en la estructura y función cardiacas podrían sobrepasar los efectos patogénicos de la grasa pericárdica.[200] Sin embargo, curiosamente, los sujetos con insuficiencia cardiaca presentan menor cantidad de TAE.[185]

A pesar del creciente interés por el TAE y a la investigación relacionada con la cuantificación de la masa del TAE mediante métodos de imagen, son todavía escasos los estudios diseñados para investigar la expresión de moléculas bioactivas en el TAE. No obstante, se ha demostrado que el TAE produce un perfil patogénico de adipoquinas en pacientes con enfermedad cardiovascular.[8, 201] Además, en estos pacientes existe una importante infiltración del TAE por células inflamatorias. Mediante análisis proteómico, nuestro grupo ha observado que el TAE presenta mayor estrés oxidativo que el TAS.[202]

Los individuos con enfermedad coronaria presentan unos niveles de adiponectina en TAE 40% inferiores a los controles[181] y también se observó una menor producción de adrenomedulina, un importante péptido vasodilatador y antioxidante, en el TAE de pacientes con enfermedad arterial coronaria.[203]

Dada su relación anatómica con el miocardio y las arterias coronarias epicárdicas, el TAE podría interactuar a nivel local mediante la modulación de la actividad paracrina o vasocrina de adipocitoquinas proinflamatorias en las arterias coronarias, pero también a nivel sistémico con la producción de hormonas relevantes en la fisiopatología de las enfermedades cardiovasculares y metabólicas.

OBJETIVOS

El objetivo principal de los trabajos que componen esta memoria es el estudio del papel del TAE en la fisiopatología de las enfermedades cardiovasculares, así como su posible influencia sobre el pronóstico a largo plazo.

Secundariamente, se han propuesto los siguientes objetivos concretos:

1. Analizar la relación entre la expresión de citoquinas inflamatorias y el estado hipertrófico adipocitario del TAE y el TAS.
2. Analizar la relación entre la expresión de adipoquinas en el TAE y la cardiopatía isquémica.
3. Analizar los efectos sistémicos de las adipoquinas expresadas en el TAE y su influencia en:
    a. Hipertensión arterial.
    b. Diabetes mellitus tipo 2.
    c. Síndrome metabólico.
4. Analizar la posible influencia pronóstica de las adipoquinas expresadas en el TAE.

CAPÍTULO 2

TAMAÑO DE LOS ADIPOCITOS EN EL TEJIDO ADIPOSO EPICÁRDICO Y EXPRESIÓN DE MCP-1

RELATIONSHIP BETWEEN EPICARDIAL ADIPOSE TISSUE ADIPOCYTE SIZE AND MCP-1 EXPRESSION.

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ABSTRACT

Adipocyte size has been associated to increase in inflammatory cytokines expression that can be related to the cardiovascular risk of obesity. Epicardial adipose tissue (EAT) was discovered to play a key role in cardiovascular diseases by producing several inflammatory adipokines. We sought to study whether EAT and subcutaneous adipose tissue (SAT) mean adipocyte sizes are related to the expression of adipokines in patients with cardiovascular diseases.

We collected EAT, SAT and blood samples from 22 patients aged 70.9 (s.d.10.3) undergoing heart surgery. Monocyte chemoattractant protein (MCP)-1, interleukin (IL)-10 and tumor necrosis factor (TNF)-α were analyzed by real time RT-PCR, ELISA or inmunohistochemistry. Hematoxylin-eosin staining was used for adipocyte area calculations. Adipocyte size is negatively correlated to MCP-1 expression ($r=-0.475$; $p=0.034$) in EAT and positively correlated in SAT ($r=0.438$; $p=0.047$). These trends persisted after stratification for sex and coronary artery disease (CAD), but only the relationship between EAT MCP-1 and adipocyte size reached statistical significance in the larger group of men with CAD. We have observed that SAT adipocyte size is correlated to BMI ($r=0.601$; $p=0.003$); whereas only a nonstatistically significant trend was observed in EAT. IL-10 and TNF-α expression were not associated to adipocyte size in EAT nor SAT. Secondarily, we found that EAT IL-10 expression is higher in patients with CAD.

These results suggest that adipocyte size is a negative determinant of MCP-1 expression in EAT and a positive determinant in SAT. These data might partly explain the different implications of EAT and SAT in cardiovascular diseases.

INTRODUCTION

Obesity, currently recognized as an inflammatory process,[13] confers an increased risk of major cardiovascular events.[204] This status is associated to adipocyte enlargement.[205] Adipose tissue consists of stromal vascular cells and adipocytes and the ratio between them can also be related to the modulation of several signalling pathways.[206] Adipocytes have a dynamic endocrine role by expressing and secreting a wide range of factors, including hormones and cytokines, that depends on cell size.[207]

Monocyte chemoattractant protein-1 (MCP-1) is mainly produced by macrophages, endothelial cells, adipocytes, and acts a potent chemotactic factor for macrophages in adipose tissue contributing to insulin resistance, hepatic steatosis in obesity[131] and atherogenesis.[125] Interleukin-10 (IL-10) and TNF-α are other cytokines secreted by adipose tissue and upregulated in the obese state.[119] IL-10 suppresses macrophage function and inhibits cytokines production. Contrarily to TNF-α, it is involved in atherogenesis by inducing proinflammatory cytokines and matrix metalloproteinases.[208] Moreover, both molecules are regulated by dietary fatty acids, which play an important role in cardiovascular diseases,[209] contributing to the inflammatory response.[210]

Recently, several studies have paid attention to EAT because it is an interesting visceral fat depot located in the atrioventricular and interventricular grooves and its volume is correlated to metabolic risk factors.[211] In addition, this tissue expresses and secretes several adipokines with pro- and anti-inflammatory properties contributing to different cardiovascular conditions. Several studies have shown that EAT and SAT present different behaviours with respect to adipokines expression.[8, 181, 212-214] In this way, regional differences in adipocyte hypertrophy and inflammatory function might suggest a different metabolic response in patients with cardiovascular disease.

In consequence, for a better understanding of the role of EAT in cardiovascular diseases we aimed to study the possible association between adipocyte size and MCP-1, IL-10 and TNF-α expression in EAT and SAT in patients with cardiovascular disease.

METHODS AND PROCEDURES

Patients.

Twenty-two patients undergoing heart surgery with sternotomy were included in the study. Exclusion criteria were previous heart surgery or concomitant infective diseases. All participants gave their informed consent. The study protocol was approved by the local ethical committee and carried according to the Declaration of Helsinki.

Clinical data were obtained upon admission to hospital before surgery. Diagnosis of coronary artery disease (CAD) was based on previous coronary angiogram. Reductions in luminal coronary artery diameters in excess of 50% were considered significant.

Adipose tissue biopsies.

EAT (0.1-1g wet weight) and SAT (2g wet weight) biopsies were obtained from upper region of the right ventricle and SAT was obtained from the thorax. Samples (43) were immediately splitted in two pieces. One piece was frozen in liquid nitrogen before storage at -80ºC until use and the other one was formalin-fixed and paraffin-embedded.

Blood samples.

Blood samples were taken out after overnight fasting before surgery. Plasma was stored at -40ºC. MCP-1 plasma levels were evaluated in duplicate by instant enzyme-linked immunosorbent assay (Bender MedSystems GmbH, Vienna, Austria). The lowest limit of sensitivity was 2.31pg/mL for MCP-1. The intra- and inter-assay CV were lower than 10%.

mRNA extraction and real time RT-PCR.

mRNA was isolated from 50-100 mg of EAT and SAT with the oligotex mRNA Spin-column kit (Quiagen GmbH, Germany). The final concentration was 1 µg tissue/1 µl solution. Then, 4.14 µl of mRNA dilution was transcribed using 200U of MMLV reverse transcriptase (Invitrogen Corp, CA, USA) in 30 µl of a pH 8.4 solution containing 20 mM Tris- HCl, 50 mM KCl, 2.5 mM de $MgCl_2$, dNTPs (1 mM each), 20 U of RNase inhibitor and random primers under the following conditions: 50 min at 37 ºC, 10 min 42ºC and 5 min at 95 ºC.

Comparative EAT and SAT MCP-1, IL-10 and TNF-α expression levels with respect to GAPDH were analyzed by real time PCR in duplicated using 8 µl of complementary DNA,

SybrGreen (Roche Diagnostics Corp, IN, USA) as fluorochrome and the primers sequence are shown in Table 2-1. The conditions of amplification were as follows: 40 cycles (30 sec at 95 ºC, 60 sec at 58 ºC for MCP-1 or 56 ºC for IL-10 and TNF-α, and 60 sec at 72 ºC).
Fluorescence curves were analysed using Chromo 4 software (MJ Research, Inc., NV, USA). Melting curves were tested in order to probe the correct amplicon. Gene expression levels were calculated with respect to GAPDH expression and represented in arbitrary units (a.u.).

Gene	Primers	Accession no.	Length (bp)	Melting T
MCP-1	5'caaactgaagctcgcactctc3' 5'gctgcagattcttgggttgtg3'	X14768	361	88
IL-10	5'gtgatgccccaagctgaga3' 5'cacggccttgctcttgtttt3'	AF043333	130	87
TNF-α	5'tcttctcgaaccccgagtga3' 5'cctctgatggcaccaccag3'	M10988	151	89
GAPDH	5'tccatgacaactttggcatcgtgg3' 5'gttgctgttgaagtcacaggagac3'	NM_002046.3	365	90

TABLE 2-1. Primers sequences.

Measurement of adipocyte size.

Adipocyte size was determined through light microscopy on formalin-fixed and paraffin-embedded biopsies from all 22 patients. After Harris' hematoxilin staining, the two dimensions (length (r_1) and width (r_2)) of ten adipocytes per each sample were measured using a graded ocular scale. The area of each adipocyte was calculated using the ellipse formula ($\pi\ r_1 r_2$). For statistical analysis, an average of 10 cells from each sample was considered.

Inmunohistochemistry.

Sections 3 µm-thick were mounted on FLEX IHC microscope slides (Dako, Glostrup, Denmark) and heated in an oven at 60ºC for 1h. The immunohistochemical technique was automatically performed using AutostainerLink 48 (Dako). After deparaffination and epitope retrieval in EnVision FLEX target retrieval solution, low pH for 20min at 97ºC, the slides were allowed to cool in PT Link to 65ºC and then in Dako wash buffer for 5 min at room temperature. The immunostaining protocol includes incubation in: (1) EnVision FLEX peroxidase-blocking reagent for 5min; (2) primary MCP-1 monoclonal antibody (R&D

Systems Wiesbaden, Germany) at a dilution of 1/20 for 30min; (3) EnVision FLEX + mouse (linker) for 15min; (4) EnVision FLEX/HRP (dextran polymer conjugated with horseradish peroxidase and affinity-isolated goat anti-mouse immunoglobulins) for 20 min; (5) substrate working solution (mix) (3,3'- diaminobenzidine tetrahydrochloride chromogen solution) for 10 min; and (6) EnVision FLEX hematoxylin for 9 min. As positive control we used sections of ductal invasive breast carcinoma.

Statistical analysis.

All results are shown as mean (s.d.). Pearson's correlation coefficient (r) was used to analyze the strength and direction of a linear relationship between continuous variables. Statistical significance was defined as $p<0.05$. All analyses were computed using SPSS 15.0 software for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

EAT and SAT biopsies were obtained from 22 patients (7 women, 15 men) aged 70.9 (s.d.10.3) years with a BMI of 27.5 (s.d. 5.1) kg/m^2. In all, 11 patients had CAD, although only 9 underwent coronary artery bypass grafting (CABG) surgery. Two of them also underwent valve surgery and 13 patients valve surgery alone. Sample characteristics are shown in Table 2-2.

The sample was split into patients with and without CAD, in order to assess significant differences between both groups. Differences were statistically significant for sex, betablocker intake and HDL cholesterol levels. As regards the prevalence of diabetes, the difference between CAD and non-CAD groups was of borderline significance.

When comparing MCP-1, TNF-α and IL-10 plasma levels and their expression levels in EAT and SAT in patients with CAD with respect to those without CAD, no significant differences were found except for EAT IL-10 expression levels, curiously higher in the group of CAD.

We found a positive correlation between adipocyte area and BMI ($r=0.601$; $p=0.003$) in SAT as Figure 2-1 shows. Also, we have observed a non-significant trend towards a positive correlation between EAT adipocyte area and BMI ($r=0.339$, $p=0.133$) (Figure 2-1). On the other hand, a negative correlation was found between adipocytes area from EAT and its MCP-1 expression ($r=-0.475$; $p=0.034$) as shown Figure 2-2A. Contrarily, SAT adipocytes area was positively associated with MCP-1 expression ($r=0.438$; $p<0.05$) (Figure 2-2B).

	Non-CAD (n=11)	CAD (n=11)	P*
Demographics and measurements			
Age (years)	70.5 (10.5)	71.3 (10.6)	0.86
Male	5/11	10/11	0.022
Body Mass Index (kg/m^2)	27.3 (4.1)	27.7 (6.2)	0.83
Waist circumference (cm)	95 (15)	97 (17)	0.73
Systolic BP (mmHg)	124 (13)	121 (22)	0.67
Diastolic BP (mmHg)	72 (10)	66 (6)	0.14
Comorbidities and cardiovascular risk factors			
Current smokers	1/11	2/11	0.71
Hypertension	9/11	8/11	0.61
Type 2 diabetes	1/11	5/11	0.056
Heart Failure	6/11	6/11	>0.99
Drugs			
ACEIs/ARBs	7/11	5/11	0.39
Statins	3/11	7/11	0.09
Beta-blockers	0/11	4/11	0.027
Calcium channel blockers	1/11	4/11	0.13
Laboratory findings			
Glucose (mg/dL)	95 (10)	110 (68)	0.48
Creatinine (mg/dl)	1.3 (0.6)	1.3 (0.5)	0.78
Triglycerides (mg/dl)	102 (29)	113 (40)	0.47
Cholesterol (mg/dl)	199 (51)	168 (40)	0.13
HDL cholesterol (mg/dl)	47 (17)	32 (9)	0.014
LDL cholesterol (mg/dl)	119 (40)	96 (38)	0.19
HbA1c (%)	5.2 (0.5)	5.7 (1.5)	0.31
Plasma MCP-1 (pg/ml)	157 (74)	163 (80)	0.87
EAT MCP-1 mRNA (a.u.)	7.8 (1.0)	7.7 (1.1)	0.79
SAT MCP-1 mRNA (a.u.)	8.0 (1.0)	7.6 (1.3)	0.40
Plasma TNF-α (pg/ml)	0.36 (0.01-75.8)	0.01 (0.01-16.36)	0.72
EAT TNF-α mRNA (a.u.)	6.7 (0.5)	6.6 (0.5)	0.67
SAT TNF-α mRNA (a.u.)	6.6 (0.8)	6.4 (0.8)	0.76
Plasma IL-10 (pg/ml)	3.6 (2.7-4.7)	2.7 (2.2-3.3)	0.10
EAT IL-10 mRNA (a.u.)	6.0 (0.8)	7.1 (1.3)	0.047
SAT IL-10 mRNA (a.u.)	6.0 (0.9)	6.1 (1.3)	0.73
EAT adipocyte size	4096 (828)	4301 (1149)	0.65
SAT adipocyte size	5111 (1868)	5312 (1647)	0.79

TABLE 2-2. Sample characteristics.

Values expressed as mean (standard deviation) or as median (percentile 25-percentile 75) for skewed variables.

* P-value referred to the comparison between groups with and without CAD.

ACEIs, angiotensin converter enzyme inhibitors; ARBs, angiotensin receptor blockers; a.u., arbitrary units; CAD, coronary artery disease; EAT, epicardial adipose tissue; HDL, high density lipoprotein; IL, interleukin; LDL, low density lipoprotein; SAT, subcutaneous adipose tissue; TNF, tumor necrosis factor.

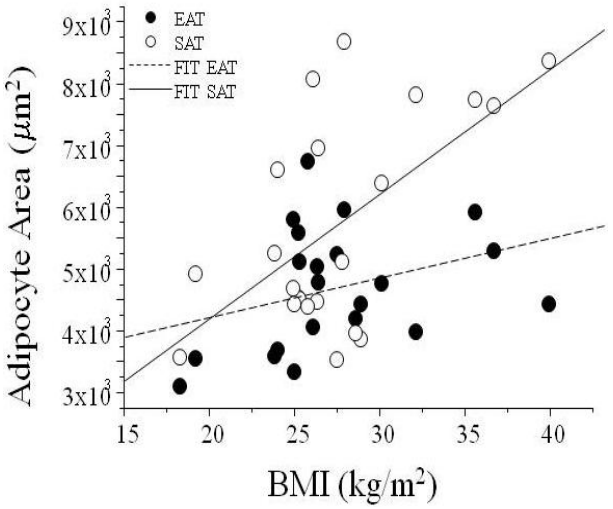

FIGURE 2-1. Correlations between adipocytes area from EAT (r=0.339;p=0.133) or SAT (r=0.601; p=0.003) and BMI are shown.

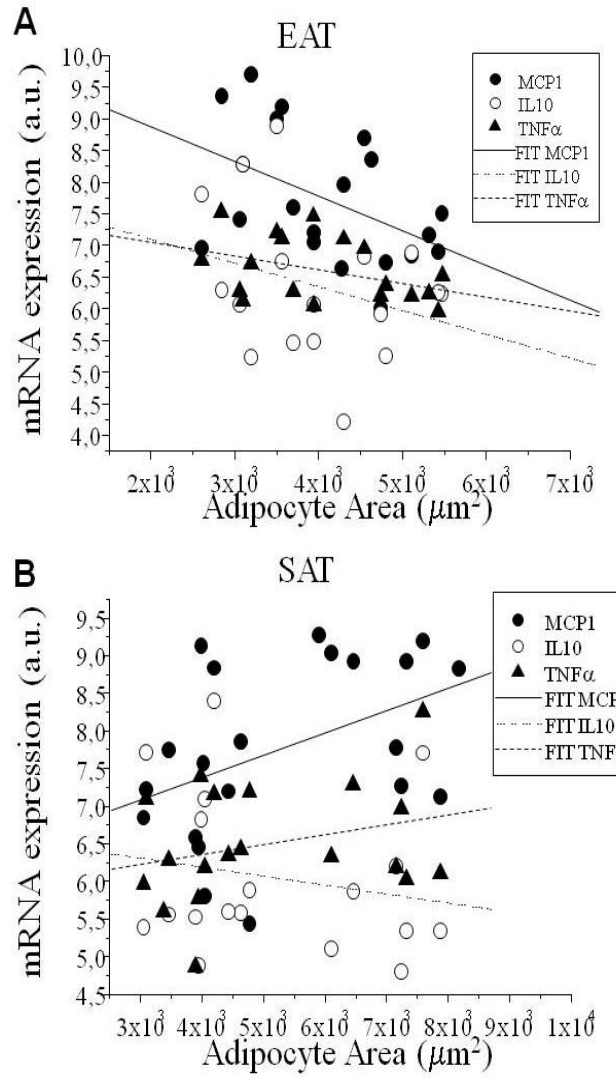

FIGURE 2-2. A Correlations between MCP-1 (r=-0.475;p=0.034), IL-10 (r=-0.292;p=0.256) and TNF-α (r=-0.406;p=0.095) expression levels with respect to GAPDH and adipocytes size in EAT. **B** Correlations between MCP-1 (r=0.438; $p<0.05$), IL-10 (r=-0.061;p=0.809) and TNF-α (r=0.285;p=0.238) and adipocytes size in SAT.

After stratification according to sex and CAD, the same trends were observed in all strata, but statistical significance was only reached in the relation between EAT MCP-1 expression and EAT adipocyte size in the larger stratum of men with CAD (r=-0.720, p=0.029).

In contrast, IL-10 and TNF-α expression showed no association with adipocytes size in EAT (Figure 2-2A) or SAT (Figure 2-2B). However, there was a slight trend to an inverse correlation between IL-10 (r=-292; p=0.256) and TNF-α expression (r=-0.406; p=0.095) to EAT adipocytes size. Nevertheless, the association between TNF-α and SAT adipocytes size tended to be positive (r=0.285; p=0.238) as Figure 2-2B shows. Moreover, we compared plasma MCP-1 levels and EAT or SAT adipocytes size and observed a positive trend in EAT (r=0.385; p=0.094) (Figure 2-3A). However, no trend was observed with respect to SAT adipocytes size (Figure 2-3B).

Our results have shown that MCP-1 expression is associated to adipocyte area in EAT as Figures 2-4A and 2-4B show. This expression is contributed by several types of cells. Thus, immunohistochemical analysis has shown that MCP-1 antibody reacts to adipocytes of EAT (Figure 2-4C). Macrophages and mast cells, as CD68 and mast cells tryptase indicate (Figure 2-4F) were strongly stained (Figure 2-4D). Moreover, one representative sample of EAT has shown positive reaction with MCP-1 in fibroblasts (Figure 2-4E) and in lymphocytes (Figures 2-4G & 2-4H).

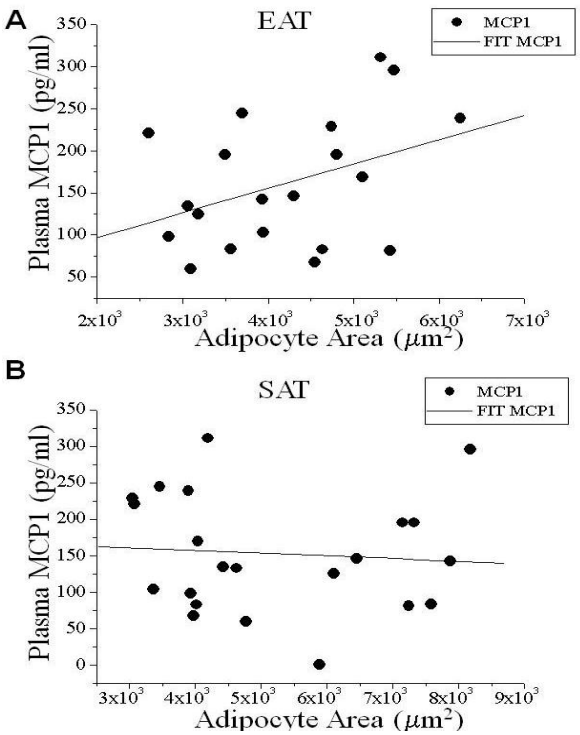

FIGURE 2-3. Correlations between plasma MCP-1 and adipocyte area in EAT (**A**) or SAT (**B**). The correlation between EAT MCP-1 levels and adipocyte size has a positive trend (r=0.385;p=0.094).

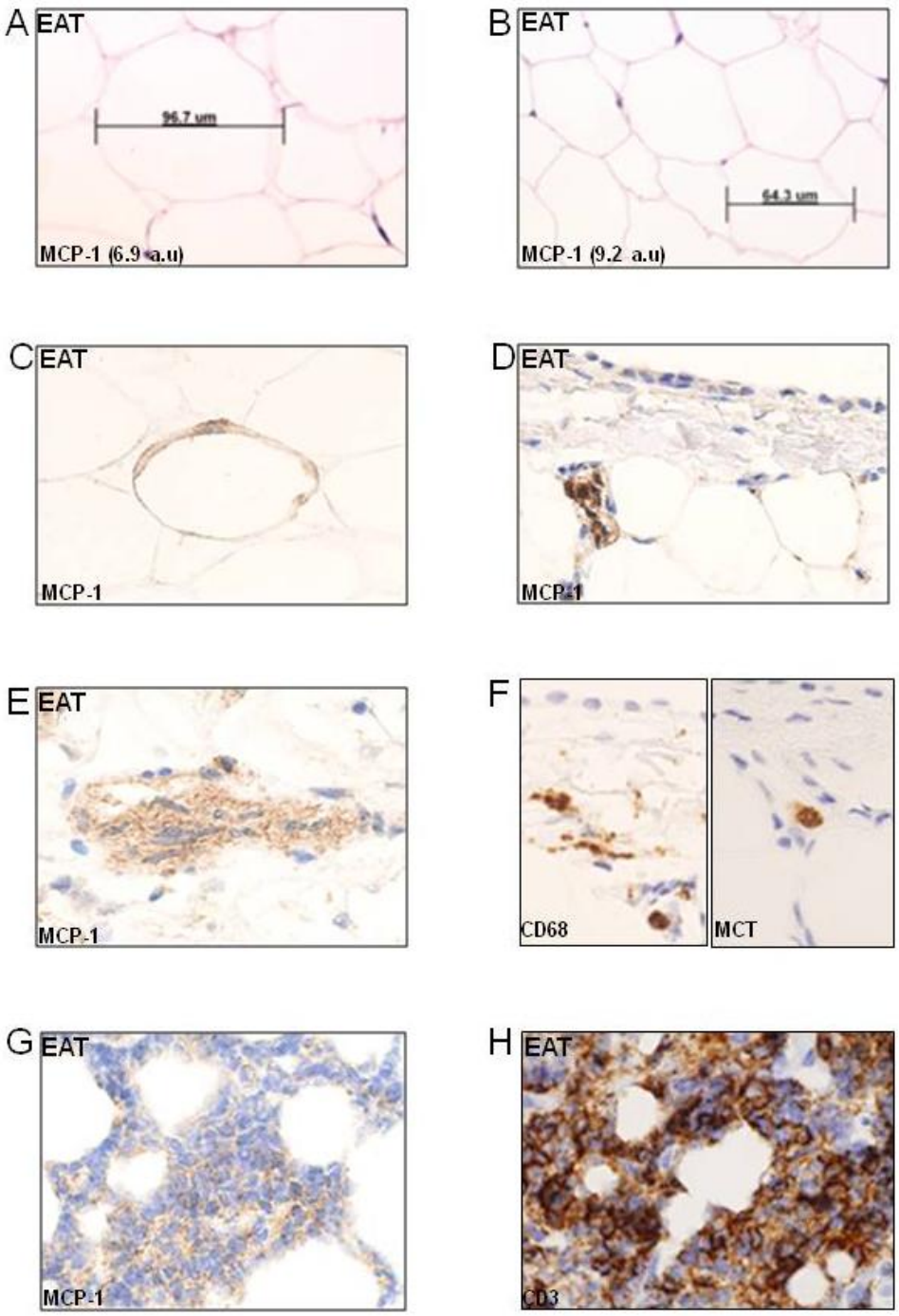

FIGURE 2-4. HE staining of EAT with low levels **(A)** and high levels **(B)** of mRNA MCP-1/GAPDH expression. Inmunohistochemical detection of MCP-1 in adipocytes **(C)**, macrophages **(D)**, fibroblasts **(E)** and lymphocytes **(G)** of EAT. Macrophages and mast cells were detected by CD68 and mast cells tryptase (MCT), respectively (**F**). Lymphocytes were detected by CD3 (**H**). Magnification 400x or 600x.

DISCUSSION

In the current study, we have extended the previous findings by demonstrating for the first time that MCP-1 EAT mRNA expression is inversely correlated to its adipocyte size, in opposite to SAT adipocytes size. Trends persisted after stratification for CAD, but statistical significance could only be demonstrated for the relationship between EAT MCP-1 and EAT adipocyte size in the larger group, namely that of men with CAD. Interestingly, no association was found between IL-10 or TNF-α expression levels and adipocyte size.

Up to date, SAT adipocytes areas were observed to be associated with BMI. Our results are in line with those findings; however, we could not find a statistically significant association between EAT adipocytes area and BMI. In addition, MCP-1 plasma levels present a positive trend with respect to EAT adipocytes size. Obesity is characterized by an increased fat mass mainly resulting from enlarged SAT adipocytes.[215] This association was evident in our results for SAT but was not so clear for EAT. In this way, enlarged SAT adipocytes depend on BMI because accretion of adipose tissue is associated with the relationship between synthesis and degradation of triacylglycerol. However, other mechanisms might contribute to the enlargement of EAT adipocytes. In line with previous reports, we found that EAT adipocytes are smaller than SAT adipocytes.[183]

Overweight exhibits elevated circulating levels of inflammatory markers such as C-reactive protein and proinflammatory cytokines like IL-6 and TNF-α.[121] Other cytokines such as IL-10 counteract with TNF-α and show anti-inflammatory properties.[216, 217] However, other studies suggested that IL-10 can reflect a proinflammatory state. Even though our study was not aimed to seek differences in EAT IL-10 expression between patients with and without CAD, very interestingly, we found that those with CAD express higher levels. Nonetheless, although Cheng and co-workers have found higher protein levels of TNF-α and IL-6 in EAT from CAD than non CAD patients,[218] we did not find differences in EAT TNF-α mRNA between patients with and without CAD.

The expression and release of inflammatory cytokines from SAT in obesity were shown to depend on adipocytes size.[184] MCP-1 is another adipokine whose expression is increased in obese animals.[219] Our data have confirmed the positive association between MCP-1 and adipocytes size in SAT, although this correlation was negative in EAT. This result suggests that EAT behaviour was different to SAT in patients with cardiovascular disease and that local

inflammation could regulate MCP-1 expression in EAT. In line with this finding, Chatterjee and co-workers reported previously that perivascular adipocytes are smaller and produce larger amounts of MCP-1 than SAT adipocytes.[220]

We also analysed MCP-1 plasma levels and observed a positive trend between circulating MCP-1 levels and EAT adipocyte size; however, no correlation was found with SAT adipocytes size. This suggests that a local effect on EAT might be the main factor to the MCP-1 expression changes. In addition, MCP-1 is expressed in SAT and induces macrophage infiltration into adipose tissue that is associated to hepatic steatosis, insulin resistance[131] and atherosclerosis.[135]

In recent years, several studies have shown that EAT is a source of inflammatory mediators and inflammatory cells depot.[8, 201] Lower levels of adiponectin, an anti-inflammatory adipokine were found in CAD[181, 213] and hypertension.[214] It would be interesting to clarify why the EAT behaviour with respect MCP-1 and adipocyte size is different than that of SAT. Local intercellular cross talk is probably more complex than suspected since adipose tissue consists of different stromal cells such as fibroblasts[221] and macrophages[222] which might contribute to an increase in MCP-1. Hence, immunohistochemistry showed that some adipocytes, macrophages, fibroblasts and lymphocytes from EAT were positive for MCP-1. The presence of interactions between immune systems and metabolism might contribute to cardiovascular pathogenesis.

In conclusion, we have found that SAT adipocytes enlargement is more depending on BMI than is EAT adipocyte size in patients with cardiovascular disease. Also, this increase is associated with higher levels of MCP-1 expression in SAT. This is in line with previous studies in patients with overweight without cardiovascular diseases. These data suggest that BMI could be a determinant factor for SAT adipocytes hypertrophy independently of other cardiovascular diseases. However, the inverse association between EAT adipocytes size, plasma MCP-1 and EAT MCP-1 expression suggests that local behaviour of EAT adipocytes and stromal vascular cells might reflect metabolic and inflammatory changes in cardiovascular disorders.

ACKNOWLEDGEMENTS

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CAPÍTULO 3

ADIPONECTINA E INTERLEUQUINA-6 EN EL TEJIDO ADIPOSO EPICÁRDICO Y EXTENSIÓN DE LA ENFERMEDAD CORONARIA

EXTENSION OF CORONARY ARTERY DISEASE IS ASSOCIATED WITH INCREASED IL-6 AND DECREASED ADIPONECTIN GENE EXPRESSION IN EPICARDIAL ADIPOSE TISSUE.

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ABSTRACT

Objective: Epicardial adipose tissue (EAT) expresses lower levels of adiponectin in patients with CAD and higher levels of inflammatory mediators such as IL-6 and leptin than subcutaneous adipose tissue. This showed one important role of EAT in coronary artery disease. However, the relationship of EAT adiponectin and IL-6 levels to the extension of coronary artery disease has not hitherto been determined. We sought to determine whether the levels of adiponectin and interleukin-6 (IL-6) mRNA in EAT are associated with the extension of coronary artery disease (CAD).

Methods: Angiographic and hormones expression were evaluated from epicardial and subcutaneous adipose tissue. 92 patients (58 CAD, 34 non-CAD) who underwent cardiac surgery. Adiponectin and IL-6 mRNA levels were measured by real time RT-PCR in epicardial and subcutaneous adipose tissue (SAT) following angiographic evaluation of their coronary arteries.

Results: We found that epicardial adipose tissue of CAD expressed lower levels of adiponectin mRNA and higher levels of IL-6 mRNA than that of non-CAD patients. As the number of injured arteries rose, adiponectin mRNA levels decreased ($r=-0.402$, $p<0.001$) and IL-6 mRNA increased ($r=0.514$, $p<0.001$) in epicardial adipose tissue.

Conclusions: The extension of CAD is significantly associated with the expression of adiponectin and IL-6 mRNA. These findings suggest that low adiponectin and high IL-6 expression by EAT may contribute to CAD extension.

INTRODUCTION

Coronary artery disease (CAD) and other atherosclerotic cardiovascular conditions jointly remain the leading cause of death in industrialized nations. A major, modifiable risk factor is obesity.[223] Adipose tissue is now recognized as an active endocrine and paracrine organ that releases several bioactive molecules, known as adipokines, that affect body weight, coagulation, fibrinolysis, insulin resistance, inflammation and atherosclerosis.[224] Particular interest has focused on epicardial adipose tissue[7] due to its proximity to coronary arteries and the consequent possibility that it may have paracrine effects on them. Moreover, this adipose tissue is related to left ventricular mass, insulin resistance, circulating low-density lipoprotein cholesterol levels, and arterial blood pressure.[191, 225] In CAD patients, EAT secretes higher levels of pro-inflammatory agents than SAT; such as IL-1ß, IL-6, TNF-α, IL-6sR and MCP-1[8] all of which influence energy metabolism, vascular function and immunologic and inflammatory responses.[9]

Recently, special interest has developed concerning the relationship between CAD and adiponectin, an adipokine with below-normal circulating levels in obese people. It is known that adiponectin has anti-diabetic and anti-atherogenic properties[226, 227] and low plasma adiponectin is associated with complex coronary lesions in men with CAD.[138] Moreover, EAT secretes less adiponectin in CAD patients than in non-CAD heart surgery patients.[181]

It has been established that the levels of adiponectin, IL-6 and other adipokines produced by EAT are altered in CAD patients. However, it has not previously been established whether these levels actually correlate with the extension to which CAD has progressed by the number of affected arteries. Here, we report the results of a study about the relationship between the extension of CAD and the levels of adiponectin and IL-6 mRNA in EAT.

MATERIALS AND METHODS

Subjects.

EAT and SAT samples were obtained from 92 patients undergoing elective heart surgery, either for coronary artery bypass grafting or for valve surgery. Biopsies were harvested just before extracorporeal circulation. Only those patients with no previous coronary angiogram or those with insufficient EAT for analysis were excluded. The study was approved by the

local Ethics Committee and written informed consent was obtained from each patient before his or her participation.

Clinical data.

Information on demographics, anthropometric measurement, cardiovascular risk factors and previous medical history was obtained retrospectively from records. Lipid profile data was obtained within 3 months prior to surgery, and all other laboratory data and the medication taken by each patient within the week prior to surgery.

Coronary artery disease assessment.

