UNIVERSIDAD AUTÓNOMA DE GUERRERO

UNIDAD ACADÉMICA DE CIENCIAS QUÍMICO BIOLÓGICAS
UNIDAD DE INVESTIGACIÓN ESPECIALIZADA EN MICROBIOLOGÍA

DOCTORADO EN CIENCIAS BIOMÉDICAS

EVALUACIÓN DEL RECEPTOR CD36 COMO MARCADOR TEMPRANO DE ENFERMEDAD CARDIOVASCULAR EN JÓVENES CON Y SIN OBESIDAD

T E S I S

Q U E P A R A O B T E N E R E L G R A D O D E

DOCTORADO EN CIENCIAS BIOMÉDICAS

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ACTA DE APROBACIÓN DE TESIS

En la ciudad de Chilpancingo, Guerrero, siendo los 9 días del mes de diciembre del dos mil trece, se reunieron los miembros del Comité Tutoral designado por la Academia de Posgrado del Doctorado en Ciencias Biomédicas, para examinar la tesis titulada **"Evaluación del receptor CD36 como marcador temprano de enfermedad cardiovascular en jóvenes con y sin obesidad"**, presentada por la alumna Luz Elena Ramos Arellano, para obtener el Grado de Doctora en Ciencias Biomédicas. Después del análisis correspondiente, los miembros del comité manifiestan su aprobación de la tesis, autorizan la impresión final de la misma y aceptan que, cuando se satisfagan los requisitos señalados en el Reglamento General de Estudios de Posgrado e Investigación Vigente, se proceda a la presentación del examen de grado.

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Evaluación del receptor CD36 como marcador temprano de enfermedad cardiovascular en jóvenes con y sin obesidad

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RESUMEN

Introducción. El receptor CD36 es una proteína que en los macrófagos sirve como un receptor *scavenger* para las LDLox y células apoptóticas, participa en el transporte de ácidos grasos en el músculo y adipocitos; contribuye al desarrollo de la lesión endotelial al unirse y endocitar a las LDLox dentro de los macrófagos llevándolos a diferenciarse a células espumosas, lo que constituye el núcleo de la lesión aterosclerótica; polimorfismos en el gen de *CD36* se han relacionado con las concentraciones de lípidos y con la presencia de enfermedades como la diabetes mellitus tipo 2. **Objetivo.** Evaluar la relación de los niveles circulantes y de expresión en la membrana de monocitos y plaquetas del receptor CD36, con el perfil de lípidos y con los polimorfismos -33137 A/G, -31118 G/A, -22674 T/C, 27645Ins/Del y 30294 G/C en el gen del receptor CD36 en jóvenes guerrerenses con y sin obesidad. **Materiales y métodos.** Se incluyeron 100 jóvenes con peso normal en los cuales se determinó el equilibrio de Hardy- Weinberg para los polimorfismos estudiados, posteriormente se incluyeron 55 jóvenes con obesidad y 133 jóvenes con peso normal. El perfil de lípidos y los niveles de glucosa fueron medidos por ensayos colorimétricos enzimáticos; los niveles séricos de LDLox y CD36 fueron cuantificados por ensayos de ELISA y los niveles de expresión de CD36 por citometría de flujo. La genotipificación de los polimorfismos fue determinada por PCR-RFLP. **Resultados.** Los jóvenes con obesidad presentaron un perfil de lípidos aterogénico con respecto al grupo de normopeso. Los jóvenes con obesidad tuvieron 5.8 veces más riesgo de tener niveles séricos de CD36 por arriba de tercer tercil (p=0.014). Los jóvenes con hipercolesterolemia, LDL-C alterado en ayuno, hipertrigliceridemia u obesidad tuvieron mayor riesgo de tener niveles séricos de LDLox por arriba del tercer tercil (p<0.05). Los niveles de expresión de CD36 en monocitos fueron más altos en los jóvenes con normopeso que en los jóvenes con obesidad (p=0.001). El genotipo -33137GG se asoció con disminución en los niveles de expresión de CD36 en monocitos y plaquetas. **Conclusión.** Los niveles séricos del receptor CD36 y LDLox se asociaron con la presencia de obesidad y dislipidemias. Pero, menor expresión de CD36 se asoció con uno de los polimorfismos en su gen, lo que sugiere un efecto protector para enfermedad cardiovascular.

Evaluación del receptor CD36 como marcador temprano de enfermedad cardiovascular en jóvenes con y sin obesidad

INTRODUCCIÓN

Las enfermedades cardiovasculares (ECV) son responsables del 30% de todas las muertes del mundo cada año; el principal trastorno cardiovascular responsable del incremento de la mortalidad es la enfermedad vascular aterosclerótica, el 80% de estas muertes ocurren en países con ingresos de medianos a bajos como China, Rusia, Polonia, Argentina e India (Bonow RO *et al.,* 2002). En México, la diabetes mellitus, las enfermedades isquémicas del corazón y las enfermedades cerebro-vasculares son responsables del 33% de las muertes en mujeres y más de 26% de las muertes en hombres; estas enfermedades comparten algunos factores de riesgo como: el sobrepeso y la obesidad, que afecta al 70% de la población de 20 años o más; el tabaquismo (21.5%), el colesterol elevado (26.5%) y la hipertensión arterial (30.8%), que también influyen en el desarrollo de las enfermedades isquémicas del corazón y las enfermedades cerebro-vasculares (Programa Nacional de Salud 2007-2012).

La base patológica de las ECV resulta de una combinación de alteraciones en el metabolismo de las lipoproteínas, estrés oxidativo, inflamación crónica y la susceptibilidad a la trombosis (Ros E, 2009). Los principales factores asociados con la ECV son: hipertensión, sobrepeso, obesidad, tabaquismo, sedentarismo, dislipidemia, antecedentes familiares de enfermedad arterial coronaria, nutrición desbalanceada, edad y género (Batsis JA y López-Jimenez F, 2010, Sailam V *et al*., 2008, Gómez BP y Bautista L, 2009). La aterosclerosis es la principal causa de ECV, es una enfermedad inflamatoria crónica progresiva, caracterizada por el incremento gradual en el grosor y endurecimiento de las arterias, que posteriormente conducen a una reducción en el diámetro del lumen y potencialmente a isquemia, seguido de la ruptura de la placa aterosclerótica (Collot-Teixeira S *et al*., 2007). Las lesiones iniciales de la aterosclerosis se caracterizan por ser placas grasosas, con un engrosamiento o hiperplasia de la pared del endotelio que incluso son observables desde edades muy tempranas: recién nacidos, niños y jóvenes (Jimenez A *et al*.,

2010). La aterosclerosis puede originarse por la acumulación de lipoproteínas modificadas en la pared arterial y la formación de macrófagos llenos de lípidos, conocidos como células espumosas (Rahaman SO *et al*., 2011). Uno de los procesos fisiopatológicos centrales en la patogénesis de la aterosclerosis es el depósito del colesterol en la pared arterial, todas las lipoproteínas están involucradas en este proceso; el colesterol es transportado por las lipoproteínas de muy baja densidad (VLDL) y lipoproteínas de baja densidad (LDL) a los tejidos periféricos; y de manera inversa el colesterol es transportado fuera de la pared arterial por las lipoproteínas de alta densidad (HDL), por lo cual se les considera que tienen un efecto antiaterogénico por su participación en el transporte inverso del colesterol en el hígado y sus propiedades antioxidantes (Yue P *et al*., 2010). La modificación de las LDL en la pared arterial en particular por la oxidación, es crucial para la captación celular de LDL en las primeras etapas de desarrollo de la placa aterosclerótica (Navarra T *et al*., 2010). Se considera que las lipoproteínas oxidadas (LDLox) son importantes biomarcadores de las ECV y se han descrito los niveles de LDLox en pacientes con enfermedades crónicas metabólicas (Lee C *et al*., 2010).

En algunos estudios en los que se han determinado los niveles de LDLox por ensayo inmunoenzimático (ELISA), se ha encontrado que los niveles circulantes de LDLox se encuentran incrementados en pacientes con infarto agudo al miocardio (34.4 ± 16.9 mg/dL), en comparación con los controles sanos (30.6 ± 12.1 mg/dL, $p<0.05$) (Lakshmy R *et al*., 2010). También se ha encontrado que los pacientes con síndrome metabólico tienen niveles significativamente más altos de LDLox (89.6 ± 33.1 U/L), en comparación con los sujetos control (68.5 ± 23.6 U/L, $p=0.007$) (Maaroos H *et al*., 2010). Sin embargo, en un estudio realizado en pacientes con aterosclerosis y en controles, no se encontraron diferencias significativas en los niveles de LDLox entre los pacientes con aterosclerosis (249.0 ± 112.0 uM/mg) y el grupo control (225.7 ± 90.6 uM/mg, $p= 0.115$), pero fueron significativamente más altos en el grupo con homocisteina elevada (255.3 ± 95.7 uM/mg), que en el grupo con homocisteina baja (161.6 ± 76.0 uM/mg, $p<0.005$) (Seo H *et al*., 2010).

Las LDLox pierden su habilidad para unirse a receptores LDL, lo cual interfiere con su procesamiento normal, las LDLox ganan afinidad por una familia de proteínas llamadas receptores carroñeros o de eliminación (receptores *scavenger*), estos receptores se localizan sobre los macrófagos y capturan e internalizan a las partículas LDLox, contribuyendo al depósito de colesterol y otros lípidos en las células; si esto se realiza por periodos prolongados, el incremento de las cantidades de LDLox internalizadas conducen a la formación de células espumosas que se acumulan y llevan a la formación de la placa aterosclerótica; estas células llenas con lípidos son más propensas a apoptosis, lo cual contribuye al crecimiento y ruptura de la placa, ya que se ha demostrado que las células espumosas interfieren en muchos eventos aterogénicos como el reclutamiento de neutrófilos y monocitos por la producción de quimiocinas, como la proteína quimioatrayente de monocitos (MCP)-1 y la interleucina (IL) -8, la formación de núcleos necróticos en las placas ateroscleróticas y la producción de metaloproteinasas de matriz (MMP), que degradan la matriz extracelular que comprende la capa fibrosa de la placa (Figura 1). (Silverstein RL, 2009, Handberg A *et al*., 2006, Paul A *et al*., 2008, Kuliczkowska *et al*., 2006, Hirose K *et al*., 2011).

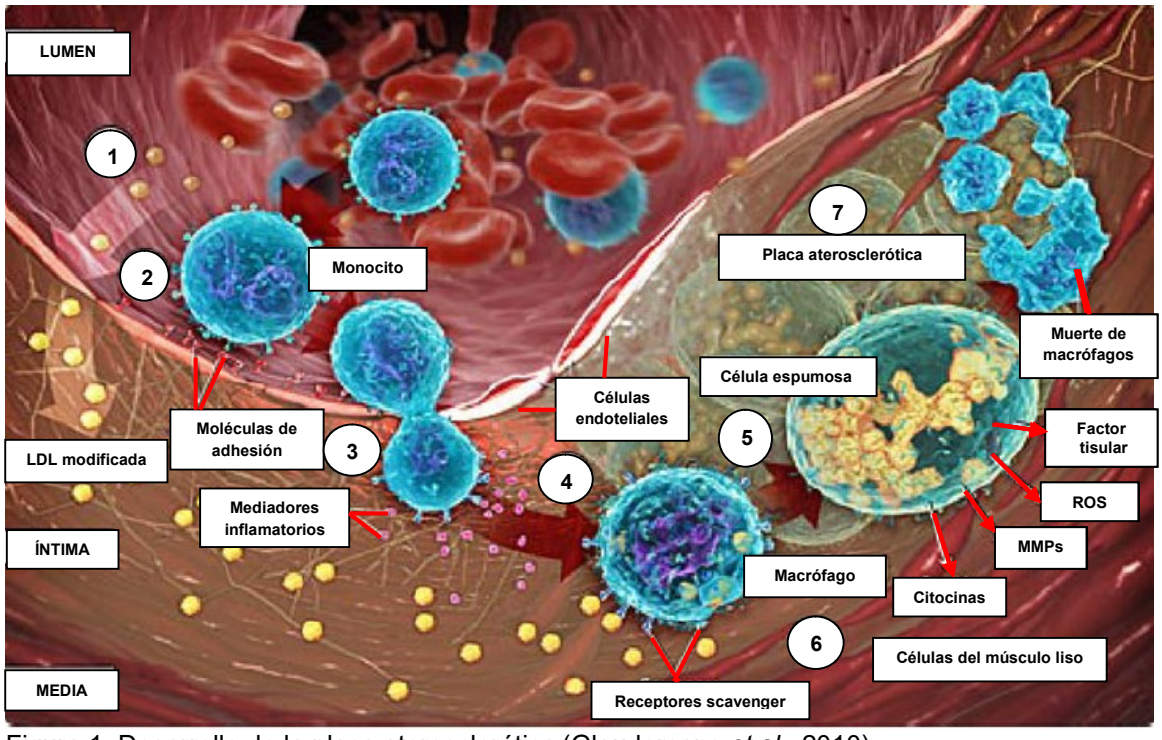

Figura.1. Desarrollo de la placa aterosclerótica (Glaudemans *et al*., 2010).

Las dislipidemias son uno de los factores de riesgo asociados con desórdenes aterotrombóticos, debido a su asociación con el estrés oxidativo y la generación de lípidos oxidados como las LDLox. Previamente se ha demostrado que la LDLox potencia la activación de las plaquetas, sin embargo, no ha sido completamente definida la forma en que las dislipidemias provocan trombosis arterial, algunas posibilidades incluyen: cambios en el colesterol de la membrana plaquetaria, daño oxidativo a los lípidos de membrana y glicoproteínas de superficie; mecanismos como estos podrían activar a las plaquetas, provocando agregados plaquetarios y formación de trombos (Jackson SP y Calkin AC, 2007).