The CAD group was defined according to the presence of at least a coronary stenosis ≥ 75% of luminal stenosis. So, this is an angiography classification rather a clinical one. Then, patients were classified as having 0, 1, 2 or 3 injured-coronary arteries (left main coronary was considered as 1 artery). Patients in groups 1, 2 and 3 were defined as CAD patients, and patients in group 0 as non-CAD.

Adipose tissue collection and RNA extraction.

EAT and SAT from each patient underwent heart surgery. EAT was immediately frozen at -80ºC pending RNA extraction. RNA was extracted by the Trizol method. The concentration and purity of the sample was estimated from the ratio between absorbances at 260 nm and 280 nm. Contaminated samples with genomic DNA were treated with DNase I. Each 5 µg of RNA was treated with 10U/µl of DNase I and 20U/µl of RNase inhibitor (both products Invitrogen Corp, CA, USA) for 2 h at 37 ºC. Then, proteins and DNA were removed from the samples with phenol, chloroform and isoamylalcohol. Finally, the RNA was precipitated with 96% ethanol and sodium acetate 0.3 M.

Reverse transcription and real time PCR.

Reverse transcription and real time polymerase chain reaction (RT-PCR) was performed using 1.2 µg of purified RNA and 200 U of MMLV reverse transcriptase (Invitrogen Corp, CA, USA) in 30 µl of a pH 8.4 solution containing 20 mM Tris- HCl, 50 mM KCl, 2.5 mM de $MgCl_2$, dNTPs (1 mM each), 20 U of RNase inhibitor and random primers under the following conditions: 50 min at 37 °C, 10 min 42 °C and 5 min at 95 °C.

The comparative analysis of adiponectin and IL-6 expression with respect to GAPDH in EAT and SAT was analyzed using 2 µl of complementary DNA by real time PCR using SybrGreen (Roche Diagnostics Corp, IN, USA) as fluorochrome and the primers listed in Table 3-1.

TABLE 3-1. Specific primers used for PCR amplification of adiponectin, IL-6 and GAPDH.

Adiponectin	sense	5'-TGGTGAGAAGGGTGAGAA-3
	antisense	5'-AGATCTTGGTAAAGCGAATG-3'
IL-6	sense	5'-GTGGCTGCAGGACATGACAA-3'
	antisense	5'-TGAGGTGCCCATGCTACATTT-3'
GAPDH	sense	5'-TCCATGACAACTTTGGCATCGTGG-3'
	antisense	5'-GTTGCTGTTGAAGTCACAGGAGAC-3'

Adiponectin and IL-6 amplification was performed as previously described.[228, 229] Genomic contamination was ruled out by means of negative controls subjected to retrotranscription conditions without MMLV. Fluorescence curves were analyzed using Chromo 4 software (MJ Research, Inc., NV, USA). Adiponectin and IL-6 mRNA levels are reported as measured levels relative to GAPDH.

Immunohistochemistry.

Paraffin-embedded tissue sections were deparaffined and rehydrated. Immunostaining was performed with antibodies directed against IL-6 (1:5 dilutions) and adiponectin (1:250) (both from Santa Cruz Biotechnology, Delaware, CA, USA) overnight. Following the protocols LSAB (Dako Diagnostics, Glostrup, Denmark) was used to detection, respectively. Immunodetection was developed with 3,3'-diaminobenzidine tetrahydrochloride kit (Dako Diagnostics, Glostrup, Denmark). Negative controls were carried out omitting the primary antibody. Inflammated appendix and SAT tissue were used as a positive control for IL-6 antibody and adiponectin antibody, respectively.

Statistical analyses.

Continuous variables are summarized as means±SD. Differences between the means of groups defined by the number of affected arteries was estimated by ANOVA.

Categorical variables are expressed as percentages and compared using chi-squared test or Fisher's exact test.

Pearson correlation coefficients were calculated to evaluate unadjusted associations between adiponectin and IL-6 mRNA levels in EAT and SAT and number of injured arteries.

Predictors of extension of CAD were identified by means of univariate polynomial logistic regression analyses with the lesion-free group (group 0) as reference, followed by multivariate analyses including variables that had proved significant in the univariate analyses and by stepwise method.

Statistical significance was defined as $p<0.05$. All analyses were performed using SPSS 11.5.1. Software for Windows (SPSS Inc., Tokyo, Japan).

RESULTS

Adiponectin and IL-6 mRNA expression levels in EAT and SAT.

Our sample consisted of 58 patients with CAD and 34 controls without angiographically proved CAD. Adiponectin mRNA levels were significantly lower in EAT of CAD patients than in patients with no CAD [13,0±3,8 as against 15,8±2,8 and IL-6 mRNA levels significantly higher, 8,1±1,5 as against 6,5±1,1 ($p<0.001$ in both cases; see Figures 3-1A and 3-1B)].

Then, 92 subjects (66 men, 26 women) included in this study were classified in four different groups according the number of affected major epicardial coronary arteries: 0 (n=34), 1 (n=11), 2 (n=18) or 3 (n=29). Table 3-2 shows baseline clinical characteristics of patients.

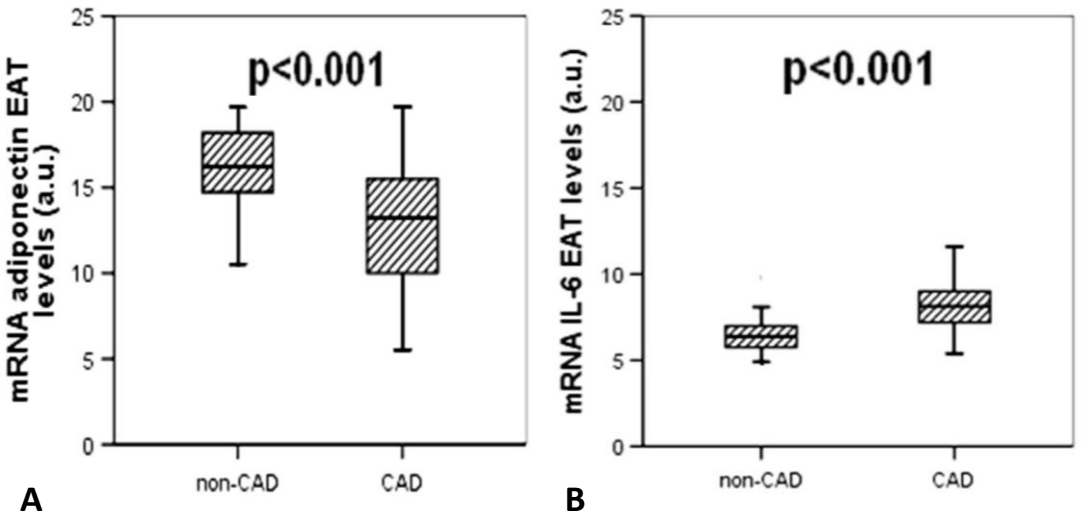

FIGURE 3-1. A. Adiponectin mRNA levels in epicardial adipose tissue (EAT) of 58 patients with coronary artery disease (CAD) and 34 without (Non-CAD) **B.** IL6 mRNA levels in EAT of 26 patients with CAD and 23 without (Non-CAD).
mRNA levels are expressed as amplified cDNA relative to GAPDH.

	Group (=number of injured arteries)				
	0 (n=34)	1(n=11)	2(n=18)	3(n=29)	p
Male (%)	53	73	89	83	0.016
Age (yr)	70±8	73±4	67±7	67±9	0.076
BMI (kg/m^2)	28±4	29±5	28±3	29±4	0.776
LVEF	65±12	67±11	56±16	53±16	0.011
Urea (mg/dL)	56±35	56±24	56±22	56±22	>0.99
Creatinine (mg/dL)	1.3±0.3	1.1±0.4	1.2±0.5	1.1±0.4	0.275
Cholesterol (mg/dL)	184±43	192±40	180±42	172±42	0.605
HDL-c(mg/dL)	36±11	40±11	34±11	36±14	0.340
LDL-c(mg/dL)	106±26	124±42	101±33	101±33	0.647
Triglycerides(mg/dL)	113±46	112±47	125±35	118±47	0.836
HT (%)	56	64	83	72	0.506
Type 2 Diabetes (%)	29	9	28	48	0.120
ACE-inhibitors (%)	32	46	33	35	0.120
Statins (%)	24	46	50	55	0.043
Betablockers (%)	21	18	39	48	0.054

TABLE 3-2. Baseline clinical characteristics of patients with 0, 1, 2 or 3 injured arteries.

p-values are for one-way ANOVA among the four groups.

BMI: body mass index, LVEF: left ventricular ejection fraction, HDL-c: high-density lipoprotein-cholesterol, LDL-c: low density lipoprotein-cholesterol, HT: arterial hypertension, ACE, angiotensin converting enzyme.

	Group (=number of injured arteries)				
	0 (n=34)	1 (n=11)	2 (n=18)	3 (n=29)	P
EAT adiponectin (a.u.)	15.8±2.8	14.8±3.4	12.7±3.4	14.1±3.8	0.001
SAT adiponectin (a.u.)	17.3±4.8	12.6±5.5	13.1±2.7	14.5±4.8	0.03
EAT IL-6 * (a.u.)	6.5±1.1	7.9±2.1	7.7±1.1	8.4±1.4	0.001

TABLE 3-3. mRNA adiponectin and IL-6 expression levels with respect to GAPDH on EAT of patients with 0, 1, 2 or 3 injured arteries.

* EAT IL6 mRNA was only measured in a subset of patients: 27 in group 0, 7 in group 1, 7 in group 2, and 12 in group 3.

p-values are for one-way ANOVA among the four groups.

EAT: epicardial adipose tissue. SAT: subcutaneous adipose tissue.

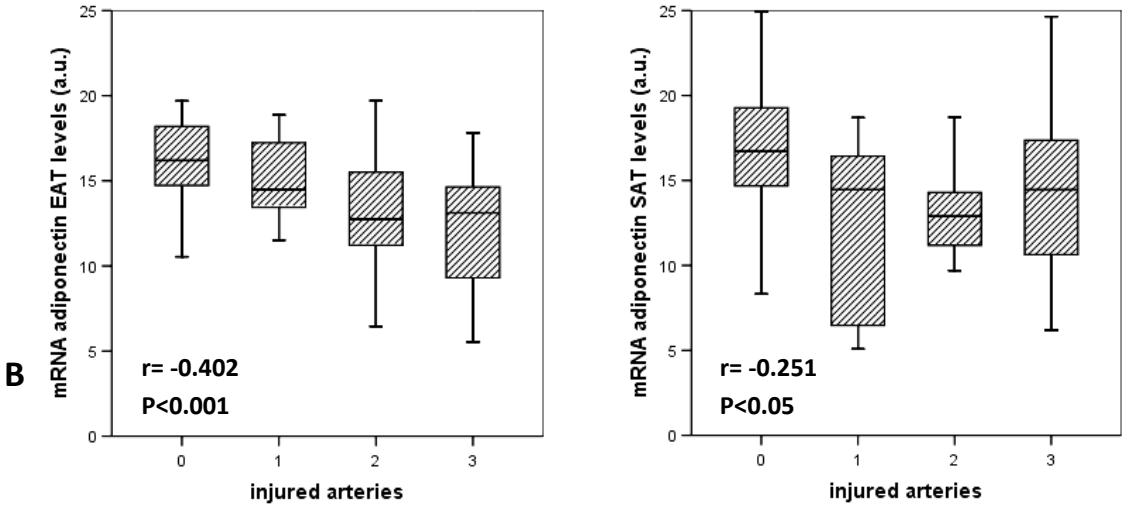

FIGURE 3-2. A. Association between EAT (**A**) and SAT (**B**) adiponectin mRNA levels and the number of injured arteries (0, 1, 2 or 3).

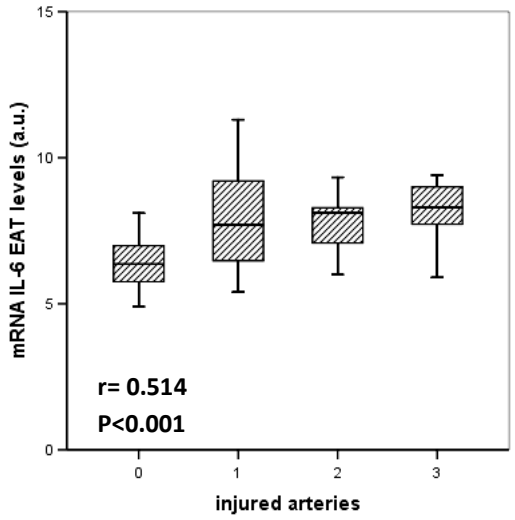

FIGURE 3-3. Association between EAT IL-6 mRNA levels and the number of injured arteries (0, 1, 2 or 3).

There were statistically significant between-group differences in the proportion of male patients and the proportion of those taking statins, both increasing with the extension of CAD ($p<0.05$), while left ventricular ejection fraction (LVEF) diminished ($p<0.05$).

Moreover, adiponectin and IL-6 mRNA levels were associated to the number of injured arteries (Table 3-3). Thus, adiponectin mRNA levels in EAT decreased as number of injured arteries increased ($r=-0.402$, $p<0.001$) (Figure 3-2A). The same association was observed in SAT ($r=-0.251$, $p<0.05$) (Figure 3-2B). On the contrary, we found a positive association between IL-6 mRNA levels in EAT and number of injured arteries ($r=0.514$, $p<0.001$) (Figure 3-3).

Adiponectin and IL-6 expression in EAT and SAT.

EAT was immunostained by IL-6 and adiponectin antibody. Human inflammated appendix and SAT were used as positive controls of IL-6 and adiponectin, respectively (Figures 3-4A, 3-4A'). We have shown the representative slides of EAT from one patient with CAD (Figures 3-4B, 3-4B') and other without CAD (Figures 3-4C, 3-4C'). Immunohistochemistry illustrates that IL-6 and adiponectin protein are expressed in EAT. However, the amount of IL-6 protein in EAT seems to be higher in the sample from a patient with multi-vessel disease (Figure 3-4C) than in the control without CAD (Figure 3-4B). Contrarily, adiponectin protein levels were lower in multi-vessel disease patient (Figure 3-4C'), than in patient without CAD (Figure 3-4B').

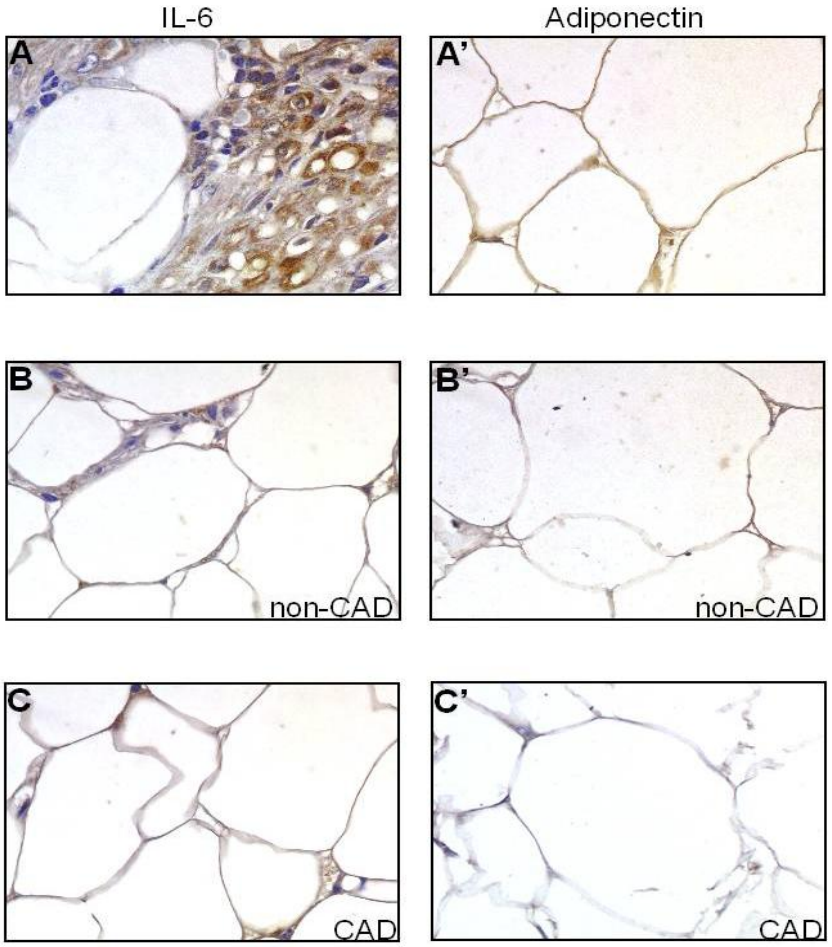

FIGURE 3-4. Original magnification x100. Immunohistochemistry of IL-6 antibody **(A, B, C)** and adiponectin antibody **(A'B'C')** in inflammated appendix tissue **(A)** in subcutaneous adipose tissue **(A')** and epicardial adipose tissue from patients without CAD **(B, B')** and with CAD (3 injured arteries) **(C,C').**

Predictors of single and multi-vessel CAD.

Univariate polynomial logistic regression analyses showed that the risk of multiple-vessel CAD fell significantly as EAT and SAT adiponectin mRNA levels rose [odds ratio (OR) 0.750, p<0.001 for EAT adiponectin; OR 0.856, p<0.05 for SAT adiponectin, see Table 3-4 for 95% confidence intervals]. Other variables associated with decreased risk in univariate analyses were female sex (OR 0.197, 95% CI 0.069-0.561; p<0.01) and LVEF (OR 0.946, 95% CI 0.909-0.984; p<0.01). Extension of CAD increased with rising EAT IL-6 mRNA

Variables	Single affected coronary		Multiple affected coronaries	
	Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
EAT adiponectin mRNA*	0.900 (0.727 – 1.114)	0.332	0.750 (0.641 – 0.878)	<0.001
SAT adiponectin mRNA*	0.789 (0.630 – 0.990)	0.040	0.856 (0.753 – 0.972)	0.017
EAT IL-6 mRNA*	2.616 (1.252 – 5.470)	0.011	2.847 (1.510 – 5.365)	0.001
Age (per 1- year incr.)	1.069 (0.949 – 1.204)	0.274	0.945 (0.889 – 1.003)	0.064
Female sex	0.422 (0.095 – 1.868)	0.256	0.197 (0.069 – 0.561)	0.002
BMI (per 1 kg/m^2 incr.)	1.073 (0.892 – 1.292)	0.454	1.015 (0.897 – 1.149)	0.811
Hypertension	1.596 (0.347 – 7.339)	0.548	2.463 (0.911 – 6.657)	0.076
Type 2 Diabetes	0.250 (0.027 – 2.286)	0.220	1.407 (0.539 – 3.674)	0.485
Dyslipemia	0.706 (0.167 – 2.989)	0.636	1.361 (0.510 – 3.632)	0.538
LVEF (per 1% incr.)	1.006 (0.946 – 1.071)	0.840	0.946 (0.909 – 0.984)	0.006
Creatinine(per 1 mg/dl incr.)	3.315 (0.280 – 39.309)	0.342	6.056 (0.911 – 40.270)	0.062
Urea (per 1 mg/dl incr.)	1.000 (0.975-1.026)	0.989	1.000 (0.983-1.017)	0.990
Cholesterol (per 1 mg/dl incr.)	1.004 (0.987 – 1.021)	0.638	0.995 (0.983 – 1.007)	0.410
Triglycerides (per 1 mg/dl incr.)	0.999 (0.981 – 1.017)	0.919	1.004 (0.992 – 1.016)	0.513
ACEI/ARA II	1.742 (0.435 – 6.977)	0.433	1.154(0.449 – 2.961)	0.766
Statins	2.708 (0.650 – 11.284)	0.171	4.062(1.514 – 10.898)	0.005

TABLE 3-4. Predictors of single- and multi-vessel CAD: results of univariate polynomial logistic regression analyses with group 0 (non-CAD patients) as reference.

* Per GAPDH unit.

EAT: epicardial adipose tissue; SAT: subcutaneous adipose tissue; IL-6: Interleukin 6; BMI: Body mass index; LEFV: left ventricular ejection fraction; ACEI/ARA II: angiotensin converting enzyme inhibitors / angiotensin receptor blockers.

levels (OR 2.847, 95% CI 1.510-5.365; p<0.001) and statins treatment (OR 4.062, 95% CI 1.514 – 10.898; p<0.001) (Table 3-5).

The risk of single-vessel CAD was significantly influenced by EAT IL-6 mRNA level, increasing as IL-6 mRNA expression increased (OR 2.616, 95% CI 1.252-5.470; p<0.05).

With all the above variables, IL-6 mRNA levels included in the analysis, multivariate logistic regression identified lower EAT adiponectin mRNA levels, lower LVEF and statin treatment as independently significant predictors of multi-vessel CAD, and statin treatment as the only independently significant associated factor to single-vessel CAD (OR 7.431, 95% CI 1.275-43.302, p<0.05) (Table 3-5). When EAT IL-6 mRNA was included in the model, it proved to be the only independent predictor of CAD.

	Single affected coronary		Multiple affected coronaries	
	Odds Ratio (95% CI)	**P**	**Odds Ratio (95% CI)**	**P**
Without EAT IL-6 mRNA in the analysis				
EAT adiponectin mRNA	0.963 (0.722 –1.285)	0.799	0.754 (0.613 – 0.928)	0.008
LVEF (×1% incr.)	0.996 (0.928 – 1.068)	0.903	0.928 (0.883 – 0.976)	0.004
Statins	7.431 (1.275 – 43.302)	0.026	5.858 (1.512– 22.700)	0.011
With EAT I-L6 mRNA in the analysis				
EAT IL-6 mRNA	2.391 (0.998 – 5.732)	0.051	2.708 (1.194 – 6.142)	0.017
EAT adiponectin mRNA	1.014 (0.614 – 1.674)	0.958	0.686 (0.442 – 1.066)	0.094
LVEF (×1% incr.)	1.033 (0.933 – 1.144)	0.535	0.933 (0.860 – 1.013)	0.098

TABLE 3-5. Impact of adiponectin and IL-6 on the extension of CAD: results of multivariate polynomial logistic regression analyses with group 0 (non-CAD patients) as reference.

CI: confidence interval; EAT: epicardial adipose tissue; SAT: subcutaneous adipose tissue; LVEF: left ventricular ejection fraction; IL-6: Interleukin 6.

DISCUSSION

EAT, like other adipose tissues, is a metabolically active organ that generates free fatty acids,[168] adiponectin,[181] variety of inflammatory cytokines that include leptin, interleukins (including IL-6) and tumor necrosis factor,[8] visfatin, omentin[230, 231] and fatty-binding protein 4.[232] It is associated with several cardiovascular risk factors, including LDL cholesterol, insulin resistance, arterial blood pressure, left ventricular mass, atrial dimensions, and diastolic function.[225] In adults it is found in the atrioventricular and interventricular grooves extending to the apex. As the amount of EAT increases it fills the space between the ventricles and spreads from the epicardial surface along the adventitia of the coronary artery branches. The proximity of EAT to coronary arteries suggests the possibility that its secretions may have paracrine effects on these vessels.

The above results show that adiponectin expression levels in the EAT of patients with CAD are significantly lower than in those of non-CAD heart surgery patients. This confirms the conclusions drawn by Iacobellis *et al.*[181] from data for a smaller patient sample (16 CAD and 6 non-CAD patients). More specifically, we found that EAT adiponectin mRNA levels tended to fall as the extension of CAD, as measured by the number of angiographically injured coronary arteries, increased, and that lower EAT adiponectin mRNA levels predicted increased risk of multi-vessel disease. In addition, we observed that EAT IL-6 mRNA levels were significantly higher in CAD than in non-CAD patients; that these levels tended to increase with the extension of CAD; and that higher EAT IL-6 mRNA levels predicted increased risk of CAD. Immunohistochemistry has shown that IL-6 and adiponectin proteins were also expressed in EAT. The representative slides from tissue sections of patients with multi-vessel and non CAD indicates that their expression proteins changes as the severity of the disease increase.

The fact that lower EAT adiponectin mRNA was *not* an independently significant predictor of multi-vessel CAD when EAT IL-6 mRNA was included in the multivariate analysis suggests that adiponectin mRNA expression may depend on IL-6 gene expression in EAT, i.e. that in EAT the pro-inflammatory cytokine IL6 downregulates EAT adiponectin, thereby promoting the amplification of vascular inflammation, plaque instability and neovascularisation,[233-235] just as exposure to IL-6 downregulates the secretion of adiponectin by SAT adipocytes.[236] This hypothesis is to some extent supported by the fact that IL-6 gene expression is more intense in

EAT than in SAT in patients with CAD.[8] In fact, the no correlation found between inflammatory cytokines expressed in EAT and plasma concentrations of circulating cytokines by Mazurek *et al.*[8] may explain that EAT IL-6 may play an important local effect on the extension of CAD.

Adiponectin, a 244-residue collagen-like protein that was discovered in 1996, is expressed mostly in adipose tissue.[22] The two adiponectin receptors that have been identified to date have been found, in varying proportions, in skeletal muscle,[36] cardiac muscle[57] and liver,[37] and in macrophages and atherosclerotic lesions.[38] The anti-obesity hormonal actions of adiponectin include its increasing fatty acid oxidation and glucose uptake and the synthesis of glucose in the liver.[36, 57] It also has anti-atherogenic and anti-inflammatory effects, accumulating in the subendothelial space of injured arteries, where it reduces the production of adhesion molecules by endothelial cells and inhibits the conversion of macrophages to foam cells by inducing the production of IL-10- and IL1-receptor blockers.[69, 181, 237] Both the anti-obesity and the anti-inflammatory actions of adiponectin are in keeping with low circulating adiponectin levels being a risk factor for CAD, and several studies have indeed shown that low systemic adiponectin levels are associated with myocardial infarction[74] and with CAD and the complexity of CAD lesions in men.[138] In this study, the EAT adiponectin mRNA levels of CAD patients were lower in patients with more extensive CAD, as measured by the number of injured arteries, and low EAT adiponectin mRNA levels were a risk factor for multi-vessel CAD independently of other risk factors except low LVEF and treatment with statins, so long as EAT IL-6 mRNA levels were not included in the analysis.

If EAT IL-6 mRNA levels were included in the multivariate analysis together with EAT adiponectin mRNA levels, they were identified as the only independently significant risk factor for CAD; this in spite of the association between the extension of CAD and treatment with statins, which lower circulating IL-6 levels.[238] The association between IL-6 and the extension of CAD is in keeping with the important role of inflammation in the pathogenesis of atheromatous plaques[239] and with the contribution of IL-6[137, 240] to the production of hs-C-Reactive Protein (CRP), which in CAD patients is associated with the presence of complex coronary lesions[138] White adipose tissue contributes about 30% of circulating IL-6[119] and the expression of this cytokine is higher in EAT than SAT in patients with CAD.[8] In fact, IL-6 might be regulating the adiponectin expression in EAT and this inflammatory property of this

tissue could lead to amplification of vascular inflammation plaque instability and neovascularisation.[233-235]

We have previously described that mRNA adiponectin and leptin expression levels were lower in EAT than in SAT.[230] But now, our results indicate that adiponectin in SAT seems to be associated with the extension of coronary artery disease; however this relationship is lower than in EAT. This association depends in part of IL-6 expression levels of this tissue.

CONCLUSION

The major findings of this study are that increased extension of CAD is associated with both lower adiponectin mRNA levels and higher IL6 mRNA levels in EAT, but that only IL-6 mRNA level is an independently significant predictor of CAD when both this variable and adiponectin mRNA level are included in the analysis. This suggests that higher levels of IL-6 mRNA might decrease adiponectin mRNA expression in EAT.

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CAPÍTULO 4

ADIPONECTINA EN EL TEJIDO ADIPOSO EPICÁRDICO E HIPERTENSIÓN ARTERIAL

EPICARDIAL ADIPOSE TISSUE EXPRESSION OF ADIPONECTIN IS LOWER IN PATIENTS WITH HYPERTENSION.

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ABSTRACT

Low plasma adiponectin levels are related to a higher risk of development of metabolic and cardiovascular disorders, including hypertension (HT). To date, there have been no studies supporting the relationship between epicardial adipose tissue (EAT) expression of adiponectin and HT.

We collected samples of EAT from 116 patients undergoing elective cardiac surgery, mostly for coronary artery bypass grafting (n=54), valve surgery (n=49) or both (n=12). Samples of subcutaneous adipose tissue (SAT) were harvested from 85 patients. After RNA isolation, the expression of adiponectin was analysed by real time retrotranscriptase (RT-PCR). Baseline clinical data were obtained from medical records. The diagnosis of HT was established mostly by the patients' general physicians following current guidelines.

We included 84 hypertensive and 32 non-hypertensive patients. Mean (±s.d.) age was 70.3±7.9 years. EAT expression levels of adiponectin were lower in hypertensives (14.0±3.6 vs 15.3±3.6 arbitrary units (a.u.), P=0.06). This difference was statistically significant (odds ratio 0.828 per a.u., P=0.020) after adjustment for age, gender, body mass index, diabetes mellitus, heart failure, coronary artery disease (CAD), total cholesterol and triglyceride levels. However, SAT adiponectin mRNA levels were similar in hypertensive and non-hypertensive patients [15.3±4.2 vs 15.3±5.0 a.u., P>0.99]. Adjustment for potential confounding factors hardly altered this result.

Our findings indicate that EAT expression of adiponectin may be associated with HT status independently of CAD or other comorbidities, whereas SAT expression does not. These results support the hypothesis that EAT is actively implicated in global cardiovascular risk, describing its association with HT.

Keywords: adiponectin; adipose tissue; blood pressure; hypertension.

INTRODUCTION

Epicardial adipose tissue (EAT) acts as an endocrine organ that produces many biologically active molecules referred to as adipocytokines.[8, 181] Most of these molecules, such as leptin, plasminogen-activator inhibitor type 1, tumour necrosis factor-α and interleukin-6, have been recognized as key factors in the pathogenesis of cardiovascular disease.[126, 241-243] They have autocrine, paracrine and endocrine effects.[244] Interestingly, their paracrine effects might have a special relevance because of the close proximity of EAT to the myocardium and coronary arteries and the absence of an anatomic barrier between EAT and these structures.[9] Accordingly, a recent study demonstrated that EAT thickness evaluated by cardiac computed tomography scan was strongly related to vascular risk factors and coronary calcification.[190]

Besides, EAT is closely related to total visceral adipose tissue, classically more linked to a higher risk of metabolic and cardiovascular disorders than "peripheral" adipose tissue. In this line, the assessment of the amount of EAT by echocardiography is a more reliable method to calculate visceral adiposity than waist circumference measurements.[171, 245]

Adiponectin is an adipocyte-derived collagen-like protein[23, 25, 26, 69] which was shown to have important effects on the cardiovascular system, particularly concerning the development of the metabolic syndrome.[68] It is related to glucose metabolism, insulin resistance and lipid metabolism.[23, 25, 26, 33, 68, 69] Low circulating adiponectin levels have been associated with diabetes mellitus (DM),[67] obesity[23] and coronary artery disease (CAD).[35, 69, 74, 75] However, in patients with congestive heart failure (CHF) reverse relationship was observed as high plasma adiponectin levels have been associated with poorer prognosis.[85, 86] Also age may influence the circulating levels of this hormone; higher levels found in the elderly[35] might be due to a phenomenon of "adiponectin resistance".

Lower plasma levels of adiponectin have also been reported in patients with arterial hypertension (HT), regardless of age, body mass index (BMI) and cholesterol levels.[79] Moreover, hypoadiponectinaemia is an independent risk factor for HT both in men and women,[246] although some studies failed to show such an association.[80] A prospective study found that low adiponectin levels at baseline are related to a higher risk of development of HT.[247] The effect of some antihypertensive drugs (ramipril and valsartan) on plasma adiponectin levels seems to be parallel to their effects on blood pressure and insulin sensitivity, in patients with metabolic syndrome.[81] Hypertension being a prevalent disorder, its impact on

cardiovascular diseases is very deep. A better knowledge of its physiopathology and its relationship with other pathological conditions can lead to a proper management of hypertensive patients.

Although the association between EAT adiponectin expression levels and gender or CAD has already been described,[181, 230] to date, its relationship with the presence of HT has not yet been elucidated. The aim of the present study was to determine whether EAT and subcutaneous adipose tissue (SAT) expression levels of adiponectin are different in hypertensive and non-hypertensive patients.

MATERIALS AND METHODS

Subjects.

Samples of EAT were obtained from 116 consecutive patients (78 men, 38 women) who underwent elective heart surgery at our hospital, for coronary artery bypass grafting (n=54), valve surgery (n=49), both (n=12) or atrial myxoma exeresis (n=1). In 85 patients, we also collected samples of SAT.

Only those patients who had undergone a previous heart surgery were excluded.

The study was approved by the local IRB and followed the guidelines proposed by the Declaration of Helsinki. Written informed consent was obtained from each patient before study participation. The participation rate was 100%.

Clinical data.

Clinical data, including the antecedent of HT, were obtained retrospectively by checking medical records. At our clinics, the diagnosis of HT was done by calculating the average of three measurements of blood pressure after a resting period of 10 min, if systolic blood pressure exceeded 140 mmHg and/or diastolic blood pressure was higher than 90 mmHg. The diagnosis of HT was performed by the patients' general physicians during regular outpatient follow-up before surgery. Non-hypertensive status was also properly assessed prior to surgery. Hypertensive patients were included in the HT group, no matter whether their blood pressure was adequately treated and targets reached at the time of inclusion in the study.

Body mass index was obtained from anthropometrical measurements upon admission to hospital. Blood samples were collected up to 1 week before surgery, except for cholesterol

levels, which were analysed within 6 months prior to surgery. The presence or absence of CAD was assessed by checking coronary angiography studies carried out up to 6 months before surgery. Only angiographically significant stenoses (more than 50% of the luminal diameter) were considered.

Collection and treatment of EAT and SAT samples.

The SAT and EAT samples were obtained before extracorporeal circulation. EAT biopsies were harvested near the proximal tract of the right coronary artery. SAT samples were obtained from the thorax. All tissues were immediately frozen at -80 °C before RNA extraction.

RNA was extracted by Trizol method. Concentration and purity of the samples were estimated by the ratio between absorbances at 260 and 280 nm. Samples contaminated with genomic DNA were treated with DNase I. Each 5 μg of RNA was treated with 10 U/μl of DNase I and 20 U/μl of RNase inhibitor (both products from Invitrogen Ltd, Paisley, UK) for 2 h at 37 °C. Proteins and DNA were removed from the samples with phenol, chloroform and isoamylalcohol. Eventually, RNA was precipitated with 96% ethanol and sodium acetate 0.3 M.

Reverse transcription and real time retrotranscriptase PCR.

Real time retrotranscriptase (RT)-PCR was performed using 1.2 μg samples of purified RNA and 200 U of MMLV reverse transcriptase (Invitrogen Ltd) in 30 μl of a solution (pH 8.4) containing 20 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, nucleotides (1 mM each), 20 U of RNase inhibitor and random primers under the following conditions: 50 min at 37 °C, 10 min 42 °C and 5 min at 95 °C.

The comparative analysis of adiponectin and glyceraldehyde 3-phosphate dehydrogenase expression in EAT was done with real time RT-PCR using SybrGreen (Roche Diagnostics Corp., Indianapolis, IN, USA) as fluorochrome and the primers previously described.[230] Adiponectin mRNA amplification was performed: 5 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 45 s at 60 °C, and 60 s at 72 °C.[228] Genomic contamination was ruled out by using negative controls at retrotranscription conditions without MMLV. Fluorescence curves were analysed using Chromo 4 software (MJ Research, Inc., Reno, NV, USA). Adiponectin mRNA was normalized to glyceraldehyde 3-phosphate dehydrogenase mRNA and expressed in arbitrary units (a.u.).

Immunohistochemistry.

Sections of EAT and SAT from hypertensive and non-hypertensive patients were paraffin-embedded and afterward deparaffined and rehydrated. Slides were incubated overnight with antibodies to adiponectin (Santa Cruz Biotechnology, Delaware, CA, USA), dilution 1:250. The LSAB protocol (Dako Diagnostics, Glostrup, Denmark) was followed. Inmunodetection was developed with 3,30-diaminobenzidine tetrahydrochloride kit (Dako Diagnostics). Negative controls were carried out by omitting the primary antibody.

Statistical analysis.

Normality assumptions of continuous variables were checked with Kolmogorov-Smirnov tests. Non-skewed variables were summarized as mean±s.d. Differences between continuous variables were tested for statistical significance by means of *t* test. Categorical variables were expressed as percentages and compared using chi-square test.

When missing data, we checked that there was no unequal distribution between groups.

We used logistic regression models to assess the association of EAT and SAT mRNA expression of adiponectin with HT, including possible confounding factors. Results are presented as ORs together with their 95% confidence intervals.

Statistical significance was defined as $P<0.05$. All analyses were performed using SPSS 15.0 software for Windows (SPSS Inc., Tokyo, Japan).

RESULTS

Patients characteristics.

The study sample included 84 hypertensive and 32 non-hypertensive patients. The main sample characteristics are given in Table 4-1. The mean (±s.d.) age was 70.3±7.9 years. Hypertensive patients were, on average, less than 2 years older than the nonhypertensive patients.

The proportion of male was higher than that of female in both groups, notably in that of non-hypertensives, although not statistically different. HT and non-HT groups were quite similar, except for use of calcium channel blockers and triglyceride levels, both significantly higher in the former group.

	All subjects	With HT (n=84)	Without HT (n=32)	P*
Demographics				
Male (%)	67	63	78	0.12
Age (years)	69.8±7.9	70.7±6.9	69.0±10.1	0.30
Comorbidities and risk factors				
Current smokers (%)	8	10	4	0.23
Body Mass Index (kg/m^2)	28.8±4.1	27.6±4.2	29.2±3.9	0.06
Type 2 diabetes (%)	36	41	24	0.13
Coronary Artery Disease (%)	61	66	48	0.10
Heart Failure (%)	30	32	25	0.45
LVEF (%)	60±15	60±14	60±15	0.91
Treatment prior to surgery				
ACEIs /ARBs (%)	41	43	35	0.63
Statins (%)	40	43	32	0.48
Beta-blockers (%)	36	38	32	0.71
Calcium channel blockers (%)	27	37	3	0.001
Laboratory findings				
Urea (mg/dL)	57±27	57±23	58±37	0.82
Creatinine (mg/dL)	1.1±0.3	1.1±0.4	1.1±0.3	0.37
Triglycerides (mg/dL)	119±50	126±51	102±42	0.023
Cholesterol (mg/dL)	181±43	178±39	183±44	0.41
HDL cholesterol (mg/dL)	37±13	36±13	38±13	0.58
LDL cholesterol (mg/dL)	108±35	106±36	112±32	0.53
EAT adiponectin mRNA (a.u.)	14.4±3.6	14.0±3.6	15.3±3.6	0.06
SAT adiponectin mRNA (a.u.) (n=85)	15.3±4.4	15.3±4.2	15.3±5.0	0.99

TABLE 4-1. Baseline characteristics of the hypertensive and the non-hypertensive groups.

Values expressed as mean±standard deviation.

* P-value referred to the comparison between HT and non-HT groups.

HT, arterial hypertension; LVEF, left ventricular ejection fraction; ACEIs, angiotensin converter enzyme inhibitors; ARBs, angiotensin receptor blockers; HDL, high density lipoprotein; LDL, low density lipoprotein; EAT, epicardial adipose tissue; mRNA, messenger ribonucleic acid; a.u., arbitrary units; SAT, subcutaneous adipose tissue.

One third of the patients of the whole group presented DM and one third – CHF. CAD was diagnosed in more than half of the study sample. However, none of these comorbidities differed significantly between both groups. The prevalence of overweight and obesity in our sample was remarkably large, with 51% subjects meeting the criteria for overweight (BMI >25 and <30 kg/m^2) and 34% meeting the criteria for obesity (BMI>30 kg/m^2). In our sample, BMI was higher in the group of non-hypertensive patients (P=0.06).

EAT adiponectin mRNA levels in hypertensive vs. non-hypertensive patients.

Hypertensive patients had lower EAT mRNA expression levels of adiponectin than non-hypertensive patients [14.0±3.6 vs. 15.3±3.6], although in this unadjusted analysis, the difference failed to reach statistical significance (P=0.06) (Figure 4-1).

However, when the association between EAT expression of adiponectin and HT was explored by means of multivariate logistic regression analysis, we found that the relationship between lower EAT adiponectin mRNA levels and HT achieved statistical significance (OR for adiponectin mRNA 0.828 per a.u., P=0.020) (Table 4-2). Variables considered in the model included possible confounding factors such as age, gender, BMI, DM, CHF, CAD and triglyceride levels. In this analysis, only older age and lower EAT adiponectin mRNA levels were found to be significantly associated with the presence of HT. Further adjustment introducing consumption of calcium channel blockers -distributed differently in both groups- led to a similar result (OR=0,825 per a.u., P=0.031).

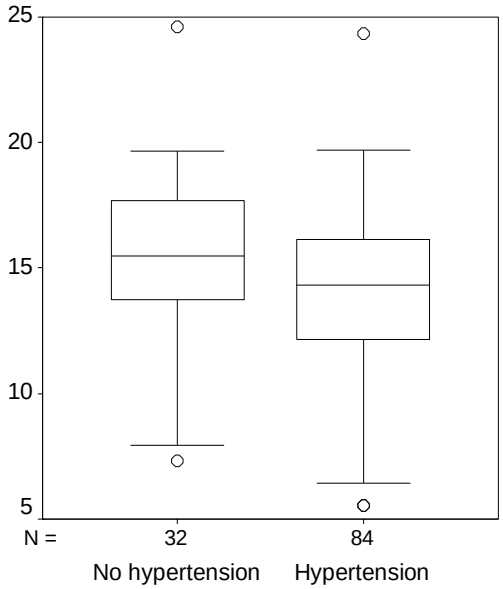

FIGURE 4-1. Box plot comparing epicardial adipose tissue (EAT) mRNA adiponectin in patients with and without hypertension.

Box-and-whiskers plot with mean values, interquartile range and lower and upper values. Outliers/extreme values are represented by the small circular symbols.
p-value of the difference of EAT mRNA adiponectin between hypertensive and non-hypertensive patients=0.06 (unadjusted).
GAPDH, glyceraldehydes 3-phosphate dehydrogenase; a.u., arbitrary units.

Variables	Odds Ratio (95% CI)	P
EAT adiponectin mRNA expression (per a.u.*)	0.828 (0.707 – 0.971)	0.020
Age (per year)	1.061 (1.000 – 1.126)	0.048
Gender (female)	2.932 (0.886 – 9.704)	0.08
Body Mass Index (per Kg/m^2)	1.111 (0.954 – 1.292)	0.18
Coronary Artery Disease	0.752 (0.247 – 2.286)	0.62
Heart Failure	0.587 (0.188 – 1.828)	0.36
Diabetes Mellitus	0.708 (0.239 – 2.102)	0.53
Triglycerides (per mg/dL)	1.010 (0.996 – 1.025)	0.17
N = 101		
R^2(Cox) = 0.183		

TABLE 4-2. Relationship between hypertension, epicardial adipose tissue adiponectin expression and other confounding factors.

Results of multivariable logistic regression analysis.

*Per GAPDH unit.

CI, confidence interval; EAT, epicardial adipose tissue; mRNA, messenger ribonucleic acid; a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

SAT adiponectin mRNA levels in hypertensive vs. non-hypertensive patients.

We found that SAT adiponectin mRNA levels were similar in hypertensive and non-hypertensive patients [15.3±4.2 vs 15.3±5.0, P>0.99] (Figure 4-2).

Adjustment for possible confounding factors as described above for EAT mRNA expression of adiponectin did not alter the result at all, as shown on Table 4-3. None of the variables introduced in this model showed to be significantly related to HT. Eventually, further adjustment including consumption of calcium channel blockers in the model hardly changed the results (OR for EAT adiponectin=0.999 per a.u., P=0.98).

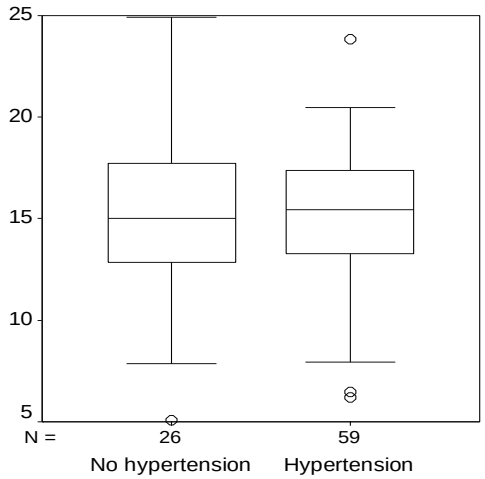

FIGURE 4-2. Box plot comparing subcutaneous adipose tissue (SAT) mRNA adiponectin in patients with and without hypertension.

Box-and-whiskers plot with mean values, interquartile range and lower and upper values. Outliers/extreme values are represented by the small circular symbols.

p-value of the difference of SAT mRNA adiponectin between hypertensive and non-hypertensive patients >0.99 (unadjusted).

a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Variables	Odds Ratio (95% CI)	P
SAT adiponectin mRNA expression (per a.u.*)	0.999 (0.880 – 1.134)	0.98
Age (per year)	1.039 (0.975 – 1.106)	0.24
Gender (female)	1.440 (0.427 – 4.850)	0.56
Body Mass Index (per Kg/m^2)	1.053 (0.904 – 1.227)	0.51
Coronary Artery Disease	0.637 (0.189 – 2.142)	0.47
Heart Failure	0.596 (0.183 – 1.940)	0.39
Diabetes Mellitus	0.611(0.193 – 1.934)	0.40
Triglycerides (per mg/dL)	1.012 (0.997 – 1.027)	0.13
N = 76		
R^2 (Cox)=0.123		

TABLE 4-3. Relationship between hypertension, subcutaneous adipose tissue adiponectin expression and other confounding factors.
Results of multivariable logistic regression analysis.
*****Per GAPDH unit.
CI, confidence interval; SAT, subcutaneous adipose tissue; mRNA, messenger ribonucleic acid; a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Immunohistochemistry.

We performed immunohistochemisty to illustrate tissue expression of adiponectin. Figure 4-3 shows adiponectin expression in EAT and SAT samples from both a hypertensive and a non-hypertensive patient.

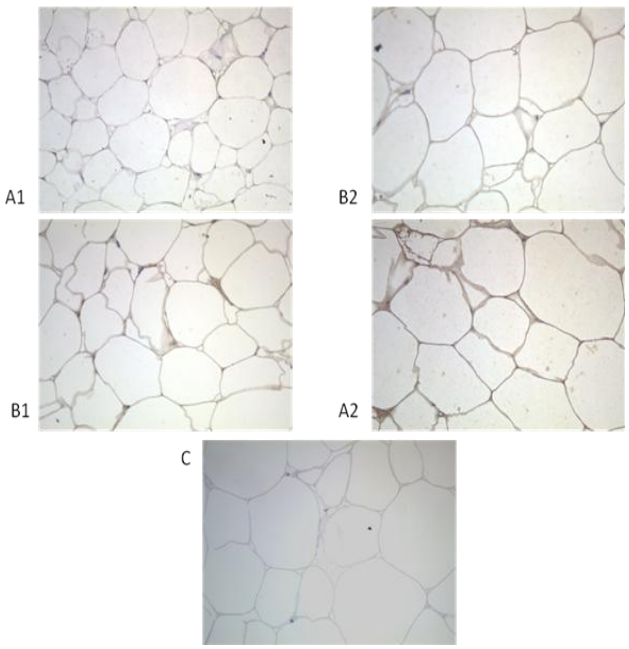

FIGURE 4-3. Immunohistochemical staining of epicardial (EAT) and subcutaneous adipose tissue (SAT) samples with antibodies to adiponectin.
A1: EAT sample from a hypertensive patient. **A2:** SAT sample from the same patient. **B1:** EAT sample from a non-hypertensive patient. **B2:** SAT sample from the same patient. **C:** Negative control with omission of primary antibody (SAT). Original magnification 40x.

DISCUSSION

This is the first study to report that EAT mRNA expression of adiponectin is lower in patients with HT, independently of other factors that can influence this cytokine's levels, such as the presence of DM, CAD or CHF.

Our results are relevant as they can have two distinct implications. The first one is that the lack of adiponectin produced by EAT may play an important role in the physiopathology of HT by the absence or reduction of its beneficial effect on the cardiovascular system, although other factors affecting adiponectin levels, specially the presence of inflammation, not evaluated here, are possibly implicated. On the other hand, a higher risk of CAD in HT patients might be related to the decrease of the protective endocrine and also local paracrine effects of adiponectin, as low levels of adiponectin detected in hypertensive patients would increase the risk of the latter. Accordingly, in our study population the prevalence of CAD although not significantly, was higher among hypertensive patients. In this regard, hypoadiponectinaemia has been recognized as a risk factor for CAD and the cluster of components of the metabolic syndrome, including HT.[68]

Interestingly, SAT expression of adiponectin does not seem to differ between hypertensive and non-hypertensive patients in our study. This observation is in accordance with prior evidence that showed no changes in SAT expression of adiponectin in patients with or without cardiovascular diseases, or even after therapeutic interventions that increase plasma adiponectin levels.[248] It is possible that, unlike SAT, visceral adipose tissue plays a relevant role in the pathogenesis of HT. And it could also partly explain why the amount of visceral fat is more closely related to the metabolic syndrome and the risk of cardiovascular diseases than that of SAT. Echocardiographic and cardiac computed tomographic scan assessment of the EAT have been shown to correlate well with the risk of cardiovascular disease.[171, 245] In this regard, a study designed by de Vos *et al.*[190] including more that 500 postmenopausal women recently demonstrated that EAT thickness measured by cardiac computed tomography has a graded relation to coronary calcification. In the same study, EAT was positively related to the use of antihypertensive drugs (P=0.007) and systolic blood pressure (P=0.03), which in some extent is in accordance with our findings. Although we did not analyse the correlation between EAT adiponectin expression levels and EAT thickness, it is likely that the increase of

the latter is associated with lower expression of this hormone, as demonstrated in other visceral adipose tissues.[249]

Regarding SAT, some proposed that post-transcriptional regulation changes of adiponectin could occur and SAT might also be relevant in the development of cardiovascular diseases.[248] But should this hypothesis be true, two different mechanisms –different expression levels and posttranscriptional regulation– could explain the role of visceral fat in the differential plasma adiponectin levels in patients with and without metabolic syndrome or cardiovascular diseases.

Hypertension is recognized as a major cardiovascular risk factor. However, epidemiological studies have demonstrated that the control of blood pressure levels, although improving, is still very poor especially in high risk patients such as those with metabolic syndrome and/or diabetes.[250-252] In these populations, blood pressure targets are reached in less than 20% of cases and usually more than three antihypertensive drugs are needed.[253] Therefore, both the promotion of the adherence to the guidelines of clinical practice and investigation on new pharmacological strategies to improve HT control and thus reduce the cardiovascular risk of these patients are relevant goals for medicine at present.[254] Accordingly, plasma adiponectin levels could be used not only as an extra tool to determine the individual metabolic and cardiovascular risk, but adiponectin could also serve as a new therapeutic target. The better understanding of the regulation and effects of adiponectin can result in the use of new interventions to prevent or treat the metabolic syndrome and cardiovascular disorders. In animal models, adiponectin infusion has beneficial effects concerning the metabolic syndrome, insulin-sensitivity and cardiovascular function. In humans, different factors can cause hyperadiponectinaemia and hence its beneficial effects: weight loss,[31] diet factors (soy protein, oils),[255] and drugs such as thiazolidinediones,[248] angiotensin converter enzyme inhibitors[96] or angiotensin receptor blockers.[256] In our sample, prescription of the latter two groups of drugs was in total almost 10% higher in hypertensive patients. This could contribute to attenuate our results, and therefore the difference in EAT adiponectin expression levels could be even slightly higher than what we described here.

Several investigations focused on the use of thiazolidinediones to treat the metabolic syndrome. These PPAR-γ agonists downregulate tumour necrosis factor-α and upregulate plasma adiponectin levels and reverse some features of the metabolic syndrome also in non-

diabetic patients.[257] Thiazolidinediones do not upregulate SAT expression of adiponectin[248] or adiponectin receptors (AdipoR1 and AdipoR2).[258] Our results are in line with these findings as we found differences in adiponectin expression in hypertensive patients in EAT but not in SAT.

The removal of EAT at the time of heart surgery could be beneficial for the prevention of cardiovascular disorders. Obviously this is just a hypothesis that should be tested as the harmlessness of these interventions has not been proved so far.

Future therapeutic strategies for the treatment of HT, especially if associated with the metabolic syndrome, should focus on adiponectin and visceral adipose tissue. However, further investigations are needed to improve our knowledge on the effects of adiponectin and its interactions with other adipocytokines.

Study limitations.

The main limitation of this cross-sectional study is that it cannot demonstrate causality but only association between variables. Besides, clinical data were collected retrospectively. The synthesis/secretion of adiponectin was not analysed, therefore we just focused on the differences in adiponectin expression levels. HT was treated categorically. Blood pressure levels at the time of inclusion in the study were not considered provided that adequately treated patients with HT were also included in the HT group.

Even though all subjects were heart surgery patients, there are possibly no reasons to believe that this could lead to a lack of validity of the results.

Conclusions.

Our findings indicate that EAT expression of adiponectin may be associated with HT status independently of CAD or other comorbidities, whereas SAT expression does not. These results support the hypothesis that EAT is actively implicated in global cardiovascular risk, describing, for the first time, its association with HT. They can also partly contribute to explain why central obesity is more related to HT than peripheral obesity.

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CONFLICTS OF INTEREST

The authors state no conflict of interest.

CAPÍTULO 5

ADIPONECTINA Y LEPTINA EN EL TEJIDO ADIPOSO EPICÁRDICO Y DIABETES MELLITUS TIPO 2

DIABETIC AND NONDIABETIC PATIENTS EXPRESS SIMILAR ADIPOSE TISSUE ADIPONECTIN AND LEPTIN LEVELS.

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ABSTRACT

Objective: Epicardial adipose tissue (EAT) is an interesting visceral fat pad with a particular location. EAT and subcutaneous adipose tissue (SAT) produce a wide range of adipokines. Some of them, including adiponectin and leptin, can influence the risk of development of diabetes and other associated metabolic and cardiovascular conditions. We sought to assess whether EAT and SAT adiponectin and leptin expression levels are different in diabetic patients with respect to nondiabetic subjects.

Subjects and methods: We collected samples of EAT from 120 patients and samples of SAT from 88 of the same group of patients undergoing elective cardiac surgery for coronary artery bypass grafting (n=69) or other procedures (n=51). After RNA isolation, adiponectin and leptin expression levels were analyzed by real time reverse transcriptase-PCR. Plasma levels were determined in small subsamples of subjects. Baseline clinical and treatment data were obtained from medical records.

Results: A total of 45 diabetic and 75 nondiabetic subjects were included in the study. Mean (s.d.) age was 70.1 (7.8) years and there were 32% women. EAT and SAT adiponectin and leptin mRNA expression levels were similar in the diabetic and the nondiabetic groups (EAT adiponectin 14.4 (4.3) *vs* 14.6 (3.4) arbitrary units (a.u.), P=0.79; SAT adiponectin 15.6 (4.7) *vs* 15.1 (3.9), P=0.54; EAT leptin 9.3(interquartile range 2.5) *vs* 9.5 (1.9) a.u., P=0.72; SAT leptin 9.9 (3.6) *vs* 10.0 (2.5) a.u., P=0.96). These findings persisted after stratification for sex and coronary artery disease. Logistic regression models including possible confounders and a combination of diabetes and impaired fasting glucose as dependent variable led to similar results. Plasma adiponectin levels were lower in diabetic patients, whereas leptin levels showed a nonsignificant trend.

Conclusion: Diabetic and nondiabetic subjects express similar EAT and SAT adiponectin and leptin levels. Counter-regulatory mechanisms of adiponectin and leptin expression in patients with established diabetes might partly account for these findings.

Keywords: adiponectin, adipose tissue, leptin, type 2 diabetes.

INTRODUCTION

Diabetes is a leading cause of morbidity and mortality and has become a major health issue worldwide. Inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, have been found to play a relevant role in its physiopathology.[243] Diabetes and metabolic syndrome often present jointly. Adiposity could well be the link between the components of this cluster of pathological conditions.

Formerly considered a mere energy depot, adipose tissue has been attributed a most interesting role in the pathogenesis of metabolic and cardiovascular diseases.[8] It produces a large amount of anti- and pro-inflammatory factors generally referred to as adipokines.[201] Classical epidemiological observations found a significant association between visceral adipose tissue –rather than subcutaneous adipose tissue (SAT)– and metabolic and cardiovascular diseases. Thus, the clustering of central obesity with insulin resistance, dyslipidemia and chronic inflammation may account for part of the cardiovascular effects of adiposity.[259] In this line, much attention has been focused on epicardial adipose tissue (EAT), an interesting representative of visceral adipose tissue. EAT extends along the major heart grooves with no anatomical fibrous layer, its products also being able to exert direct effects on the main epicardial coronary arteries and the myocardium. As shown by different groups, EAT expresses a pathogenic profile of adipokines.[8, 213, 214]

In recent years, much attention has been focused on adiponectin, the most abundant protein secreted by adipocytes. Initial investigations showed its positive effects as an insulin sensitizer and a protective cardiovascular hormone.[39] Epidemiological and laboratory studies showed a beneficial effect of adiponectin on preventing atherosclerosis and coronary artery disease (CAD).[74] However, more recent research and a comprehensive meta-analysis failed to confirm these findings and led to rather different conclusions, namely that baseline adiponectin circulating levels do not affect the risk of development of CAD significantly.[73]

Diabetic patients, especially those with macroangiopathy, have lower adiponectin plasma levels than control subjects.[67] Different studies have shown that lower plasma adiponectin levels are strongly correlated with insulin resistance[260] and predict the development of insulin resistance[261] and diabetes,[260, 262-264] irrespective of baseline measures of obesity.Leptin, mainly secreted by adipocytes,[119] is positively correlated to total body fat and has an important role in

the regulation of appetite and energy balance. Plasma leptin levels are closely associated with body fat storage and may respond to changes in energy expenditure.

Leptin deficiency produces severe obesity and metabolic, immunological and neuroendocrinological dysfunctions.[108] Leptin resistance is far more common and virtually present in all obese subjects.[265] Leptin is associated with insulin resistance[266] and predicts the development of diabetes.[111]

In vitro research showed that it also exerts some pro-atherosclerotic effects, such as endothelial activation and migration,[267, 268] smooth muscle cell proliferation and calcification,[269] and activation of monocytes.[270] Even though some studies showed an association between high leptin levels and CAD,[112] others failed to do so,[115] despite finding an association between leptinemia and inflammatory markers.[271, 272]

The aim of the present study is to explore the relationship between EAT and SAT adiponectin and leptin expression and established type 2 diabetes.

SUBJECTS AND METHODS

Subjects.

Samples of EAT and SAT were collected from 120 patients (82 men, 38 women) who underwent elective cardiac surgery at our hospital, for coronary artery bypass grafting (N=53), valve surgery (N=49), both (N=16), myxoma exeresis (N=1) or repair of atrial septal defect (N=1). Exclusion criteria were: previous cardiothoracic surgery, infective diseases and type 1 diabetes mellitus, because of its demonstrated different relationship to adiponectin levels.[273]

The study was approved by the local institutional review board and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from every patient before inclusion. The participation rate was 100%.

Clinical data.

Clinical data were obtained from medical records. The diagnosis of type 2 diabetes was accurately assessed following theAmerican Diabetes Association current criteria.[274] Patients were classified as diabetic or nondiabetic. Abnormal fasting glucose, as defined by fasting concentrations ≥ 5.6 mmol/l, was included as dependent variable in the logistic regression

model, together with diabetes. Oral glucose tolerance testing was not routinely applied and thereby impaired glucose tolerance was not ruled out.

Body mass index was calculated from anthropometrical measurements on admission to hospital. Overweight and obesity were defined as body mass index ≥ 25 and ≥ 30 kg/m^2, respectively.

Blood samples were collected after overnight fasting up to 1 week before surgery and analyzed using standard methods, but cholesterol levels were determined within 6 months before surgery. Significant CAD was discarded or confirmed by noninvasive testing and/or coronary angiogram performed within 6 months before surgery. Cutoff point for angiographically significant coronary artery stenosis was 50% of the lumen diameter.

As regards treatment, we included data concerning the drugs that patients were taking during the weeks immediately before sample collection. Former treatments were not considered.

Collection of adipose tissue samples.

SAT and EAT samples were obtained before starting extracorporeal circulation. EAT biopsies were harvested from the area close to the proximal tract of the right coronary artery. SAT samples were obtained from the thorax, at the sternotomy incision. All tissue samples were immediately frozen and stored at -80 °C until laboratory processing.

RNA was extracted and purified by the Trizol method. The concentration and purity of the samples were estimated by the ratio between absorbances at 260 and 280 nm. Samples were treated with DNase I to avoid genomic DNA contamination. Each 5 ug of RNA was treated with 10 U of DNase I and 20 U of RNase inhibitor (both manufactured by Invitrogen Ltd, Paisley, UK) for 2 h at 37 °C. Phenol, chloroform and isoamylalcohol were used to remove proteins and DNA from the samples. RNA was precipitated with 96% ethanol and sodium acetate 0.3 M.

Freezing-thawing cycles were avoided whenever possible to ensure maximum quality of the determinations.

Reverse transcription and real time PCR.

Real-time reverse transcriptase PCR was performed using 1.2 μg samples of purified mRNA and 200 U reverse transcriptase (Malooney Murine Leukemia Virus, MMLV) (Invitrogen Ltd, Paisley, UK) in 30 μl of a pH 8.4 solution containing 20 mM Tris-HCl, 50 mM KCl, 2.5 mM

$MgCl_2$, nucleotides (1mM each), 20 U of RNase inhibitor and random primers under the following conditions: 50 min at 37 °C, 10 min 42 °C and 5 min at 95 °C.

The comparative analysis of adiponectin and glyceraldehyde 3-phosphate dehydrogenase expression in EAT was performed with real time reverse transcriptase PCR using SYBRGreen (Roche Diagnostics Corp, Indianapolis, IN, USA) as fluorochrome and the primers previously described.[230] Adiponectin mRNA amplification was performed as follows: 5 min at 95 °C, then 40 cycles of 30 s at 95 °C, 45 s at 60 °C, and 60 s at 72 °C.[228] Genomic contamination was ruled out by using negative controls without MMLV at retrotranscription conditions. Fluorescence curves were analyzed with Chromo 4 software (MJ Research, Inc., Reno, NV, USA). Gene expression levels were obtained by calculating the antilogarithm of the inverse adiponectin/GAPDH ratio and presented in arbitrary units (a.u.).

All laboratory measurements were made blind to participants' disease status, with samples randomly distributed for analysis.

Plasma adiponectin and leptin determinations.

Plasma levels of adiponectin and leptin were analyzed in duplicate using commercially available human high sensitivity enzyme-linked immunosorbent assay (ELISA) kits (SPI-bio, Montigny le Bretonneux, France; and R&D Systems, Inc., Minneapolis, MN, USA, respectively). The lowest limits of sensitivity were 0.5 ng/ml for adiponectin and 7.8 pg/ml for leptin, and the intra- and inter-assay variance coefficients were lower than 10%.

Western blot analysis.

In all, 100-150 mg of EAT and SAT were rinsed in 5 ml of phosphate saline solution containing 0.5mM EDTA, 5mM KCl, 10mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic (HEPES) acid, 2mM $MgCl_2$, 10mM $NaHCO_3$, 0.5 mM KH_2PO_4, 0.5 mM NaH_2PO_4, 10 mM glucose, 110 mM NaCl and, 0.16 mM $CaCl_2$ at pH=7.4, and then centrifuged at 300 g for 1 min to remove residual blood. Tissues were resuspended (weight/volume) in a lysis buffer (125 mM Tris pH 6.8, 10% glycerol, 2% SDS, 100 mM dithiothreitol, 1 anti-protease cocktail from Sigma-Aldrich, St Louis, MO, USA) and sheared by homogenizer pestle using sample grinding kit (GE Healthcare, Waukesha, WI, USA). Then, the proteins were precipitated with 20% trichloroacetic acid in acetone. Finally, samples were resuspended at a final concentration of 1ug of homogenized tissue per 1ul in Laemmli buffer. Protein

separation (40ug) was carried out in 12% SDS-polyacrylamide gel electrophoresis gel and transferred on a polyvinylidene fluoride membrane for 45 min at 280mA. Membranes were blocked for 2h at room temperature with 5% of milk in Tris-buffered saline tween-20 containing: 20mM Tris-HCl (pH 7.6), 150 mM NaCl and 0.1% Tween 20. The membranes were then exposed with goat adiponectin antibody (1:500 dilution) and goat GAPDH antibody (1:1000) (Santa Cruz Biotechnology, Delaware, CA, USA) overnight, and then to peroxidase-conjugated rabbit anti-goat IgG. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Amersham Pharmacia Ltd, London, UK) and quantified by densitometry Image J software. Adiponectin protein levels were evaluated in duplicate and quantified with respect to GAPDH.

Statistical analysis.

Categorical variables are expressed as percentages and compared using χ^2-test or Fisher's exact test.

We used the Kolmogorov-Smirnov test to check the normality of continuous variables. Non-skewed variables are summarized as the mean (standard deviation) and those with skewed distribution as the median (interquartile range). Differences between continuous variables were tested for statistical significance by means of Student's *t*test. Mann-Whitney test was performed whenever nonparametrical testing was required.

To discard the influence of sex, CAD, hypertension (HT) and statin treatment, we stratified the sample for these variables and repeated the described analysis across strata.

Logistic regression models including possible confounders were used to assess the association between EAT and SAT adiponectin and leptin mRNA levels and diabetes or impaired fasting glucose.

In case of missing data, we checked that there was no unequal distribution between groups.

Statistical significance was defined as $P<0.05$. All analyses were computed using SPSS 15.0 software for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Patient characteristics.

In all, 45 diabetic patients and 75 nondiabetic western European subjects were included in this study. The prevalence of diabetes was almost 38% in our sample. Table 5-1 presents the main characteristics of the study sample. Mean age was 70.1 years (s.d. 7.7).

Diabetic and nondiabetic groups did not differ significantly for most clinical variables. There was a very high prevalence of obesity in both groups, with overall ratios of overweight and obesity of 55 and 30%, respectively. As for other comorbidities, such as HT and heart failure, the prevalence was higher in the diabetic group, though not significant. CAD was more prevalent in the group of diabetics, with borderline statistical significance.

Concerning drug intake, there were only slight differences in calcium channel blocker consumption, higher in the group of diabetics, and differences in statin consumption, more prevalent in the same group. In all, 24 diabetic patients were on oral antidiabetic agents, mostly sulfonylureas (n=14) or metformin (n=8), alone or in combination.

Fasting glucose levels were higher in the group of diabetics, despite treatment. Diabetic patients had lower cholesterol levels than the nondiabetic group, though the difference was not statistically significant.

EAT and SAT adipokine mRNA expression in diabetic vs. *nondiabetic subjects.*

Diabetic patients had similar EAT and SAT mRNA adiponectin (Figure 5-1) and leptin (Figure 5-2) levels than nondiabetic patients. We stratified the sample according to sex and CAD, but no significant differences were found concerning adiponectin (Table 5-2) and leptin (Table 5-3) mRNA expression in different strata when comparing diabetic and nondiabetic groups. Further stratification for statin consumption or HT led to similar results (data not shown). Conversely, statin treatment did not show a significant effect on adipokine expression in any stratum. As regards the influence of oral antidiabetic drug on EAT and SAT adipokine expression, no significant difference was found when compared with the group of diabetics free from this treatment (EAT adiponectin 14.5 (2.6) *vs.* 14.0 (5.9) a.u., P=0.68; EAT leptin 9.2 (8.3-10.7) *vs.* 9.6 (7.6-10.8) a.u., P=0.96; SAT adiponectin 15.3 (5.4) *vs.* 15.7 (4.1) a.u., P=0.83; SAT leptin 8.9 (7.7-12.6) *vs.* 10.4 (8.6-11.6) a.u., P=0.77).

	DM (n=45)	Non-DM (n=75)	P*
Demographics			
Age (years)	70.4 (6.9)	69.9 (8.3)	0.74
Female (%)	36	29	0.48
Comorbidities and risk factors			
Current smokers (%)	7	9	0.89
Body Mass Index (kg/m^2)	28.9 (4.1)	28.3 (4.0)	0.39
Hypertension (%)	78	64	0.07
Coronary Artery Disease (%)	71	53	0.05
Heart Failure (%)	35	30	0.59
Drugs			
ACEIs /ARBs (%)	48	39	0.72
Calcium channel blockers (%)	59	36	0.01
Statins (%)	37	36	0.99
Beta-blockers (%)	33	22	0.47
Oral antidiabetics (%)	53	-	-
Laboratory findings			
Glucose (mg/dl)	6.8 (5.4-9.2)	5.1 (4.8-5.5)	<0.001
Urea (mg/dL)	8.5 (7.2-11.3)	7.7 (6.7-10.0)	0.10
Creatinine (mg/dL)	97 (80-115)	88 (80-106)	0.32
Triglycerides (mg/dL)	1.3 (0.9-1.7)	1.2 (0.9-1.5)	0.46
Cholesterol (mg/dL)	4.4 (1.1)	4.7 (1.0)	0.09
HDL cholesterol (mg/dL)	0.9 (0.7-1.2)	0.9 (0.8-1.3)	0.45
LDL cholesterol (mg/dL)	2.7 (0.8)	2.9 (1.0)	0.22
EAT adiponectin mRNA (a.u.)	14.4 (4.3)	14.6 (3.4)	0.79
EAT leptin mRNA (a.u.)	9.3 (8.2-10.7)	9.5 (8.5-10.4)	0.72
EAT adiponectin/GAPDH protein expression (a.u.) (n=10)	0.84 (0.24)	1.01 (0.25)	0.32
SAT adiponectin mRNA (a.u.) (n=88)	15.6 (4.7)	15.1 (3.9)	0.54
SAT leptin mRNA (a.u.) (n=88)	9.9 (8.4-12.0)	10.0 (8.7-11.2)	0.96
SAT adiponectin/GAPDH protein expression (a.u.) (n=10)	1.12 (0.56)	0.60 (0.50)	0.16
Plasma adiponectin (µg/ml) (n=12)	7.2 (3.1)	15.4 (6.7)	0.02
Plasma leptin (ng/ml) (n=26)	4.9 (3.4)	6.3 (5.7)	0.55

TABLE 5-1. Sample characteristics.