Los receptores *scavenger* son un grupo de proteínas transmembranales que participan en funciones celulares, como la adhesión y la eliminación de células apoptóticas y lipoproteínas modificadas como las LDLox. Los receptores *scavenger* de clase A, (SRA, clase A), CD36 (clase B) y el receptor de LDLox tipo lectina 1 (LOX-1, clase E) son responsables de aproximadamente 90% de la captación de LDLox (Rasouli N *et al*., 2009). El receptor CD36 es una proteína integral de membrana expresada sobre monocitos/macrófagos, plaquetas, endotelio microvascular, adipocitos, músculo cardiaco y esquelético, reticulocitos, microglía, queratinocitos, epitelio del pigmento retinal, epitelio renal, intestino, glándula mamaria, células dendríticas y hepatocitos (Abumrad NA, 2005, Omi K, *2003*, Febbraio M y Silverstein RL, 2007, Silverstein, 2009). Inicialmente se identificó como receptor para la trombospondina-1 (TSP-1) y eritrocitos infectados de malaria, ahora se conoce que es un receptor scavenger de clase B que reconoce varios ligandos que no están relacionados, incluyendo TSP-1 y fosfolípidos oxidados expresados sobre las LDLox y en la superficie de células apoptóticas, ácidos grasos de cadena larga, péptidos amiloidogénicos, y componentes específicos de la pared celular microbiana o de superficies celulares (Chen K *et al*., 2008, Erdman *et al*., 2009).

El receptor CD36 está involucrado en una variedad de procesos biológicos incluyendo el metabolismo de lípidos, inflamación, aterosclerosis, y angiogénesis, dependiendo de la naturaleza del ligando al cual esté expuesto y al tipo de tejido o célula sobre la cual se expresa, como se ha demostrado en estudios recientes, las

plaquetas se unen a las LDLox vía CD36 y su interacción conduce a la activación plaquetaria, contribuyendo a un estado protrombótico en la etapa de hiperlipidemia (Chen K *et al*., 2008). El receptor CD36 es una proteína compleja multifuncional que en los macrófagos sirve como un receptor *scavenger* para las LDLox y células apoptóticas, participa en el transporte de ácidos grasos en el músculo y adipocitos (Handberg *et al*, 2006), contribuye al desarrollo de la lesión endotelial al unirse y endocitar a las LDLox dentro de los macrófagos llevándolos a diferenciarse a células espumosas, lo que constituye el núcleo de la lesión aterosclerótica (Collot-Teixeira S *et al*., 2007).

La secuencia de nucleótidos del gen del receptor CD36 origina una proteína de 471 aa con un peso molecular aproximado de 53 kDa, sin embargo, la presencia de 10 sitios de N-glucosilación en CD36 aumenta el peso molecular de la proteína entre 78 y 90 kDa, dependiendo del tipo celular y de las glucosilaciones postraduccionales que protegen a la proteína contra la proteólisis (Rac ME *et al*., 2007, Demers A, 2009). El receptor CD36 consiste de un dominio extracelular, dos transmembranales y dos citoplásmicos, correspondientes al C-terminal y N-terminal de la molécula; el codón de inicio de la traducción se localiza a 289 nucleótidos río abajo del extremo 5´ del mRNA del receptor CD36, no hay cisteínas libres en el receptor CD36, las seis cisteínas agrupadas están unidas por puentes disulfuro; la proteína tiene dos segmentos intracelulares cortos (de 1-6 y 461-472 residuos de aa) que pueden sufrir acilación y dos dominios transmembranales (de 7-28 y 439-460 residuos de aa) potencialmente acilados, la parte restante del receptor CD36 es extracelular, comprende 7 sitios de glucosilación y 3 puentes disulfuro (Handberg *et al*., 2006, Chen K *et al*., 2008).

El gen del receptor CD36 se localiza en el brazo largo del cromosoma 7, en la región 11.2 (7q11.2), abarca 36 Kb, comprende 15 exones, la región 5´no traducida (5´-UTR) consiste de los exones 1a, 1b, 1c, 1e, 1f, exón 2 y una parte del exón 3; la otra parte restante del exón 3, los exones 4 al 13 y parte del exón 14 codifican para la proteína CD36, el resto del segmento del exón 14 y el exón 15 comprenden la región

3´no traducida (3´-UTR) (Figura 2) (Banerjee M et al., 2010, Collot-Teixeira S *et al*., 2007).

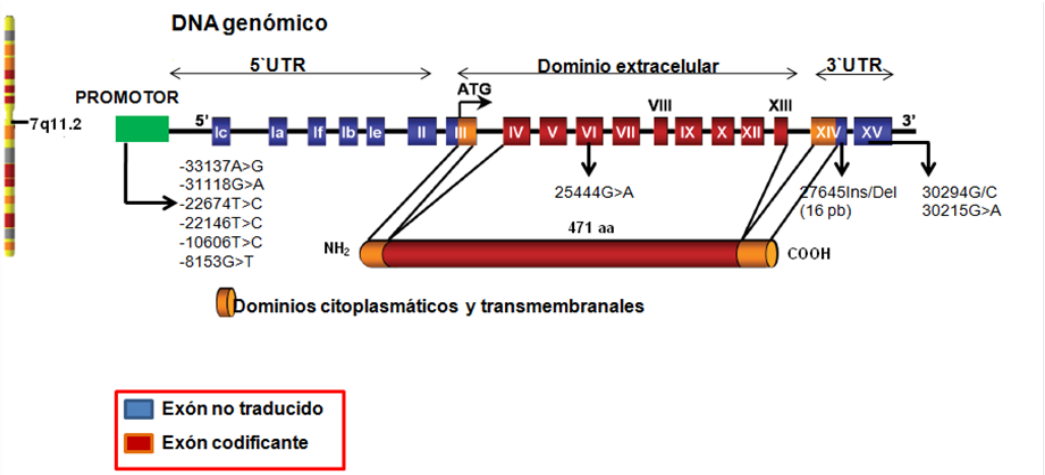

Figura 2. Representación esquemática del gen del receptor.

La expresión del gen del receptor CD36 es dependiente del tejido, en los adipocitos, el receptor nuclear activado por el proliferador de peroxisomas γ (PPARγ) es el principal regulador del gen; en los monocitos, la expresión del receptor puede estar sobre regulada por factores como el factor estimulante de colonias de macrófagos (M-CSF), factor estimulante de granulocitos/macrófagos (GM-CSF), LDL nativas y modificadas, colesterol celular, interleucina 4, insulina y glucosa; mientras que su expresión se inhibe por el factor de crecimiento transformante β (TGF-β), corticoesteroides, HDL, y lipopolisacáridos (LPS); en el músculo cardiaco y esquelético la expresión del receptor CD36 se incrementa por los triglicéridos y ácidos grasos, también se regula por los requerimientos energéticos del tejido, el ejercicio e insulina (Rac ME *et al*., 2007, Silverstein RL y Febbraio M, 2010, Rahaman SO *et al*, 2006). Existe también un aumento de la expresión del receptor CD36 debido a niveles altos de glucosa a través de mecanismos no transcripcionales que puede contribuir al estado proaterosclerótico asociado a diabetes (Silverstein RL, 2009).

La LDLox interactúa con el receptor CD36 e induce una cascada de señalización que lleva a la activación de cinasas de la familia Src (lyn, Fyn), las cuales fosforilan y activan a la cinasa de adhesión focal (FAK) provocando la polimerización de la

actina; las interacciones de las LDLox con el receptor CD36 también conducen a la generación de especies reactivas de oxígeno (ROS) intracelular mediada por la NADPH oxidasa, provocando la inactivación oxidativa de SHP-2, que induce la activación permanente de FAK y afecta el desensamblaje del citoesqueleto, favoreciendo la inhibición de la migración de la célula, por lo que los macrófagos quedan atrapados en la neoíntima endotelial (Park YM *et al*., 2009).

Las LDLox también inducen la pérdida de la polaridad celular e inhibición de la locomoción del macrófago de manera dependiente del receptor CD36; la interacción de LDLox/CD36 activa a la cinasa Rac a través de VAv, a su vez Rac activada inhibe a la miosina II no muscular, la miosina II inactivada no puede generar la fuerza de atracción lamelipodial dando lugar al retroceso lamelipodial, lo que produce pérdida de la polaridad celular que es esencial para la migración de los macrófagos (Park YM, 2010). La vía de señalización en las plaquetas activada por el receptor CD36, es similar a la que se lleva a cabo en los macrófagos, la interacción de la LDLox con el receptor CD36 induce el reclutamiento y fosforilación de Fyn y Lyn, provocando la activación rápida de Vav y de los miembros de la familia JNK, promoviendo la trombosis (Silverstein LR *et al*., 2010). Las LDLox por medio del receptor CD36 también activan a las MAP cinasas ERK, JNK y p38, se ha confirmado que p38 y JNK participan en la señalización para la internalización de las LDLox, la transactivación del PPARγ por p38 aumenta la expresión de CD36 y esta autorregulación positiva es característica de las células espumosas, la cinasa Akt incrementa la expresión de CD36 en respuesta a las LDLox, mientras que PKC está involucrada en la activación del PPARγ por las LDLox vía CD36 en el macrófago; la activación de PKC después de la interacción CD36-LDLox también activa al factor de transcripción NF-κB que desencadena la producción de una gran variedad de citocinas inflamatorias, como TNFα/β, IL-1β, IL-6, IFN β/γ las cuales amplifican la respuesta inflamatoria local de la placa de ateroma, la apoptosis de las células espumosas presentes en el sitio de la lesión depende también de la interacción LDLox-CD36 por la activación de la caspasa-3 (Figura 3) (Demers A, 2009).

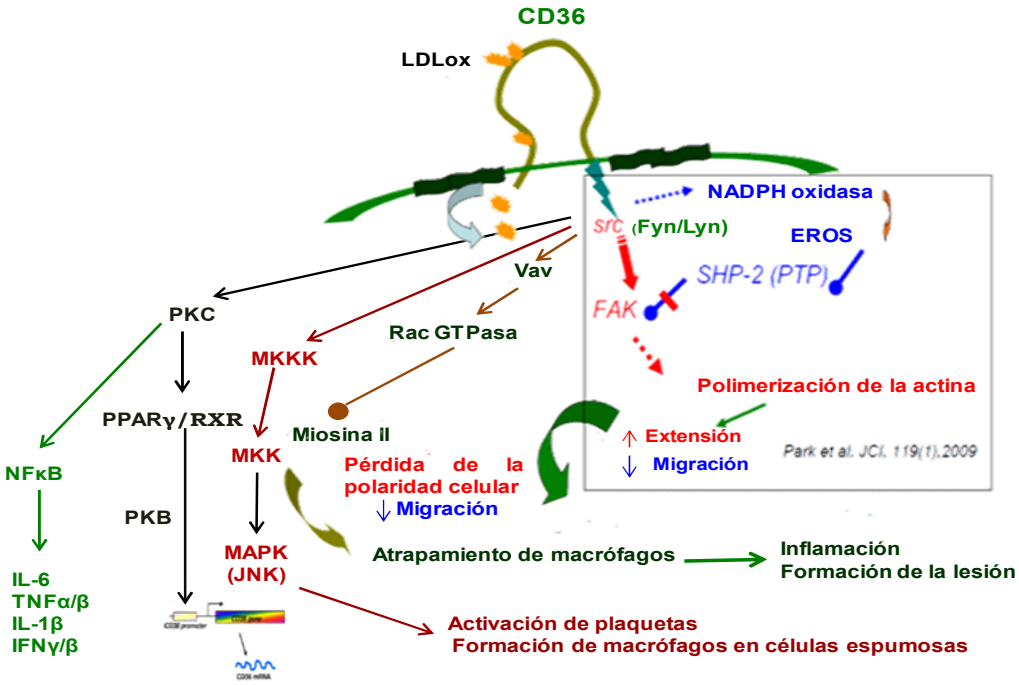

Figura 3. Vías de señalización desencadenadas por la interacción LDLox/CD36 en macrófagos y plaquetas.

Los mecanismos de liberación de CD36 en su forma circulante en la actualidad son poco claros, pero se ha sugerido que los niveles plasmáticos del receptor CD36 podrían servir como un biomarcador de la expresión alterada del receptor CD36 celular, de los niveles elevados de lipoproteínas modificadas y de la inflamación de bajo grado; por otra parte, la apoptosis de células espumosas se ha relacionado a la desestabilización de la placa y a la formación de trombos con el subsecuente desarrollo de eventos isquémicos agudos; también se ha sugerido que la apoptosis de macrófagos cargados de lípidos puede dar lugar a una mayor liberación del receptor CD36 circulante (Handberg *et al*., 2008). Se ha propuesto que el receptor CD36 o una forma truncada de la proteína, podría ser liberada a la circulación como parte del estado inflamatorio de bajo grado en resistencia a la insulina o en la apoptosis de células espumosas, por lo tanto micropartículas asociadas al receptor CD36 pueden ser liberadas a la circulación proporcionando así un marcador de expresión del receptor CD36 en células donde se expresa como plaquetas, monocitos/macrófagos, adipocitos o después de la activación de estas células por diversos estímulos, por lo tanto, CD36 circulante puede ser medido en muestras de sangre con técnicas sencillas (Handberg *et al*, 2006).