Values expressed as mean (standard deviation) or as median (percentile25-percentile75) for variables not following normal distribution. * P-value referred to the comparison between diabetic and nondiabetic groups.

ACEIs, angiotensin converter enzyme inhibitors; ARBs, angiotensin receptor blockers; a.u., arbitrary units; EAT, epicardial adipose tissue; SAT; subcutaneous adipose tissue.

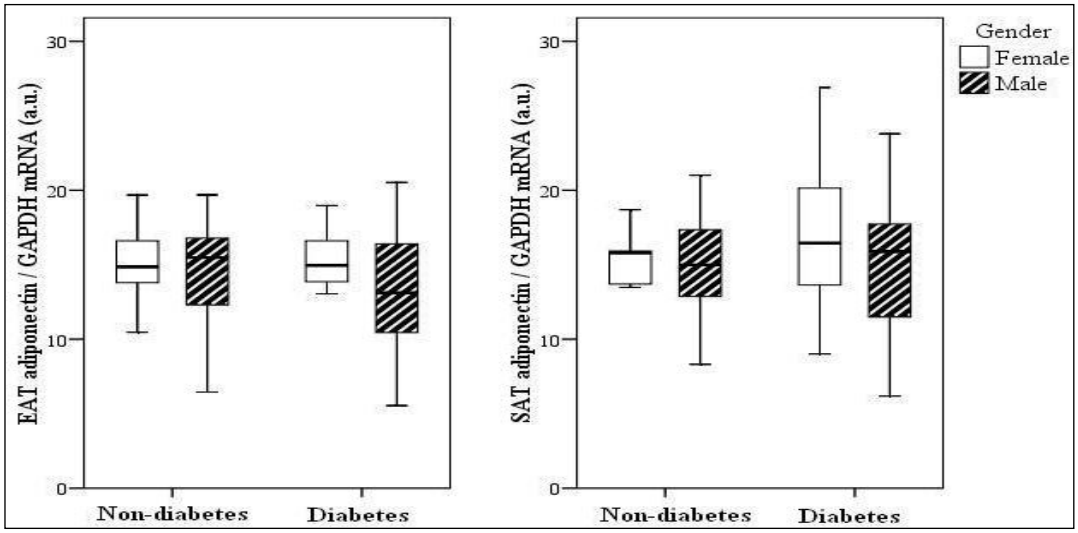

FIGURE 5-1. Box plots comparing epicardial (EAT) and subcutaneous (SAT) adiponectin expression between diabetic and non-diabetic subjects.
Horizontal lines represent minimum, percentiles 25, 50, 75 and maximum values. P-values for the comparison between diabetic and non-diabetic subjects: P=0.19 (EAT/male); P=0.23 (EAT/female); P=0.79 (SAT/male); P=0.18 (SAT/female). P-values for the comparison between male and female: P=0.039 (EAT), P=0.34 (SAT). N=120 (EAT); N=88 (SAT).
a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

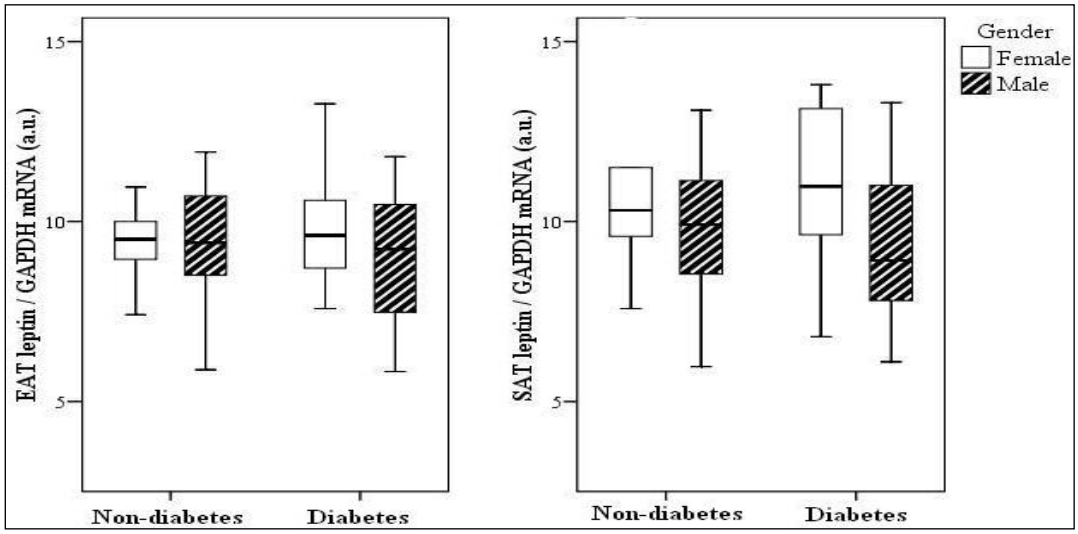

FIGURE 5-2. Box plots comparing epicardial (EAT) and subcutaneous (SAT) leptin expression between diabetic and non-diabetic subjects.
Horizontal lines represent minimum, percentiles 25, 50, 75 and maximum values. P-values for the comparison between diabetic and non-diabetic subjects: P=80 (EAT/male); P=0.60 (EAT/female); P=0.75 (SAT/male); P=0.55 (SAT/female). P-values for the comparison between male and female: P=0.61 (EAT), P=0.04 (SAT). N=119 (EAT); N=86 (SAT).
a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

		Male						Female					
		Non-CAD			*CAD*			*Non-CAD*			*CAD*		
		N	Mean (SD)	P	*N*	Mean (SD)	P	*N*	Mean (SD)	P	*N*	Mean (SD)	P
EAT mRNA adiponectin (a.u.)	*NonDM*	*20*	15.5 (3.1)	0.98	*33*	13.9 (3.9)	0.23	*15*	15.1 (3.3)	0.19	*7*	14.7 (2.2)	0.47
	DM	*8*	15.5 (3.1)		*21*	12.5 (3.9)		*5*	18.1 (6.6)		*11*	15.7 (3.1)	
SAT mRNA adiponectin (a.u.)	*NonDM*	*18*	16.2 (4.0)	0.92	*22*	14.3 (4.1)	0.93	*6*	16.1 (1.9)	0.19	*7*	13.8 (3.8)	0.47
	DM	*5*	16.3 (1.8)		*18*	14.4 (4.9)		*5*	20.2 (5.3)		*7*	15.0 (3.7)	

TABLE 5-2. Comparison of adiponectin expression levels between diabetic and nondiabetic patients across different strata according to sex and CAD.

Results from *t*-test.

a.u., arbitrary units; CAD, coronary artery disease; DM, type 2 diabetes mellitus; EAT, epicardial adipose tissue; SAT, subcutaneous adipose tissue.

		Male						Female					
		Non-CAD			*CAD*			*Non-CAD*			*CAD*		
		N	Median (p25-p75)	P	*N*	Median (p25-p75)	P	*N*	Median (p25-p75)	P	*N*	Median (p25-p75)	P
EAT mRNA leptin (a.u.)	*Non-DM*	*20*	9.4 (8.3-10.8)	0.88	*33*	9.5 (8.4-10.8)	0.47	*14*	9.4 (8.7-10.0)	0.64	*7*	9.5 (9.0-10.2)	0.82
	DM	*8*	9.5 (7.2-10.8)		*21*	8.9 (7.4-10.7)		*5*	9.6 (8.7-15.6)		*11*	9.6 (8.7-10.2)	
SAT mRNA leptin (a.u.)	*Non-DM*	*17*	10.3 (8.9-11.3)	0.91	*21*	9.7 (8.3-10.9)	0.59	*6*	12.9 (9.3-18.7)	0.86	*7*	10.0 (8.9-11.0)	0.23
	DM	*5*	10.7 (8.4-14.1)		*18*	8.7 (7.5-11.4)		*5*	10.8 (9.6-16.3)		*7*	12.0 (8.6-13.2)	

TABLE 5-3. Comparison of leptin expression levels between diabetic and nondiabetic patients across different strata according to sex and CAD.

Results from Mann-Whitney test.

a.u., arbitrary units; CAD, coronary artery disease; DM, type 2 diabetes mellitus; EAT, epicardial adipose tissue; p25-p75, percentile25-percentile75; SAT, subcutaneous adipose tissue.

It should be noted that only one patient was on thiazolidinediones, and therefore the influence of this treatment could not be studied.

Besides, logistic regression models including possible confounders such as gender, age, body mass index, total cholesterol, HT and CAD failed to show EAT and SAT adiponectin and leptin mRNA expression differences in the group of patients with diabetes mellitus or abnormal fasting glucose compared with the control group, as shown in Table 5-4.

EAT and SAT adiponectin protein levels in diabetic vs. *nondiabetic subjects.*

We performed western blot analyses in a subsample of patients (five diabetic and five nondiabetic patients). We did not find significant differences in adiponectin protein expression between both groups, as shown in Table 5-1, although a slight trend can be observed toward lower protein levels in SAT in nondiabetic subjects.

Plasma adiponectin and leptin concentrations in diabetic vs. *nondiabetic subjects.*

Small subsamples of patients were studied to assess differences in plasma adiponectin (n=12) and leptin levels (n=26) between diabetic and nondiabetic subjects (Table 5-1). As expected, diabetic patients presented lower adiponectin plasma levels when compared with nondiabetic subjects. However, no significant differences were found in leptin levels, possibly due to small sample size.

Variables	Odds Ratio (95% CI)	P
EAT adiponectin (per a.u.)*	1.02 (0.90-1.05)	0.76
Gender (male)	2.72 (0.97-7.67)	0.058
Age (per year)	1.02 (0.96-1.08)	0.49
Body mass index (per kg/m2)	1.23 (1.07-1.47)	0.003
Total cholesterol (per mg/dl)	0.99 (0.98-1.00)	0.29
Hypertension	3.56 (1.27-9.98)	0.016
Coronary artery disease	1.69 (0.64-4.50)	0.29
N = 104 R2 (Nagelkerke) = 0.286		
SAT adiponectin (per a.u.)*	0.96 (0.84-1.11)	0.60
Gender (male)	3.82 (0.99-14.74)	0.052
Age (per year)	1.11 (0.95-1.09)	0.60
Body mass index (per kg/m2)	1.26 (1.06-1.51)	0.009
Total cholesterol (per mg/dl)	1.00 (0.98-1.01)	0.64
Hypertension	4.08 (1.23-13.52)	0.021
Coronary artery disease	1.55 (0.47-5.03)	0.47
N = 77 R^2 (Nagelkerke) = 0.344		
EAT leptin (per a.u.)*	1.01 (0.91-1.27)	0.41
Gender (male)	3.30 (1.13-9.64)	0.029
Age (per year)	1.02 (0.96-1.08)	0.54
Body mass index (per kg/m2)	1.28 (1.10-1.48)	0.002
Total cholesterol (per mg/dl)	0.99 (0.98-1.00)	0.20
Hypertension	4.23 (1.47-12.21)	0.008
Coronary artery disease	1.56 (0.58-4.21)	0.38
N = 103 R^2 (Nagelkerke) = 0.327		
SAT leptin (per a.u.)*	1.07 (0.81-1.28)	0.48
Gender (male)	3.09 (0.80-11.91)	0.10
Age (per year)	1.02 (0.95-1.09)	0.67
Body mass index (per kg/m2)	1.25 (1.05-1.48)	0.011
Total cholesterol (per mg/dl)	1.00 (0.98-1.01)	0.62
Hypertension	4.57 (1.35-15.43)	0.015
Coronary artery disease	1.90 (0.58-6.24)	0.29
N = 76 R^2 (Nagelkerke) = 0.349		

TABLE 5-4. Relationship between EAT, SAT adiponectin and leptin and other variables and diabetes or impaired fasting glucose.

Results of multivariate logistic regression analysis. Dependent variable: type 2 diabetes or impaired fasting glucose.

*Per GAPDH mRNA unit.

a.u., arbitrary units; CI, confidence interval; EAT, epicardial adipose tissue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SAT, subcutaneous adipose tissue.

DISCUSSION

This study, based on the largest series of EAT reported, is the first to show that EAT and SAT adiponectin and leptin levels are similar in patients with established diabetes or abnormal fasting glucose as compared with nondiabetic subjects with normal fasting glucose, irrespective of sex, age and other possible confounders. This finding adds complexity to the controversial body of evidence showing that lower plasma adiponectin levels and higher plasma leptin levels are associated with metabolic and cardiovascular conditions.

Previous prospective investigations showed a very narrow association between hypoadiponectinemia and the development of insulin resistance[261] and diabetes,[260, 262-264] strongly suggesting a relevant role for this adipokine in the physiopathology of diabetes. Laboratory research also resulted in promising conclusions, reinforcing the role of adiponectin as an insulin sensitizer antidiabetic hormone.[39] In this line, consistent with previous research,[67] and even though the subsample analyzed was small, we found that patients with diabetes present significantly lower adiponectin plasma levels as compared with those without diabetes, despite expressing similar adipose tissue mRNA levels. Factors other than adiponectin expression from these fat depots can obviously influence plasma levels.

Curiously, though a beneficial antiatherogenic effect of adiponectin was initially observed, recent studies showed that in patients with CAD higher adiponectin levels are associated with increased risk of cardiovascular events and of cardiovascular and non-cardiovascular mortality.[275] An explanation postulated for this apparent paradox is that adiponectin concentrations are elevated in a counterregulatory manner as a mechanism of response to excessive proatherosclerotic processes.[276] In this scenario, high adiponectin levels would not result in net beneficial effects. It is likely that in patients with diabetes there is also counterregulation of adiponectin expression in visceral adipose tissue and SAT. This mechanism would be intense enough to equal the visceral adipose tissue and SAT adiponectin expression levels with those of nondiabetic patients, as we find in this study.

Another plausible explanation that can overlap the latter is that in this set of elderly obese patients, insulin resistance is possibly highly prevalent even in nondiabetic subjects. To minimize this limitation, we also conducted logistic regression analyses and defined diabetes or abnormal fasting glucose as a dependent variable. Again, no statistically significant differences concerning adipokine expression were found.

A recent study showed an association between EAT thickness and impaired glucose tolerance.[277] Differences in the size of fat pads between diabetic and nondiabetic patients might well be one of the reasons why, despite similar adiponectin and leptin expression levels, plasma levels differ between both groups.[67] Although EAT is relatively small in size, it is a representative of visceral adipose tissue[278] and its anatomical location suggests a major role in the physiology and physiopathology of cardiovascular diseases.

Our group showed lower EAT adiponectin levels in patients with more severe established CAD [213], in line with previous observations.[181] Very interestingly, adiponectin levels were decreased in EAT but not in SAT, suggesting a major implication of EAT in the physiopathology of coronary atherosclerosis and CAD. EAT would then act as an endocrine organ directly affecting the underlying coronary arteries.

In accordance with previous findings reported,[213, 230] women and non-CAD individuals express higher EAT adiponectin and leptin levels. We also described an association between lower EAT adiponectin expression levels in patients with HT, but similar SAT levels.[214] SAT could be less involved in the physiopathology of HT than EAT, and very likely a lack of counter-regulation in EAT adiponectin expression could occur in HT.

In this study, we do not find differences in EAT and SAT leptin expression in diabetics with respect to nondiabetic subjects. Prospective studies focused on plasma levels showed a higher rate of development of diabetes in patients with higher baseline leptin levels.[111] We did not find significant differences in leptin plasma levels between diabetic and nondiabetic patients either, but the subsample analyzed was too small to draw any conclusion.

However, concerning leptin and atherosclerosis, laboratory and epidemiological research led to heterogeneous results,[111, 115, 267-270] suggesting a very complex regulation of the synthesis and secretion of this hormone. Metabolic changes in patients with diabetes might be responsible for the regulation of leptin expression by EAT and SAT.

As for the effect of treatment on adiponectin and leptin expression, only statins and obviously oral antidiabetic agents were differently distributed in diabetic and nondiabetic groups. No relevant trends were observed regarding adipokine expression. The similar adiponectin expression levels in patients under statin treatment, though requiring confirmation by larger studies specifically designed for this purpose, can seem controversial. Statins could have a slight effect, not strong enough to increase adiponectin expression and to decrease leptin

expression in this set of patients. Or maybe they could have posttranscriptional rather than transcriptional effects. However, should this be correct, adipokine expression levels would probably change as a result of feedback stimulation. Previous studies focused on the effect of statins on insulin sensitivity and adipokine levels are controversial, not allowing any definite reliable conclusions.[96, 279-282]

It would have been very interesting to check the effect of peroxisome proliferator-activated receptor PPAR-γ inhibitors thiazolidinediones on EAT and SAT adipokine levels, but only one patient was taking them, as the use of these drugs is very limited in our area. Other oral antidiabetic drugs do not seem to have significant effects on EAT and SAT adipokine levels.

Inflammatory cytokines, together with leptin and adiponectin, have a crucial role in the pathogenesis of metabolic and cardiovascular diseases. We are starting to understand the importance of these hormones, but their intricate relationship and their accurate effects remain still unclear.

Study limitations.

This study follows a cross-sectional design, and hence cannot explain causality but only association between variables. Owing to obvious ethical concerns, only patients undergoing elective heart surgery were included in the study. This is the reason why the mean age of the sample is quite high.

We assessed fasting glucose impairment but oral glucose tolerance test was not routinely applied and therefore it was not possible to study the whole spectrum of clinical glucose disturbances. This is especially important in the case of CAD patients, as almost one third can present with IGT [283, 284]. However, differences in adipokine expression were not found in non-CAD patients, whose prevalence of undiagnosed abnormal glucose regulation is presumably very low.

We mostly focused on EAT and SAT mRNA expression levels rather than on protein tissue levels as the latter could have a different origin and not reflect adipose tissue adiponectin and leptin production properly. In any case, no differences were found in adiponectin protein levels between diabetic and nondiabetic patients in the small subsample studied. Leptin protein levels were not determined.

Overall, we consider that the findings of this study are still fully valid, especially on the basis of an elderly population.

CONCLUSIONS

EAT and SAT adiponectin and leptin mRNA levels do not differ between diabetic and nondiabetic patients. Counterregulatory mechanisms of adiponectin and leptin expression in patients with diabetes might partly account for these findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CAPÍTULO 6

ADIPONECTINA EN EL TEJIDO ADIPOSO EPICÁRDICO Y SÍNDROME METABÓLICO

LOWER EPICARDIAL ADIPOSE TISSUE ADIPONECTIN IN PATIENTS WITH METABOLIC SYNDROME.

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(enviado para publicación)

ABSTRACT

Background: Adiponectin is an anti-atherogenic insulin-sensitizer hormone whose plasma concentration is lower in patients with metabolic syndrome (MS). Visceral adiposity, including epicardial adipose tissue (EAT), is closely related to the development of MS and coronary artery disease (CAD). We sought to study whether EAT and subcutaneous adipose tissue (SAT) adiponectin mRNA levels are similar in patients with and without MS.

Methods: EAT, SAT and blood samples were collected from patients undergoing elective cardiac surgery, for revascularization (n=19) or other procedures (n=27). Plasma adiponectin was measured using ELISA. mRNA was purified and adiponectin mRNA quantified by real time RT-PCR.

Results: Mean (SD) age was 71.6 (9.6) years. Patients who met Adult Treatment Panel III MS criteria (n=29) presented lower plasma adiponectin concentrations (11.2 (7.4) *vs.* 19.6 (8.4) mg/l, P=0.004), lower EAT adiponectin mRNA (12.7 (3.0) *vs.* 15.1 (3.7) a.u., P=0.029) and similar SAT adiponectin mRNA levels (13.7 (4.2) *vs.* 15.6 (5.7) a.u., P=0.25) than those without MS. After adjusting for age, sex, CAD and heart failure, the association with MS remained statistically significant for plasma adiponectin (OR 0.862 (0.762-0.974)), was of borderline significance for EAT adiponectin mRNA (OR 0.796 (0.630-1.005)) and not significant for SAT adiponectin mRNA (OR 0.958 (0.818-1.122)). Patients in the lower quartiles of EAT adiponectin mRNA and plasma adiponectin presented a higher mean of components of the MS.

Conclusion: Subjects with MS present lower EAT adiponectin mRNA levels than those without MS, whereas SAT adiponectin mRNA levels do not seem to differ between both groups. EAT might be the link between MS and its atherothrombotic cardiac complications.

INTRODUCTION

The metabolic syndrome (MS) is a cluster of well documented risk factors for cardiovascular diseases and diabetes (DM) that frequently present jointly and are associated to pro-inflammatory pro-atherogenic states.[259, 285] Its definition remaining still controversial, MS has become a major health concern worldwide.[259]

Central obesity is usually regarded as a key component of the MS. Epicardial adipose tissue (EAT) is a very interesting representative of visceral adiposity, due to its anatomical proximity to the coronary arteries and the myocardium. EAT is closely related to total visceral adiposity; in fact, the amount of EAT has been demonstrated to reflect visceral adiposity more accurately than waist circumference measurement.[171] EAT thickness as evaluated by cardiac computed tomography scan is associated to vascular risk factors and coronary calcification and to MS.[197] EAT produces a large amount of pro- and anti-inflammatory cytokines[8] and the absence of a fibrous layer would likely allow their paracrine effects on the myocardium and coronary arteries.

Adiponectin, a collagen-like protein, is mainly produced by adipocytes and represents their most abundant circulating product.[23] Adiponectin is an insulin-sensitizer[34, 286] adipokine that exhibits diverse protective anti-inflammatory,[287] anti-atherogenic[32, 288] and vasodilatory effects.[289] Lower plasma adiponectin levels are present in men and have also been associated with obesity,[23] DM,[67, 290] coronary artery disease (CAD),[70] hypertension (HT)[79] and heart failure (HF).[84]

However, in patients with established CAD, high plasma adiponectin levels failed to lead to better prognosis.[73] The explanation for this paradoxical observation would be that in this set of patients, hyperadiponectinemia is possibly due to counter-regulatory mechanisms and its beneficial effects are unable to reverse such an advanced inflammatory atherogenic process.[291]

Hypoadiponectinemia has been associated to each defining component of the MS and to the MS itself, independently of other factors.[68, 292] This association was much stronger than that of pro-inflammatory markers, such as TNF-alpha, IL-6 and C-reactive protein.[293] Adiponectin concentrations can predict the risk of development of the MS and DM.[294]

Patients with established DM appear to present similar EAT adiponectin levels,[295] and these findings raise the possibility of a counter-regulatory mechanism, similar to the one explained above. Recent studies have demonstrated lower EAT adiponectin expression in men[230] and in

patients with HT,[214] and also enhanced IL-6 and decreased adiponectin expression levels in EAT in patients with CAD.[181, 213] Interestingly, these levels relate to the extension of CAD as measured by the number of injured arteries.[213]

However, up to date the relationship between EAT adiponectin levels and MS remained still unknown. We sought to determine whether EAT and SAT adiponectin expression levels are different in patients with MS with respect to those without it.

MATERIALS AND METHODS

Study population.

Fifty Caucasian patients undergoing elective cardiac surgery at our hospital were invited to participate in the study. Exclusion criteria were prior cardiothoracic surgery or concomitant infective or neoplastic diseases.

The study was approved by the local Ethics Committee and has been carried out in accordance with the principles of the Declaration of Helsinki as revised in 2000. Participation was voluntary. Forty-six patients gave written informed consent and were recruited for the study.

Clinical data.

Upon admission to hospital, clinical data were obtained both through direct interview and by checking medical records. Anthropometric measurements were also registered at that time. As for blood pressure, mean of three separate measurements at rest was calculated. Metabolic syndrome was diagnosed following Adult Treatment Panel (ATP) III most recent criteria, and patients were classified as with or without MS. Exclusion/diagnosis of CAD was based on previous ischemia detection tests and/or coronary angiogram. Patients were also classified as with or without HF, irrespective of its cause.

Prior treatments were considered only if continued during the week before surgical procedure.

Sample collection.

Blood samples for lipid profile, urea and creatinine measurements were collected after overnight fasting up to three days before surgery and analyzed using standard methods at the hospital central laboratory.

Blood samples for adiponectin were collected in EDTA tubes on the same day of surgical procedure and before it early in the morning, then centrifuged to separate plasma and stored at -40ºC until assay.

EAT biopsies were harvested near the proximal tract of the right coronary artery, whereas SAT samples were obtained from the thorax. All tissue samples were stored at -80ºC until processing at the research laboratory.

Freezing-thawing cycles were avoided whenever possible in order to ensure optimal conditions of preservation.

Plasma adiponectin analysis.

Plasma levels of adiponectin were analyzed in duplicate using a commercially available human high sensitivity ELISA kit (SPI-bio, Montigny-le-Bretonneux, France). The lowest limit of sensitivity was 0.5 ng/ml, and the intra- and inter-assay variance coefficients were lower than 10%.

mRNA purification and real time RT-PCR.

mRNA was isolated from 50-120 mg of EAT and SAT using the oligotex mRNA spin-column kit (Qiagen GmbH, Germany). Reverse transcription was performed using 4.14 µl of mRNA dilution and 200U of MMLV reverse transcriptase (Invitrogen Corp, CA, USA) in 30 µl of a pH 8.4 solution containing 20 mM Tris-HC, 50 mM KCl, 2.5 mM $MgCl_2$, deoxynucleotides (1 mM each), 20 U of RNase inhibitor and random primers under the following conditions: 50 min at 37ºC, 10 min at 42ºC and 5 min al 95 ºC.

The analysis of EAT and SAT adiponectin mRNA expression was performed with respect to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA expression by real time PCR using 8 µl of complementary DNA, 2 µl of SybrGreen (Roche Diagnostics Cor, IN, USA) as fluorochrome and the primers previously described.[230] The conditions of amplification were: 5 min at 95 ºC and then 40 cycles of 30 sec at 95ºC, 45 sec at 60 ºC and 60 sec at 72 ºC. Chromo 4 software (MJ Research, inc., NV, USA) was used to analyze fluorescence curves. Melting curves were tested to assess the correct amplicon. Gene expression was calculated using the antilogarithm of inverse adiponectin/GAPDH ratio and presented in arbitrary units (a.u.).

Statistical analysis.

Categorical variables were expressed as percentages and differences between groups tested using χ^2-test or Fisher's exact test.

Kolmogorov-Smirnov method was used to check the normality of continuous variables. Non-skewed variables were summarized as mean (standard deviation) and those with skewed distribution as median (interquartile range). Differences between continuous variables were tested for statistical significance by means of *t* test. Mann-Whitney test was performed whenever non-parametrical testing was required.

Logistic regression models were computed to assess the association between EAT adiponectin mRNA expression, SAT adiponectin mRNA expression and plasma adiponectin concentration levels and MS, independently of other variables. Results are presented as odds ratios (ORs) together with their 95% confidence intervals (CI).

Pearson's test was used to assess correlations between EAT adiponectin mRNA expression, SAT adiponectin mRNA expression and plasma adiponectin concentration levels.

Whenever missing data, we checked that there was no unequal distribution between groups.

Statistical significance was defined as $p<0.05$. All analyses were computed using SPSS 15.0 software for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Patients characteristics.

Twenty-nine patients with MS as diagnosed following ATP-III criteria and 17 patients not meeting such criteria were included in the study. Table 6-1 shows main sample characteristics. Mean age was 71.6(9.6). There were 67% male in the whole sample, with a higher ratio in the group with MS.

The prevalence of obesity was remarkably high in our sample, as 52% subjects had overweight (BMI >25 kg/m^2 and < 30 kg/m^2) and 33% obesity (BMI >30 kg/m^2). The prevalence of CAD and HF were quite high too. Notably, CAD was more prevalent in patients with MS whereas HF was more prevalent in those without MS, though differences failed to reach statistical significance in both cases.

	Non-MS (n=17)	MS (n=29)	P*
Demographics and measurements			
Age (years)	73.3 (7.2)	70.5 (10.8)	0.36
Male (%)	53	76	0.10
Body Mass Index (kg/m^2)	25.8 (5.2)	30.5 (4.5)	0.002
Waist circumference (cm)	88 (17)	105 (8)	<0.001
Systolic BP (mmHg)	125 (13)	129 (20)	0.46
Diastolic BP (mmHg)	70 (8)	70 (7)	0.93
Comorbidities and risk factors			
Current smokers (%)	6	24	0.28
Hypertension (%)	59	90	0.025
Type 2 diabetes (%)	6	48	0.003
Coronary Artery Disease (%)	41	59	0.20
Heart Failure (%)	59	31	0.17
Drugs			
ACEIs (%)	12	24	0.27
ARBs (%)	29	52	0.12
Statins (%)	41	83	0.005
Beta-blockers (%)	18	45	0.059
Calcium channel blockers (%)	29	21	0.37
Laboratory findings			
Urea (mg/dL)	66 (29)	54 (24)	0.12
Creatinine (mg/dL)	1.1 (1.0-1.3)	1.1 (1.0-1.2)	0.62
Triglycerides (mg/dL)	89 (20)	125 (70)	0.045
Cholesterol (mg/dL)	194 (46)	166 (41)	0.036
HDL cholesterol (mg/dL)	48 (16)	34 (10)	0.001
LDL cholesterol (mg/dL)	115 (41)	100 (31)	0.15
VLDL cholesterol (mg/dL)	18 (4)	25 (14)	0.038
Plasma adiponectin (n=36)	19.6 (8.4)	11.2 (7.4)	0.004
EAT adiponectin mRNA (a.u.) (n=40)	15.1 (3.7)	12.7 (3.0)	0.029
SAT adiponectin mRNA (a.u.) (n=40)	15.6 (5.7)	13.7 (4.2)	0.25

TABLE 6-1. Sample characteristics.

Values expressed as mean (standard deviation) or as median (percentile25-percentile75) for variables not following normal distribution pattern. * P-value referred to the comparison between groups with and without MS.

ACEIs, angiotensin converter enzyme inhibitors; ARBs, angiotensin receptor blockers; a.u., arbitrary units; EAT, epicardial adipose tissue; MS, metabolic syndrome; SAT, subcutaneous adipose tissue.

Conditions and biochemical findings included in the definition of MS (HT, DM, increased waist circumference, total cholesterol and triglyceride levels and decreased HDL cholesterol levels) or closely related to it were obviously more prevalent in the group of patients with MS. However, mean blood pressure was similar in both groups.

As regards treatment, a larger proportion of patients with MS received angiotensin converter enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), beta-blockers and statins, with respect to patients without MS. However, such differences were only statistically significant for statins and of borderline significance for beta-blockers.

EAT adiponectin mRNA expression levels in patients with and without MS.

Patients with MS express significantly lower EAT adiponectin mRNA than those without MS (12.7(3.0) *vs.* 15.1 (3.7) a.u., P=0.029) (Figure 6-1A). When including age, sex, CAD and HF in a logistic regression model, we found that the association between lower EAT adiponectin mRNA and MS was of borderline statistical significance (OR 0.796 (0.630-1.005), per a.u.), as shown on Table 6-2.

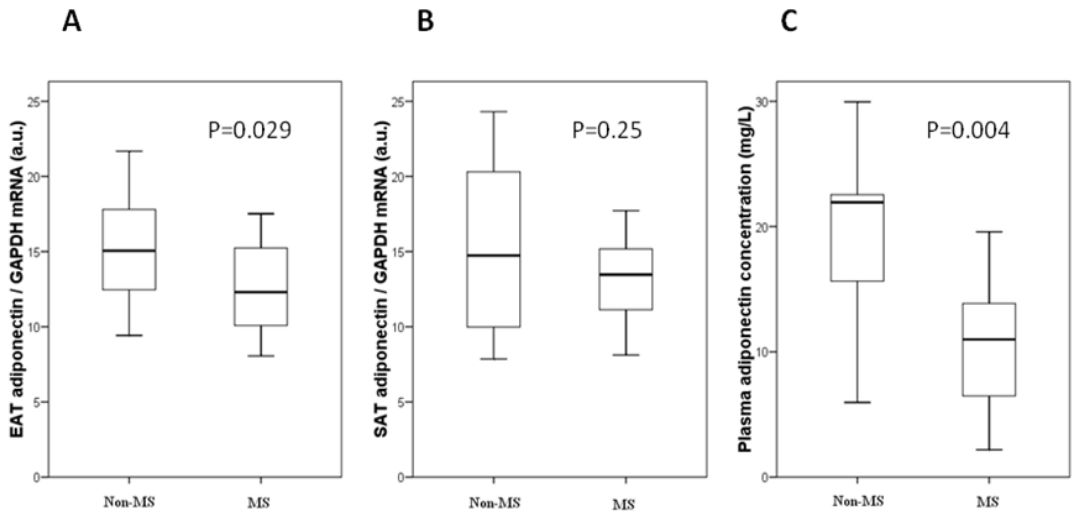

FIGURE 6-1 A-C. Box plots representing epicardial adipose tissue (EAT) and subcutaneous adipose tissue (SAT) adiponectin expression levels and plasma adiponectin concentrations in patients with and without metabolic syndrome (MS).
P-values for the comparison between the groups with and without MS.
a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

SAT adiponectin mRNA expression levels in patients with and without MS.

SAT adiponectin mRNA expression levels were not significantly different in patients with MS than in those without MS, although a slight trend was found towards lower adiponectin expression in patients with MS (13.7 (4.2) *vs.* 15.6 (5.7) a.u., P=0.25) (Figure 6-1B).

However, when adding age, sex, CAD and HF in a logistic regression model, no association was found between SAT adiponectin mRNA and MS at all (OR 0.958 (0.818-1.122), per a.u.), as shown on Table 6-2.

Variables	Odds Ratio (95% CI)	P
EAT adiponectin (per a.u.)*	0.796 (0.630-1.005)	0.055
Gender (male)	0.435 (0.080-2.366)	0.34
Age (per year)	1.004 (0.920-1.096)	0.92
Coronary artery disease	1.017 (0.191-5.412)	0.99
Heart failure	2.211 (0.497-9.834)	0.30
N = 40		
R^2(Nagelkerke) = 0.252		
SAT adiponectin (per a.u.)*	0.958 (0.818-1.122)	0.95
Gender (male)	0.425 (0.079-2.275)	0.32
Age (per year)	1.001 (0.923-1.086)	0.97
Coronary artery disease	1.182 (0.247-5.664)	0.84
Heart failure	2.572 (0.589-11.242)	0.95
N = 40		
R^2(Nagelkerke) = 0.165		
Plasma adiponectin (per mg/L)	0.862 (0.762-0.974)	0.018
Gender (male)	0.513 (0.055-4.778)	0.56
Age (per year)	1.009 (0.901-1.131)	0.87
Coronary artery disease	1.101 (0.147-8.228)	0.93
Heart failure	4.705 (0.762-29.530)	0.10
N = 36		
R^2(Nagelkerke) = 0.394		

TABLE 6-2. Relationship between EAT, SAT and plasma adiponectin and other variables and the metabolic syndrome.

Results of multivariate logistic regression analysis. *Per GAPDH mRNA unit.

a.u., arbitrary units; CI, confidence interval; EAT, epicardial adipose tissue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Plasma adiponectin concentrations in patients with and without MS.

Patients with MS express significantly lower plasma adiponectin concentration levels than those without MS (11.2 (7.4) *vs.* 19.6 (8.4) mg/l, P=0.004) (Figure 6-1C). When including age, sex, CAD and HF in a logistic regression model, we found that the association between lower plasma adiponectin concentration levels and MS remained statistically significant (OR 0.862 (0.762-0.974), per mg/l), as shown on Table 6-2.

Correlation between EAT and SAT adiponectin mRNA levels and plasma adiponectin concentrations.

EAT adiponectin mRNA levels are positively correlated to plasma adiponectin levels (Pearson's r=0.370, P=0.034) (Figure 6-2A) and SAT adiponectin mRNA levels (Pearson's r=0.390, P=0.017) (Figure 6-2C). Figure 6-2B shows a weaker not statistically significant correlation between SAT adiponectin mRNA levels and plasma adiponectin levels (Pearson's r=0.280, P=0.13).

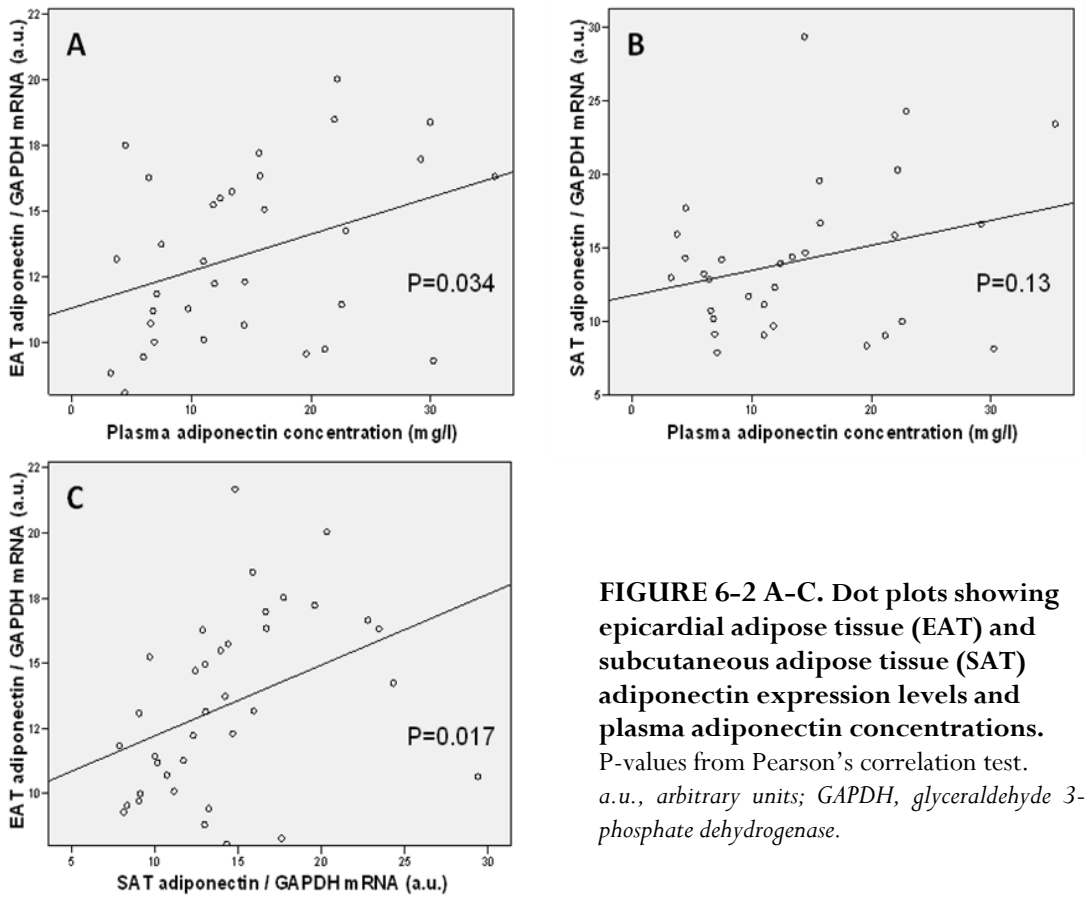

FIGURE 6-2 A-C. Dot plots showing epicardial adipose tissue (EAT) and subcutaneous adipose tissue (SAT) adiponectin expression levels and plasma adiponectin concentrations. P-values from Pearson's correlation test. *a.u., arbitrary units; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.*

Comparison of the number of components of the MS in groups of patients with different EAT, SAT and plasma adiponectin levels.

The mean number of defining components of the MS in patients with EAT adiponectin mRNA levels on the lowest quartile (>10.7 a.u.) was significantly higher than that of patients whose levels were on the upper quartile (>16.3 a.u.) (3.6 (1.4) *vs.* 1.9 (1.2), P=0.008), whereas differences in other variables tested including age, sex, CAD and HF were not significantly different between both groups.

Likewise, the mean number of components of the MS was higher in patients with plasma adiponectin concentrations on the lowest quartile (<6.8 mg/dl) when compared to those whose levels were on the upper quartile (>21.7 mg/dl) (3.6 (0.9) *vs.* 1.9 (0.8), P=0.001) again with no significant differences in the distribution of the variables mentioned above.

This was not the case when comparing patients whose SAT adiponectin mRNA levels were on the lowest quartile (<10.8 a.u.) with respect to those with levels on the upper quartile (>16.5 a.u.) (3.0 (1.3) *vs.* 2.7 (1.1), P=0.58), but age distribution was also different in both groups (70.3 (5.9) *vs.* 76.1 (6.1), P=0.045).

DISCUSSION

Although there existed prior research linking EAT adiponectin expression to some components of the MS and cardiovascular disease, namely HT[214] and CAD,[181, 213] this is the first study to focus on EAT and SAT adiponectin levels in patients with the MS itself.

Our main finding is that patients with MS express lower EAT adiponectin levels than those without it. Although CAD and HF could confound our results, after adjustment for these variables and also for age and sex, the association between EAT adiponectin levels and MS remained of borderline statistical significance. Besides, we demonstrated a relationship between lower EAT adiponectin levels and the presence of more defining components of the MS, which increases the strength of our results notably.

In line with previous research,[68] we found that plasma adiponectin levels are lower in patients with MS and that the group of patients with lower plasma levels presents a higher mean of components of the MS. Matsushita and coworkers[293] demonstrated that plasma adiponectin levels were more strongly associated to MS than CRP and IL-6, independently of its clustering components, and also to each of them individually. They also found that plasma TNF-alpha levels were not associated to MS at all, possibly because the effects of this potent pro-inflammatory cytokine are mostly paracrine, and its plasma levels do not necessarily change even if its tissue concentrations do. Adiponectin inhibits TNF-alpha, and conversely TNF-alpha downregulates adiponectin production.[296]

An interesting hypothesis that arises from these findings would be that adiponectin is on the basis of the proinflammatory state observed in patients with MS. If so, adiponectin would be a key factor in the development of MS and its vascular complications. Adiponectin is an insulin-sensitizer protective adipokine that exhibits multiple beneficial metabolic and cardiovascular effects.[34, 286, 290] Though produced by adipose tissue, adiponectin levels are lower in obese subjects.[23] Lacking adiponectin would then result in deleterious effects, as its positive actions would be suppressed. This situation would lead to the development of some features of the MS, including insulin resistance, lipid metabolism impairment and a pro-inflammatory pro-atherogenic state.

EAT could play a special role as it can exert direct paracrine effects on the coronary arteries and the myocardium due to its close proximity to them. EAT adipokines might have a very intense local activity on the heart. A pathogenic profile of cytokines was observed in patients

with CAD,[8] and differences in EAT but not in SAT adiponectin expression are associated to the extension of CAD, as we demonstrated in a different set of patients.[213] Therefore, though EAT is a relatively small fat pad, it could be regarded as a crucial organ that might influence heart metabolism and local inflammatory state.

ConceRning SAT, a trend was found for adiponectin expression but it did not reach statistical significance, possibly due to the high variability of SAT adiponectin expression. However, we found significant positive correlations between EAT and SAT adiponectin mRNA levels and between EAT adiponectin mRNA levels and plasma adiponectin levels. These findings suggest that all three could be related to MS, the association being less strong for SAT adiponectin and actually non-significant for the correlation between SAT and plasma adiponectin. Overall, these results could very likely reflect a weaker more variable contribution of this tissue to the physiopathology of MS than that of visceral adipose tissue.

As regards the role of SAT, it has been postulated that posttranscriptional changes[248] could occur in SAT, and if so SAT would possibly have a more direct implication in the physiopathology of MS.Even then, these changes could also be present in visceral adipose tissue and enhance its physiopathologic role as well.

Factors clustered in the MS were treated independently so far. The understanding of the physiopathology of the MS can be helpful to develop a comprehensive approach to the MS and new strategies for its management and the prevention of metabolic complications and cardiovascular adverse outcomes. Enhancing adiponectin production in selected patients with hypoadiponectinemia at early stages might be a suitable strategy to prevent the development of the MS and its metabolic and cardiovascular complications. PPAR-gamma agonists[29] such as thiazolidinediones and some ARBs might be useful to increase adiponectinemia, although their net beneficial effects in this scenario are yet to be demonstrated.

However, though the role of EAT adipokines in the development of MS and CAD seems promising, up to date the accurate functions of EAT and the complex interactions between adiponectin and other cytokines remain still unclear. Future research will surely shed some more light on this exciting field.

Limitations.

This study follows a cross-sectional design, and hence is hypothesis generating but cannot demonstrate causality. Prospective studies should be carried for this purpose. The mean age of

our sample is quite high and cardiovascular diseases were present in all cases, as subjects were recruited from a heart surgery department. However, we consider that the validity of these results could extend at least to similar elderly populations and likely to the whole adult population. As explained above, the influence of CAD, HF and other potential confounders was appropriately ruled out by adjusting for these variables. EAT and SAT adiponectin mRNA expression levels were preferred to tissue adiponectin levels as the latter would reflect EAT and SAT adiponectin production less accurately.

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CONFLICTS OF INTEREST/DISCLOSURES STATEMENT

None.

CAPÍTULO 7

ADIPONECTINA Y LEPTINA EN EL TEJIDO ADIPOSO EPICÁRDICO Y PRONÓSTICO CARDIOVASCULAR

EPICARDIAL ADIPOSE TISSUE ADIPONECTIN LEVELS PREDICT CARDIOVASCULAR EVENTS. A LONG-TERM FOLLOW-UP STUDY.

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(enviado para publicación)

ABSTRACT

Background: Epicardial adipose tissue (EAT) produces a wide range of adipokines and has recently been linked to the physiopathology of cardiovascular (CV) and metabolic diseases. We aimed to study whether EAT and subcutaneous (SAT) adiponectin and leptin expression levels are associated with CV outcomes during long-term follow-up.

Methods: We included 137 patients undergoing elective cardiac surgery -mainly for CABG (n=62), valve surgery (n=60) or both (n=13)- between 2004 and 2007. Samples of EAT and SAT were obtained during surgery. RNA was purified and adiponectin and leptin expression levels analyzed by real time RT-PCR. Plasma adiponectin levels were determined in a subsample of subjects (n=43). Patients were followed up to assess CV events, defined as stroke, coronary acute syndrome, admission for heart failure, need for revascularization or CV death.

Results: Mean age was 69.9 (s.d.8.2) years and there were 31% women. In all, 34 patients developed CV events during 41.4 (s.d. 23.3) months of mean follow-up. Patients with CV events had lower EAT and SAT adiponectin levels at baseline (12.4 (3.0) vs. 15.7 (3.8) a.u., P=0.001; and 13.7 (2.6) vs. 15.7 (4.4)a.u., P=0.048, respectively). However, baseline EAT and SAT leptin levels and plasma adiponectin levels were not significantly different between patients with and without cardiovascular events during follow-up. Cox proportional hazards models adjusting for covariates in stages revealed that only EAT adiponectin levels and heart failure could predict CV events.

Conclusions: Baseline EAT adiponectin levels are strong predictors of CV outcomes in patients with CV diseases. EAT might play a major role in the development of CV complications through local effects.

Keywords: adiponectin, cardiovascular, epicardial adipose tissue, prognosis.

INTRODUCTION

In recent years, adipose tissue has been recognized as a complex endocrine organ which expresses and secretes bioactive molecules generally known as adipokines.[2] Adipokines can exert local as well as systemic effects, through autocrine, paracrine and endocrine mechanisms, and play a relevant role in the development of cardiovascular (CV) and metabolic diseases.

Adiponectin is an insulin-sensitizer[32] anti-inflammatory[287] anti-atherogenic[33] adipokine which is mainly secreted by adipocytes and represents their most abundant circulating product.[23] Lower plasma adiponectin levels are related to obesity,[23] metabolic syndrome,[68] hypertension (HT),[79] diabetes,[67] coronary artery disease (CAD)[70] and heart failure (HF).[84] Adiponectin concentrations can predict the risk of development of metabolic syndrome and diabetes.[294]

Despite the known beneficial effects of adiponectin, several studies showed that higher plasma levels are associated with worse prognosis in patients with CAD,[297] although others found the opposite.[74] However, in these patients hyperadiponectinemia could be due to feedback stimulation, and the beneficial effects of the hormone would be unable to reverse such an advanced inflammatory atherogenic process.[291] Hyperadiponectinemia is also associated with worse prognosis in patients with chronic HF, possibly either owing to deleterious energy expenditure or because it is a marker of weight loss.[298] Besides, higher adiponectin plasma levels have also curiously been found to predict worse CV outcomes in the elderly.[299]

Leptin, a hormone mainly produced by adipocytes,[119] is positively correlated to total body fat and exerts pro-atherosclerotic effects.[269] Leptinemia and inflammatory markers are also related.[272] However, even though some studies showed an association between high leptin levels and CAD,[112] others failed to link leptin levels and CV outcome[115] or simply found that they do not provide more information on prognosis than BMI alone.[300]

Epicardial adipose tissue (EAT) is a particularly interesting fat pad due to its anatomical location close to the myocardium and the major coronary arteries. The absence of a fibrous layer and the fact that they share the same microvasculature led to hypothesize that the wide range of adipokines produced by EAT[8] could directly affect the structure and function of the heart. EAT adiponectin expression is lower in patients with HT[214] and EAT adiponectin and interleukin-6 levels are also related to the extension of CAD.[213] Nevertheless, curiously no

association was found between EAT and SAT adiponectin and leptin levels and the presence of diabetes.[295]

EAT could be the link between metabolic and CV conditions and influence CV prognosis. We sought to explore whether EAT and SAT adiponectin and leptin expression levels could predict CV events during long-term follow-up.

SUBJECTS AND METHODS

Subjects.

We included 137 patients who underwent elective cardiac surgery between 2004 and 2007 at our hospital, mostly for coronary artery bypass grafting (n=62), valve surgery (n=60) or both (n=13). Exclusion criteria were previous cardiothoracic surgery, active infective diseases and diabetes mellitus type 1.

The study was approved by the local Ethics Committee and conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from every patient before participation in the study.

Baseline data and follow-up.

Both baseline clinical data and follow-up data were obtained by checking medical records. Outcomes were assessed as of May 6th 2010 in 100% of patients. CV events were defined as stroke, coronary acute syndrome, heart failure, need for revascularization or CV death during follow-up.

Blood samples were collected after overnight fasting up to one week prior to surgery -except for cholesterol levels, determined within 6 months before surgery- and analyzed at the hospital laboratory using standard methods. Significant coronary artery disease was diagnosed by coronary angiogram prior to surgery. Coronary artery stenoses in excess of 50% of lumen diameter were considered angiographycally significant.

Collection of adipose tissue samples.

SAT and EAT samples were obtained before starting extracorporeal circulation and processed as described previously.[295] EAT biopsies were collected from the area near the proximal tract of the right coronary artery. SAT samples were harvested from the thorax. Tissue samples were immediately frozen and stored at -80 °C until laboratory processing.

Trizol method was used for RNA purification. The concentration and purity of the samples were estimated by the ratio between absorbances at 260 and 280 nm. Genomic DNA contamination was avoided by treating samples with DNase I. Each 5 ug of RNA was treated with 10 U of DNase I and 20 U of RNase inhibitor (both manufactured by Invitrogen Ltd, Paisley, UK) for 2 h at 37 °C. Phenol, chloroform and isoamylalcohol were used to remove proteins and DNA from the samples. The RNA was precipitated with 96% ethanol and sodium acetate 0.3 M.

Freezing-thawing cycles were avoided whenever possible in order to maximize the quality of the determinations.

Reverse transcription and real time polymerase chain reaction (real time RT-PCR).

Real time RT-PCR was performed using 1.2 µg samples of purified mRNA and 200 U reverse transcriptase (MMLV) (Invitrogen Ltd) in 30 µl of a pH 8.4 solution containing 20 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, nucleotides (1mM each), 20 U of RNase inhibitor and random primers under the following conditions: 50 min at 37 °C, 10 min 42 °C and 5 minutes at 95 °C.

The comparative analysis of adiponectin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression in EAT was performed with real time RT-PCR using SybrGreen (Roche Diagnostics Corp, In, USA) as fluorochrome and the primers previously described.[230] Adiponectin mRNA amplification was performed as follows: 5 minutes at 95 °C, then 40 cycles of 30 seconds at 95 °C, 45 seconds at 60 °C, and 60 seconds at 72 °C.[228] Genomic contamination was ruled out by using negative controls without MMLV under retrotranscription conditions. Fluorescence curves were analyzed with Chromo 4 software (MJ Research, Inc., Reno, NV, USA). Gene expression levels were obtained by calculating the antilogarithm of inverse adiponectin/GAPDH ratio and presented in arbitrary units (a.u.).

All laboratory measurements were made blind to participants' disease status, with samples randomly distributed for analysis.

Plasma adiponectin determinations.

Plasma adiponectin levels were analyzed in duplicate in a random subsample of 43 patients by using a commercially available human high sensitivity enzyme-linked immunosorbent assay

(ELISA) kit (SPI-bio, Montigny le Bretonneux, France). The lowest limit of sensitivity was 0.5 ng/ml and intra- and inter-assay variance coefficients was below 10%.

Statistical analysis.

Categorical variables are expressed as percentages and compared using χ^2-test or Fisher's exact test when applicable.

Kolmogorov-Smirnov test was used to check normality assumptions of continuous variables. Non-skewed variables are summarized as mean (standard deviation) and those with skewed distribution as median (interquartile range). Differences between continuous variables were tested for statistical significance by means of *t* test. Mann-Whitney test was performed whenever non-parametrical testing was required.

We used Kaplan-Meier test to compare cumulative survival rates free from CV events in different groups of patients according to baseline parameters. Cox proportional regression multivariable stepwise models were computed to examine associations between adipose tissue adiponectin and leptin levels and CV outcomes. We included covariates most likely to predict CV events (age, gender, BMI, HT, CABG surgery, creatinine levels) at the first step.

Spearman test was used to study possible correlations between plasma adiponectin concentrations and EAT and SAT adiponectin expression levels.

When missing data, we checked that there was no unequal distribution between groups. Statistical significance was defined as $p<0.05$. All analyses were computed using SPSS 17.0 software for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Patients' characteristics.

In all, 137 patients (31% women) with a mean age of 69.9 years (s.d. 8.2) were included in the study. The main sample characteristics are shown on Table 7-1. Of note, there was a very high prevalence of obesity, with overall ratios of overweight and obesity of 54% and 30%, respectively. Besides, the prevalence of HT, CAD and HF was also quite high in our sample.

During a mean follow-up of 41.4 (s.d. 23.3) months, 34 patients suffered from CV events, defined as heart failure (n=17), coronary acute syndrome (n=7), CV mortality (n=7), need for revascularization (n=2) and stroke (n=1). Patients with CV events during follow-up were

	No CV events (n=103)	CV events (n=34)	P*
Demographics			
Age (years)	70.2 (8.0)	68.8 (8.7)	0.40
Female (%)	33	24	0.48
Current smokers (%)	14	8	0.63
Body Mass Index (kg/m^2)	28.5 (4.2)	28.6 (3.6)	0.94
Hypertension (%)	64	88	0.008
Type 2 diabetes (%)	33	41	0.41
Coronary Artery Disease (%)	53	71	0.080
CABG surgery (%)	51	71	0.037
Heart Failure (%)	24	60	0.002
LVEF (%)	61 (14)	50 (18)	0.002
Mortality (%)	17	38	0.010
Drugs			
ACEIs / ARBs (%)	38	53	0.30
Statins (%)	43	38	0.55
Beta-blockers (%)	33	41	0.69
Calcium channel blockers (%)	22	35	0.25
Laboratory Findings			
Urea (mg/dL)	55 (28)	61 (21)	0.23
Creatinine (mg/dL)	1.0 (0.9-1.1)	1.2 (1.1-1.3)	0.026
Triglycerides (mg/dL)	115 (79-143)	105 (80-121)	0.27
Cholesterol (mg/dL)	185 (43)	173 (41)	0.17
HDL cholesterol (mg/dL)	32 (28-43)	33 (28-52)	0.53
LDL cholesterol (mg/dL)	111 (38)	108 (32)	0.71
Plasma adiponectin (µg/mL) (n=43)	6.5 (4.4-16.6)	7.5 (4.1-11.6)	0.80
EAT adiponectin mRNA (a.u.)	15.1 (3.8)	12.4 (3.0)	0.001
SAT adiponectin mRNA (a.u.)	15.7 (4.4)	13.7 (2.6)	0.048
EAT leptin mRNA (a.u.)	9.6 (8.8-10.8)	9.0 (7.6-10.1)	0.063
SAT leptin mRNA (a.u.)	10.3 (8.8-11.4)	8.9 (8.0-10.7)	0.075

TABLE 7-1. Sample characteristics and comparison between patients with and without cardiovascular events during follow-up.

Values expressed as mean (standard deviation) or as median (percentiles 25-75) for variables not following normal distribution pattern.

* P-values referring to the comparison between groups with and without metabolic syndrome.

ACEIs, angiotensin converter enzyme inhibitors; ARBs, angiotensin receptor blockers; a.u., arbitrary units; CABG, coronary artery bypass grafting; CV, cardiovascular; EAT, epicardial adipose tissue; HDL, high density lipoprotein; LDL, low density lipoprotein; LVEF, left ventricle ejection fraction; SAT; subcutaneous adipose tissue.

more likely to have HT, CABG surgery, HF, lower left ventricle ejection fraction and higher plasma creatinine levels, when compared with patients without CV events. There was also a nonstatistically significant trend towards a higher prevalence of coronary artery disease.

No differences between both groups were found in terms of drug intake at baseline, but treatment changes during follow-up were not considered. Eventually, as expected, all-cause mortality was higher in the group with CV events.

EAT and SAT adipokine mRNA expression and CV prognosis.

Patients with CV events during follow-up had lower EAT and SAT adiponectin expression levels at baseline (Table 7-1, Figures 7-1A & 7-1B). There was also a nonstatistically significant trend towards higher EAT and SAT leptin expression levels in a univariate analysis (Table 7-1, Figures 7-1C & 7-1D). After splitting the sample in three groups according to EAT adiponectin expression and comparing Kaplan-Meier cumulative survival rates free from CV events, we found that patients in the lower tertile presented with higher rates of CV events (P=0.005 for the comparison with the higher tertile, P=0.003 for the trend), as shown on Figure 7-2. Similarly, patients in the lowest tertile of SAT adiponectin expression had higher rates of CV events (P=0.021 for the comparison with the higher tertile, P=0.038 for the trend) (Figure 7-3).

Cox proportional regression stepwise models revealed that only HF and EAT adiponectin expression could predict CV events (Table 7-2), whereas SAT adiponectin and leptin levels and EAT leptin levels could not. Concerning CV mortality and all-cause mortality, both adiponectin and leptin adipose tissue expression levels failed to predict outcomes (data not shown).

Plasma adiponectin concentrations and CV prognosis.

No trend was observed towards differential plasma adiponectin levels at baseline in patients with and without CV events during follow-up (Table 7-1 & Figure 7-4), although the subsample studied was small. Moreover, plasma adiponectin concentrations were not significantly correlated to EAT or SAT adiponectin expression levels (correlation coefficients 0.122 and 0.001, P=0.54 and P=0.99, respectively).

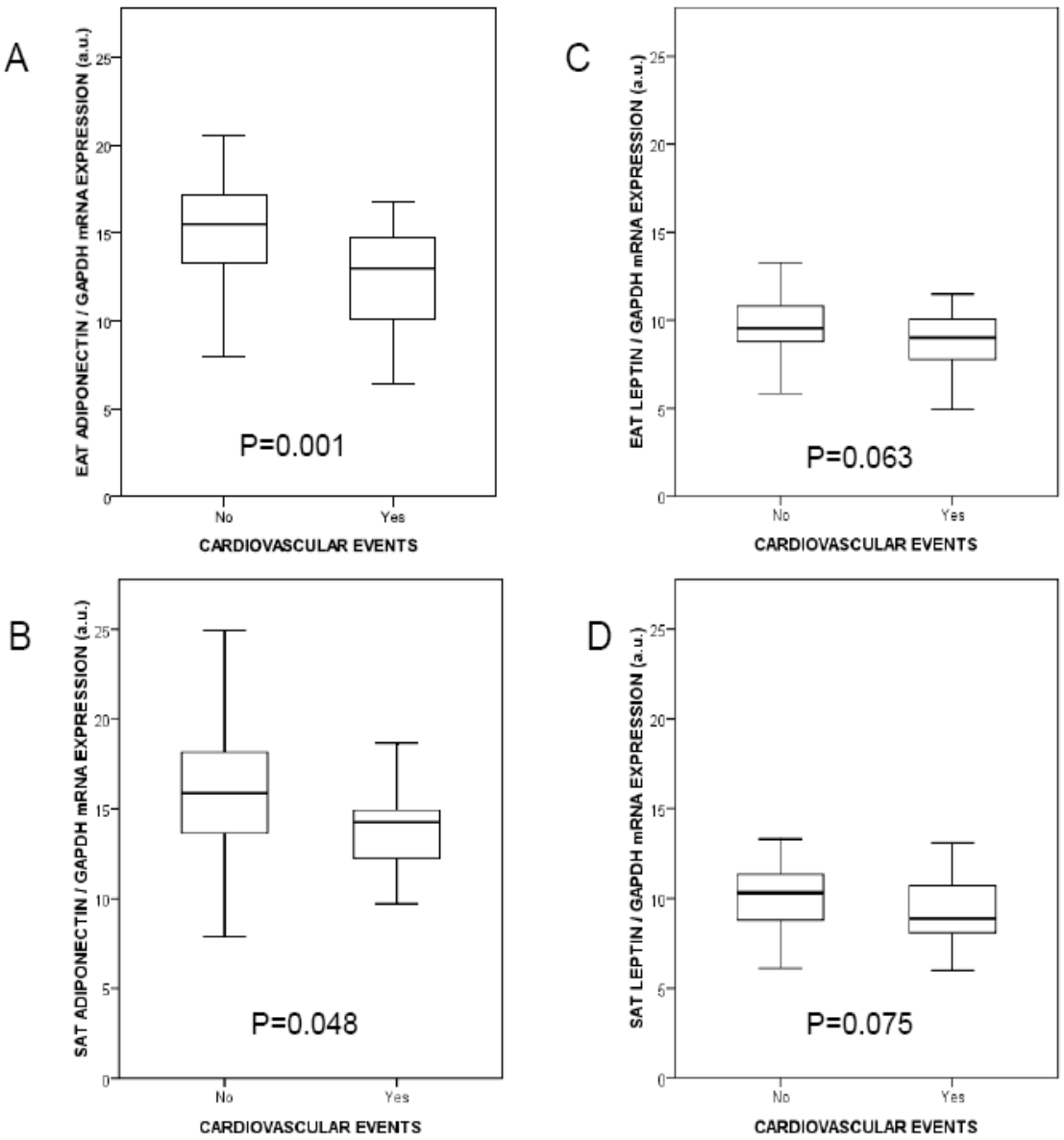

FIGURE 7-1 A-D. Comparison between EAT and SAT adiponectin and leptin levels in patients with and without cardiovascular events during follow-up.

Box-plots showing minimum, percentiles 25-50-75 and maximum values. Extreme values/outliers are not represented. * P-value referring to the comparison between groups with and without cardiovascular events during follow-up.

EAT, epicardial adipose tissue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SAT, subcutaneous adipose tissue.

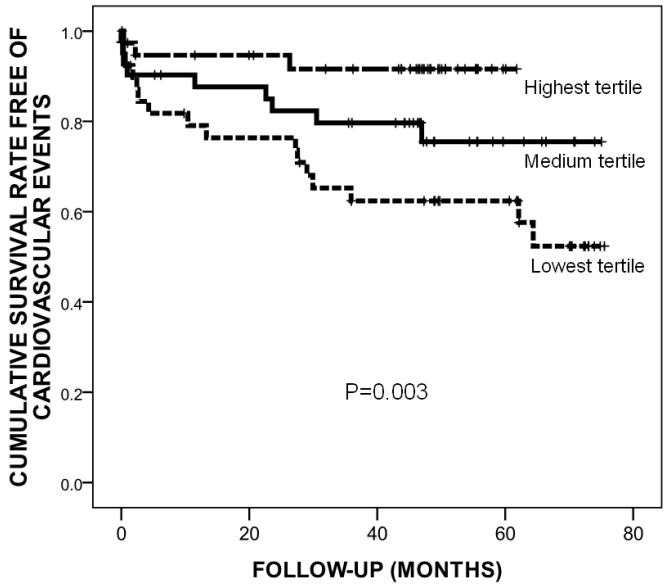

FIGURE 7-2. Kaplan-Meier cumulative survival curves free of cardiovascular events according to EAT adiponectin levels.

Comparison between tertiles of patients according to their EAT adiponectin/GAPDH levels. * P-value referring to the comparison between tertiles.

EAT, epicardial adipose tissue; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

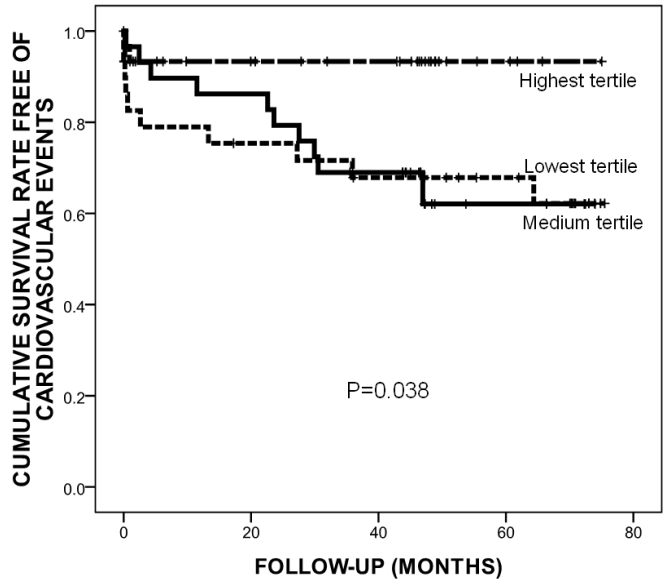

FIGURE 7-3. Kaplan-Meier cumulative survival curves free of cardiovascular events according to SAT adiponectin levels.

Comparison between tertiles of patients according to their SAT adiponectin/GAPDH levels. * P-value referring to the comparison between tertiles.

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; SAT, subcutaneous adipose tissue.

	Hazard ratio (95% CI)	P-value
Model 1		
Age (per year)	0.970 (0.917-1.026)	0.29
Sex (male)	1.603 (0.545-4.717)	0.39
Hypertension	1.690 (0.482-5.921)	0.41
CABG surgery	1.848 (0.676-5.051)	0.23
Heart Failure	4.066 (1.702-9.709)	0.002
Creatinine (per mg/dL)	1.278 (0.376-4.348)	0.70
EAT adiponectin mRNA (per a.u.)	0.896 (0.800-1.003)	0.057
Model 2		
Heart Failure	3.659 (1.596-8.392)	0.002
EAT adiponectin mRNA (per a.u.)	0.883 (0.792-0.984)	0.024

TABLE 7-2. Association between EAT adiponectin levels and cardiovascular events.

Results of Cox proportional regression models. Variables for model 2 where obtained by means of forward stepwise method.

CABG, coronary artery bypass grafting; CI, confidence interval; EAT, epicardial adipose tissue.

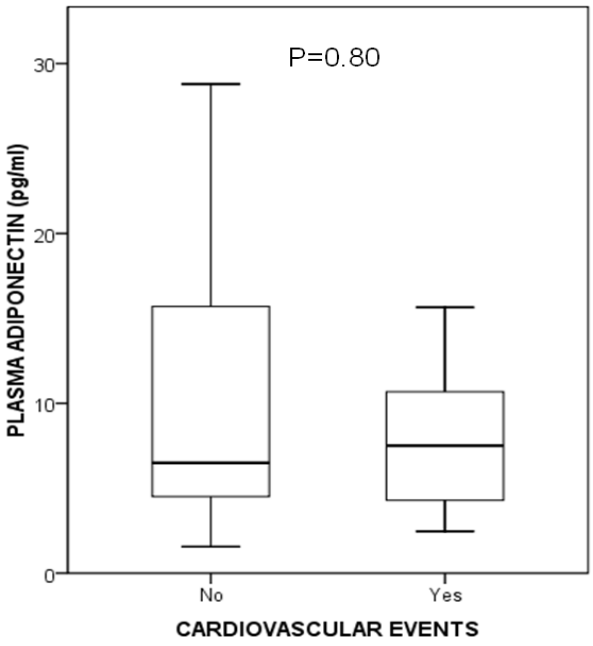

FIGURE 7-4. Comparison between plasma adiponectin levels in patients with and without cardiovascular events during follow-up.

Box-plots showing minimum, percentiles 25-50-75 and maximum values. Extreme values/outliers are not represented. * P-value referring to the comparison between groups with and without cardiovascular events during follow-up.

DISCUSSION

This is the first study to examine the influence of EAT and SAT adiponectin levels on CV prognosis. Although based on a relatively small sample size –however, the largest series of EAT reported so far, to the best of our knowledge–, we found that only EAT adiponectin levels, together with HF, a classical prognostic factor, could predict CV events during long-term follow-up. EAT adipokines could have relevant systemic effects, but local effects on the myocardium and the coronary arteries are more likely to account for these results.

On the contrary, even though patients with lower SAT adiponectin levels at baseline had more CV events during follow-up, this association seems to be completely influenced by other factors. Thus, we found that SAT adiponectin levels and SAT and EAT leptin levels did not predict CV outcomes after adjustment for covariates.

Besides, in line with some previous reports , we could not find an association between lower plasma adiponectin levels and worse CV prognosis.[297] Very interestingly, not even a correlation between plasma and EAT or SAT adiponectin levels could be demonstrated, although previous research based on other populations observed an association between adipose tissue and plasma levels.[301] Therefore, plasma adiponectin levels are not associated with CV prognosis and do not correlate to SAT or EAT levels; but the latter do seem to be associated with CV events during follow-up in our study, irrespective of possible confounders.