Recientemente, se propuso un modelo sobre la fisiopatología de los niveles incrementados del receptor CD36 circulante en plasma en el síndrome metabólico, el cual propone que la forma circulante del receptor CD36 refleja su nivel de expresión en tejido, en particular su nivel de expresión en monocitos y macrófagos; los niveles elevados del receptor CD36 circulante puede ser un marcador de un incremento en la expresión del receptor CD36 por una serie de tejidos que se asocian con el síndrome metabólico (Koonen *et al*, 2011). Los últimos reportes con respecto al origen de la forma circulante del receptor CD36 han demostrado que el receptor CD36 circulante se encuentra asociado a micropartículas que son originadas principalmente de plaquetas en sujetos normales y en un menor porcentaje de células endoteliales y monocitos; también se ha observado que estas micropartículas están elevadas en individuos japoneses delgados que tienen diabetes mellitus tipo 2, originadas por un incremento de lipoproteínas remanentes de colesterol que pueden activar a las plaquetas para liberar micropartículas (Alkhatatbeh M *et al*, 2011).

En estudios realizados en personas con obesidad, diabetes tipo 2 y en personas sanas se ha observado que los niveles circulantes del receptor CD36 se encuentran marcadamente elevados (4.5 veces más) en diabéticos tipo 2, comparado con los de peso normal (0.71±0.35 vs 0.16±0.09 unidades relativas; $p<0.0005$) y 3 veces más alto en personas obesas no diabéticas, comparado con personas de peso normal (0.46 ± 0.25 vs 0.16 ±0.09 unidades relativas; $p<0.030$) (Handberg A *et al.*, 2006). En cuanto a la expresión del receptor CD36, se ha encontrado una fuerte inmunotinción del receptor CD36 en macrófagos cargados de lípidos en el núcleo graso de la placa aterosclerótica de pacientes con síntomas de desestabilización de la placa en comparación con los pacientes asintomáticos, lo que refuerza la relación entre el receptor CD36 y la aterosclerosis avanzada (Handberg A *et al*., 2008).

Variaciones en el gen del receptor CD36 se han asociado con los niveles de lípidos, ácidos grasos libres, resistencia a insulina, obesidad y enfermedad coronaria, sin embargo, se carece de evidencia que vinculen estas variaciones genéticas con las concentraciones plasmáticas del receptor CD36 circulante (Koonen D *et al*, 2011). En estudios realizados en diferentes poblaciones han reportado que polimorfismos en el

gen del receptor CD36 se relacionan con las concentraciones de lípidos y con la presencia de enfermedades como la diabetes mellitus tipo 2 (Ma X *et al*., 2004, Goyenechea E *et al*., 2008, Love L *et al*., 2008, Morii *et al*., 2009, Banerjee M *et al*., 2010).

Considerando los antecedentes mencionados, el objetivo de este trabajo de investigación fue evaluar la relación de los niveles circulantes y de expresión en la membrana de monocitos y plaquetas del receptor CD36, con el perfil de lípidos y con los polimorfismos -33137 A/G, -31118 G/A, -22674 T/C, 27645Ins/Del y 30294 G/C en el gen del receptor CD36 en jóvenes guerrerenses con y sin obesidad. Por lo cual este trabajo se divide en tres capítulos que se describen a continuación:

CAPÍTULO I

CD36 haplotypes are strongly associated with lipid profile in normal-weight subjects

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LIPIDS IN HEALTH AND DISEASE

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CD36 haplotypes are associated with lipid profile in normal-weight subjects

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Abstract

Background: Dyslipidemia is a common metabolic disorder that may result from abnormalities in the synthesis, processing and catabolism of lipoprotein particles. Disorders of lipoprotein concentrations and elevated concentration of oxidized lipoproteins (oxLDL) are risk factors in the pathogenesis of cardiovascular diseases (CVD). CD36 plays an important role in lipid metabolism and polymorphisms in the *CD36* gene are related to cardiovascular risk factors. The purpose of this study was to evaluate whether there is an association between genotypes and haplotypes of five polymorphisms in the *CD36* gene with lipid levels in young normal-weight subjects.

Methods: A total of 232 unrelated subjects with normal-weight of 18 to 25 years old (157 women and 75 men) were randomly selected. The lipid profile and glucose levels were measured by enzymatic colorimetric assays. Genotyping of the polymorphisms -33137A/G (rs1984112), -31118G/A (rs1761667), -22674 T/C (rs2151916), 27645 Ins/Del (rs3840546) and 30294G/C (rs1049673) in the *CD36* receptor gene was performed by polymerase chain reaction and restriction fragment length polymorphism, linkage disequilibrium analysis among the five polymorphisms and an analysis of haplotype were estimated.

Results: HDL-C levels was lower in men than in women ($P = 0.03$). However, the median oxLDL levels in men was higher than in women ($P = 0.05$). There was no significant difference in the levels of TC, TG, LDL-C and glucose ($P > 0.05$). HDL-C levels were lower in the subjects with TC genotype of polymorphism -22674 T/C ($P = 0.04$), but the carriers of TT genotype had lower oxLDL levels ($P = 0.01$). LDL-C levels were higher in young carriers of CC genotype for 30294G/C polymorphism than non-carriers ($P = 0.03$). The subjects carrying the AATDC haplotype had 3.2 times presumably higher risk of LDL-C > 100 mg/dL than the carrying the AGTIG haplotype ($P = 0.02$), whereas the subjects carrying the AATIC haplotype had 2.0 times presumably higher risk of TC > 200 mg/dL than the carrying the AGTIC haplotype ($P = 0.02$).

Conclusion: The study provides evidence of a genetic association of *CD36* haplotypes with the variability in LDL-C and TC levels in a sample of normal-weight subjects.

Keywords: Dyslipidemia, *CD36* gene, Polymorphisms, Haplotypes

Background

Dyslipidemia is a common metabolic disorder that may result from abnormalities in the synthesis, processing and catabolism of lipoprotein particles [1]. Lipoproteins are the macromolecular vehicles for transport of hydrophobic lipids throughout the aqueous environment of the circulatory system; they are composed of various lipid species aggregated with specific proteins (apolipoproteins), which act as receptor ligands, stabilize the emulsion and confer structural properties to the lipoprotein particle [2].

Lipid transport and metabolism involves three general pathways: (1) the exogenous pathway, whereby chylomicrons are synthesized by the small intestine, and dietary triglycerides (TGs) and cholesterol are transported to various cells of the body; (2) the endogenous pathway, whereby the very low-density lipoprotein cholesterol (VLDL-C) and TGs are synthesized by the liver for transport to various tissues; in the plasma, the TGs in VLDLs are hydrolyzed by lipoprotein lipase (LPL),

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generating a series of smaller, cholesterol enriched lipoproteins: intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL); (3) and the reverse cholesterol transport, whereby high-density lipoprotein (HDL) in a series of metabolic steps facilitates the removal of cholesterol from the peripheral tissues for delivery to the liver and steroidogenic organs [3]. Disorders of lipoprotein concentrations such as elevated LDL-C (≥160 mg/dL), low HDL-C (<40 mg/dL), increase TG (≥150 mg/dL) and elevated oxidized lipoproteins (oxLDL) levels are risk factors in the pathogenesis of cardiovascular diseases (CVD) [4]. However, it has been shown that atherosclerosis can begin with LDL-C levels ≥100 mg/dL, therefore modification of lipid and lipoprotein classification identifies to LDL-C <100 mg/dL as optimal [5].

CD36 is a polypeptide from 78 to 88 kDa of molecular weight (50 kDa deglycosylated) depending on the cell type [6]. CD36 receptor is involved in a variety of biological processes including lipid metabolism, inflammation, atherosclerosis, angiogenesis, innate immune responses, uptake of apoptotic cells, oxidized lipids and advanced glycation end products, transforming growth factor-β activation, insulin resistance, diabetes and thrombosis, depending on nature of the ligand and tissue or cell type on which it is expressed [7-10]. CD36 contributes to oral fat perception and intestinal chylomicron formation [11]. CD36 is a multi-ligand scavenger receptor expressed on a variety of cell types including adipocytes, myocytes, monocytes, macrophages, platelets, hepatocytes and vascular epithelial cells [12,13]. Given the many functions of CD36 including long chain fatty acid transport (LCFA), changes in CD36 expression and protein may lead to several disturbances including insulin resistance and dyslipidemia [14]. This receptor is up regulated by oxLDL in macrophages and contributes to the formation and accumulation of foam cells at sites of arterial lesions during early and late atherosclerosis [15].

The *CD36* gene is located on chromosome 7 q11.2, is encoded by 15 exons and spans 36 Kb, CD36 plays an important role in lipid metabolism and its gene polymorphisms are associated to cardiovascular risk factors [16,17]. Genome-wide linkage scans have identified nearby regions of chromosome 7 that are associated with features of metabolic syndrome, such as triglyceride concentrations, HDL-C, and triglyceride/HDL ratio [18].

The aim of this study was to evaluate whether there is an association between genotypes and haplotypes of five polymorphisms in the *CD36* gene with lipid levels in normal-weight young men and women.

Results

General and biochemical characteristics

General and biochemical characteristics of study subjects according to gender are shown in Table 1. The measurements of body weight, height, systolic blood pressure, prevalence of hypertension and oxLDL levels were higher in men than in women ($P < 0.05$), whereas HDL-C levels was lower in men than in women ($P = 0.03$). There was no significant difference in the levels of TC, TG, LDL-C and glucose ($P > 0.05$).

Table 1 Clinical characteristics and lipid levels by gender

Variables	Female (n = 157)	Male (n = 75)	*P* value
Age (years)	21 (19–22)	20 (20–22)	0.433
Weight (kg)	52 (48–56)	63 (57–68)	0.001
Height (cm)	156 (152–159)	169 (166–173)	0.001
BMI (kg/m^2)	22 (20–23)	22 (20–23)	0.411
SBP (mmHg)	102 (97–106)	110 (103–119)	0.001
DBP (mmHg)	66 (60–71)	68 (61–72)	0.299
Hypertension (%)	4 (3)	8 (11)	0.009
TC (mg/dL)	75 (58–102)	77 (57–19)	0.926
HDL-C (mg/dL)	48 (38–58)	42 (37–52)	0.032
LDL-C (mg/dL)	93 (67–118)	89 (70–116)	0.311
oxLDL (U/L)	34 (27–46)	39 (31–53)	0.053
TG (mg/dL)	75 (58–103)	77 (57–109)	0.926
Glucose (mg/dL)	81 (75–88)	81 (75–88)	0.942

SBP, Systolic blood pressure; *DBP*, Diastolic blood pressure; *TC*, Total cholesterol; *HDL-C*, High-density lipoprotein cholesterol; *LDL-C*, Low-density lipoprotein cholesterol; *oxLDL*, Oxidized low-density lipoprotein; *TG*, Triglyceride. The values were presented as median (percentile 25-75th). The difference between genders was determined by the Wilcoxon-Mann–Whitney test. Data of hypertension were presented in (n) and the percentages; the difference between genders was determined by chi-square test.

Genotypic and allelic frequencies

Table 2 shows the genotype and allelic distribution of five *CD36* polymorphisms in normal-weight subjects, genotype frequencies of each polymorphism were in Hardy-Weinberg equilibrium. The 27645Ins/Del polymorphism was found high linkage disequilibrium (LD) with -31118G/A and -22674 T/C polymorphisms; while the -33137A/G and -31118G/A polymorphisms were also found in high LD (Figure 1).

As shown in Table 3, the levels of HDL-C were lower in the −22674 TC carriers ($P = 0.04$), but the subjects with TT genotype for this same polymorphism had lower oxLDL levels than non-carriers ($P = 0.01$). LDL-C levels were higher in 30294 CC carriers than non-carriers ($P = 0.03$).

Haplotypes and dyslipidemia susceptibility

To examine the combined effect of five variants -33137A/G (rs1984112), -31118G/A (rs1761667), -22674 T/C (rs2151916), 27645Ins/Del (rs3840546) and 30294G/C (rs1049673) in the *CD36* receptor gene locus, we performed haplotype analysis considered the combinations most frequent (Table 4). In particular, the subjects with AATDC (12122) haplotype had 3.2 times higher risk of LDL-C >100 mg/dL than the carrying the

Table 2 Allele and genotype frequencies in normal-weight subjects

Polymorphism	Genotype n (%)	Allele	n (%)	HWE X^2 (*P value*)	Location
−33137A/G					5′flanking exon 1A
AA	117 (50)	A	328 (0.71)	0.11 (0.73)	
AG	94 (41)	G	136 (0.29)		
GG	21 (9)				
−31118G/A					5′flanking exon 1A
GG	46 (20)	G	196 (0.42)	1.53 (0.21)	
GA	104 (45)	A	268 (0.58)		
AA	82 (35)				
−22674 T/C					Promoter
TT	106 (46)	T	309 (0.67)	0.84 (0.35)	
TC	97 (42)	C	155 (0.33)		
CC	29 (12)				
27645Ins/Del					Exon 14 (3′-UTR)
Ins/Ins	206 (89)	Ins	437 (0.94)	0.06 (0.79)	
Ins/Del	25 (10.6)	Del	27 (0.06)		
Del/Del	1 (0.4)				
30294G/C					Exon 15 (3′-UTR)
GG	13 (6)	G	111 (0.24)	0.01 (0.92)	
GC	85 (36)	C	353 (0.76)		
CC	134 (58)				

HWE, Hardy-Weinberg Equilibrium.

AGTIG (11111) haplotype ($P = 0.02$), whereas the subjects carrying the AATIC (12112) haplotype had 2.0 times presumably higher risk of TC > 200 mg/dL than the carrying the AGTIC (11112) haplotype ($P = 0.02$).

Discussion

The results of the present study show that the levels of serum HDL-C in a Mexican young population with normal-weight were higher in women than in men. This gender difference in HDL-C may be attributed to high endogenous estrogen levels in women, as it has been demonstrated that estrogen concentrations may increase HDL-C, which confers a protective effect of cardiovascular disease to premenopausal women [19]. However, also has been shown that the difference in HDL-C between women and men is an androgen effect, but not an estrogen effect, therefore, at puberty, concurrent with the rise in endogenous testosterone levels, the HDL-C levels in young men decline to the adulthood [20].