Several reasons could account for the difference between adipose tissue and plasma adiponectin levels in this set of patients. Firstly, EAT and SAT gene expression levels might not accurately reflect actual protein levels. Posttranscriptional changes might partially fade this association, but such changes are purely speculative and have not been assessed so far. However, even if they play a role in patients with CV diseases, an explanation to clarify why gene expression levels are lower in patients with worse CV outcome would be needed.

More interestingly, the difference between EAT and plasma adiponectin levels could reflect local effects of adiponectin directly on the heart. The absence of an anatomic layer separating the EAT from the rest of the heart would allow EAT products to influence myocardial function directly and also to be crucial in the physiopathology of CAD.[8] Our findings would support the hypothesis that EAT has a major implication in the physiopathology of CV diseases and their complications, possibly through the above mentioned local effects. Moreover, they

could explain the previous conflicting evidence showing that higher plasma adiponectin levels in patients with CV disease did not affect CV prognosis or could even worsen it.[297] If plasma levels do not reflect local adiponectin concentrations, these results would no longer be contradictory.

The lack of an independent association between SAT adiponectin levels and CV outcomes is also interesting. We hypothesize that SAT adiponectin levels might play a role in CV prognosis to some extent, but they would be more influenced by systemic factors than EAT adiponectin levels. EAT is a visceral fat pad with a particular location and could act as an endocrine organ directly affecting the underlying coronary arteries and the myocardium and being mostly influenced by them. EAT levels are lower in patients with CAD,[181] especially in those with more severe CAD.[213] Remarkably, those levels were elevated in EAT but *not* in SAT, which suggested a major implication of EAT in CV physiopathology.

We could not find an association between adiponectin levels and CV mortality or all-cause mortality. Obviously, the relatively small number of events may account for these results.

In what concerns leptin, no association was found between its EAT or SAT levels and CV outcomes. These results are hardly surprising, considering that leptin has many different biological effects and has failed to show a clear net effect on CV diseases in some previous studies.[115, 300]

The role of adipokines in the physiopathology of CV diseases remains still to be fully elucidated. The initial vision of adiponectin as a beneficial hormone has recently been questioned. Although plasma adiponectin could be a marker of worse CV prognosis in some circumstances that need to be cleared, our results support the classical evidence that the role of adiponectin is mainly protective. Thus, plasma adiponectin might not accurately reflect local levels within the heart, and these levels rather than systemic ones could truly influence CV prognosis.

Study limitations.

Owing to ethical concerns, only patients undergoing elective heart surgery were included in the study.

We focused on EAT and SAT mRNA expression levels rather than on protein tissue levels as the latter could have a different origin and not reflect adipose tissue adiponectin and leptin

production properly. Plasma adiponectin levels were analyzed only in a small subsample of patients and plasma leptin was not analyzed at all in this study.

Treatment changes during follow-up were not considered and therefore their influence could not be tested.

Conclusions.

EAT adiponectin levels are strong predictors of CV outcomes in patients with CV diseases. EAT might play a major role in the development of CV complications.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST/DISCLOSURES STATEMENT

None.

CAPÍTULO 8

DISCUSIÓN GENERAL

Los trabajos que componen la presente memoria aportan información sobre la expresión diferencial de varias adipoquinas en el TAE y en el TAS, y su asociación con la hipertrofia adipocitaria, con la patología cardiovascular y metabólica, y con el pronóstico cardiovascular.

El estudio de la grasa corporal ha demostrado que este tejido presenta una intensa actividad metabólica y que actúa como un auténtico órgano endocrino.[1] Clásicamente, se ha observado que el tejido adiposo visceral posee propiedades diferentes respecto al tejido adiposo subcutáneo, y que participa en la patogenia del llamado síndrome metabólico y de las enfermedades cardiovasculares asociadas al mismo.

El TAE es también un compartimento adiposo visceral, pero su peculiar situación anatómica, desfavorable para la mecánica cardiaca, ha despertado un interés especial en los últimos años. Se ha demostrado que este tejido produce una gran variedad de moléculas bioactivas,[8] y mediante técnicas de imagen se ha encontrado también una fuerte asociación entre la cantidad de TAE y la enfermedad cardiovascular.[193-195]

IMPLICACIONES DEL TAE A NIVEL LOCAL

Aunque el TAE representa tan sólo una mínima proporción de la grasa corporal total, dada la estrecha relación anatómica con el miocardio y las arterias coronarias, se postuló que las adipoquinas producidas por el mismo podrían ejercer efectos paracrinos y vasocrinos relevantes.

En este sentido, resulta muy interesante la ausencia de una fascia que separe el TAE del miocardio y de las arterias coronarias epicárdicas, así como el hecho de que compartan la misma microvasculatura,[9] lo que sugiere que estas estructuras podrían estar interrelacionadas funcionalmente.

Relación entre el estado hipertrófico adipocitario y la expresión de adipoquinas: comparación entre el TAE y el TAS.

La obesidad es un proceso inflamatorio[13] que se asocia a la hipertrofia adipocitaria[205] y que incrementa el riesgo de eventos cardiovasculares.[204] El tejido adiposo consta de células del estroma vascular y de adipocitos, y la ratio entre ellos puede asociarse a la modulación de diversas vías de señalización.[206] Asimismo, se ha observado que el patrón de secreción de los adipocitos depende en gran parte del tamaño de los mismos.[207] Por otra parte, MCP-1 actúa como un potente factor de la quimiotaxis de macrófagos en el tejido adiposo y contribuye a la resistencia a la insulina, a la esteatosis hepática en la obesidad[131] y a la aterogénesis.[125] IL-10 y TNF-α son otras citoquinas secretadas por el tejido adiposo cuya producción se incrementa en los sujetos obesos.[119]

No obstante, hasta el momento no disponíamos de información sobre la relación entre el tamaño de los adipocitos en el TAE y su patrón de expresión de adipoquinas. Por este motivo, decidimos estudiar la posible relación de la hipertrofia adipocitaria en el TAE y en el TAS con el índice de masa corporal, así como con los niveles de expresión de citoquinas proinflamatorias, concretamente de TNF-α y de MCP-1, y de una citoquina con efectos antiinflamatorios, IL-10 (capítulo 2).

En línea con estudios previos, que asocian la obesidad a un incremento de la masa adiposa debida fundamentalmente a la hipertrofia adipocitaria,[215] observamos que el tamaño medio de los adipocitos en el TAS se correlaciona directamente con el IMC. Sin embargo, no ocurre lo mismo en el TAE, donde el tamaño medio de los adipocitos es menor que en el TAS, lo cual concuerda también con observaciones anteriores,[183] y no se asocia al IMC. Estos resultados apoyan la hipótesis de que las propiedades de ambos tejidos son muy diferentes. Los mecanismos de la hipertrofia adipocitaria parecen ser distintos en el TAE y en el TAS, como se desprende también de los estudios que muestran que el volumen de TAE se correlaciona mejor con la masa ventricular que con la cantidad total de grasa corporal.[171, 172]

Por otra parte, mientras que el tamaño de los adipocitos en el TAS se asocia directamente a su expresión de MCP-1, curiosamente en el TAE la correlación entre el tamaño de los adipocitos y la expresión de MCP-1 es inversa. Esta tendencia persiste tras estratificar por la presencia de enfermedad arterial coronaria, pero sólo con diferencias significativas en el grupo más

numeroso, el de pacientes varones con enfermedad arterial coronaria, posiblemente por falta de potencia en el resto de grupos debido a un tamaño muestral pequeño.

Estudios previos realizados con adipocitos procedentes del TAS demostraron que la expresión y liberación de citoquinas inflamatorias depende del volumen celular,[184] y también se observó que la expresión de MCP-1 se incrementa en animales obesos.[219] Nuestros resultados confirman la correlación positiva entre los niveles de MCP-1 y el tamaño de los adipocitos en el TAS, pero no en el TAE, lo cual sugiere de nuevo que el comportamiento de ambos tejidos es diferente, por lo menos en los pacientes cardiópatas, que constituyen nuestra población de estudio, en los que la inflamación local podría regular la expresión de MCP-1 por el TAE.

La correlación inversa descrita entre el tamaño de los adipocitos y la expresión de MCP-1 resulta *a priori* sorprendente, pero concuerda en cierto modo con observaciones previas que señalaban que los adipocitos perivasculares son de menor tamaño y producen mayores niveles de MCP-1 que los del TAS.[220] Por tanto, la regulación de la expresión de MCP-1 –y probablemente de otras adipoquinas– debe de ser distinta en el TAE y en el TAS, lo cual implica la existencia de diferencias en el metabolismo de ambos tejidos o bien en la regulación a nivel local.

Mediante inmunohistoquímica, comprobamos que, en ambos tipos de tejido, los adipocitos, los macrófagos y los mastocitos expresan MCP-1, si bien en el TAE también se observa MCP-1 en los fibroblastos y en los linfocitos. Es posible que exista una compleja interacción entre las distintas células de este tejido, que podría tener implicaciones en la fisiopatología cardiovascular en determinadas circunstancias.

A pesar de que los pacientes con exceso de peso presentan niveles circulantes elevados de marcadores inflamatorios como la PCR y de citoquinas proinflamatorias como IL-6 y TNF-α, no encontramos ninguna asociación entre los niveles de expresión de IL-10 o TNF-α y el tamaño de los adipocitos. IL-10 es una citoquina antiinflamatoria que contrarresta los efectos de TNF-α aunque, por el contrario, otros estudios sugieren que los niveles de IL-10 podrían reflejar un estado proinflamatorio.[216, 217]

Por otra parte, observamos que los pacientes con enfermedad arterial coronaria presentan niveles de ARNm de IL-10 más elevados, si bien este no era el objetivo primario de nuestro estudio, por lo que el resultado debe considerarse con cautela. Sin embargo, mientras que otros autores describen concentraciones superiores de TNF-α en el TAE de los pacientes con

enfermedad coronaria,[218] no encontramos diferencias en cuanto a la expresión génica de TNF-α en el TAE de pacientes con o sin enfermedad coronaria.

Al analizar los niveles plasmáticos de MCP-1, hallamos una tendencia a la asociación con el tamaño de los adipocitos en el TAE, pero no con los del TAS, lo cual sugiere que los efectos locales en el TAE podrían ser un factor determinante de las diferencias de expresión del MCP-1 en este tejido.

En conclusión, en los pacientes cardiópatas, el tamaño medio de los adipocitos del TAS se relaciona directamente con el IMC y con los niveles de expresión de MCP-1, en línea con lo descrito en otras poblaciones.[184] Sin embargo, el tamaño medio de los adipocitos en el TAE no se asocia con el IMC, y se correlaciona de manera inversa con los niveles de MCP-1. El TAE y TAS presentan diferentes componentes celulares que participan en la expresión de la proteína quimiotáctica MCP-1, responsable de la infiltración de células inflamatorias. Estos datos sugieren que el comportamiento de ambos tejidos es diferente, y que probablemente también poseen implicaciones distintas en la fisiopatología cardiovascular y metabólica.

Enfermedad arterial coronaria

Por su relación anatómica con las arterias coronarias epicárdicas, parte de la investigación sobre el TAE se ha centrado en estudiar su implicación en la fisiopatología de la enfermedad coronaria. En los últimos años, múltiples estudios de imagen han demostrado la relación entre la cantidad de TAE y la aterosclerosis coronaria.[193-195] No obstante, debido a la dificultad de obtener biopsias de este tejido, existen muchos menos datos sobre el patrón de producción de adipoquinas por el mismo y la enfermedad coronaria. Un estudio pionero mostró que el TAE de los pacientes sometidos a cirugía de revascularización presenta un perfil patogénico de expresión de adipoquinas similar al tejido graso visceral, y mayor infiltración macrofágica;[201] pero no se comparó con el TAE de controles sin cardiopatía isquémica. Iacobellis y colaboradores[181] publicaron un estudio con un número pequeño de pacientes sometidos a cirugía cardiaca, en el que observaron niveles de expresión de adiponectina en TAE más bajos en los sujetos con enfermedad coronaria, respecto a los que no la presentaban. Es probable que los individuos con mayor cantidad de TAE presenten menores niveles plasmáticos de adiponectina, tal como se ha observado que ocurre con los niveles plasmáticos de la hormona en relación con la grasa abdominal.[249] De este modo, los pacientes con mayor cantidad de

TAE, asociado directamente a la cantidad de grasa visceral, expresarían menores niveles locales de adiponectina, y por lo tanto tendrían una mayor susceptibilidad a la aterosclerosis coronaria.

Partiendo de estos trabajos previos, decidimos estudiar los niveles de expresión de adiponectina y de IL-6 en el TAE de pacientes con enfermedad coronaria en una muestra mayor que la publicada previamente,[181] e investigar también si dichos niveles se correlacionan con la extensión de la enfermedad coronaria, medida según el número de arterias coronarias epicárdicas afectas (capítulo 3). Como grupo control, seleccionamos pacientes sin enfermedad arterial coronaria sometidos a cirugía cardiaca por otros motivos. Comprobamos así que los pacientes con enfermedad arterial coronaria sometidos a cirugía cardiaca presentan niveles de expresión de adiponectina en el TAE menores que los sujetos control. Además, los pacientes con mayor número de arterias coronarias afectas presentan en el TAE niveles significativamente menores de expresión de adiponectina y niveles superiores de IL-6. El hecho de que IL-6 atenúe la asociación entre los niveles de adiponectina en el TAE y la extensión de la enfermedad coronaria quizá pueda explicarse por la interrelación que existe entre las distintas adipoquinas. Es decir, si bien los niveles de expresión de adiponectina en el TAE se asocian a la extensión de la enfermedad coronaria, esta relación podría verse también influida por el efecto de otras adipoquinas.

No obstante, en una muestra menor de pacientes, no encontramos asociación entre los niveles de expresión de TNF-α y la presencia de enfermedad arterial coronaria, tal como se describe en el capítulo 2, lo cual se opone a los hallazgos de Cheng y colaboradores.[218] Por tanto, a la luz de estos resultados, la implicación de TNF-α en la fisiopatología de la enfermedad arterial coronaria parece ser menor que la de adiponectina, o bien más compleja.

Observamos también que los niveles de IL-10 son mayores en pacientes con cardiopatía isquémica. Aunque IL-10 es un inhibidor de TNF-α y sus efectos conocidos son predominantemente antiinflamatorios,[216] en esta situación podría reflejar un estado inflamatorio, lo cual explicaría estos hallazgos. No obstante, teniendo en cuenta que el número de pacientes es pequeño y que el estudio no fue diseñado con ese propósito, este hallazgo ha de considerarse con cautela y precisa confirmación mediante estudios específicos, como se ha señalado previamente.

En cuanto a los niveles de adiponectina en el TAS, también se asocian a la extensión de la enfermedad coronaria, aunque en menor medida que los niveles en el TAE. Obviamente, la expresión de adiponectina en el TAS contribuye de forma importante a los niveles plasmáticos de la hormona, pero el interés de los niveles de expresión de adiponectina en el TAE radica sobre todo en los efectos antiinflamatorios y antiaterogénicos locales que esta adipoquina puede ejercer directamente sobre las arterias coronarias. De este modo, se postula que pueden ser las acciones vasocrinas y paracrinas de la adiponectina sobre las arterias coronarias y el miocardio las que tengan mayor relevancia en la patogenia y fisiopatología de la enfermedad arterial coronaria.

Por otra parte, cabe destacar de nuevo la relación que existe entre el volumen de TAE, los factores de riesgo cardiovascular y la propia aterosclerosis coronaria.[193, 194, 211] El TAE no se asocia a la cantidad total de grasa corporal, sino que representa un marcador bastante preciso de la grasa visceral total, asociada en mayor medida al síndrome metabólico y a sus complicaciones cardiovasculares, entre ellas la enfermedad arterial coronaria. Así, el TAE podría ser una pieza clave en la relación existente entre los componentes del síndrome metabólico y la enfermedad arterial coronaria.

Estudios recientes han mostrado que, en los pacientes con cardiopatía isquémica, los niveles plasmáticos de adiponectina elevados se asocian a un peor pronóstico cardiovascular,[297] y que lo mismo ocurre en pacientes con arteriopatía periférica.[92] Dado que la adiponectina ejerce efectos beneficiosos desde el punto de vista metabólico, es muy probable que esta asociación se deba a que, en estadios avanzados de la enfermedad, los niveles plasmáticos de adiponectina aumenten por un mecanismo de contrarregulación. En esta situación, las propiedades antiinflamatorias y antiaterogénicas de la hormona ya no serían suficientes para revertir la situación. Es posible que los niveles de expresión de adiponectina en el TAE tengan más importancia que las concentraciones plasmáticas como predictores del pronóstico cardiovascular, ya que podrían reflejar de forma más precisa los niveles locales de la adipoquina.

IMPLICACIONES DEL TAE A NIVEL SISTÉMICO

Posiblemente, además de efectos locales, las adipoquinas secretadas por el TAE ejercen efectos sistémicos de gran relevancia. El TAE podría así encontrarse en el centro de una encrucijada que relaciona la obesidad –especialmente el patrón de obesidad central– y la patología que se asocia a ella, ya sea de índole marcadamente sistémica, como el síndrome metabólico, o principalmente local, como la enfermedad arterial coronaria.

Por tanto, más allá de la asociación entre los niveles de adiponectina y de citoquinas proinflamatorias en el TAE y la enfermedad arterial coronaria, decidimos investigar la relación entre la expresión de adipoquinas en este tejido y otras enfermedades en las que su papel resultaba *a priori* menos aparente.

Hipertensión arterial.

El TAE se asocia a la cantidad total de masa miocitaria, y observaciones recientes mostraron también que los pacientes hipertensos presentan mayor cantidad de TAE medida por resonancia magnética.[190, 302] Por otra parte, algunos estudios revelaron que los pacientes con hipertensión arterial presentan niveles plasmáticos de adiponectina menores que los no hipertensos.[79] Los niveles plasmáticos bajos de adiponectina se asocian también a la hipertrofia ventricular izquierda, aunque es probable que la propia hipertensión arterial desempeñe un papel importante en esta asociación.[82]

Partiendo de estos estudios previos, decidimos estudiar si los pacientes con hipertensión arterial presentan niveles de adiponectina en el TAE distintos respecto a los pacientes no hipertensos (capítulo 4). Así, observamos que los pacientes con hipertensión arterial expresan menos adiponectina en el TAE que los no hipertensos, independientemente de otros factores que podrían influir en los niveles de expresión de la hormona.

Por otro lado, comprobamos también que la expresión de adiponectina en TAS no se asocia a la hipertensión arterial. Esto concuerda con estudios previos que no hallaron diferencias en la expresión de adiponectina en el TAS en pacientes con o sin enfermedad cardiovascular, o incluso tras intervenciones terapéuticas para incrementar los niveles de adiponectina plasmática.[248] No obstante, puesto que determinamos los niveles de expresión genética de adiponectina y no los niveles de proteína, pueden existir cambios postranscripcionales que regulen los niveles de adiponectina y que la implicación del TAS en la fisiopatología de la

hipertensión arterial sea mayor. Sin embargo, no sólo no se ha demostrado que tales cambios postranscripcionales existan, sino que, de ser así, podrían ocurrir también en el TAE y reforzar la implicación de este tejido en la fisiopatología de la hipertensión arterial.

Estas observaciones se pueden integrar con los conocimientos previos, y permiten realizar una interpretación conjunta de la relación entre el TAE y la hipertensión arterial. Los pacientes con mayor cantidad de TAE podrían presentar menor expresión de adiponectina por este tejido y quizá también por el tejido adiposo visceral en general. El déficit de producción de adiponectina se asociaría a una mayor prevalencia de hipertensión arterial,[79] probablemente debido a los efectos de adiponectina en la regulación del metabolismo del óxido nítrico y de la función endotelial.[41, 42]

Pero nuestros hallazgos tienen también otras implicaciones que conviene destacar. Por una parte, el tejido adiposo visceral podría tener un papel más importante en la fisiopatología de la hipertensión arterial que el TAS, lo cual concuerda con observaciones clásicas que asocian la obesidad central y la hipertensión arterial, así como el resto de componentes del llamado síndrome metabólico. Por otro lado, el riesgo más elevado de enfermedad arterial coronaria en pacientes con hipertensión arterial podría deberse al déficit de adiponectina a nivel sistémico, pero también a nivel local, debido a una menor producción de la adipoquina por el TAE en los pacientes hipertensos.

De esta forma, el TAE podría ser el nexo que enlaza la obesidad central y algunos de los factores de riesgo cardiovasculares principales –en este caso la hipertensión arterial– con la enfermedad arterial coronaria.

Diabetes mellitus tipo 2.

Estudios previos han demostrado que la masa de TAE se asocia a la resistencia a la insulina y a la diabetes mellitus tipo 2.[191, 192] Los niveles plasmáticos de adiponectina también se relacionan con la resistencia a la insulina, y predicen el desarrollo de la misma y de diabetes mellitus tipo 2, independientemente de las medidas basales de obesidad.[260-263] Además, los pacientes diabéticos, sobre todo aquellos con macroangiopatía, presentan niveles plasmáticos menores de adiponectina.[67] Por otra parte, la leptina se correlaciona con la cantidad total de grasa corporal y se ha asociado a la resistencia a la insulina y de forma dispar a enfermedades cardiovasculares y metabólicas.[108, 112] Partiendo de estas observaciones, decidimos comparar

los niveles de expresión de adiponectina y leptina en TAE y TAS en pacientes diabéticos, respecto a no diabéticos (capítulo 5).

Al contrario de lo que cabría esperar teniendo en cuenta los efectos beneficiosos de la adiponectina sobre el metabolismo de la glucosa,[36, 39, 66] observamos que los pacientes con DM tipo 2 establecida o glucemia basal alterada presentan niveles similares de adiponectina en el TAE y en el TAS respecto a los pacientes sin DM tipo 2 ni glucemia basal alterada. Quizá estos resultados se deban a un mecanismo de contrarregulación en la expresión de adiponectina por el tejido adiposo, similar al que se ha postulado para explicar la asociación entre los niveles plasmáticos elevados de adiponectina y un peor pronóstico en pacientes con IAM.[275, 276] Estos mecanismos de contrarregulación podrían ser menos importantes en la hipertensión arterial, ya que es posible que la ausencia del efecto protector de la adiponectina frente a la disfunción endotelial no genere un *feedback* capaz de incrementar la expresión de adiponectina. Así, de acuerdo con nuestros hallazgos, mientras que los niveles de expresión de adiponectina continuarían siendo bajos en los pacientes con hipertensión arterial, en el caso de la diabetes mellitus no ocurriría lo mismo, puesto que la propia glucemia y la insulina influirían en la expresión de adiponectina.

No obstante, en línea con estudios previos,[36, 66, 67, 260] y aunque solamente se analizó un pequeño subgrupo de pacientes seleccionados de manera aleatoria, los niveles plasmáticos de adiponectina sí son inferiores en los pacientes diabéticos, lo cual podría deberse a diferencias en el tamaño de los depósitos adiposos o a cambios postranscripcionales u otros mecanismos de regulación de los niveles plasmáticos que todavía no han sido elucidados.

También analizamos si los niveles de expresión de adiponectina y leptina eran distintos en los pacientes que recibían tratamiento con estatinas respecto a los que no lo recibían, pero no encontramos diferencias significativas entre ambos grupos. De todas formas, estudios previos diseñados para analizar el efecto de las estatinas sobre la sensibilidad a la insulina y los niveles de adipoquinas resultaron controvertidos, con resultados inconsistentes.[103, 104]

En cuanto a los niveles de leptina, tampoco observamos diferencias entre los pacientes diabéticos y no diabéticos tanto en el TAE como en el TAS, lo que sugiere la existencia de una compleja regulación de la expresión de la hormona.

Síndrome metabólico.

El síndrome metabólico es un conjunto de cofactores que aumentan el riesgo cardiovascular. Aunque las distintas definiciones de síndrome metabólico son bastante recientes y poco homogéneas, en su patogenia subyace la presencia de obesidad visceral y de cierto grado de resistencia a la insulina.[259, 285] Teniendo en cuenta además la relación descrita previamente entre la expresión de adiponectina en el TAE, la hipertensión arterial y la enfermedad coronaria, cabe pensar que este tejido puede desempeñar un papel crucial en el desarrollo del síndrome metabólico y en su asociación con la cardiopatía isquémica. Por este motivo, aunque previamente habíamos analizado los niveles de expresión de adiponectina en el TAE y en el TAS en relación con la hipertensión arterial y con la DM tipo 2, así como los niveles de leptina en relación con la DM tipo 2, decidimos estudiar, en otro grupo de pacientes, si dichos niveles se asocian a la presencia de síndrome metabólico (capítulo 6).

Nuestros resultados muestran que los pacientes con síndrome metabólico presentan niveles menores de adiponectina en el TAE, y se refuerzan por el hallazgo que los pacientes en el cuartil inferior de expresión de adiponectina por el TAE presentan un mayor número de criterios que definen el síndrome metabólico.

Además, en línea con observaciones previas,[68] los pacientes con síndrome metabólico presentan niveles plasmáticos menores de adiponectina. En este punto, conviene destacar que otros estudios demostraron que los niveles plasmáticos de adiponectina se asocian con más fuerza que los niveles de proteína C reactiva y de IL-6 al síndrome metabólico y a cada uno de sus componentes por separado.[292, 294] Los niveles plasmáticos de TNF-α no se relacionan con el síndrome metabólico, lo cual podría deberse en parte a que los efectos de esta citoquina proinflamatoria son principalmente paracrinos, y quizá sus concentraciones plasmáticas no reflejen adecuadamente los niveles tisulares locales. No obstante, como se ha comentado previamente, nuestro grupo tampoco ha encontrado diferencias en cuanto a los niveles de expresión de TNF-α en el tejido adiposo de pacientes con cardiopatía isquémica respecto a los que no la presentan. Una vez más, es preciso señalar que los niveles de expresión génica podrían no reflejar adecuadamente los niveles de actividad de la citoquina.

En todo caso, teniendo en cuenta que la adiponectina es el principal producto proteico secretado por los adipocitos y que existe una estrecha interrelación entre adiponectina y TNF-α, resulta muy atractiva la idea de que sea la adiponectina del TAE la que desempeñe un papel

más relevante en la fisiopatología del síndrome metabólico y de las enfermedades cardiovasculares. Hay que destacar que, si bien TNF-α disminuye los niveles de adiponectina,[49] también la adiponectina influye en los niveles de TNF-α,[29] y probablemente esto tenga mayor importancia desde un punto de vista patogénico.

En el TAS, no parece existir una expresión diferencial de adiponectina tan clara en los pacientes con síndrome metabólico respecto a los que no lo presentan. Este hallazgo resulta especialmente interesante, puesto que de nuevo pondría de manifiesto diferencias en la implicación de tejidos adiposos relativamente distintos en la fisiopatología del síndrome metabólico. Obviamente, esto no significa que el TAS, cuantitativamente muy importante, no tenga relevancia en el desarrollo del síndrome metabólico, pero sí apunta a que el TAE y el tejido adiposo visceral desempeñen un papel especial y aporta un argumento más a las observaciones epidemiológicas clásicas que asocian la obesidad visceral a un mayor riesgo metabólico y cardiovascular.

Una hipótesis interesante que se formula a partir de estos resultados sería que la adiponectina es la base del estado proinflamatorio observado en pacientes con síndrome metabólico. Así, la hormona podría actuar como un factor clave en el desarrollo del síndrome metabólico y de sus complicaciones vasculares. Los sujetos obesos presentan niveles de adiponectina menores, por lo que podrían carecer de los efectos beneficiosos de la adipoquina, lo cual propiciaría el desarrollo de algunas de las características del síndrome metabólico: resistencia a la insulina, alteración del metabolismo lipídico e instauración de un estado proinflamatorio y proaterogénico.[259, 285]

Por tanto, es posible que el tejido adiposo visceral sea el principal componente responsable de esta situación, pero, a nivel local, el TAE podría ejercer un papel importante no sólo por su contribución como parte del tejido adiposo visceral, sino por su capacidad de influencia directa sobre las arterias coronarias, de tal manera que puede propiciar el desarrollo de las placas de ateroma. De este modo, por su consabida peculiaridad anatómica, el TAE podría ser un elemento importante como nexo que vincule el síndrome metabólico y la enfermedad cardiovascular.

EXPRESIÓN DE ADIPOQUINAS EN EL TAE Y PRONÓSTICO CARDIOVASCULAR

Teniendo en cuenta la relación que existe entre las adipoquinas producidas por el TAE y la patología cardiovascular y metabólica, decidimos estudiar si estas adipoquinas, concretamente adiponectina y leptina, podían ejercer también alguna influencia sobre el pronóstico cardiovascular a largo plazo (capítulo 7). Para ello, realizamos el seguimiento de 137 pacientes de los cuales se habían obtenido muestras de TAE, durante un periodo medio de 41,4 meses. Se recogieron también muestras de plasma y de TAS de algunos pacientes, previamente y durante la intervención quirúrgica, respectivamente, para comprobar también si podrían influir en el pronóstico cardiovascular.

Hasta el momento, no existía ningún estudio que evaluase la asociación entre la producción de adipoquinas por el TAE y el pronóstico cardiovascular, y resultó muy interesante comprobar que, efectivamente, los pacientes con menores niveles basales de expresión de adiponectina en el TAE presentan una mayor tasa de eventos cardiovasculares durante el seguimiento a largo plazo. Estos eventos se habían definido como ictus, episodio de insuficiencia cardiaca aguda, síndrome coronario agudo, necesidad de revascularización o muerte cardiovascular. Además, mediante análisis multivariado se observó que los niveles de adiponectina en el TAE y la presencia de insuficiencia cardiaca son los únicos predictores independientes del desarrollo de eventos cardiovasculares durante el seguimiento.

Sin embargo, si bien los pacientes con niveles bajos de adiponectina en el TAS también presentan una mayor tasa de eventos cardiovasculares durante el seguimiento realizado, esta asociación parece depender por completo de otros factores, puesto que desaparece al ajustar por los mismos. Por otra parte, no encontramos ninguna asociación entre los niveles de adiponectina en el TAS o en el plasma o los niveles de leptina en el TAE o en el TAS y el pronóstico cardiovascular.

Estos hallazgos no se oponen a observaciones previas que asociaban la hiperadiponectinemia en pacientes con enfermedad coronaria[291] o insuficiencia cardiaca congestiva[84] a un peor pronóstico cardiovascular, sino que más bien sugieren que probablemente son los niveles de adiponectina locales los que desempeñan un papel crucial en la fisiopatología de las enfermedades cardiovasculares. De hecho, el conocimiento derivado de estudios anteriores resultaba aparentemente contradictorio, ya que los efectos biológicos de la adiponectina son

principalmente beneficiosos, mientras que sus valores plasmáticos elevados parecían asociarse a un peor pronóstico, lo cual sugería un efecto deletéreo de la hormona.[84, 291]

La explicación que se ha postulado para justificar estas observaciones es la existencia de un mecanismo de contrarregulación similar al que ocurre en muchos otros procesos biológicos. Este mecanismo daría lugar a concentraciones elevadas de adiponectina en pacientes con un marcado estado inflamatorio, pero que ya no sería suficiente para revertir la situación. Así, la hiperadiponectinemia podría servir como un marcador de mal pronóstico en situaciones de enfermedad coronaria avanzada, mientras que en sujetos sin enfermedad o bien en estadios más precoces actuaría como un factor protector, debido a sus efectos antiinflamatorios, antiateroscleróticos y de sensibilización a la insulina, fundamentalmente. En cuanto a los pacientes con insuficiencia cardiaca, se ha observado también que los niveles plasmáticos elevados de adiponectina se asocian a una mayor severidad de la enfermedad y a una mayor mortalidad, probablemente en relación con la caquexia, que resulta deletérea en estos pacientes.[84]

Sin embargo, nuestros resultados sugieren que los pacientes con niveles basales elevados de expresión tisular de adiponectina en el TAE presentan un mejor pronóstico cardiovascular, mientras que los niveles en el TAS no predicen el pronóstico cardiovascular tras ajustar por posibles factores de confusión. Tampoco encontramos asociación entre las concentraciones plasmáticas de adiponectina y el pronóstico cardiovascular, aunque en este caso sólo se estudió un pequeño subgrupo de pacientes. De nuevo, estos hallazgos apoyan la hipótesis de que el TAE desempeña un papel especial en la fisiopatología cardiovascular.

La explicación más plausible, por tanto, es que la adiponectina presente en las arterias coronarias y en el miocardio provenga principalmente del TAE, y que sean los niveles locales de adiponectina los que tengan una verdadera importancia pronóstica. De este modo, los niveles plasmáticos de adiponectina carecerían de valor pronóstico, o bien incluso la hiperadiponectinemia podría servir como marcador de mal pronóstico en determinadas situaciones –en estadios de enfermedad avanzada–, pero a nivel fisiopatológico los niveles bajos de adiponectina en el TAE y, por ende, en las arterias coronarias y el miocardio, serían los que marcarían un peor pronóstico cardiovascular, quizá no sólo en este subgrupo de pacientes sino en la población general.

Aunque hay que tener en cuenta la heterogenicidad de la muestra incluida en nuestro estudio, los resultados se mantenían tras ajustar por la presencia de cardiopatía isquémica y por otras variables que podrían influir en la asociación de adiponectina con el pronóstico cardiovascular.
Por otro lado, también es preciso reiterar que analizamos los niveles de expresión génica de adiponectina, y no los niveles de proteína. Si bien estos últimos podrían diferir respecto a los niveles de ARNm, todavía no existen estudios a este respecto y, a la luz de los conocimientos actuales, la hipótesis que describimos resulta más plausible.
En cuanto a la leptina, no encontramos ninguna asociación entre sus niveles en el TAE o en el TAS y el pronóstico cardiovascular, en línea con observaciones previas que tampoco mostraron relación entre los niveles plasmáticos de la hormona y el pronóstico cardiovascular.[115, 300] Realmente, los estudios diseñados para estudiar la asociación entre los niveles plasmáticos de leptina y la enfermedad cardiovascular han aportado resultados contradictorios, lo cual sugiere que la regulación de la leptina es muy compleja y concuerda con la gran diversidad de efectos biológicos que ejerce esta hormona.[107]

IMPLICACIONES CLÍNICAS

El creciente interés por el estudio del tejido adiposo en los últimos años, tras el descubrimiento de sus propiedades secretoras, ha llevado a importantes avances en este campo. Los estudios sobre la expresión de adipoquinas en el TAE y su relación con diversas enfermedades, así como la investigación con técnicas de imagen cardiaca, nos están ayudando a comprender el papel crucial que el TAE puede desempeñar en la fisiopatología cardiovascular.
En su conjunto, los resultados que presentamos sugieren que la función del TAE no se limita a servir como un simple depósito energético y como un sistema regulador de ácidos grasos libres para el metabolismo miocárdico, sino también como un auténtico órgano productor de citoquinas inflamatorias y de adipoquinas con efectos paracrinos, vasocrinos e incluso sistémicos.
Estos hallazgos apoyan la hipótesis de que el TAE tiene mayor relevancia que el TAS en la fisiopatología cardiovascular y metabólica. Es probable que las diferencias entre el TAS y el tejido adiposo visceral conformen el sustrato fisiopatológico que explica la clásica asociación entre la obesidad central, el síndrome metabólico y el desarrollo de eventos cardiovasculares.