There was no significant difference in the levels of TC, TG, and LDL-C between genders. These findings are in agreement with previous studies in other populations [21-23]. In this research, oxLDL levels were higher in men than women; but there is a lack of published data about the oxLDL levels by gender. However, it has been shown that men have a higher oxidative stress compared to women, due to an increased generation of reactive oxygen species (ROS) and/or reduced activity of antioxidants, considering that under healthy conditions, cellular respiration in the mitochondria is the dominant source of ROS, therefore, a higher baseline metabolic rate in men than in women might contribute to a higher level of oxidative stress in men [24]. It is also known that an increase in oxLDL, as a consequence of increased oxidative stress and reduced antioxidant defenses, is a key event in the atherogenic process and a cardiovascular disease risk factor, which may be reflected in an increase in circulating levels of oxLDL [25].

CD36 gene is highly polymorphic; data from Ensembl Variation Build 60 (which is based on dbSNP Release 131) describe 2935 common genetic variants within 5 kb of the gene. Some involve putative transcription factor binding sites or sites in the 5′-untranslated region, which are of potential significance because translational efficiency of the CD36 mRNA and thereby CD36 protein expression levels have been shown to be regulated by variants in the 5′-untranslated region [26]. A study on *CD36* gene variants showed that subjects with GG homozygote genotype of the variant 80121259A > G (rs3211849) had a higher triglyceride level (99.16 ± 2.61 mg/dL) compared with non-carriers (89.27 ± 1.45 mg/dL, $P = 0.001$). In addition, compared with non-carriers, subjects with CT heterozygous and TT homozygous genotypes of the variant 80122878C > T

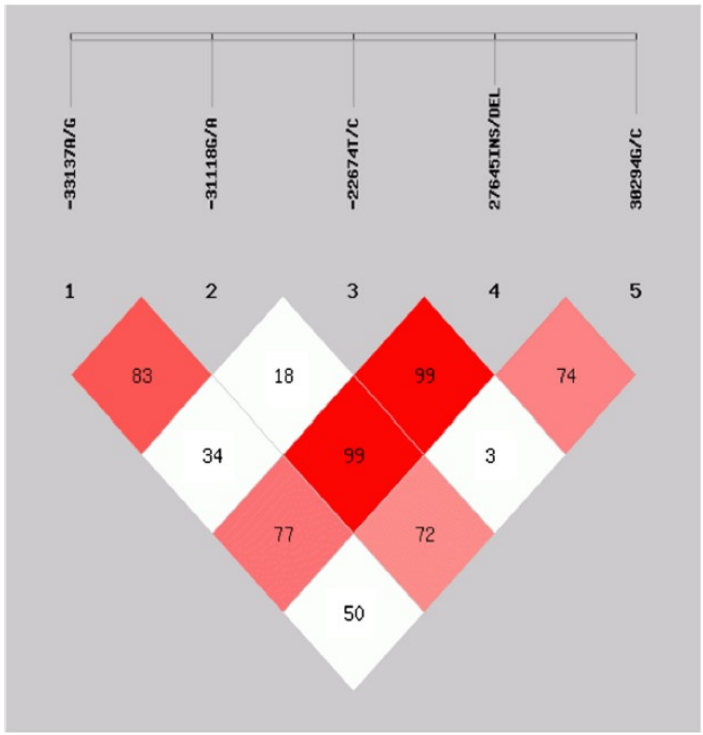

Figure 1 Linkage disequilibrium test of *CD36* gene polymorphisms (D′).

(rs1054516) had a significantly lower HDL-C level (46.6 ± 0.46 mg/dL for non-carriers, 44.6 ± 0.36 mg/dL for heterozygous, and 44.3 ± 0.56 mg/dL for homozygous, $P = 0.0008$) [27].

Previous genetic studies showed various effects between *CD36* locus and dyslipidemias, a genome-wide linkage scan among 418 individuals from 27 extended Mexican American families found two different locations on chromosome 7 were suggested as linked to susceptibility loci influencing in HDL cholesterol and triglycerides levels, however, it has been reported a major susceptibility locus in chromosome 15q influencing in TG levels in a Mexican American population [28]. In addition, among non-diabetic Mexican American families, quantitative trait locus study showed a strong linkage of two factors metabolic syndrome related, HDL-C and triglycerides to chromosome 7 (LOD score up to 3.2) [29].

A meta-analysis showed that *CD36* gene locus (7p11-q21.11) was significantly linked to triglycerides and triglycerides/HDL-C ratio, but not linked to LDL or total cholesterol [30]. In addition, a study in 61 *CD36*-deficient patients and 25 controls showed that the HDL-C concentrations in the *CD36*-deficient patients were significantly higher than in the control subjects, however, nondiabetic *CD36*-deficient patients had higher triglyceride concentrations than the control subjects, and triglyceride concentrations were higher in the diabetic CD36-deficient patients than in the nondiabetic *CD36*-deficient patients [31].

In this research the genotype frequencies of each polymorphism were in Hardy-Weinberg equilibrium. However, genotype and allele frequencies found in this study for each of the polymorphisms in the *CD36* gene are different to those reported in Caucasian population [32,33]. Mexicans are an admixed population, descended from a recent mix of Amerindian and European ancestry with a small proportion of African ancestry [34]. The influence of various races in our genetic background as Mexican population may explain the allele and genotype differences with respect to other populations.

In this study, the 27645Ins/Del polymorphism was found in high LD with polymorphisms -31118G/A, -22674 T/C and 30294G/C. These results were similar to those previously reported by Ma X, *et al.* in Caucasians [32].

Here we show an association between the *CD36* polymorphisms and serum lipid levels in Mexican subjects. In this study, the subjects with -22674TC genotype had lower HDL-C levels than non-carriers. However, oxLDL levels were lower in the TT subjects; these results are different to those reported by Goyenechea *et al.*, 2008 in the Spanish population, they shown that subjects carrying the CC genotype had higher levels of HDL-C and

Table 3 Blood lipid profile according to *CD36* polymorphisms

Polymorphism	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	oxLDL (U/L)
−33137A/G					
AA	150 (140–178)	70 (53–93)	43 (34–52)	86 (59–124)	32 (26–39)
AG	157 (138–180)	79 (56–109)	41 (35–49)	73 (48–109)	30 (24–38)
GG	154 (136–176)	66 (52–109)	41 (35–50)	63 (52–89)	33 (26–35)
P value	*0.64*	*0.40*	*0.88*	*0.19*	*0.70*
−31118G/A					
GG	149 (131–171)	70 (53–102)	44 (34–59)	82 (55–103)	30 (26–35)
GA	160 (144–181)	79 (63–109)	41 (36–48)	75 (51–109)	31 (26–45)
AA	152 (140–172)	63 (53–95)	42 (34–51)	87 (57–137)	30 (25–38)
P value	*0.18*	*0.09*	*0.71*	*0.20*	*0.34*
−22674 T/C					
TT	152 (137–169)	65 (52–109)	43 (37–58)	77 (51–114)	28 (25–35)
TC	154 (138–179)	77 (62–102)	38 (32–48)	89 (61–116)	34 (28–43)
CC	153 (145–175)	71 (53–92)	44 (34–52)	73 (52–106)	34 (28–46)
P value	*0.66*	*0.71*	*0.04*	*0.60*	*0.01*
27645Ins/Del					
Ins/Ins	153 (139–179)	72 (53–103)	42 (34–50)	78 (55–105)	31 (26–38)
Ins/Del	152 (136–169)	68 (56–108)	42 (31–50)	111 (54–145)	29 (26–34)
Del/Del	149 (149–149)	129 (129–129)	34 (34–34)	123 (123–123)	-
P value	*0.66*	*0.43*	*0.60*	*0.31*	*0.51*
30294G/C					
GG	134 (114–150)	77 (63–107)	46 (39–59)	72 (56–103)	33 (26–47)
GC	153 (133–179)	76 (58–106)	46 (36–57)	89 (67–112)	35 (28–46)
CC	150 (130–175)	75 (56–103)	46 (38–56)	96 (75–121)	36 (28–48)
P value	0.06	0.89	0.76	0.03	0.72

TC, Total cholesterol; *TG*, Triglyceride; *HDL-C*, High-density lipoprotein cholesterol; *LDL-C*, Low-density lipoprotein cholesterol; *oxLDL*, Oxidized low-density lipoprotein. The values were presented as median (percentile 5-95th). *P* values among genotypes by Kruskal Wallis test.

lower LDL-C, this difference may be due to they did a study with dietary intervention where participants were under a low calorie diet for 8 weeks and the sample size, also their sample size was larger than ours, they concluded that the association with lower LDL-C level observed in response to the low calorie diet may devolve from reduced ability of −22674CC homozygotes to take up fatty acids from the intestine, to synthesize triglycerides and secrete LDL-C. Our study also found that 30294CC carriers had higher LDL-C concentrations compared with subjects with the other genotypes; CC genotype was associated with high levels of free fatty acids in Caucasian population [9].

Although it is not clear how -33137A/G, -31118G/A -22674 T/C, 27645Ins/Del and 30294G/C polymorphisms can modulate lipid metabolism, has been demonstrated that -22674 T/C SNP is located upstream of the promoter, 14 bases 5′of the transcription start site, which is a binding element for the transcriptional repressor GFI1B, this SNP was in complete LD with the -33137A/G SNP in Caucasians from the general population, thus so has been reported that the allele A at position -33137A/G is in complete LD with the in3$(TG)_{13}$ variant, which determines the expression of an alternative spliced, inactive transcript lacking exons 4 and 5 [32]. In addition the allele A of −31118 G/A SNP has been associated with reduced CD36 expression, lies between two alternative CD36 promoters, 1C and 1A [35]. It has been suggested that the 27645Ins/Del and 30294G/C polymorphisms located in the 3′-UTR could determine decreased mRNA stability [32].

In this research, five polymorphisms in the *CD36* gene in haplotypes combinations were associated with high LDL-C and TC levels. This study showed that a haplotype analysis with five variants in the *CD36* receptor gene may explain the lipid profile variation more than a single variant. Similarly, in the Caucasian population have studied the five polymorphisms in the *CD36*

Table 4 Association of *CD36* haplotypes with lipid levels

Haplotype	Cases (n = 96) LDL-C > 100 mg/dL	Controls (n = 134)	OR (95% CI)	*P* value
AGTIG	(0.046)	(0.035)	1.28 (0.50-3.28)	0.59
11111				
AGTIC	(0.088)	(0.081)	1.06 (0.54-2.08)	0.84
11112				
AATIC	(0.365)	(0.340)	1.08 (0.73-1.61)	0.68
12112				
AATDC	(0.060)	(0.019)	3.25 (1.12-9.40)	0.02
12122				
AACID	(0.116)	(0.145)	0.75 (0.42-1.31)	0.31
12212				
GGTIG	(0.045)	(0.099)	0.42 (0.19-0.92)	0.02
21111				
GGTIC	(0.042)	(0.034)	1.21 (0.46-3.20)	0.69
21112				
GGCIG	(0.050)	(0.086)	0.55 (0.25-1.19)	0.12
21211				
GGCIC	(0.112)	(0.066)	1.74 (0.90-3.37)	0.09
21212				
Haplotype	**Cases (n = 26) Cholesterol total > 200 mg/dL**	**Controls (n = 206)**	**OR (95% CI)**	***P* value**
AGTIC	(0.052)	(0.087)	0.60 (0.16-2.15)	0.43
11112				
AGCIC	(0.031)	(0.012)	2.86 (0.46-17.50)	0.23
11212				
AATIC	(0.472)	(0.332)	2.02 (1.09-3.74)	0.02
12112				
AATDC	(0.020)	(0.044)	0.46 (0.06-3.43)	0.44
12122				
AACIC	(0.109)	(0.131)	0.85 (0.33-2.13)	0.72
12212				
GGTIG	(0.021)	(0.081)	0.25 (0.037-1.74)	0.13
21111				
GGTIC	(0.022)	(0.039)	0.58 (0.086-3.95)	0.57
21112				
GGCIG	(0.083)	(0.068)	1.30 (0.45-3.77)	0.62
21211				
GGCIC	(0.080)	(0.088)	0.94 (0.32-2.74)	0.92
21212				

1 = Major allele, 2 = minor allele. Markers: -33137A/G, -31118G/A, -22674 T/C, 27645Ins/Del, 30294G/C polymorphisms of *CD36* gene.

receptor gene and were found in high linkage disequilibrium and a common haplotype at the *CD36* locus was associated with high free fatty acid levels and increased cardiovascular risk [32]. However, further investigations are needed to confirm our findings and demonstrate the mechanisms underlying such associations.

Nonetheless, the results of association studies must always be interpreted with caution, especially when multiple comparisons are performed, and replication in other populations is needed before a link between *CD36* variants, dyslipidemias and cardiovascular disease.

Finally, some limitations of our study should be considered. Although this study had sufficient statistical power to detected large effects resulting from common alleles, the power to evaluate small effects due to rare alleles was limited. However, small genetic effects can be expected because of the complexity of lipid metabolism. Second, we could not completely exclude the influence of factors such as consumption of alcohol and tobacco on lipid levels.

Conclusions

The study provides evidence of a genetic association of *CD36* haplotypes with the variability in LDL-C and TC levels in a sample of normal-weight subjects. The *CD36* gene may be a candidate susceptibility to dyslipidemia in Mexican population.

Methods

Subjects

A total de 232 unrelated normal-weight subjects of 18 to 25 years old, from the state of Guerrero, Mexico. The participants were randomly selected considering their body mass index (BMI) of 18.5 to 24.9 kg/m^2. There were 157 women and 75 men, none of them had medication with lipid-lowering drugs such as statins or fibrates. The participants signed informed consent forms, and the protocol was approved by the Research Ethics Committee of the University of Guerrero.