Así, por su proximidad a las arterias coronarias y al miocardio, el TAE podría ejercer efectos de extraordinaria importancia en el desarrollo de la aterosclerosis coronaria, pero también ser el nexo de unión entre los factores de riesgo cardiovascular y las enfermedades asociadas, e influir en el pronóstico cardiovascular. De esta forma, en el estudio del TAE podría encontrarse la clave de la clásica asociación entre la obesidad central, el síndrome metabólico y las enfermedades cardiovasculares.

Por otra parte, nuestros resultados sugieren también que la adiponectina es una adipoquina con una gran influencia en la enfermedad cardiovascular y que sus efectos son generalmente beneficiosos. Observamos que los pacientes con mayor expresión de adiponectina en el TAE presentan mayor prevalencia de hipertensión arterial y de síndrome metabólico, y mayor prevalencia y severidad de enfermedad arterial coronaria. Aunque, por tratarse de estudios transversales, no era posible establecer una relación temporal entre los niveles de adiponectina y el desarrollo de la patología descrita, también llevamos a cabo un estudio prospectivo que mostró que los pacientes con menores niveles basales de expresión de adiponectina en el TAE presentan una mayor tasa de complicaciones cardiovasculares durante el seguimiento a largo plazo.

Si bien estudios previos otorgaban un papel primordial a las citoquinas proinflamatorias, como TNF-α, en la fisiopatología cardiovascular, es posible que la adiponectina preceda al estado proinflamatorio. De esta forma, los individuos con obesidad central y mayor cantidad de tejido adiposo visceral presentarían menores niveles de adiponectina, lo cual conllevaría la sobreexpresión de otras citoquinas[29] y el desarrollo de un estado proinflamatorio característico del síndrome metabólico. A nivel local, el déficit de adiponectina propiciaría la ateromatosis coronaria y explicaría la asociación entre el síndrome metabólico y la cardiopatía isquémica.

El propio estado inflamatorio podría inducir la expresión de adiponectina en determinadas circunstancias, pero en estadios avanzados sus efectos antiinflamatorios y antiaterogénicos serían insuficientes para revertir la situación. Es probable que este fenómeno explique los similares niveles de expresión de adiponectina en el TAE de los sujetos diabéticos y no diabéticos, así como el peor pronóstico de los pacientes con cardiopatía isquémica e hiperadiponectinemia que reportan otros autores.[297]

El conocimiento de la fisiopatología del TAE nos permite especular acerca del beneficio de posibles estrategias terapéuticas encaminadas fundamentalmente a estimular la síntesis de

adiponectina en determinados pacientes con elevado riesgo metabólico y cardiovascular. El ejercicio físico y la pérdida de peso han demostrado disminuir la cantidad de TAE, pero hoy día todavía no disponemos de un tratamiento farmacológico eficaz. Los agonistas PPAR-γ, como las tiazolidinedionas, y algunos betabloqueantes y bloqueantes de los receptores de angiotensina pueden ser útiles para incrementar la adiponectinemia,[96, 97, 101, 106] pero o bien sus efectos adversos superan el beneficio que pueden aportar, o sus beneficios en este contexto clínico todavía se desconocen.

PERSPECTIVAS FUTURAS

El interés que ha despertado el estudio del TAE, junto con los alentadores resultados de los trabajos más recientes y su potencial como diana terapéutica del síndrome metabólico y de las enfermedades cardiovasculares, continuarán motivando el estudio intensivo de este tejido en los próximos años. Entre los campos que merecerán mayor atención se encuentran los siguientes:

-Particularidades del TAE en comparación con otros compartimentos de tejido adiposo visceral, y del propio TAE en distintas localizaciones.

-Metabolismo de la adiponectina e implicación fisiopatológica de las distintas formas de la hormona (monómeros, trímeros, etc.). Estudio de posibles cambios postranscripcionales y su trascendencia.

-Efectos biológicos de las distintas adipoquinas y citoquinas, e interrelación entre ellas.

-Estrategias terapéuticas dirigidas a mejorar el perfil metabólico y a prevenir los eventos cardiovasculares, mediante intervención directa sobre el TAE.

No cabe duda de que todavía quedan muchos aspectos por esclarecer hasta llegar a comprender por completo la compleja interrelación entre las adipoquinas expresadas en el TAE y su implicación exacta en el desarrollo de las enfermedades cardiovasculares. No obstante, el estudio de este tejido arrojará probablemente mucha luz al conocimiento fisiopatológico, y abrirá nuevas alternativas de prevención integral de los factores de riesgo cardiovascular y de tratamiento de la patología cardiovascular y metabólica.

CAPÍTULO 9

CONCLUSIONES

- ***Conclusión 1:*** Al contrario de lo que ocurre en el TAS, el tamaño de los adipocitos en el TAE no se asocia al IMC y se correlaciona inversamente con los niveles de expresión de MCP-1. No se ha observado asociación entre los niveles de expresión de TNF-α y de IL-10 y el tamaño de los adipocitos en el TAE ni en el TAS. Es probable que los mecanismos de hipertrofia adipocitaria y la regulación de MCP-1 sean distintos en el TAE y en el TAS.

- ***Conclusión 2:*** Los pacientes con mayor extensión de la enfermedad arterial coronaria expresan niveles bajos de mRNA de adiponectina y niveles elevados de mRNA de IL-6 en el TAE. Sin embargo, no existen diferencias significativas en cuanto a los niveles de expresión de adiponectina ni de IL-6 en el TAS. El TAE podría tener una implicación directa en la fisiopatología de la enfermedad arterial coronaria.

- ***Conclusión 3:*** Los pacientes con hipertensión arterial presentan niveles bajos de expresión de adiponectina en el TAE y similares niveles de expresión de adiponectina en el TAS respecto a los no hipertensos, independientemente de otros factores. Este hallazgo podría explicar en parte la relación entre la hipertensión arterial y la obesidad central.

- ***Conclusión 4:*** Los niveles de expresión de adiponectina y leptina en el TAE y el TAS son similares en los pacientes con diabetes mellitus tipo 2 respecto a los no diabéticos. Este hallazgo podría deberse a un fenómeno de contrarregulación, o bien a otros mecanismos todavía no dilucidados.

- ***Conclusión 5:*** Los pacientes con síndrome metabólico presentan menores niveles de mRNA en el TAE que los sujetos sin síndrome metabólico, mientras que los niveles

en el TAS no difieren entre ambos grupos. Los pacientes con niveles bajos de expresión de adiponectina en el TAE presentan un mayor número de los componentes que definen el síndrome metabólico. El TAE puede ser uno de los nexos entre el síndrome metabólico y sus complicaciones cardiovasculares.

- ***Conclusión 6:*** Los pacientes con menores niveles de expresión de adiponectina en el TAE y en el TAS presentan más eventos cardiovasculares durante el seguimiento a largo plazo. Los niveles de adiponectina en el TAE y la insuficiencia cardiaca son predictores independientes del pronóstico cardiovascular tras la cirugía cardiaca. Sin embargo, no se ha encontrado asociación entre la expresión de leptina en el tejido adiposo y el pronóstico cardiovascular, ni entre la expresión de adiponectina y leptina y la mortalidad por cualquier causa durante el seguimiento.

BIBLIOGRAFÍA

1. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab*. 2000;11:327-332
2. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab*. 2004;89:2548-2556
3. Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. *Int J Obes Relat Metab Disord*. 2003;27:875-888
4. Leow MK, Addy CL, Mantzoros CS. Clinical review 159: Human immunodeficiency virus/highly active antiretroviral therapy-associated metabolic syndrome: Clinical presentation, pathophysiology, and therapeutic strategies. *J Clin Endocrinol Metab*. 2003;88:1961-1976
5. Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H, Eckel RH. The metabolic syndrome. *Endocr Rev*. 2008;29:777-822
6. Dusserre E, Moulin P, Vidal H. Differences in mrna expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. *Biochim Biophys Acta*. 2000;1500:88-96
7. Third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii) final report. *Circulation*. 2002;106:3143-3421
8. Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG, Martin J, Goldstein BJ, Shi Y. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation*. 2003;108:2460-2466
9. Iacobellis G, Corradi D, Sharma AM. Epicardial adipose tissue: Anatomic, biomolecular and clinical relationships with the heart. *Nat Clin Pract Cardiovasc Med*. 2005;2:536-543
10. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science*. 1993;259:87-91
11. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest*. 1995;95:2111-2119
12. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112:1796-1808
13. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest*. 2003;112:1785-1788
14. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest*. 2007;117:175-184
15. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA. Expression of cd68 and

macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: Association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes*. 2005;54:2305-2313

16. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res*. 2005;46:2347-2355
17. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, 2nd, DeFuria J, Jick Z, Greenberg AS, Obin MS. Adipocyte death, adipose tissue remodeling, and obesity complications. *Diabetes*. 2007;56:2910-2918
18. Trayhurn P, Wood IS. Adipokines: Inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr*. 2004;92:347-355
19. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, Shimomura I. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes*. 2007;56:901-911
20. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, Schraw T, Durand JL, Li H, Li G, Jelicks LA, Mehler MF, Hui DY, Deshaies Y, Shulman GI, Schwartz GJ, Scherer PE. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest*. 2007;117:2621-2637
21. Agarwal AK, Garg A. Genetic disorders of adipose tissue development, differentiation, and death. *Annu Rev Genomics Hum Genet*. 2006;7:175-199
22. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to c1q, produced exclusively in adipocytes. *J Biol Chem*. 1995;270:26746-26749
23. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun*. 1999;257:79-83
24. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. Cdna cloning and expression of a novel adipose specific collagen-like factor, apm1 (adipose most abundant gene transcript 1). *Biochem Biophys Res Commun*. 1996;221:286-289
25. Kishida K, Nagaretani H, Kondo H, Kobayashi H, Tanaka S, Maeda N, Nagasawa A, Hibuse T, Ohashi K, Kumada M, Nishizawa H, Okamoto Y, Ouchi N, Maeda K, Kihara S, Funahashi T, Matsuzawa Y. Disturbed secretion of mutant adiponectin associated with the metabolic syndrome. *Biochem Biophys Res Commun*. 2003;306:286-292
26. Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. Structure-function studies of the adipocyte-secreted hormone acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. *J Biol Chem*. 2003;278:9073-9085
27. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kda adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A*. 2001;98:2005-2010

28. Motoshima H, Wu X, Sinha MK, Hardy VE, Rosato EL, Barbot DJ, Rosato FE, Goldstein BJ. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: Effects of insulin and rosiglitazone. *J Clin Endocrinol Metab*. 2002;87:5662-5667
29. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y. Ppargamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes*. 2001;50:2094-2099
30. Han SH, Quon MJ, Kim JA, Koh KK. Adiponectin and cardiovascular disease: Response to therapeutic interventions. *J Am Coll Cardiol*. 2007;49:531-538
31. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab*. 2001;86:3815-3819
32. Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida K, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class a scavenger receptor expression in human monocyte-derived macrophages. *Circulation*. 2001;103:1057-1063
33. Berg AH, Combs TP, Scherer PE. Acrp30/adiponectin: An adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab*. 2002;13:84-89
34. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med*. 2001;7:941-946
35. Nakamura Y, Shimada K, Fukuda D, Shimada Y, Ehara S, Hirose M, Kataoka T, Kamimori K, Shimodozono S, Kobayashi Y, Yoshiyama M, Takeuchi K, Yoshikawa J. Implications of plasma concentrations of adiponectin in patients with coronary artery disease. *Heart*. 2004;90:528-533
36. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*. 2003;423:762-769
37. Kharroubi I, Rasschaert J, Eizirik DL, Cnop M. Expression of adiponectin receptors in pancreatic beta cells. *Biochem Biophys Res Commun*. 2003;312:1118-1122
38. Chinetti G, Zawadski C, Fruchart JC, Staels B. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors pparalpha, ppargamma, and lxr. *Biochem Biophys Res Commun*. 2004;314:151-158
39. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein acrp30 enhances hepatic insulin action. *Nat Med*. 2001;7:947-953

40. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Hormonal regulation of adiponectin gene expression in 3t3-l1 adipocytes. *Biochem Biophys Res Commun*. 2002;290:1084-1089
41. Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M, Igura T, Inui Y, Kihara S, Nakamura T, Yamashita S, Miyagawa J, Funahashi T, Matsuzawa Y. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Horm Metab Res*. 2000;32:47-50
42. Shibata R, Ouchi N, Kihara S, Sato K, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of amp-activated protein kinase signaling. *J Biol Chem*. 2004;279:28670-28674
43. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, Funahashi T, Walsh K. Adiponectin stimulates angiogenesis by promoting cross-talk between amp-activated protein kinase and akt signaling in endothelial cells. *J Biol Chem*. 2004;279:1304-1309
44. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res*. 2004;94:e27-31
45. Shibata R, Skurk C, Ouchi N, Galasso G, Kondo K, Ohashi T, Shimano M, Kihara S, Murohara T, Walsh K. Adiponectin promotes endothelial progenitor cell number and function. *FEBS Lett*. 2008;582:1607-1612
46. Ohashi K, Kihara S, Ouchi N, Kumada M, Fujita K, Hiuge A, Hibuse T, Ryo M, Nishizawa H, Maeda N, Maeda K, Shibata R, Walsh K, Funahashi T, Shimomura I. Adiponectin replenishment ameliorates obesity-related hypertension. *Hypertension*. 2006;47:1108-1116
47. Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, Shin HK, Moskowitz MA, Ouchi N. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. *Circulation*. 2008;117:216-223
48. Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. Adiponectin reduces atherosclerosis in apolipoprotein e-deficient mice. *Circulation*. 2002;106:2767-2770
49. Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H, Hotta K, Nishida M, Takahashi M, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial nf-kappab signaling through a camp-dependent pathway. *Circulation*. 2000;102:1296-1301
50. Kumada M, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation*. 2004;109:2046-2049
51. Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, Kihara S, Walsh K. Adiponectin modulates inflammatory reactions via calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest*. 2007;117:375-386

52. Tao L, Gao E, Jiao X, Yuan Y, Li S, Christopher TA, Lopez BL, Koch W, Chan L, Goldstein BJ, Ma XL. Adiponectin cardioprotection after myocardial ischemia/reperfusion involves the reduction of oxidative/nitrative stress. *Circulation*. 2007;115:1408-1416
53. Shibata R, Sato K, Pimentel DR, Takemura Y, Kihara S, Ohashi K, Funahashi T, Ouchi N, Walsh K. Adiponectin protects against myocardial ischemia-reperfusion injury through ampk- and cox-2-dependent mechanisms. *Nat Med*. 2005;11:1096-1103
54. Ouedraogo R, Wu X, Xu SQ, Fuchsel L, Motoshima H, Mahadev K, Hough K, Scalia R, Goldstein BJ. Adiponectin suppression of high-glucose-induced reactive oxygen species in vascular endothelial cells: Evidence for involvement of a camp signaling pathway. *Diabetes*. 2006;55:1840-1846
55. Shinmura K, Tamaki K, Saito K, Nakano Y, Tobe T, Bolli R. Cardioprotective effects of short-term caloric restriction are mediated by adiponectin via activation of amp-activated protein kinase. *Circulation*. 2007;116:2809-2817
56. Shibata R, Sato K, Kumada M, Izumiya Y, Sonoda M, Kihara S, Ouchi N, Walsh K. Adiponectin accumulates in myocardial tissue that has been damaged by ischemia-reperfusion injury via leakage from the vascular compartment. *Cardiovasc Res*. 2007;74:471-479
57. Pineiro R, Iglesias MJ, Gallego R, Raghay K, Eiras S, Rubio J, Dieguez C, Gualillo O, Gonzalez-Juanatey JR, Lago F. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Lett*. 2005;579:5163-5169
58. Skurk C, Wittchen F, Suckau L, Witt H, Noutsias M, Fechner H, Schultheiss HP, Poller W. Description of a local cardiac adiponectin system and its deregulation in dilated cardiomyopathy. *Eur Heart J*. 2008;29:1168-1180
59. Liao Y, Takashima S, Maeda N, Ouchi N, Komamura K, Shimomura I, Hori M, Matsuzawa Y, Funahashi T, Kitakaze M. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of ampk and glucose metabolism. *Cardiovasc Res*. 2005;67:705-713
60. Shibata R, Ouchi N, Ito M, Kihara S, Shiojima I, Pimentel DR, Kumada M, Sato K, Schiekofer S, Ohashi K, Funahashi T, Colucci WS, Walsh K. Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nat Med*. 2004;10:1384-1389
61. Fujita K, Maeda N, Sonoda M, Ohashi K, Hibuse T, Nishizawa H, Nishida M, Hiuge A, Kurata A, Kihara S, Shimomura I, Funahashi T. Adiponectin protects against angiotensin ii-induced cardiac fibrosis through activation of ppar-alpha. *Arterioscler Thromb Vasc Biol*. 2008;28:863-870
62. Fujioka D, Kawabata K, Saito Y, Kobayashi T, Nakamura T, Kodama Y, Takano H, Obata JE, Kitta Y, Umetani K, Kugiyama K. Role of adiponectin receptors in endothelin-induced cellular hypertrophy in cultured cardiomyocytes and their expression in infarcted heart. *Am J Physiol Heart Circ Physiol*. 2006;290:H2409-2416
63. Shibata R, Izumiya Y, Sato K, Papanicolaou K, Kihara S, Colucci WS, Sam F, Ouchi N, Walsh K. Adiponectin protects against the development of systolic dysfunction following myocardial infarction. *J Mol Cell Cardiol*. 2007;42:1065-1074

64. Salmenniemi U, Ruotsalainen E, Pihlajamaki J, Vauhkonen I, Kainulainen S, Punnonen K, Vanninen E, Laakso M. Multiple abnormalities in glucose and energy metabolism and coordinated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. *Circulation*. 2004;110:3842-3848
65. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. Adiponectin and protection against type 2 diabetes mellitus. *Lancet*. 2003;361:226-228
66. Yamamoto Y, Hirose H, Saito I, Tomita M, Taniyama M, Matsubara K, Okazaki Y, Ishii T, Nishikai K, Saruta T. Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the japanese population. *Clin Sci (Lond)*. 2002;103:137-142
67. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol*. 2000;20:1595-1599
68. Santaniemi M, Kesaniemi YA, Ukkola O. Low plasma adiponectin concentration is an indicator of the metabolic syndrome. *Eur J Endocrinol*. 2006;155:745-750
69. Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, Hotta K, Nishida M, Takahashi M, Nakamura T, Yamashita S, Funahashi T, Matsuzawa Y. Novel modulator for endothelial adhesion molecules: Adipocyte-derived plasma protein adiponectin. *Circulation*. 1999;100:2473-2476
70. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, Matsuzawa Y. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol*. 2003;23:85-89
71. Lindsay RS, Resnick HE, Zhu J, Tun ML, Howard BV, Zhang Y, Yeh J, Best LG. Adiponectin and coronary heart disease: The strong heart study. *Arterioscler Thromb Vasc Biol*. 2005;25:e15-16
72. Lawlor DA, Davey Smith G, Ebrahim S, Thompson C, Sattar N. Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. *J Clin Endocrinol Metab*. 2005;90:5677-5683
73. Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, Danesh J, Whincup PH. Adiponectin and coronary heart disease: A prospective study and meta-analysis. *Circulation*. 2006;114:623-629
74. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA*. 2004;291:1730-1737
75. Wolk R, Berger P, Lennon RJ, Brilakis ES, Davison DE, Somers VK. Association between plasma adiponectin levels and unstable coronary syndromes. *Eur Heart J*. 2007;28:292-298

76. Kojima S, Funahashi T, Sakamoto T, Miyamoto S, Soejima H, Hokamaki J, Kajiwara I, Sugiyama S, Yoshimura M, Fujimoto K, Miyao Y, Suefuji H, Kitagawa A, Ouchi N, Kihara S, Matsuzawa Y, Ogawa H. The variation of plasma concentrations of a novel, adipocyte derived protein, adiponectin, in patients with acute myocardial infarction. *Heart*. 2003;89:667
77. Kojima S, Funahashi T, Otsuka F, Maruyoshi H, Yamashita T, Kajiwara I, Shimomura H, Miyao Y, Fujimoto K, Sugiyama S, Sakamoto T, Yoshimura M, Ogawa H. Future adverse cardiac events can be predicted by persistently low plasma adiponectin concentrations in men and marked reductions of adiponectin in women after acute myocardial infarction. *Atherosclerosis*. 2007;194:204-213
78. Shibata R, Numaguchi Y, Matsushita K, Sone T, Kubota R, Ohashi T, Ishii M, Kihara S, Walsh K, Ouchi N, Murohara T. Usefulness of adiponectin to predict myocardial salvage following successful reperfusion in patients with acute myocardial infarction. *Am J Cardiol*. 2008;101:1712-1715
79. Iwashima Y, Katsuya T, Ishikawa K, Ouchi N, Ohishi M, Sugimoto K, Fu Y, Motone M, Yamamoto K, Matsuo A, Ohashi K, Kihara S, Funahashi T, Rakugi H, Matsuzawa Y, Ogihara T. Hypoadiponectinemia is an independent risk factor for hypertension. *Hypertension*. 2004;43:1318-1323
80. Cesari M, Pessina AC, Zanchetta M, De Toni R, Avogaro A, Pedon L, Dorigatti F, Maiolino G, Rossi GP. Low plasma adiponectin is associated with coronary artery disease but not with hypertension in high-risk nondiabetic patients. *J Intern Med*. 2006;260:474-483
81. Yilmaz MI, Sonmez A, Caglar K, Celik T, Yenicesu M, Eyileten T, Acikel C, Oguz Y, Yavuz I, Vural A. Effect of antihypertensive agents on plasma adiponectin levels in hypertensive patients with metabolic syndrome. *Nephrology (Carlton)*. 2007;12:147-153
82. Hong SJ, Park CG, Seo HS, Oh DJ, Ro YM. Associations among plasma adiponectin, hypertension, left ventricular diastolic function and left ventricular mass index. *Blood Press*. 2004;13:236-242
83. Kozakova M, Muscelli E, Flyvbjerg A, Frystyk J, Morizzo C, Palombo C, Ferrannini E. Adiponectin and left ventricular structure and function in healthy adults. *J Clin Endocrinol Metab*. 2008;93:2811-2818
84. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, Hildebrandt P. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation*. 2005;112:1756-1762
85. George J, Patal S, Wexler D, Sharabi Y, Peleg E, Kamari Y, Grossman E, Sheps D, Keren G, Roth A. Circulating adiponectin concentrations in patients with congestive heart failure. *Heart*. 2006;92:1420-1424
86. Tsutamoto T, Tanaka T, Sakai H, Ishikawa C, Fujii M, Yamamoto T, Horie M. Total and high molecular weight adiponectin, haemodynamics, and mortality in patients with chronic heart failure. *Eur Heart J*. 2007;28:1723-1730
87. McEntegart MB, Awede B, Petrie MC, Sattar N, Dunn FG, MacFarlane NG, McMurray JJ. Increase in serum adiponectin concentration in patients with heart

failure and cachexia: Relationship with leptin, other cytokines, and b-type natriuretic peptide. *Eur Heart J*. 2007;28:829-835

88. Anker SD, Rauchhaus M. Insights into the pathogenesis of chronic heart failure: Immune activation and cachexia. *Curr Opin Cardiol*. 1999;14:211-216
89. Ingelsson E, Riserus U, Berne C, Frystyk J, Flyvbjerg A, Axelsson T, Lundmark P, Zethelius B. Adiponectin and risk of congestive heart failure. *JAMA*. 2006;295:1772-1774
90. Lin HV, Kim JY, Pocai A, Rossetti L, Shapiro L, Scherer PE, Accili D. Adiponectin resistance exacerbates insulin resistance in insulin receptor transgenic/knockout mice. *Diabetes*. 2007;56:1969-1976
91. Golledge J, Leicht A, Crowther RG, Clancy P, Spinks WL, Quigley F. Association of obesity and metabolic syndrome with the severity and outcome of intermittent claudication. *J Vasc Surg*. 2007;45:40-46
92. Iwashima Y, Horio T, Suzuki Y, Kihara S, Rakugi H, Kangawa K, Funahashi T, Ogihara T, Kawano Y. Adiponectin and inflammatory markers in peripheral arterial occlusive disease. *Atherosclerosis*. 2006;188:384-390
93. Monzillo LU, Hamdy O, Horton ES, Ledbury S, Mullooly C, Jarema C, Porter S, Ovalle K, Moussa A, Mantzoros CS. Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. *Obes Res*. 2003;11:1048-1054
94. Miyazaki T, Shimada K, Mokuno H, Daida H. Adipocyte derived plasma protein, adiponectin, is associated with smoking status in patients with coronary artery disease. *Heart*. 2003;89:663
95. Iwashima Y, Katsuya T, Ishikawa K, Kida I, Ohishi M, Horio T, Ouchi N, Ohashi K, Kihara S, Funahashi T, Rakugi H, Ogihara T. Association of hypoadiponectinemia with smoking habit in men. *Hypertension*. 2005;45:1094-1100
96. Koh KK, Quon MJ, Han SH, Ahn JY, Jin DK, Kim HS, Kim DS, Shin EK. Vascular and metabolic effects of combined therapy with ramipril and simvastatin in patients with type 2 diabetes. *Hypertension*. 2005;45:1088-1093
97. Koh KK, Quon MJ, Han SH, Chung WJ, Ahn JY, Seo YH, Kang MH, Ahn TH, Choi IS, Shin EK. Additive beneficial effects of losartan combined with simvastatin in the treatment of hypercholesterolemic, hypertensive patients. *Circulation*. 2004;110:3687-3692
98. Guerre-Millo M, Gervois P, Raspe E, Madsen L, Poulain P, Derudas B, Herbert JM, Winegar DA, Willson TM, Fruchart JC, Berge RK, Staels B. Peroxisome proliferator-activated receptor alpha activators improve insulin sensitivity and reduce adiposity. *J Biol Chem*. 2000;275:16638-16642
99. Koh KK, Han SH, Quon MJ, Yeal Ahn J, Shin EK. Beneficial effects of fenofibrate to improve endothelial dysfunction and raise adiponectin levels in patients with primary hypertriglyceridemia. *Diabetes Care*. 2005;28:1419-1424
100. Choi KC, Ryu OH, Lee KW, Kim HY, Seo JA, Kim SG, Kim NH, Choi DS, Baik SH, Choi KM. Effect of ppar-alpha and -gamma agonist on the expression of visfatin, adiponectin, and tnf-alpha in visceral fat of oletf rats. *Biochem Biophys Res Commun*. 2005;336:747-753

101. Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, Olefsky JM. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes*. 2002;51:2968-2974
102. Phillips SA, Ciaraldi TP, Kong AP, Bandukwala R, Aroda V, Carter L, Baxi S, Mudaliar SR, Henry RR. Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes*. 2003;52:667-674
103. Paolisso G, Gualdiero P, Manzella D, Rizzo MR, Tagliamonte MR, Gambardella A, Verza M, Gentile S, Varricchio M, D'Onofrio F. Association of fasting plasma free fatty acid concentration and frequency of ventricular premature complexes in nonischemic non-insulin-dependent diabetic patients. *Am J Cardiol*. 1997;80:932-937
104. Mrc/bhf heart protection study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: A randomised placebo-controlled trial. *Lancet*. 2002;360:7-22
105. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase a in 3t3-l1 adipocytes. *FEBS Lett*. 2001;507:142-146
106. Celik T, Iyisoy A, Kursaklioglu H, Kardesoglu E, Kilic S, Turhan H, Yilmaz MI, Ozcan O, Yaman H, Isik E, Fici F. Comparative effects of nebivolol and metoprolol on oxidative stress, insulin resistance, plasma adiponectin and soluble p-selectin levels in hypertensive patients. *J Hypertens*. 2006;24:591-596
107. Guzik TJ, Mangalat D, Korbut R. Adipocytokines - novel link between inflammation and vascular function? *J Physiol Pharmacol*. 2006;57:505-528
108. Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, t cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest*. 2002;110:1093-1103
109. Buettner C, Pocai A, Muse ED, Etgen AM, Myers MG, Jr., Rossetti L. Critical role of stat3 in leptin's metabolic actions. *Cell Metab*. 2006;4:49-60
110. Buettner C, Muse ED, Cheng A, Chen L, Scherer T, Pocai A, Su K, Cheng B, Li X, Harvey-White J, Schwartz GJ, Kunos G, Rossetti L. Leptin controls adipose tissue lipogenesis via central, stat3-independent mechanisms. *Nat Med*. 2008;14:667-675
111. Welsh P, Murray HM, Buckley BM, de Craen AJ, Ford I, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Sattar N. Leptin predicts diabetes but not cardiovascular disease: Results from a large prospective study in an elderly population. *Diabetes Care*. 2009;32:308-310
112. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the west of scotland coronary prevention study (woscops). *Circulation*. 2001;104:3052-3056
113. Soderberg S, Stegmayr B, Ahlbeck-Glader C, Slunga-Birgander L, Ahren B, Olsson T. High leptin levels are associated with stroke. *Cerebrovasc Dis*. 2003;15:63-69
114. Soderberg S, Ahren B, Jansson JH, Johnson O, Hallmans G, Asplund K, Olsson T. Leptin is associated with increased risk of myocardial infarction. *J Intern Med*. 1999;246:409-418

115. Sattar N, Wannamethee G, Sarwar N, Chernova J, Lawlor DA, Kelly A, Wallace AM, Danesh J, Whincup PH. Leptin and coronary heart disease: Prospective study and systematic review. *J Am Coll Cardiol*. 2009;53:167-175
116. Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A. Leptin-replacement therapy for lipodystrophy. *N Engl J Med*. 2002;346:570-578
117. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: A randomized, controlled, dose-escalation trial. *JAMA*. 1999;282:1568-1575
118. Ruan H, Lodish HF. Insulin resistance in adipose tissue: Direct and indirect effects of tumor necrosis factor-alpha. *Cytokine Growth Factor Rev*. 2003;14:447-455
119. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. *Endocrinology*. 2004;145:2273-2282
120. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking tnf-alpha function. *Nature*. 1997;389:610-614
121. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab*. 2001;280:E745-751
122. Plomgaard P, Bouzakri K, Krogh-Madsen R, Mittendorfer B, Zierath JR, Pedersen BK. Tumor necrosis factor-alpha induces skeletal muscle insulin resistance in healthy human subjects via inhibition of akt substrate 160 phosphorylation. *Diabetes*. 2005;54:2939-2945
123. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an engineered human anti-tnf-alpha antibody (cdp571) on insulin sensitivity and glycemic control in patients with niddm. *Diabetes*. 1996;45:881-885
124. Tam LS, Tomlinson B, Chu TT, Li TK, Li EK. Impact of tnf inhibition on insulin resistance and lipids levels in patients with rheumatoid arthritis. *Clin Rheumatol*. 2007;26:1495-1498
125. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999;340:115-126
126. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation*. 2000;101:1767-1772
127. Bruunsgaard H, Andersen-Ranberg K, Jeune B, Pedersen AN, Skinhoj P, Pedersen BK. A high plasma concentration of tnf-alpha is associated with dementia in centenarians. *J Gerontol A Biol Sci Med Sci*. 1999;54:M357-364
128. Skoog T, Dichtl W, Boquist S, Skoglund-Andersson C, Karpe F, Tang R, Bond MG, de Faire U, Nilsson J, Eriksson P, Hamsten A. Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J*. 2002;23:376-383

129. Castellanos M, Castillo J, Garcia MM, Leira R, Serena J, Chamorro A, Davalos A. Inflammation-mediated damage in progressing lacunar infarctions: A potential therapeutic target. *Stroke*. 2002;33:982-987
130. Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: Results of the anti-tnf therapy against congestive heart failure (attach) trial. *Circulation*. 2003;107:3133-3140
131. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, Kitazawa S, Miyachi H, Maeda S, Egashira K, Kasuga M. Mcp-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest*. 2006;116:1494-1505
132. Inouye KE, Shi H, Howard JK, Daly CH, Lord GM, Rollins BJ, Flier JS. Absence of cc chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. *Diabetes*. 2007;56:2242-2250
133. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*. 2003;112:1821-1830
134. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, Jones KL, Kawamori R, Cassis LA, Tschop MH, Bruemmer D. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. *J Clin Invest*. 2007;117:2877-2888
135. Libby P. Atheroma: More than mush. *Lancet*. 1996;348 Suppl 1:s4-7
136. Kanda T, Takahashi T. Interleukin-6 and cardiovascular diseases. *Jpn Heart J*. 2004;45:183-193
137. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, Matsuzawa Y. Reciprocal association of c-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation*. 2003;107:671-674
138. Otsuka F, Sugiyama S, Kojima S, Maruyoshi H, Funahashi T, Matsui K, Sakamoto T, Yoshimura M, Kimura K, Umemura S, Ogawa H. Plasma adiponectin levels are associated with coronary lesion complexity in men with coronary artery disease. *J Am Coll Cardiol*. 2006;48:1155-1162
139. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? *Atherosclerosis*. 2000;148:209-214
140. Tsutamoto T, Hisanaga T, Wada A, Maeda K, Ohnishi M, Fukai D, Mabuchi N, Sawaki M, Kinoshita M. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. *J Am Coll Cardiol*. 1998;31:391-398
141. Biasucci LM, Liuzzo G, Fantuzzi G, Caligiuri G, Rebuzzi AG, Ginnetti F, Dinarello CA, Maseri A. Increasing levels of interleukin (il)-1ra and il-6 during the first 2 days

of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation*. 1999;99:2079-2084

142. Zdanov A, Schalk-Hihi C, Menon S, Moore KW, Wlodawer A. Crystal structure of epstein-barr virus protein bcrf1, a homolog of cellular interleukin-10. *J Mol Biol*. 1997;268:460-467
143. Anguera I, Miranda-Guardiola F, Bosch X, Filella X, Sitges M, Marin JL, Betriu A, Sanz G. Elevation of serum levels of the anti-inflammatory cytokine interleukin-10 and decreased risk of coronary events in patients with unstable angina. *Am Heart J*. 2002;144:811-817
144. Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Boersma E, Simoons ML, Zeiher AM. Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. *Circulation*. 2003;107:2109-2114
145. Fichtlscherer S, Breuer S, Heeschen C, Dimmeler S, Zeiher AM. Interleukin-10 serum levels and systemic endothelial vasoreactivity in patients with coronary artery disease. *J Am Coll Cardiol*. 2004;44:44-49
146. Sprengers ED, Kluft C. Plasminogen activator inhibitors. *Blood*. 1987;69:381-387
147. Song CZ, Siok TE, Gelehrter TD. Smad4/dpc4 and smad3 mediate transforming growth factor-beta (tgf-beta) signaling through direct binding to a novel tgf-beta-responsive element in the human plasminogen activator inhibitor-1 promoter. *J Biol Chem*. 1998;273:29287-29290
148. Alessi MC, Peiretti F, Morange P, Henry M, Nalbone G, Juhan-Vague I. Production of plasminogen activator inhibitor 1 by human adipose tissue: Possible link between visceral fat accumulation and vascular disease. *Diabetes*. 1997;46:860-867
149. Mertens I, Verrijken A, Michiels JJ, Van der Planken M, Ruige JB, Van Gaal LF. Among inflammation and coagulation markers, pai-1 is a true component of the metabolic syndrome. *Int J Obes (Lond)*. 2006;30:1308-1314
150. Agirbasli M. Pivotal role of plasminogen-activator inhibitor 1 in vascular disease. *Int J Clin Pract*. 2005;59:102-106
151. Stoney RM, O'Dea K, Herbert KE, Dragicevic G, Giles GG, Cumpston GN, Best JD. Insulin resistance as a major determinant of increased coronary heart disease risk in postmenopausal women with type 2 diabetes mellitus. *Diabet Med*. 2001;18:476-482
152. Trost S, Pratley R, Sobel B. Impaired fibrinolysis and risk for cardiovascular disease in the metabolic syndrome and type 2 diabetes. *Curr Diab Rep*. 2006;6:47-54
153. Meigs JB, O'Donnell C J, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, Nathan DM, Sullivan LM, D'Agostino RB, Wilson PW. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: The framingham offspring study. *Diabetes*. 2006;55:530-537
154. Klein RL, Semler AJ, Baynes JW, Thorpe SR, Lyons TJ, Jenkins AJ. Glycation does not alter ldl-induced secretion of tissue plasminogen activator and plasminogen activator inhibitor-1 from human aortic endothelial cells. *Ann N Y Acad Sci*. 2005;1043:379-389