Blood pressure

Blood pressure was measured in the sitting position with the use of an automatic sphygmomanometer on the left arm after 10 min rest. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from two readings with a minimal interval of 10 min. Hypertension was defined as mean SBP ≥140 mmHg and/or DBP ≥90 mm Hg [36].

Biochemical analysis

A venous blood sample of 5 mL was obtained from each subject after at least a 12 hours fasting. All serum lipid levels and glucose were determined by enzymatic methods with commercially available kits (spinreact).

Abnormal biochemical levels were identified when total-cholesterol (TC) > 200 mg/dL, TG ≥ 150 mg/dL, LDL-C > 100 mg/dL, HDL-C < 40 mg/dL and glucose >100 mg/dL, based on the criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) [5].

Determination of serum oxLDL

An enzyme-linked immunosorbant assay (ELISA) for oxLDL (Mercodia Oxidized LDL ELISA) was performed, according to the manufacturer's instructions using coated microtitration strips of 96-well plates, serum was diluted 1/6561, and incubated at room temperature for 2 h in plates precoated with oxLDL-lgG. After six washings, the plates were incubated with an anti-apolipoprotein B (apoB) monoclonal antibody at room temperature for 30 minutes. After the removal of unbound conjugates by washing the samples six times, tetramethylbenzidine (TMB) was added to the wells as a chromogenic substrate. The mixture was incubated at room temperature in the dark for 15 minutes. Color development was stopped via a stopping solution, and absorbency was measured at 450 nm within 30 minutes. The oxLDL was calculated by constructing a standard curve using the standards included in the kit. The oxLDL concentrations in the samples were quantified in biomedical units as defined by the manufacturer.

Genotyping

Genomic DNA was extracted from leukocytes in samples of whole blood and was stored at −20°C until analysis. Genotyping of the five polymorphisms -33137A/G (rs1984112), -31118G/A (rs1761667), -22674 T/C (rs2151916), 27645 Ins/Del (rs3840546) and 30294G/C (rs1049673) was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). The five polymorphisms in the *CD36* gene were selected for genotyping based on previously reported association with free fatty acids and triglycerides, whose minor allele frequency >5% and one insertion/deletion polymorphism [32]. Primers for the polymorphims were designed and restriction enzymes (REs) were identified using the Primer 3 and NEB cutter softwares respectively. Unique National Center for Biotechnology Information (NCBI) Build 37 chromosome and base pair locations may be obtained from Ensembl Variation Build 59 (which is based on dbSNP Release 131). Details including the location of polymorphisms in the CD36 gene, primer sequences and REs with product sizes are presented in Table 5.

Each reaction system of a total volume of 24 μL, containing 100 ng of genomic DNA, 0.2 mM of each primer, 0.2 mM dNTPs, and 2.0U of *Taq* polymerase (Invitrogen Life Technologies). The PCR products were digested with the respective restriction enzymes, and were visualized on 6% polyacrylamide gels stained with silver nitrate 2%.

Table 5 - Characteristics of the polymorphisms studied in the *CD36* gene

Polymorphisms	Primer sequence	Annealing temp. (°C)	Product size (bp)	Restriction enzyme/allele sizes
−33137A/G	F: 5′-CATGCAGCTCTGTTTTATGTGAG-3′	60	159	*MseI*
				AA 67, 56, 29, 7
(rs1984112)	R: 5′-CCCCATCTCTTAGGCCCGTGACA-3′			AG 85, 67, 56, 29, 7
				GG 85, 67, 7
−31118G/A	F: 5′-CAAAATCACAATCTATTCAAGACCA-3′	58	190	*HhaI*
				AA 190
(rs1761667)	R: 5′-TTTTGGGAGAAATTCTGAAGAG-3′			GA 190, 138, 52
				GG 138, 52
−22674 T/C	F: 5-′TCTTGCTGGGCCCTGCCCA-3′	68	452	*Hpy 188I*
				TT 452
(rs2151916)	R: 5′-TGTTTGCCCCAAGTGCTGGGTC-3′			TC 452, 430, 22
				CC 430, 22
27645Ins/Del16	F: 5′-GGGACCATTGGTGATGAGAAGG-3′	68	563	Ins/Ins
			563, 547	Ins/Del
(rs3840546)	R: 5′–TTGGAAAATGCACGGCCAGCA-3′		547	Del/Del
30294G/C	F: 5′-ACGCTTGGCATCTTCAGAATGCT-3′	60	465	*MnlI*
				GG 331, 134
(rs1049673)	R: 5′TGAACCCCTGCTCAAGAAACAGAGT-3′			GC 331, 265, 134, 66
				CC 265, 134, 66

Statistical analysis

The statistical analyses were done with the statistical software package SPSS 15.0 and STATA software 9.0. Quantitative variables were expressed as medians and 25^{th} to 75^{th} percentiles or 5^{th} to 95^{th} percentiles; the significance of differences between groups was determined using Wilcoxon-Mann Whitney or Kruskal Wallis test. Qualitative variables were expressed as percentages. Allele and genotype frequencies were determined by calculating Hardy-Weinberg equilibrium, difference in genotype distribution between the groups female and male was obtained using the chi-square test, a P value of less than 0.05 was considered statistically significant. For analysis linkage disequilibrium of polymorphisms in *CD36* gene and the association between haplotypes and lipid phenotypes were analysed using the software program SHEsis (http://analysis.bio-x.cn/myAnalysis.php) [37].

Competing interests

HBM is Editor-in-Chief of the Journal of Foot and Ankle Research. It is journal policy that editors are removed from the editorial decision making processes for papers they have co-authored. The remaining authors declare that they have no competing interests.

Authors' contributions

LERA carried out genetic analysis and writing the manuscript. ABSB performed laboratory measurements and quality control. IPGG performed the statistical analysis. LSG and JFMV participated in the critical revision of the manuscript. IPR conceived the study and participated in manuscript preparation. All authors read and approved the final manuscript.

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CAPÍTULO II

Circulating CD36 and oxLDL levels are associated with cardiovascular risk factors in young subjects: a case-control study

Circulating CD36 and oxLDL levels are associated with cardiovascular risk factors in young subjects: a case-control study

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Abstract

Background

The cardiovascular disease (CVD) results from a combination of abnormalities in lipoprotein metabolism, oxidative stress, chronic inflammation, and susceptibility to thrombosis. Atherosclerosis is the major cause of CVD. CD36 has been shown to play a critical role in the development of atherosclerotic lesions by its capacity to bind and promote endocytosis of oxidized low-density lipoprotein (oxLDL) and it is implicated in the formation of foam cells. The purpose of this research was to evaluate whether there is an association of sCD36 and oxLDL levels with cardiovascular risk factors in young subjects.

Methods

A total of 188 subjects, 18 to 25 years old, 133 normal-weight and 55 obese subjects from the state of Guerrero, Mexico were recruited in the study. The lipid profile and glucose levels were measured by enzymatic colorimetric assays. Enzyme-linked immunosorbant assays (ELISA) for oxLDL and sCD36 were performed. Wilcoxon- Mann Whitney, chi-square and multinomial regression were used in the statistical analyses.

Results

The levels of TC, LDL-C, TG, oxLDL and sCD36 were higher in obese subjects than in normal-weight controls, as well as, monocyte and platelet count ($P<0.05$). Obese subjects had 5.8 times higher risk of sCD36 in third tertil (>97.8 ng/mL) than normal-weight controls ($P=0.014$) and 7.4 times higher risk of oxLDL levels in third tertile (>48.0 U/L) than control group. The subjects with hypercholesterolemia, hypertriglyceridemia, fasting impaired LDL-C had a higher risk of oxLDL levels in third tertile (>48.0 U/L) than control group ($P<0.05$).

Conclusions

Circulating CD36 and oxLDL levels are associated with cardiovascular risk factors in young subjects and may be potential early markers for cardiovascular disease (CVD).

Keywords

CD36, oxLDL, cardiovascular risk factors, obesity.

Background

The cardiovascular disease (CVD) risk factors including advanced age, obesity, smoking, hyperlipidemia, diabetes, and hypertension, account for 30–40% of the disease prevalence in the world [1]. The pathological basis of CVD results from a combination of abnormalities in lipoprotein metabolism, oxidative stress, chronic inflammation, and susceptibility to thrombosis [2]. Atherosclerosis is the major cause of CVD [3], is a chronic inflammatory disease of the arterial wall that underlies many of the common causes of cardiovascular morbidity and mortality, including myocardial infarction (MI), cerebrovascular and peripheral vascular disease [4].

A main process central to the pathogenesis of atherosclerosis is the deposition of cholesterol in the arterial wall, nearly all lipoproteins are involved in this process, including cholesterol carried by very low-density (VLDL), remnant lipoproteins and low-density lipoproteins (LDL), particularly the small and dense form; conversely, cholesterol is carried away from the arterial wall by high-density lipoprotein (HDL) [5].

Oxidative modification of LDL (oxLDL) in the arterial wall is central to the pathogenesis of atherosclerosis [6]. OxLDL was associated with carotid intimal-media thickness, unstable plaques in the coronary and carotid arteries, impaired brachial and coronary endothelial function, and coronary artery disease [7]. OxLDL loses its ability to bind to LDL receptors, which interferes with its normal processing, gains affinity for a family of proteins called scavenger receptors [8], leading to macrophage activation, foam-cell formation, secretion of growth factors and proinflammatory cytokines, thereby promoting plaque formation [9,10].

CD36, an 88 kDa glycoprotein, was originally described as platelet receptor glycoprotein and belongs to the class B scavenger receptor family [11,12]. CD36 is expressed on an extensive range of cells and tissues, including microvascular endothelial cells, monocytes and macrophages, dendritic cells, adipocytes, keratinocytes, cardiac and skeletal muscle, retinal pigment epithelium, microglia, reticulocytes, breast, gut, renal epithelium, platelets, hepatocytes, smooth muscle cells and binds a diverse array of ligands [13-16]. CD36 is the best characterized free fatty acid transporter involved in different biological processes like angiogenesis, inflammation, lipid metabolism, atherosclerosis and platelet activation [17].

Monocyte/macrophage CD36 has been shown to play a critical role in the development of atherosclerotic lesions by its capacity to bind and promote endocytosis of oxLDL, and it is implicated in the formation of foam cells [18,19]. The pathogenic role of oxLDL in atherosclerosis largely depends on CD36 [20].

A soluble form of CD36 (sCD36), a marker of altered tissue CD36 expression, was recently identified in human plasma, and elevated levels were found in obesity and type 2 diabetes; sCD36 is up to 4-fold higher in plasma from obese T2D-patients compared with lean healthy control subjects [21]. The circulating concentration of CD36 is associated with markers of liver injury in subjects with altered glucose tolerance [22]. A recent study revealed that plasma soluble CD36 correlates significantly with markers of atherosclerosis, insulin resistance and fatty liver in a no diabetic healthy population [23]. Due to the wide-spread tissue expression of CD36 and its broad range of functions it is difficult to foresee which specific pathological processes may reflect alterations in sCD36 [24]. However, a recent study showed that sCD36 is not a proteolytic product, but rather is associated with a specific subset of circulating microparticles (MPs) that can readily be analyzed; originate mainly from platelets in normal subjects [25]. The aim of this research was to evaluate whether there is an association of sCD36 and oxLDL levels with cardiovascular risk factors in young subjects.

Methods

Subjects

A total of 188 subjects were randomly selected, 18 to 25 years old, 133 normal-weight controls (BMI 18.5 to 24.9 kg/m2) and 55 obese subjects (BMI ≥30 kg/m2) from the state of Guerrero, Mexico. There were 117 women and 71 men; none of the participants were taking any medication or had evidence of metabolic disease other than obesity. All subjects gave their written informed consent after the purpose of the study had been explained to them. The protocol was approved by the Research Ethics Committee of the University of Guerrero.

Blood pressure

Blood pressure was measured in the sitting position with the use of an automatic sphygmomanometer on the left arm after 10 min rest. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from two readings with a minimal

interval of 10 min. Hypertension was defined as mean SBP ≥140 mmHg and/or DBP ≥90 mm Hg [26].

Biochemical analysis

A venous blood sample of 5 mL was obtained from each subject after at least a 12 hours fasting. All serum lipid levels and glucose were determined by enzymatic methods with commercially available kits (Spinreact). Abnormal biochemical levels were identified when total-cholesterol (TC) ≥ 200 mg/dL, triglycerides (TG) ≥ 150 mg/dL, low-density lipoprotein cholesterol (LDL-C) >100 mg/dL, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL and glucose >100 mg/dL, based on the criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) [27].

Determination of sCD36 and oxLDL levels

Enzyme-linked immunosorbant assays (ELISA) for oxLDL (Mercodia Oxidized LDL ELISA) and sCD36 (Human soluble CD36 ELISA, kit-Aviscera Bioscience) were performed, according to the manufacturer's instructions, with an intra-assay CV <6% and interassay CV <7% for oxLDL ELISA assay, and an intra-assay CV <5% and interassay CV <9% for CD36 ELISA assay.

Statistical analysis

The statistical analyses were done with the statistical software package SPSS 15.0 and STATA software 9.0. Quantitative variables were expressed as medians and 25th to 75th percentiles and the significance of differences between groups was determined using Wilcoxon-Mann Whitney test. Qualitative variables were expressed as percentages and the differences between groups were determined using the chi-square test. For analysis of association between serum levels of oxLDL and sCD36 with lipid phenotypes were analysed using multinomial regression, a P value <0.05 was considered statistically significant.

Results

General and biochemical characteristics

General and biochemical characteristics of the study subjects are shown in table 1. The measurements of body weight, height, systolic and diastolic blood pressure, prevalence of hypertension, and TC, LDL-C, TG, sCD36 and oxLDL levels were higher in obese subjects than in normal-weight controls, as well as, monocyte and platelet count ($P<0.05$). There were no significant differences by gender ($P= 0.162$).

Correlations between sCD36 and oxLDL levels with selected variables

In all subjects studied, sCD36 was significantly correlated with weight, BMI, waist, hip, waist-to-hip ratio, % fat, % fat mass, LDL-C, oxLDL and monocyte count. The obese subjects showed a high correlation among BMI and sCD36 (r=0.50, P=0.028) (table 2). The oxLDL levels were correlated positively with weight, BMI, waist, hip, waist-to-hip ratio, % fat, % fat mass, and TC, TG, LDL-C, sCD36 and monocyte count, in all subjects. In normal-weight and obese groups, oxLDL levels showed a high correlation with weight, BMI, waist, waist-to-hip ratio, % fat mass, and TG, LDL-C levels and monocyte count (table 3).

Circulating CD36 and oxLDL levels according to metabolic abnormalities

The subjects with hypertriglyceridemia and hypertension had high sCD36, while oxLDL levels were higher in subjects with hypercholesterolemia, hypertriglyceridemia, fasting impaired LDL-C and hypertension compared to those without these abnormalities ($P<0.05$) (table 4).

Association of cardiovascular risk factors with sCD36 and oxLDL levels

For the analysis of association with some cardiovascular risk factors, sCD36 was classified into tertiles (first tertile <23.3 ng/mL, second tertile 23.3 to 97.8 ng/mL and third tertile >97.8 ng/mL), because there are no reference values established. It was observed that obese subjects had 5.8 times higher risk of sCD36 in third tertile than normal weight controls, adjusted for age and gender (P=0.014) (table 5). The oxLDL levels were also classified into tertiles (first tertile <31.9 U/L, second tertile 31.9 a 48.0 U/L and third tertile >48.0 U/L) to analyze its association with cardiovascular risk factors. The table 5 also shows that subjects with hypercholesterolemia had 7.5 times higher risk of oxLDL levels

in third tertile than subjects without these abnormalities, individuals with fasting impaired LDL-C had 4.5 times higher risk of oxLDL levels in third tertile and subjects with hypertriglyceridemia had 17.9 times higher risk of oxLDL levels in third tertile, adjusted for age, gender and BMI. While obese individuals had 7.4 times higher risk of oxLDL levels in third tertile than controls, adjusted for age and gender.

Discussion

In this research, we found a higher prevalence of hypertension in the obese subjects (32.7%) than in normal weight subjects (6.1%), with a prevalence of 13.6% in the total participants in the study. These data are similar to that reported in another study in U.S. teenagers; they reported a prevalence of hypertension of 30% in obese subjects [28]. It is known that the increase sodium reabsorption induced by angiotensin II produced by adipocytes affects renal natriuresis, so that obese subjects need blood pressure levels higher than normal weight subjects to maintain a balance between the intake sodium and renal diuresis [29].

In this study, as in previous studies the lipid profile (TC, LDL-C and TG) was higher in obese subjects than normal weight controls. This may be due to the increased adiposity, adipose tissue undergoes morphological and physiological changes, including the release of proinflammatory cytokines such as tumour necrosis factor alpha (TNF-α), which decrease insulin sensitivity and increase lipolysis, these changes contribute to insulin resistance and dyslipidaemia [30].

An interesting finding, in this study was that obese subjects have a higher number of monocytes and platelets than the normal weight subjects. The leukocyte count is considered as an indicator of inflammatory status in obesity [31]. In addition, research has shown that adults and children with obesity have higher levels of leukocytes, mainly monocytes, compared with adults and children of normal weight [32-34]. Regarding increased platelet count in subjects with obesity, similar findings have been shown in other studies, where the platelet count is higher in adolescents and adults with obesity than normal weight subjects [35-36]. It has been shown that interleukin-6 (IL-6) induces differentiation of megakaryocytes into platelets, IL-6 is produced by adipose tissue [37,38]. Furthermore, has been reported that obese individuals have increased levels of IL-6 [39], which may explain the increase in platelets in obese state.

In this study, we observed that sCD36 was higher in obese subjects than in normal weight subjects (143.3 ng/mL vs. 32.3 ng/mL, *P*=0.002), these results are congruent to the reported in previous studies [21,40]. This may be due to obese subjects showed an increased number of platelets and monocytes, as was recently reported that the circulating form of the CD36 receptor is associated with microparticles mainly originated of platelets, leukocytes and endothelial cells as a result of stimuli or apoptosis [8,25]. These microparticles have been found increased in subjects with insulin resistance and obese with type 2 diabetes, due to low-grade inflammation that present [41-43]. In this study, we observed that serum oxLDL levels were higher in obese subjects than in control group (51.5 U/L vs. 35.4 U/L), these results are consistent with those reported in other studies [44]. This may be due to the increase oxidative stress in obese state, which favors the oxidation of LDL-C [45,46].

We observed a high correlation of sCD36 with BMI in obese subjects (r =50, *P*=0.028), similar results have been reported in previous studies [47]. While oxLDL levels showed a strong correlation with BMI, TG and LDL-C in subjects with and without obesity. Such correlations are similar to those reported in other studies [48,49].

In this research, sCD36 in third tertile (>97.8 ng/mL) were associated with obesity, although there is a lack of studies that support this association, sCD36 have been correlated with BMI [47,50]. It has also been reported higher sCD36 in obese subjects than in normal weight subjects in other studies [21,43]. Furthermore, it is proposed that high CD36 levels may be a marker of increased CD36 expression known from a number of tissues that are associated with the metabolic syndrome; macrophage infiltration and low-grade inflammation in abdominal obesity, which may lead to dyslipidemia and peroxidation of lipoproteins [24].

We also found that oxLDL levels in third tertile (>48.0 U/L) were associated with hypercholesterolemia, fasting impaired LDL-C, hypertriglyceridemia and obesity. The association between dyslipidemia and oxidation of LDL has been demonstrated in individuals in the pre-diabetic state [48]. It has also been observed in middle-aged people that obesity and dyslipidemia are the strongest predictors of oxLDL levels [51]. The association between cardiovascular disease (CVD) and oxLDL has been demonstrated in others studies [52-55]. Considering the associations shown in this study sCD36 and oxLDL levels with traditional cardiovascular risk factors such as obesity, hypercholesterolemia,

hypertriglyceridemia and fasting impaired LDL-C, measuring sCD36 and oxLDL levels can be incorporated into cardiovascular risk factors in young subjects for diagnosis early of cardiovascular disease.

Our research has some limitations. We cannot study the associations between sCD36 and oxLDL levels with early atherosclerosis. As our study is comprised of young subjects without clinical atherosclerotic diseases, we only were able to study associations between traditional cardiovascular risk factors with sCD36 and oxLDL levels. Whether the increase of these markers in young subjects is associated with silent phase of atherosclerosis remains to be elucidated.

Conclusions

In this research, sCD36 and oxLDL levels are associated with cardiovascular risk factors, particularly with obesity, hypercholesterolemia, fasting impaired LDL-C and HDL-C and hypertriglyceridemia. Therefore, sCD36 and oxLDL levels may be potential early markers for CVD. However, these associations should be investigated in further studies.

Competing interests

No conflicts of interest.

Authors' contributions

LERA performed ELISA assays, statistical analysis and writing the manuscript. UDCM carried out the immunoassays. ABSB performed biochemical measurements and quality control. JFMV and NCA participated in the critical revision of the manuscript. IPR conceived the study and participated in manuscript preparation. All authors read and approved the final manuscript.

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Tables and captions

Table 1. Anthropometric and biochemical variables by group.

Variables	Normal weight	Obese	*P* value
N	133	55	-
Age (years)	20 (19-22)	21 (20-23)	0.005
Gender			0.162
Female (%)	87 (65)	30 (55)	
Male (%)	46 (35)	25 (45)	
Weight (kg)	54.5 (49.2-59.4)	90.9 (79.1-99.9)	0.001
Height(cm)	158.7 (153.0-167.5)	163.5 (155-171)	0.036
BMI (kg/m^2)	21.5 (20.0-23.4)	33.1 (31.3-35.3)	0.001
SBP (mmHg)	104 (98-112)	115 (112-121)	0.001
DBP (mmHg)	68 (61-73)	70 (67-77)	0.003
Hypertension			0.001
No	124 (93.9)	35 (67.3)	
Yes	8 (6.1)	17 (32.7)	
TC (mg/dL)	152 (136-173)	160 (137-195)	0.057
HDL-C (mg/dL)	43 (38-54)	44 (38-47)	0.717
LDL-C (mg/dL)	89 (61-116)	117 (88-157)	0.001
TG (mg/dL)	72 (56-98)	121 (75-89)	<0.001
Glucose (mg/dL)	83 ((75-89)	83 (77-90)	0.386
Monocytes (%)	7 (5-9)	9 (7-11)	0.0005
Platelet ($10^3/mm^3$)	247 (215-289)	265 (232-307)	0.048
CD36 (ng/mL)	32.3 (16.8-102.4)	146.3 (76.7-694.7)	0.002
oxLDL (U/L)	35.4 (27.9-47.1)	51.5 (38.0-59.7)	<0.001

BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; TG,Triglyceride; oxLDL, oxidized Low-Density Lipoprotein. The values were presented as median (percentile 25-75^{th}). The difference between genders was determined by the Wilcoxon-Mann-Whitney test. Data of hypertension were presented in (n) and the percentages; the difference between genders was determined by chi-square test.

Table 2. Correlation between sCD36 and selected variables.

Variables	All subjects		Normal weight		Obese	
	r	*p*	r	*p*	r	*p*
Weight (kg)	0.29	0.004	0.14	0.217	0.41	0.073
Height (cm)	0.03	0.774	0.04	0.724	0.15	0.534
BMI (kg/m^2)	0.32	0.001	0.17	0.150	0.50	0.028
Waist (cm)	0.30	0.003	0.13	0.2474	0.30	0.210
Hip (cm)	0.31	0.002	0.18	0.1190	0.30	0.201
Waist-to-hip ratio	0.23	0.026	0.001	0.988	0.02	0.931
% Fat	0.32	0.001	0.19	0.097	-0.02	0.909
% Fat mass	0.35	0.0006	0.22	0.058	0.30	0.203
Glucose (mg/dL)	-0.15	0.132	-0.16	0.168	-0.01	0.954
TC (mg/dL)	-0.05	0.609	-0.15	0.201	0.16	0.479
TG (mg/dL)	0.15	0.134	-0.001	0.991	0.33	0.151
HDL-C (mg/dL)	0.07	0.456	0.10	0.373	-0.06	0.771
LDL-C (mg/dL)	0.21	0.038	0.02	0.803	0.23	0.325
oxLDL (U/L)	0.25	0.024	0.06	0.623	0.29	0.334
Monocytes (%)	0.33	0.001	0.22	0.056	0.20	0.376
Platelet ($10^3/mm^3$)	0.15	0.142	0.03	0.741	0.23	0.309

r= Spearman correlation coefficient; *p*= *p* value

Table 3. Correlation between oxLDL levels and selected variables.

Variables	All subjects		Normal weight		Obese	
	r	*p*	r	*p*	r	*p*
Weight (kg)	0.43	0.0001	0.23	0.009	0.31	0.026
Height (cm)	0.05	0.438	-0.06	0.499	0.17	0.234
BMI (kg/m^2)	0.51	0.0001	0.40	<0.001	0.37	0.009
Waist (cm)	0.52	0.0001	0.39	<0.001	0.38	0.007
Hip (cm)	0.36	0.0001	0.10	0.225	0.21	0.139
Waist-to-hip ratio	0.53	0.0001	0.42	<0.001	0.30	0.034
% Fat	0.32	0.0001	0.13	0.124	0.09	0.516
% Fat mass	0.41	0.0001	0.21	0.014	0.39	0.005
Glucose (mg/dL)	-0.10	0.182	-0.19	0.027	0.12	0.398
TC (mg/dL)	0.32	<0.001	0.17	0.053	0.67	<0.001
TG (mg/dL)	0.47	<0.001	0.38	<0.001	0.33	0.018
HDL-C (mg/dL)	0.04	0.557	0.04	0.621	0.11	0.422
LDL-C (mg/dL)	0.43	<0.001	0.33	0.0001	0.53	0.0001
CD36 (ng/mL)	0.25	0.024	0.06	0.623	0.29	0.334
Monocytes (%)	0.40	<0.001	0.31	0.0003	0.34	0.020
Platelets ($10^3/mm^3$)	0.01	0.889	0.01	0.879	-0.15	0.293

r= Spearman correlation coefficient; *p*= *p* value

Table 4. sCD36 and oxLDL levels according to metabolic abnormalities.

Variables (N)	sCD36 levels (ng/mL)	oxLDL levels (U/L)
Fasting normal glucose (>100 mg/dL)		
No (176)	47.7 (18.4-219.9)	37.8 (29.5-50.6)
Yes (12)	62.2 (16.7-200.4)	52.7 (30.1-60.5)
	P=0.93	*P*=0.20
Hypercholesterolemia (≥200 mg/dL)		
No (162)	44.9 (17.3-223.6)	36.7 (29.1-48.5)
Yes (26)	74.8 (25.7-98.7)	54.5 (45.5-65.0)
	P=0.55	*P*=0.0002
Fasting impaired LDL-C (>100 mg/dL)	47.7 (17.2-208.2)	32.4 (27.8-45.6)
No (95)	74.8 (21.8-270.4)	47.1 (35.8-57.1)
Yes (93)	*P*=0.30	*P*=0.0001
Fasting impaired HDL-C (<40 mg/dL)	61.1 (19.6-262)	45.6 (31.9-54.3)
No (88)	46.7 (17.9-127.2)	34.6 (29-48.5)
Yes (100)	*P*=0.49	*P*= 0.06
Hypertriglyceridemia (≥150 mg/dL)	43.1 (16.7-190.6)	36.1 (28.2-48.1)
No (161)	180.3 (66.01-2295)	52.5(48.5-59.7)
Yes (27)	*P*=0.01	*P*=0.0001
Hypertension (SBP ≥140 mmHg, DBP ≥ 90 mmHg)		
No (161)	34.1 (16.7-190.6)	37.6 (29-119.0)
Yes (27)	79.8 (66.01-200.4)	48.0 (31.5-58.8)
	P=0.03	*P*= 0.1218

The values were presented as median (percentile 25-75th). The difference between genders was determined by the Wilcoxon-Mann-Whitney test.