155. Nordt TK, Sawa H, Fujii S, Sobel BE. Induction of plasminogen activator inhibitor type-1 (pai-1) by proinsulin and insulin in vivo. *Circulation*. 1995;91:764-770
156. Sobel BE, Taatjes DJ, Schneider DJ. Intramural plasminogen activator inhibitor type-1 and coronary atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2003;23:1979-1989
157. Sebestjen M, Keber I, Zegura B, Simcic S, Bozic M, Fressart MM, Stegnar M. Statin and fibrate treatment of combined hyperlipidemia: The effects on some novel risk factors. *Thromb Haemost*. 2004;92:1129-1135
158. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA. The hormone resistin links obesity to diabetes. *Nature*. 2001;409:307-312
159. Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, Smith SR. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab*. 2004;89:1844-1848
160. Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, Orlova C, Mantzoros CS. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: Cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab*. 2003;88:4848-4856
161. Weikert C, Westphal S, Berger K, Dierkes J, Mohlig M, Spranger J, Rimm EB, Willich SN, Boeing H, Pischon T. Plasma resistin levels and risk of myocardial infarction and ischemic stroke. *J Clin Endocrinol Metab*. 2008;93:2647-2653
162. Butler J, Kalogeropoulos A, Georgiopoulou V, de Rekeneire N, Rodondi N, Smith AL, Hoffmann U, Kanaya A, Newman AB, Kritchevsky SB, Vasan RS, Wilson PW, Harris TB. Serum resistin concentrations and risk of new onset heart failure in older persons: The health, aging, and body composition (health abc) study. *Arterioscler Thromb Vasc Biol*. 2009;29:1144-1149
163. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cdna encoding a novel human pre-b-cell colony-enhancing factor. *Mol Cell Biol*. 1994;14:1431-1437
164. Varma V, Yao-Borengasser A, Rasouli N, Bodles AM, Phanavanh B, Lee MJ, Starks T, Kern LM, Spencer HJ, 3rd, McGehee RE, Jr., Fried SK, Kern PA. Human visfatin expression: Relationship to insulin sensitivity, intramyocellular lipids, and inflammation. *J Clin Endocrinol Metab*. 2007;92:666-672
165. Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M, Bluher M. Plasma visfatin concentrations and fat depot-specific mrna expression in humans. *Diabetes*. 2005;54:2911-2916
166. Dahl TB, Yndestad A, Skjelland M, Oie E, Dahl A, Michelsen A, Damas JK, Tunheim SH, Ueland T, Smith C, Bendz B, Tonstad S, Gullestad L, Froland SS, Krohg-Sorensen K, Russell D, Aukrust P, Halvorsen B. Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: Possible role in inflammation and plaque destabilization. *Circulation*. 2007;115:972-980
167. Ho E, Shimada Y. Formation of the epicardium studied with the scanning electron microscope. *Dev Biol*. 1978;66:579-585

168. Marchington JM, Mattacks CA, Pond CM. Adipose tissue in the mammalian heart and pericardium: Structure, foetal development and biochemical properties. *Comp Biochem Physiol B*. 1989;94:225-232
169. Sacks HS, Fain JN. Human epicardial adipose tissue: A review. *Am Heart J*. 2007;153:907-917
170. Reiner L, Mazzoleni A, Rodriguez FL. Statistical analysis of the epicardial fat weight in human hearts. *AMA Arch Pathol*. 1955;60:369-373
171. Iacobellis G, Ribaudo MC, Assael F, Vecci E, Tiberti C, Zappaterreno A, Di Mario U, Leonetti F. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: A new indicator of cardiovascular risk. *J Clin Endocrinol Metab*. 2003;88:5163-5168
172. Corradi D, Maestri R, Callegari S, Pastori P, Goldoni M, Luong TV, Bordi C. The ventricular epicardial fat is related to the myocardial mass in normal, ischemic and hypertrophic hearts. *Cardiovasc Pathol*. 2004;13:313-316
173. Iacobellis G, Ribaudo MC, Zappaterreno A, Iannucci CV, Leonetti F. Relation between epicardial adipose tissue and left ventricular mass. *Am J Cardiol*. 2004;94:1084-1087
174. Kim MK, Tomita T, Kim MJ, Sasai H, Maeda S, Tanaka K. Aerobic exercise training reduces epicardial fat in obese men. *J Appl Physiol*. 2009;106:5-11
175. Iacobellis G, Singh N, Wharton S, Sharma AM. Substantial changes in epicardial fat thickness after weight loss in severely obese subjects. *Obesity (Silver Spring)*. 2008;16:1693-1697
176. Kim MK, Tanaka K, Kim MJ, Matuso T, Endo T, Tomita T, Maeda S, Ajisaka R. Comparison of epicardial, abdominal and regional fat compartments in response to weight loss. *Nutr Metab Cardiovasc Dis*. 2009;19:760-766
177. Marchington JM, Pond CM. Site-specific properties of pericardial and epicardial adipose tissue: The effects of insulin and high-fat feeding on lipogenesis and the incorporation of fatty acids in vitro. *Int J Obes*. 1990;14:1013-1022
178. Sacks HS, Fain JN, Holman B, Cheema P, Chary A, Parks F, Karas J, Optican R, Bahouth SW, Garrett E, Wolf RY, Carter RA, Robbins T, Wolford D, Samaha J. Uncoupling protein-1 and related messenger ribonucleic acids in human epicardial and other adipose tissues: Epicardial fat functioning as brown fat. *J Clin Endocrinol Metab*. 2009;94:3611-3615
179. Mattacks CA, Pond CM. Site-specific and sex differences in the rates of fatty acid/triacylglycerol substrate cycling in adipose, tissue and muscle of sedentary and exercised dwarf hamsters (phodopus sungorus). *Int J Obes*. 1988;12:585-597
180. Lauer MN MP, Quinn DW, McTernan CL, Harte AL, Barnett AH, Bosner RS, Kumar S Agt, pai and resistin gene expression in human epicardial fat *Diabetologia (Abstract 38th EASD Annual Meeting of the European-Association-for-the-Study-of-Diabetes. BUDAPEST, HUNGARY, SEP 01-05, 2002)*. 2002;45:A36
181. Iacobellis G, Pistilli D, Gucciardo M, Leonetti F, Miraldi F, Brancaccio G, Gallo P, di Gioia CR. Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease. *Cytokine*. 2005;29:251-255

182. Silaghi A, Achard V, Paulmyer-Lacroix O, Scridon T, Tassistro V, Duncea I, Clement K, Dutour A, Grino M. Expression of adrenomedullin in human epicardial adipose tissue: Role of coronary status. *Am J Physiol Endocrinol Metab*. 2007;293:E1443-1450
183. Rabkin SW. Epicardial fat: Properties, function and relationship to obesity. *Obes Rev*. 2007;8:253-261
184. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab*. 2007;92:1023-1033
185. Fluchter S, Haghi D, Dinter D, Heberlein W, Kuhl HP, Neff W, Sueselbeck T, Borggrefe M, Papavassiliu T. Volumetric assessment of epicardial adipose tissue with cardiovascular magnetic resonance imaging. *Obesity (Silver Spring)*. 2007;15:870-878
186. Iacobellis G, Willens HJ. Echocardiographic epicardial fat: A review of research and clinical applications. *J Am Soc Echocardiogr*. 2009;22:1311-1319; quiz 1417-1318
187. Malavazos AE, Ermetici F, Cereda E, Coman C, Locati M, Morricone L, Corsi MM, Ambrosi B. Epicardial fat thickness: Relationship with plasma visfatin and plasminogen activator inhibitor-1 levels in visceral obesity. *Nutr Metab Cardiovasc Dis*. 2008;18:523-530
188. Ding J, Kritchevsky SB, Harris TB, Burke GL, Detrano RC, Szklo M, Jeffrey Carr J, Multi-Ethnic Study of A. The association of pericardial fat with calcified coronary plaque. *Obesity (Silver Spring)*. 2008;16:1914-1919
189. Natale F, Tedesco MA, Mocerino R, de Simone V, Di Marco GM, Aronne L, Credendino M, Siniscalchi C, Calabro P, Cotrufo M, Calabro R. Visceral adiposity and arterial stiffness: Echocardiographic epicardial fat thickness reflects, better than waist circumference, carotid arterial stiffness in a large population of hypertensives. *Eur J Echocardiogr*. 2009;10:549-555
190. de Vos AM, Prokop M, Roos CJ, Meijs MF, van der Schouw YT, Rutten A, Gorter PM, Cramer MJ, Doevendans PA, Rensing BJ, Bartelink ML, Velthuis BK, Mosterd A, Bots ML. Peri-coronary epicardial adipose tissue is related to cardiovascular risk factors and coronary artery calcification in post-menopausal women. *Eur Heart J*. 2008;29:777-783
191. Iacobellis G, Leonetti F. Epicardial adipose tissue and insulin resistance in obese subjects. *J Clin Endocrinol Metab*. 2005;90:6300-6302
192. Wang CP, Hsu HL, Hung WC, Yu TH, Chen YH, Chiu CA, Lu LF, Chung FM, Shin SJ, Lee YJ. Increased epicardial adipose tissue (eat) volume in type 2 diabetes mellitus and association with metabolic syndrome and severity of coronary atherosclerosis. *Clin Endocrinol (Oxf)*. 2009;70:876-882
193. Djaberi R, Schuijf JD, van Werkhoven JM, Nucifora G, Jukema JW, Bax JJ. Relation of epicardial adipose tissue to coronary atherosclerosis. *Am J Cardiol*. 2008;102:1602-1607
194. Greif M, Becker A, von Ziegler F, Lebherz C, Lehrke M, Broedl UC, Tittus J, Parhofer K, Becker C, Reiser M, Knez A, Leber AW. Pericardial adipose tissue determined by dual source ct is a risk factor for coronary atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2009;29:781-786

195. Alexopoulos N, McLean DS, Janik M, Arepalli CD, Stillman AE, Raggi P. Epicardial adipose tissue and coronary artery plaque characteristics. *Atherosclerosis*.210:150-154
196. Sade LE, Eroglu S, Bozbas H, Ozbicer S, Hayran M, Haberal A, Muderrisoglu H. Relation between epicardial fat thickness and coronary flow reserve in women with chest pain and angiographically normal coronary arteries. *Atherosclerosis*. 2009;204:580-585
197. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, O'Donnell CJ, Fox CS. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: The framingham heart study. *Circulation*. 2008;117:605-613
198. Liu J, Fox CS, Hickson D, Sarpong D, Ekunwe L, May WD, Hundley GW, Carr JJ, Taylor HA. Pericardial adipose tissue, atherosclerosis and cardiovascular disease risk factors: The jackson heart study. *Diabetes Care*.
199. Mahabadi AA, Massaro JM, Rosito GA, Levy D, Murabito JM, Wolf PA, O'Donnell CJ, Fox CS, Hoffmann U. Association of pericardial fat, intrathoracic fat, and visceral abdominal fat with cardiovascular disease burden: The framingham heart study. *Eur Heart J*. 2009;30:850-856
200. Fox CS, Gona P, Hoffmann U, Porter SA, Salton CJ, Massaro JM, Levy D, Larson MG, D'Agostino RB, Sr., O'Donnell CJ, Manning WJ. Pericardial fat, intrathoracic fat, and measures of left ventricular structure and function: The framingham heart study. *Circulation*. 2009;119:1586-1591
201. Baker AR, Silva NF, Quinn DW, Harte AL, Pagano D, Bonser RS, Kumar S, McTernan PG. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol*. 2006;5:1
202. Salgado-Somoza A, Teijeira-Fernandez E, Fernandez AL, Gonzalez-Juanatey JR, Eiras S. Proteomic analysis of epicardial and subcutaneous adipose tissue reveals differences in proteins involved in oxidative stress. *Am J Physiol Heart Circ Physiol*.
203. Iacobellis G, di Gioia CR, Di Vito M, Petramala L, Cotesta D, De Santis V, Vitale D, Tritapepe L, Letizia C. Epicardial adipose tissue and intracoronary adrenomedullin levels in coronary artery disease. *Horm Metab Res*. 2009;41:855-860
204. Knudson JD, Dick GM, Tune JD. Adipokines and coronary vasomotor dysfunction. *Exp Biol Med (Maywood)*. 2007;232:727-736
205. Hirsch J, Batchelor B. Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab*. 1976;5:299-311
206. Farnier C, Krief S, Blache M, Diot-Dupuy F, Mory G, Ferre P, Bazin R. Adipocyte functions are modulated by cell size change: Potential involvement of an integrin/erk signalling pathway. *Int J Obes Relat Metab Disord*. 2003;27:1178-1186
207. Marques BG, Hausman DB, Martin RJ. Association of fat cell size and paracrine growth factors in development of hyperplastic obesity. *Am J Physiol*. 1998;275:R1898-1908
208. Lee WH, Kim SH, Lee Y, Lee BB, Kwon B, Song H, Kwon BS, Park JE. Tumor necrosis factor receptor superfamily 14 is involved in atherogenesis by inducing proinflammatory cytokines and matrix metalloproteinases. *Arterioscler Thromb Vasc Biol*. 2001;21:2004-2010

209. Erkkila A, de Mello VD, Riserus U, Laaksonen DE. Dietary fatty acids and cardiovascular disease: An epidemiological approach. *Prog Lipid Res*. 2008;47:172-187
210. Bradley RL, Fisher FF, Maratos-Flier E. Dietary fatty acids differentially regulate production of tnf-alpha and il-10 by murine 3t3-l1 adipocytes. *Obesity (Silver Spring)*. 2008;16:938-944
211. Wang TD, Lee WJ, Shih FY, Huang CH, Chang YC, Chen WJ, Lee YT, Chen MF. Relations of epicardial adipose tissue measured by multidetector computed tomography to components of the metabolic syndrome are region-specific and independent of anthropometric indexes and intraabdominal visceral fat. *J Clin Endocrinol Metab*. 2009;94:662-669
212. Kremen J, Dolinkova M, Krajickova J, Blaha J, Anderlova K, Lacinova Z, Haluzikova D, Bosanska L, Vokurka M, Svacina S, Haluzik M. Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: Possible role in postoperative insulin resistance. *J Clin Endocrinol Metab*. 2006;91:4620-4627
213. Eiras S, Teijeira-Fernandez E, Shamagian LG, Fernandez AL, Vazquez-Boquete A, Gonzalez-Juanatey JR. Extension of coronary artery disease is associated with increased il-6 and decreased adiponectin gene expression in epicardial adipose tissue. *Cytokine*. 2008;43:174-180
214. Teijeira-Fernandez E, Eiras S, Grigorian-Shamagian L, Fernandez A, Adrio B, Gonzalez-Juanatey JR. Epicardial adipose tissue expression of adiponectin is lower in patients with hypertension. *J Hum Hypertens*. 2008;22:856-863
215. Maffeis C, Silvagni D, Bonadonna R, Grezzani A, Banzato C, Tato L. Fat cell size, insulin sensitivity, and inflammation in obese children. *J Pediatr*. 2007;151:647-652
216. Akdis CA, Blaser K. Mechanisms of interleukin-10-mediated immune suppression. *Immunology*. 2001;103:131-136
217. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. *Annu Rev Immunol*. 2004;22:929-979
218. Cheng KH, Chu CS, Lee KT, Lin TH, Hsieh CC, Chiu CC, Voon WC, Sheu SH, Lai WT. Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. *Int J Obes (Lond)*. 2008;32:268-274
219. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A*. 2003;100:7265-7270
220. Chatterjee TK, Stoll LL, Denning GM, Harrelson A, Blomkalns AL, Idelman G, Rothenberg FG, Neltner B, Romig-Martin SA, Dickson EW, Rudich S, Weintraub NL. Proinflammatory phenotype of perivascular adipocytes: Influence of high-fat feeding. *Circ Res*. 2009;104:541-549
221. Andrade ZA, de-Oliveira-Filho J, Fernandes AL. Interrelationship between adipocytes and fibroblasts during acute damage to the subcutaneous adipose tissue of rats: An ultrastructural study. *Braz J Med Biol Res*. 1998;31:659-664
222. Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (at): Implication of macrophages resident in the at. *J Clin Endocrinol Metab*. 2005;90:2282-2289

223. Eckel RH, Kahn R, Robertson RM, Rizza RA. Preventing cardiovascular disease and diabetes: A call to action from the american diabetes association and the american heart association. *Diabetes Care*. 2006;29:1697-1699
224. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature*. 2006;444:875-880
225. Iacobellis G, Leonetti F, Singh N, A MS. Relationship of epicardial adipose tissue with atrial dimensions and diastolic function in morbidly obese subjects. *Int J Cardiol*. 2007;115:272-273
226. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: Molecular links between obesity and atheroslcerosis. *Am J Physiol Heart Circ Physiol*. 2005;288:H2031-2041
227. Matsuzawa Y. The metabolic syndrome and adipocytokines. *FEBS Lett*. 2006;580:2917-2921
228. Liu YM, Lacorte JM, Viguerie N, Poitou C, Pelloux V, Guy-Grand B, Coussieu C, Langin D, Basdevant A, Clement K. Adiponectin gene expression in subcutaneous adipose tissue of obese women in response to short-term very low calorie diet and refeeding. *J Clin Endocrinol Metab*. 2003;88:5881-5886
229. Juarranz MG, Santiago B, Torroba M, Gutierrez-Canas I, Palao G, Galindo M, Abad C, Martinez C, Leceta J, Pablos JL, Gomariz RP. Vasoactive intestinal peptide modulates proinflammatory mediator synthesis in osteoarthritic and rheumatoid synovial cells. *Rheumatology (Oxford)*. 2004;43:416-422
230. Iglesias MJ, Eiras S, Pineiro R, Lopez-Otero D, Gallego R, Fernandez AL, Lago F, Gonzalez-Juanatey JR. [gender differences in adiponectin and leptin expression in epicardial and subcutaneous adipose tissue. Findings in patients undergoing cardiac surgery]. *Rev Esp Cardiol*. 2006;59:1252-1260
231. Fain JN, Sacks HS, Buehrer B, Bahouth SW, Garrett E, Wolf RY, Carter RA, Tichansky DS, Madan AK. Identification of omentin mrna in human epicardial adipose tissue: Comparison to omentin in subcutaneous, internal mammary artery periadventitial and visceral abdominal depots. *Int J Obes (Lond)*. 2008
232. Vural B, Atalar F, Ciftci C, Demirkan A, Susleyici-Duman B, Gunay D, Akpinar B, Sagbas E, Ozbek U, Buyukdevrim AS. Presence of fatty-acid-binding protein 4 expression in human epicardial adipose tissue in metabolic syndrome. *Cardiovasc Pathol*. 2008
233. Miyata K, Shimokawa H, Kandabashi T, Higo T, Morishige K, Eto Y, Egashira K, Kaibuchi K, Takeshita A. Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo. *Arterioscler Thromb Vasc Biol*. 2000;20:2351-2358
234. Shimokawa H, Ito A, Fukumoto Y, Kadokami T, Nakaike R, Sakata M, Takayanagi T, Egashira K, Takeshita A. Chronic treatment with interleukin-1 beta induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest*. 1996;97:769-776
235. Prescott MF, McBride CK, Court M. Development of intimal lesions after leukocyte migration into the vascular wall. *Am J Pathol*. 1989;135:835-846

236. Simons PJ, van den Pangaart PS, Aerts JM, Boon L. Pro-inflammatory delipidizing cytokines reduce adiponectin secretion from human adipocytes without affecting adiponectin oligomerization. *J Endocrinol*. 2007;192:289-299
237. Wolf AM, Wolf D, Avila MA, Moschen AR, Berasain C, Enrich B, Rumpold H, Tilg H. Up-regulation of the anti-inflammatory adipokine adiponectin in acute liver failure in mice. *J Hepatol*. 2006;44:537-543
238. Ascer E, Bertolami MC, Venturinelli ML, Buccheri V, Souza J, Nicolau JC, Ramires JA, Serrano CV, Jr. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177:161-166
239. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111:3481-3488
240. Greenberg AS, Nordan RP, McIntosh J, Calvo JC, Scow RO, Jablons D. Interleukin 6 reduces lipoprotein lipase activity in adipose tissue of mice in vivo and in 3t3-l1 adipocytes: A possible role for interleukin 6 in cancer cachexia. *Cancer Res*. 1992;52:4113-4116
241. Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor-alpha and increased risk of recurrent coronary events after myocardial infarction. *Circulation*. 2000;101:2149-2153
242. Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation to long-term mortality in unstable coronary artery disease. Frisc study group. Fragmin during instability in coronary artery disease. *N Engl J Med*. 2000;343:1139-1147
243. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA*. 2001;286:327-334
244. Trayhurn P, Beattie JH. Physiological role of adipose tissue: White adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc*. 2001;60:329-339
245. Iacobellis G, Assael F, Ribaudo MC, Zappaterreno A, Alessi G, Di Mario U, Leonetti F. Epicardial fat from echocardiography: A new method for visceral adipose tissue prediction. *Obes Res*. 2003;11:304-310
246. Lee HS, Lee M, Joung H. Adiponectin represents an independent risk factor for hypertension in middle aged korean women. *Asia Pac J Clin Nutr*. 2007;16:10-15
247. Chow WS, Cheung BM, Tso AW, Xu A, Wat NM, Fong CH, Ong LH, Tam S, Tan KC, Janus ED, Lam TH, Lam KS. Hypoadiponectinemia as a predictor for the development of hypertension: A 5-year prospective study. *Hypertension*. 2007;49:1455-1461
248. Rasouli N, Yao-Borengasser A, Miles LM, Elbein SC, Kern PA. Increased plasma adiponectin in response to pioglitazone does not result from increased gene expression. *Am J Physiol Endocrinol Metab*. 2006;290:E42-E46
249. Ogawa Y, Kikuchi T, Nagasaki K, Hiura M, Tanaka Y, Uchiyama M. Usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese japanese children. *Hypertens Res*. 2005;28:51-57
250. Gonzalez-Juanatey JR, Alegria-Ezquerra E, Aznar-Costa J, Bertomeu-Martinez V, Franch-Nadal J, Palma-Gamizf JL. [knowledge and implementation of cardiovascular

risk clinical practice guidelines by general practitioners and specialists]. *Rev Esp Cardiol*. 2006;59:801-806

251. Banegas JR, Segura J, Ruilope LM, Luque M, Garcia-Robles R, Campo C, Rodriguez-Artalejo F, Tamargo J. Blood pressure control and physician management of hypertension in hospital hypertension units in spain. *Hypertension*. 2004;43:1338-1344
252. Llisterri Caro JL, Rodriguez Roca GC, Alonso Moreno FJ, Lou Arnal S, Divison Garrote JA, Santos Rodriguez JA, Raber Bejar A, de Castellar Sanso R, Ruilope Urioste LM, Banegas Banegas JR. [blood pressure control in spanish hypertensive patients in primary health care centres. Prescap 2002 study]. *Med Clin (Barc)*. 2004;122:165-171
253. Bakris GL. A practical approach to achieving recommended blood pressure goals in diabetic patients. *Arch Intern Med*. 2001;161:2661-2667
254. Sans S, Paluzie G, Balana L, Puig T, Balaguer-Vintro I. [trends in prevalence, awareness, treatment and control of arterial hypertension between 1986 and 1996: The monica-catalonia study]. *Med Clin (Barc)*. 2001;117:246-253
255. Nagasawa A, Fukui K, Funahashi T, Maeda N, Shimomura I, Kihara S, Waki M, Takamatsu K, Matsuzawa Y. Effects of soy protein diet on the expression of adipose genes and plasma adiponectin. *Horm Metab Res*. 2002;34:635-639
256. Furuhashi M, Ura N, Higashiura K, Murakami H, Tanaka M, Moniwa N, Yoshida D, Shimamoto K. Blockade of the renin-angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension*. 2003;42:76-81
257. Esposito K, Ciotola M, Carleo D, Schisano B, Saccomanno F, Sasso FC, Cozzolino D, Assaloni R, Merante D, Ceriello A, Giugliano D. Effect of rosiglitazone on endothelial function and inflammatory markers in patients with the metabolic syndrome. *Diabetes Care*. 2006;29:1071-1076
258. Li W, Tonelli J, Kishore P, Owen R, Goodman E, Scherer PE, Hawkins M. Insulin-sensitizing effects of thiazolidinediones are not linked to adiponectin receptor expression in human fat or muscle. *Am J Physiol Endocrinol Metab*. 2007;292:E1301-1307
259. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709-2716
260. Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J. Adiponectin and development of type 2 diabetes in the pima indian population. *Lancet*. 2002;360:57-58
261. Yamamoto Y, Hirose H, Saito I, Nishikai K, Saruta T. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: Two-year follow-up study in japanese population. *J Clin Endocrinol Metab*. 2004;89:87-90
262. Daimon M, Oizumi T, Saitoh T, Kameda W, Hirata A, Yamaguchi H, Ohnuma H, Igarashi M, Tominaga M, Kato T. Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the japanese population: The funagata study. *Diabetes Care*. 2003;26:2015-2020

263. Duncan BB, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponectin and the development of type 2 diabetes: The atherosclerosis risk in communities study. *Diabetes*. 2004;53:2473-2478
264. Mather KJ, Funahashi T, Matsuzawa Y, Edelstein S, Bray GA, Kahn SE, Crandall J, Marcovina S, Goldstein B, Goldberg R. Adiponectin, change in adiponectin, and progression to diabetes in the diabetes prevention program. *Diabetes*. 2008;57:980-986
265. Unger RH. Hyperleptinemia: Protecting the heart from lipid overload. *Hypertension*. 2005;45:1031-1034
266. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. *Science*. 1996;274:1185-1188
267. Park HY, Kwon HM, Lim HJ, Hong BK, Lee JY, Park BE, Jang Y, Cho SY, Kim HS. Potential role of leptin in angiogenesis: Leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro. *Exp Mol Med*. 2001;33:95-102
268. Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzman M, Brownlee M. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase a. *J Biol Chem*. 2001;276:25096-25100
269. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL. Leptin enhances the calcification of vascular cells: Artery wall as a target of leptin. *Circ Res*. 2001;88:954-960
270. Santos-Alvarez J, Goberna R, Sanchez-Margalet V. Human leptin stimulates proliferation and activation of human circulating monocytes. *Cell Immunol*. 1999;194:6-11
271. Brennan AM, Li TY, Kelesidis I, Gavrila A, Hu FB, Mantzoros CS. Circulating leptin levels are not associated with cardiovascular morbidity and mortality in women with diabetes: A prospective cohort study. *Diabetologia*. 2007;50:1178-1185
272. van Dielen FM, van't Veer C, Schols AM, Soeters PB, Buurman WA, Greve JW. Increased leptin concentrations correlate with increased concentrations of inflammatory markers in morbidly obese individuals. *Int J Obes Relat Metab Disord*. 2001;25:1759-1766
273. Imagawa A, Funahashi T, Nakamura T, Moriwaki M, Tanaka S, Nishizawa H, Sayama K, Uno S, Iwahashi H, Yamagata K, Miyagawa J, Matsuzawa Y. Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes. *Diabetes Care*. 2002;25:1665-1666
274. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2008;31 Suppl 1:S55-60
275. Cavusoglu E, Ruwende C, Chopra V, Yanamadala S, Eng C, Clark LT, Pinsky DJ, Marmur JD. Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain. *Eur Heart J*. 2006;27:2300-2309
276. Rathmann W, Herder C. Adiponectin and cardiovascular mortality: Evidence for "reverse epidemiology". *Horm Metab Res*. 2007;39:1-2

277. Iacobellis G, Barbaro G, Gerstein HC. Relationship of epicardial fat thickness and fasting glucose. *Int J Cardiol*. 2008;128:424-426
278. Singh N, Singh H, Khanijoun HK, Iacobellis G. Echocardiographic assessment of epicardial adipose tissue - a marker of visceral adiposity. *Mcgill J Med*. 2007;10:26-30
279. Saito S, Fujiwara T, Matsunaga T, Minagawa K, Fukui K, Fukuda I, Osanai T, Okumura K. Increased adiponectin synthesis in the visceral adipose tissue in men with coronary artery disease treated with pravastatin: A role of the attenuation of oxidative stress. *Atherosclerosis*. 2008;199:378-383
280. Chu CH, Lee JK, Lam HC, Lu CC, Sun CC, Wang MC, Chuang MJ, Wei MC. Atorvastatin does not affect insulin sensitivity and the adiponectin or leptin levels in hyperlipidemic type 2 diabetes. *J Endocrinol Invest*. 2008;31:42-47
281. Forst T, Pfutzner A, Lubben G, Weber M, Marx N, Karagiannis E, Koehler C, Baurecht W, Hohberg C, Hanefeld M. Effect of simvastatin and/or pioglitazone on insulin resistance, insulin secretion, adiponectin, and proinsulin levels in nondiabetic patients at cardiovascular risk--the piostat study. *Metabolism*. 2007;56:491-496
282. Takagi T, Matsuda M, Abe M, Kobayashi H, Fukuhara A, Komuro R, Kihara S, Caslake MJ, McMahon A, Shepherd J, Funahashi T, Shimomura I. Effect of pravastatin on the development of diabetes and adiponectin production. *Atherosclerosis*. 2008;196:114-121
283. Norhammar A, Tenerz A, Nilsson G, Hamsten A, Efendic S, Ryden L, Malmberg K. Glucose metabolism in patients with acute myocardial infarction and no previous diagnosis of diabetes mellitus: A prospective study. *Lancet*. 2002;359:2140-2144
284. Bartnik M, Ryden L, Ferrari R, Malmberg K, Pyorala K, Simoons M, Standl E, Soler-Soler J, Ohrvik J. The prevalence of abnormal glucose regulation in patients with coronary artery disease across europe. The euro heart survey on diabetes and the heart. *Eur Heart J*. 2004;25:1880-1890
285. Executive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). *JAMA*. 2001;285:2486-2497
286. Tschritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, Staiger H, Maerker E, Haring H, Stumvoll M. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes*. 2003;52:239-243
287. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood*. 2000;96:1723-1732
288. Tian L, Luo N, Klein RL, Chung BH, Garvey WT, Fu Y. Adiponectin reduces lipid accumulation in macrophage foam cells. *Atherosclerosis*. 2009;202:152-161
289. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem*. 2003;278:45021-45026

290. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab*. 2001;86:1930-1935
291. Dekker JM, Funahashi T, Nijpels G, Pilz S, Stehouwer CD, Snijder MB, Bouter LM, Matsuzawa Y, Shimomura I, Heine RJ. Prognostic value of adiponectin for cardiovascular disease and mortality. *J Clin Endocrinol Metab*. 2008;93:1489-1496
292. Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin--a key adipokine in the metabolic syndrome. *Diabetes Obes Metab*. 2006;8:264-280
293. Matsushita K, Yatsuya H, Tamakoshi K, Wada K, Otsuka R, Takefuji S, Sugiura K, Kondo T, Murohara T, Toyoshima H. Comparison of circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in japanese men. *Arterioscler Thromb Vasc Biol*. 2006;26:871-876
294. Choi KM, Lee J, Lee KW, Seo JA, Oh JH, Kim SG, Kim NH, Choi DS, Baik SH. Serum adiponectin concentrations predict the developments of type 2 diabetes and the metabolic syndrome in elderly koreans. *Clin Endocrinol (Oxf)*. 2004;61:75-80
295. Teijeira-Fernandez E, Eiras S, Grigorian-Shamagian L, Salgado-Somoza A, Martinez-Comendador JM, Gonzalez-Juanatey JR. Diabetic and nondiabetic patients express similar adipose tissue adiponectin and leptin levels. *Int J Obes (Lond)*. 2010 Jul;34:1200-1208.
296. Arita Y, Kihara S, Ouchi N, Maeda K, Kuriyama H, Okamoto Y, Kumada M, Hotta K, Nishida M, Takahashi M, Nakamura T, Shimomura I, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-bb-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation*. 2002;105:2893-2898
297. Schnabel R, Messow CM, Lubos E, Espinola-Klein C, Rupprecht HJ, Bickel C, Sinning C, Tzikas S, Keller T, Genth-Zotz S, Lackner KJ, Munzel TF, Blankenberg S. Association of adiponectin with adverse outcome in coronary artery disease patients: Results from the atherogene study. *Eur Heart J*. 2008;29:649-657
298. Kistorp C, Faber J, Galatius S, Gustafsson F, Frystyk J, Flyvbjerg A, Hildebrandt P. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. *Circulation*. 2005;112:1756-1762
299. Poehls J, Wassel CL, Harris TB, Havel PJ, Swarbrick MM, Cummings SR, Newman AB, Satterfield S, Kanaya AM. Association of adiponectin with mortality in older adults: The health, aging, and body composition study. *Diabetologia*. 2009;52:591-595
300. Lieb W, Sullivan LM, Harris TB, Roubenoff R, Benjamin EJ, Levy D, Fox CS, Wang TJ, Wilson PW, Kannel WB, Vasan RS. Plasma leptin levels and incidence of heart failure, cardiovascular disease, and total mortality in elderly individuals. *Diabetes Care*. 2009;32:612-616
301. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: Relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. *Diabetes*. 2003;52:1779-1785

302. Sironi AM, Pingitore A, Ghione S, De Marchi D, Scattini B, Positano V, Muscelli E, Ciociaro D, Lombardi M, Ferrannini E, Gastaldelli A. Early hypertension is associated with reduced regional cardiac function, insulin resistance, epicardial, and visceral fat. *Hypertension*. 2008;51:282-288

APÉNDICE

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Relationship between epicardial adipose tissue adipocyte size and MCP-1 expression

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Extension of coronary artery disease is associated with increased IL-6 and decreased adiponectin gene expression in epicardial adipose tissue

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ORIGINAL ARTICLE

Epicardial adipose tissue expression of adiponectin is lower in patients with hypertension

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ORIGINAL ARTICLE

Diabetic and nondiabetic patients express similar adipose tissue adiponectin and leptin levels

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