Table 5. Association of cardiovascular risk factors with sCD36 and oxLDL levels in the to third tertile

Variables	sCD36 levels			oxLDL levels		
	OR (95% CI)	R^2	*p value*	OR (95% CI)	R^2	*p value*
Hypercholesterolemia	0.9 (0.14-5.75)	0.098	0.932*	7.5 (1.80-31.15)	0.155	0.006*
Fasting impaired LDL-C	0.7 (0.22-2.45)	0.085	0.622*	4.5 (1.79-11.31)	0.152	0.001*
Fasting impaired HDL-C	0.4 (0.15-1.46)	0.103	0.195*	0.3 (0.10-0.63)	0.147	0.003*
Hypertriglyceridemia	5.3 (0.50-55.87	0.094	0.164*	17.9 (2.03-158.92)	0.154	0.009*
Hypertension	4.5 (0.45-44.63)	0.098	0.197*	0.51 (0.13-1.98)	0.117	0.336*
Obesity	5.8 (1.43-24.05)	0.070	0.014**	7.4 (2.82-19.60)	0.80	<0.001**

* Adjusted for age, gender and BMI, **Ajusted for age and gender

CAPÍTULO III

Circulating CD36, polymorphisms and its expression on monocytes and platelets in young subjects

Circulating CD36, polymorphisms and its expression on monocytes and platelets in young subjects

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Abstract

CD36 is an integral membrane protein implicated in hemostasis, lipid metabolism, atherogenesis, diabetes and cardiomyopathy. To date 1372 SNPs have been reported in human *CD36* gene, however, only few among them have showed association with dyslipidemia, atherosclerosis and type 2 diabetes. The aim of this study was to evaluate whether there is a relationship between five polymorphisms in the *CD36* gene with its serum levels and both monocyte and platelet expression. This study included 133 nonobese and 55 obese young subjects. The lipids and glucose levels were measured by enzymatic colorimetric assays. Genotyping of rs1984112, rs1761667, rs2151916, rs3840546 and rs1049673 in *CD36* gene polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism. Enzyme-linked immunosorbant assays for oxLDL and CD36 were performed. Monocyte and platelet CD36 expression was measured by flow cytometry. The levels of TC, LDL-C, TG, oxLDL, CD36, and monocyte and platelet count were higher in obese than nonobese subjects ($P<0.05$). Monocyte CD36 expression was higher in nonobese than in obese group. In particular -33137GG genotype was associated with a decrease in monocyte and platelet CD36 expression levels. Our results show that *CD36* gene polymorphisms might be associated with monocyte and platelet CD36 expression in young subjects.

1. Introduction

CD36 is a class B scavenger receptor originally identified as a platelet glycoprotein [1], is an glycoprotein expressed on various human cells, such as platelets, monocytes/ macrophages, adipocytes, erythrocyte precursors, spleen tissue, smooth muscles, cardiac and skeletal muscles, capillary endothelial cells, cells of the retina, mammary gland and small intestine [2-4]. CD36 is a receptor for several ligands, including oxidized low-density lipoprotein (oxLDL), long chain fatty acids, thrombospondin, collagen, apoptotic neutrophils, *Plasmodium falciparum* infected erythrocytes, anionic phospholipids and advanced glycation end products [5-7]. CD36 is an integral membrane protein that has been implicated in hemostasis, thrombosis, malaria, inflammation, lipid metabolism, atherogenesis, insulin resistance, diabetes and cardiomyopathy [8,9]. CD36 is also known as FAT (fatty acid translocase) because it binds long chain free fatty acids and facilitates their transport into cells, in myocytes this serves to supply the cells with an energy source

for beta-oxidation, in adipocytes it results in lipid storage, in the gut participates in fat and fat-soluble vitamin absorption [10]. CD36 promotes platelet activation in response to oxidized phospholipids, including oxLDL [11]. Monocyte/macrophage CD36 has been shown to play a critical role in the development of atherosclerotic lesions by its capacity to bind and endocytose oxLDL, and it is implicated in the formation of foam cells [12-13]. CD36 is an 88 kDa heavily glycosylated transmembrane protein, it consists of an extracellular domain flanked by two transmembrane and two cytoplasmic domains, a domain located between amino acids 155 and 183 of CD36 involves in ox-LDL binding and other ox-LDL binding sites have also been reported such as amino acids 28–93 and possibly 120–155 [14]. CD36 expression in monocytes has been shown to be regulated by peroxisome proliferator‑activated receptor-γ (PPARγ) and certain cytokines, including interleukin-4 (IL-4), in a transcriptional manner and by hyperglycemia in a post-transcriptional manner [15]. A soluble form of CD36 (sCD36), a marker of altered tissue CD36 expression was recently identified in human plasma and elevated levels were found in obesity and type 2 diabetes [16]. The circulating concentration of CD36 is associated with markers of liver injury in subjects with altered glucose tolerance [17].

The *CD36* gene is encoded by 15 exons that extend more than 46 Kb on chromosome 7q11.2 [18]. The exons 1, 2, and 15 are noncoding, exons 3 and 14 encode N-terminal and C-terminal domains of the CD36 protein, respectively, the 5′-untranslated region of CD36 mRNA is encoded by 3 exons, exon 3 contains the last 89 nucleotides of the 5′-untranslated region and encodes the N-terminal cytoplasmic and transmembrane domains, the 3′-untranslated region is contained in exon 14 only or in exons 14 and 15 [19]. To date 1372 SNPs have been reported in human *CD36* gene, however, only few among them have showed physiological and clinical association with disorders such as dyslipidemia, hyperlipidemia, atherosclerosis and T2DM [20]. The aim of this study was to evaluate whether there is a relationship between five polymorphisms in the *CD36* gene with its serum levels and both monocyte and platelet expression in young subjects.

2. Materials and Methods

2.1. Subjects

This study included 188 subjects, 18 to 25 years old, 133 nonobese subjects with a body mass index (BMI) of 18.5 to 24.9 kg/m^2 and 55 obese subjects with a BMI ≥30 kg/m^2 from

the state of Guerrero, Mexico. The subjects were randomly selected; none of them had medication with lipid-lowering drugs such as statins or fibrates. The participants signed informed consent forms, and the protocol was approved by the Research Ethics Committee of the University of Guerrero.

2.2. Blood pressure

Blood pressure was measured in the sitting position with the use of an automatic sphygmomanometer on the left arm after 10 min rest. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from two readings with a minimal interval of 10 min. Hypertension was defined as mean SBP ≥140 mmHg and/or DBP ≥90 mm Hg [21].

2.3. Biochemical analysis

Blood samples were obtained from each subject after at least a 12 hours fasting. The serum lipid levels and glucose were determined by enzymatic methods with commercially available kits (Spinreact). Abnormal biochemical levels were identified when TC ≥ 200 mg/dL, TG ≥ 150 mg/dL, LDL-C >100 mg/dL, HDL-C < 40 mg/dL and glucose >100 mg/dL, based on the criteria of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) [22].

2.4. Determination of serum levels of oxLDL and CD36

Enzyme-linked immunosorbant assays (ELISA) for oxLDL (Mercodia Oxidized LDL ELISA) and CD36 (Human soluble CD36 ELISA kit, Aviscera Bioscience) were performed, according to the manufacturer's instructions, with an intra-assay CV of <6% and interassay CV of <7% for oxLDL assay and an intra-assay CV of <5% and interassay CV of <9% for CD36 assay.

2.5. Monocyte and platelet CD36 expression levels

Fasting venous blood was collected in K2-EDTA tubes and evaluated for monocyte and platelet CD36 expression by flow cytometry (guava easyCyte™ Flow Cytometer, Merck Millipore) running guavaSoft (Merck Millipore, version 2.2.3) acquisition and analysis software. Instrument setup and calibration were performed daily with Guava Easy Check

Kit beads according to the manufacturer's recommendations (Merck Millipore). The compensation was manually adjusted by an experienced operator. CD36 expression on 5,000 monocytes was measured using 25 µL of whole blood collected in EDTA anticoagulant was incubated at room temperature (18-25 ºC) for 30 min protected from light and antibodies (BioLegend): 5 µL anti-CD36-PE (clone 5-271) and 5 µL of anti-CD14-FITC (clone HCD14) and fluorochrome conjugated isotype controls; the cell suspension was lysed with lysing solution according to the manufacturer's directions (Sigma-Aldrich). The lysed sample was then washed in phosphate buffered saline (PBS) and resuspended in PBS with 2% paraformaldehyde prior to analysis. The CD36 fluorescence expression considering the median fluorescence intensity (MFI) for CD36-PE was recorded for each sample. Platelet CD36 expression was performed using 25 µL of whole blood collected in EDTA anticoagulant and was incubated at room temperature (18-25 ºC) for 30 min protected from light and antibodies (BioLegend): 5 µL anti-CD36-PE (clone 5-271) and 5 µL of anti-CD41-PE/Cy5 (clone HIP8), unrealized erythrocyte lysis, cells were resuspended in PBS and were acquired until 5,000 events populated the platelet region and the MFI recorded.

2.6. Genotyping

Genomic DNA was extracted from leukocytes in samples of whole blood and was stored at -20°C until analysis. Genotyping of the five polymorphisms -33137A/G (rs1984112), -31118G/A (rs1761667), -22674T/C (rs2151916), 27645 Ins/Del (rs3840546) and 30294G/C (rs1049673) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers for the polymorphims were designed and restriction enzymes (REs) were identified using the Primer 3 and NEB cutter software respectively. Details including the location of polymorphisms in the *CD36* gene, primer sequences and REs with product sizes are presented in Table 1.

3. Results

3.1. Anthropometric and biochemical characteristics

Anthropometric and metabolic characteristics of study participants are given in Table 2. The systolic and diastolic blood pressure, prevalence of hypertension, levels of TC, LDL-C, TG, oxLDL, CD36, and monocyte and platelet count were higher in obese than

nonobese subjects (P<0.05). However, monocyte CD36 expression level was higher in nonobese than in obese group (448 vs. 371, P=0.001).

3.2.Genotypic and allelic frequencies

In this study the genotype distribution of five polymorphisms -33137A/G, -31118G/A, -22674T/C, 27645Ins/Del and 30294G/C in the *CD36* gene were in Hardy-Weinberg equilibrium in the nonobese subjects (P=0.784, P=0.076, P=0.185, P=0.553, P=0.658, respectively). Table 3 shows significant differences in the distribution of genotype and allele frequencies for the polymorphism -31118G/A between obese and non obese subjects. However, there were no significant differences in the distribution of genotype and allele frequencies for the polymorphisms -33137A/G, -22674T/C, 27645Ins/Del and 30294G/C between the study groups.

3.3. Genotypes and serum lipid levels

The triglyceride levels were lower in subjects -31118AA carriers than non-carriers (P=0.028). There were no significant differences in the levels of TC, HDL-C, LDL-C and oxLDL for the polymorphisms -33137A/G, -22674T/C, 27645Ins/Del and 30294G/C in the *CD36* gene (data not shown).

3.4. Serum levels and CD36 expression by genotypes

Table 4 shows that subjects -33137GG carriers had a lower CD36 expression on monocyte and platelet compared with non-carriers (P=0.029). In this research, -33137GG genotype was associated with a decrease CD36 expression on monocyte and platelet, adjusted for age, gender, glucose, oxLDL and BMI (Table 5).

4. Discussion

In this study, we assessed whether there is a relationship between five polymorphisms in the *CD36* gene with its serum levels and both monocyte and platelet expression. As in previous studies, results of the present study show that obese subjects had higher TC, LDL and triglycerides levels than nonobese subjects [23,24]. This may be due to the increased adiposity, adipose tissue undergoes morphological and physiological changes, including the release of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), which decrease insulin sensitivity and increase lipolysis, these changes contribute to

insulin resistance and dyslipidemia [25]. Also known that increasing the lipolysis velocity leads an increased mobilization and increasing circulating levels of free fatty acids, thus the excess fatty acids on the liver stimulates triglyceride synthesis, assembly and secretion of lipoproteins very low density rich in cholesterol (VLDL-C) and increasing of the blood cholesterol levels [26].

Interestingly, in this study we observed that obese subjects had high monocyte and platelet count than nonobese group. However, there is a lack of published data about these findings, but has been reported that leukocyte count is considered as an indicator of inflammatory status in obesity, as has been positively correlated with BMI in other studies [27]. It is also known that interleukin-6 (IL-6) induces differentiation of megakaryocytes into platelets and the IL-6 is produced by adipose tissue [28,29]. In addition, has been reported that obese subjects have increased IL-6 levels [30].

We observed that circulating CD36 levels were higher in obese than in nonobese subjects (143.3 vs. 32.3 ng/mL, P=0.002), these results are consistent to the reported in previous studies [16,31]. This may be due to obese subjects showed an increased number of platelets and monocytes, as was recently reported that circulating CD36 is associated with microparticles mainly originated of platelets, leukocytes and endothelial cells as a result of stimuli or apoptosis [32,33]. In addition, these microparticles have also been found increased in patients with insulin resistance and in obese with type 2 diabetes, due to low grade inflammation that present [34-36].

In this research, oxLDL levels were higher in obese than in nonobese group (51.5 vs. 35.4 U/L), these results are similar with published data in other study [37]. This may be due to the increase of oxidative stress in obese state, which favors the oxidation of LDL-C [38,39]. The oxLDL accumulates in the sub-endothelial space of the arterial wall and activates endothelial cells, smooth muscle cells and macrophages, resulting chronic inflammatory response contributes to atherosclerotic plaque progression [40]. It is also known that an increase in oxLDL, as a consequence of increased oxidative stress and reduced antioxidant defenses, is a key event in the atherogenic process and a cardiovascular disease risk factor, which may be reflected in an increase in circulating oxLDL levels [41].

Our results show that monocyte CD36 expression was higher in nonobese than in obese subjects. This finding is in agreement with a previous study in Polish population, expression of scavenger receptor CD36 on monocytes in obese women was significantly lower than in lean individuals [42]. Another study observed that CD36 expression in blood cells was inversely correlated with BMI, the authors concluded that CD36 levels were influenced by genetic factors [43]. However, these results are in apparent contradiction to a study performed in Sweden by Gertow et al., who found a positive correlation between CD36 expression levels in subcutaneous adipose tissue with BMI, body fat and subcutaneous fat [44]. This discrepancy may due to that the control of CD36 expression in adipocytes is different in monocytes and other cell types [19].

CD36 gene is highly polymorphic; data from Ensembl Variation Build 60 (which is based on dbSNP Release 131) describe 2935 common genetic variants within 5 kb of the gene. Some involve putative transcription factor binding sites or sites in the 5`-untranslated region, which are of potential significance because translational efficiency of the CD36 mRNA and thereby CD36 protein expression levels have been shown to be regulated by variants in the 5`-untranslated region [45]. In this study the genotype frequencies of each polymorphism were in Hardy-Weinberg equilibrium. However, genotype and allele frequencies found in this study for each of the polymorphisms in the *CD36* gene are different to those reported in Caucasian population [46,47]. The influence of various races in our genetic background as Mexican population may explain the allele and genotype differences with respect to other populations. Mexicans are an admixed population, descended from a recent mix of Amerindian and European ancestry with a small proportion of African ancestry [48]. Although it is not clear how -33137A/G, -31118G/A -22674 T/C, 27645Ins/Del and 30294G/C polymorphisms can modulate the CD36 expression, has been demonstrated that -22674 T/C SNP is located upstream of the promoter, 14 bases 5`of the transcription start site, which is a binding element for the transcriptional repressor GFI1B, this SNP was in complete LD with the -33137A/G SNP in Caucasians from the general population, thus so has been reported that the allele A at position -33137A/G is in complete LD with the in3(TG)13 variant, which determines the expression of an alternative spliced, inactive transcript lacking exons 4 and 5 [46]. In addition the allele A of -31118 G/A SNP has been associated with reduced CD36 expression, lies between two alternative CD36 promoters, 1C and 1A [49]. It has been

suggested that the 27645Ins/Del and 30294G/C polymorphisms located in the 3`-UTR could determine decreased mRNA stability [46].

In this research the -33137GG genotype was associated with reduced levels of monocyte and platelet CD36 expression. However, it lacks of information about how this genotype may influence on CD36 expression. Further studies in other populations are needed to confirm such association.

5. Conclusions

The present study shows that polymorphisms in *CD36* gene may be related with both monocyte and platelet CD36 expression. Circulating CD36 levels were not associated with *CD36* gene polymorphisms.

Conflict of interests

No conflicts of interest.

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Preparation of Tables

Table 1: Characteristics of the polymorphisms studied in the *CD36* gene

Polymorphisms	Primer sequence	Annealing temp. (°C)	Product size (bp)	Restriction enzyme/allele sizes
-33137A/G (rs1984112)	F: 5`-CATGCAGCTCTGTTTTATGTGAG-3'	60	159	*MseI*
	R: 5`-CCCCATCTCTTAGGCCCGTGACA-3`			AA 67, 56, 29, 7
				AG 85, 67, 56, 29, 7
				GG 85, 67, 7
-31118G/A (rs1761667)	F: 5`-CAAAATCACAATCTATTCAAGACCA-3´	58	190	*HhaI*
	R: 5`-TTTTGGGAGAAATTCTGAAGAG-3´			AA 190
				GA 190, 138, 52
				GG 138, 52
-22674T/C (rs2151916)	F: 5`-TCTTGCTGGGCCCTGCCCA-3´	68	452	*Hpy 188I*
	R: 5`-TGTTTGCCCCAAGTGCTGGGTC-3´			TT 452
				TC 452, 430, 22
				CC 430, 22
27645Ins/Del16 (rs3840546)	F: 5`-GGGACCATTGGTGATGAGAAGG-3´	68	563	Ins/Ins
	R: 5`-TTGGAAAATGCACGGCCAGCA -3´		563, 547	Ins/Del
			547	Del/Del
30294G/C (rs1049673)	F: 5`-ACGCTTGGCATCTTCAGAATGCT-3´	60	465	*MnlI*
	R: 5`TGAACCCCTGCTCAAGAAACAGAGT-3´			GG 331, 134
				GC 331, 265, 134, 66
				CC 265, 134, 66

Table 2: Anthropometric and biochemical characteristics by group.

Variables	Nonobese	Obese	*P* value
n	133	55	-
BMI (kg/m^2)	21.5 (20.0-23.4)	33.1 (31.3-35.3)	0.001
SBP (mmHg)	104 (98-112)	115 (112-121)	0.001
DBP (mmHg)	68 (61-73)	70 (67-77)	0.003
Hypertension			0.001
No	124 (93.9)	35 (67.3)	
Yes	8 (6.1)	17 (32.7)	
TC (mg/dL)	152 (136-173)	160 (137-195)	0.057
HDL-C (mg/dL)	43 (38-54)	44 (38-47)	0.717
LDL-C (mg/dL)	89 (61-116)	117 (88-157)	0.001
TG (mg/dL)	72 (56-98)	121 (75-89)	<0.001
Glucose (mg/dL)	83 ((75-89)	83 (77-90)	0.386
Monocytes (%)	7 (5-9)	9 (7-11)	0.0005
Platelet ($10^3/mm^3$)	247 (215-289)	265 (232-307)	0.048
CD36 (ng/mL)	32.3 (16.8-102.4)	146.3 (76.7-694.7)	0.002
oxLDL (U/L)	35.4 (27.9-47.1)	51.5 (38.0-59.7)	<0.001
Monocyte CD36 expression (MFI)	448 (356-605)	371 (318-452)	0.001
Platelet CD36 expression (MFI)	132 (102-162)	130 (98-147)	0.259

BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; TG, Triglyceride; oxLDL, oxidized Low-Density Lipoprotein; MFI, Median Fluorescence Intensity. The values were presented as median (percentile 25-75^{th}). The difference between genders was determined by the Wilcoxon-Mann-Whitney test. Data of hypertension were presented in (n) and the percentages; the difference between genders was determined by chi-square test.

Table 3: Allelic and genotypic frequencies of *CD36* gene polymorphisms by group.

Polymorphism	Nonobese (133) n (%)	Obese (55) n (%)	*P* value
-33137 A/G			
Genotype			
AA	65 (48.9)	25 (45.5)	0.636
AG	55 (41.3)	22 (40.0)	
GG	13 (9.8)	8 (14.5)	
Allele			
A	185 (70)	72 (65)	0.654
G	81 (30)	38 (35)	
-31118 G/A			
Genotype			
GG	29 (21.8)	15 (27.3)	0.021
GA	55 (41.4)	31 (56.4)	
AA	49 (36.8)	9 (16.4)	
Allele			
G	113 (0.42)	61 (0.55)	0.022
A	153 (0.58)	49 (0.45)	
-22674 T/C			
Genotype			
TT	61 (45.9)	23 (41.8)	0.611
TC	53 (39.8)	26 (47.3)	
CC	19 (14.3)	6 (10.9)	
Allele			
T	175 (0.66)	72 (0.65)	0.854
C	91 (0.34)	38 (0.35)	
27645 Ins/Del			
Genotype			
Ins/Ins	120 (90.2)	49 (89.1)	0.814
Ins/Del	13 (9.8)	6 (10.9)	
Del/Del	-	-	
Allele			
Ins	253 (0.95)	104 (0.95)	0.819
Del	13 (0.05)	6 (0.05)	
30294 G/C			
Genotype			
GG	7 (5.3)	8 (14.6)	0.102
GC	51 (38.3)	19 (34.5)	
CC	75 (56.4)	28 (50.9)	
Allele			
G	65 (24)	35 (32)	0.141
C	201 (76)	75 (68)	

P value by chi-square test

Table 4: Serum levels and CD36 expression by genotypes.

Polymorphism	Serum levels (ng/mL)	Monocyte CD36 Expression (MFI)	Platelet CD36 expression(MFI)
-33137 A/G	*P= 0.302*	*P= 0.007*	*P= 0.012*
AA	57.6 (16.8-219.9)	450 (377-595)	137 (114-162)
AG	55.1 (21.7-261)	388 (327-504)	120 (76-166)
GG	27.1 (7.3-98.7)	315 (303-345)	95 (91-101)
-31118 G/A	*P= 0.597*	*P= 0.066*	*P= 0.231*
GG	26.5 (16.5-235.1)	326 (293-374)	162 (101-187)
GA	56.8 (22.1-208. 9)	426 (366-506)	130 (91-162)
AA	57.6 (14.8-200.4)	524 (403-628)	128 (113-147)
-22674 T/C	*P= 0.378*	*P= 0.070*	*P= 0.087*
TT	54.4 (25.7-261.0)	435 (346-591)	149 (100-173)
TC	46.7 (16.2-102.3)	416 (360-540)	127 (98-146)
CC	67.7 (15.4-190.6)	365 (298-392)	104 (96-130)
27645 Ins/Del	*P= 0.990*	*P= 0. 853*	*P= 0.177*
Ins/Ins	55.1 (19.4-200.4)	419 (346-536)	132 (98-166)
Ins/Del	51.7 (13.2-470.8)	435 (305-591)	100 (89-134)
Del/Del	-	-	-
30294 G/C	*P= 0.697*	*P= 0.424*	*P= 0.640*
GG	48.0 (15.4-90.1)	352 (305-491)	95 (83-149)
GC	35.0 (17.6-417.2)	407 (346-539)	132 (100-166)
CC	58 (22.4-)190.6	429 (345-540)	128 (99 -162)

P values by Kruskal Wallis test.

Table 5: Association of -33137A/G polymorphism with CD36 expression levels

	Monocytes					
	Model without adjusted			Model ajusted*		
Genotype	β	*P* value	95% IC	β	*P* value	95% IC
AG	-57.5	0.092	-124.7, 9.6	-31.7	0.398	-106.0, 42.5
GG	-128.1	0.027	-241.4, -14.7	-138.9	0.029	-263.6,-14.2
			Platelets			
AG	-17.0	0.077	-36.0, 1.8	-17.4	0.109	-38.9, 3.9
GG	-41.9	0.011	-73.9, -9.9	-46.1	0.013	-82.1, -10.1

*Adjusted by age, gender, glucose, oxLDL and BMI.

DISCUSIÓN

En este trabajo de investigación los polimorfismos -33137 A/G, -31118 G/A, -22674 T/C, 27645 Ins/Del y 30294 G/C en el gen del receptor CD36 se encontraron en equilibrio de Hardy-Weinberg (*P*>0.05) en el grupo control (232 jóvenes con peso normal) como se describe en el capítulo I, lo que nos permitió analizarlos como probables marcadores genéticos de susceptibilidad para dislipidemias y enfermedad cardiovascular en población guerrerense.

En el cuanto a los resultados obtenidos en los 188 sujetos que incluyó a jóvenes con y sin obesidad, en este trabajo al igual que en estudios anteriores el perfil de lípidos y la prevalencia de dislipidemias y glucosa alterada en ayuno fue mayor en los jóvenes con obesidad que los jóvenes con normopeso (Lima S *et al*., 2004, Romero E *et al*., 2007). Esto puede deberse a que con el incremento de la adiposidad, el tejido adiposo sufre cambios morfológicos y fisiológicos, incluyendo la liberación de citocinas proinflamatorias como el factor de necrosis tumoral alfa (TNF-α), que disminuyen la sensibilidad a la insulina e incrementan la lipólisis; estos cambios contribuyen a la resistencia a insulina y a la dislipidemia (Martin LJ *et al.*, 2005). Además, al incrementar la velocidad de la lipólisis provoca una mayor movilización y aumento de los niveles circulantes de ácidos grasos libres, el exceso de ácidos grasos sobre el hígado estimula la síntesis de triglicéridos, el ensamblaje y la secreción de lipoproteínas de muy baja densidad ricas en colesterol (VLDL-C), incrementando los niveles de colesterol en sangre (Contreras EA y Santiago J, 2011).

En este trabajo se observó que los niveles séricos de LDLox fueron mayores en los jóvenes con obesidad que en los jóvenes con peso normal (51.5 U/L vs 35.4 U/L); estos resultados son congruentes con lo reportado en otros estudios (Neuparth MJ *et al*., 2013); debido probablemente a la inflamación de bajo grado y al aumento del estrés oxidativo que se presenta en estado de obesidad, lo que incrementa la oxidación de las LDL (Fernández-Sánchez A *et al*., 2011, Savini I *et al*., 2013). Por género, los hombres tuvieron una mayor concentración de LDLox que las mujeres.

Sin embargo, se carece de datos publicados en cuanto a los niveles de LDLox por género; pero se ha documentado que los hombres presentan mayor estrés oxidativo en comparación con las mujeres, debido a un incremento en la generación de especies reactivas de oxígeno (EROS) y a una reducida actividad de antioxidantes, tomando en cuenta que bajo condiciones saludables, la respiración celular en la mitocondria origina EROS; una tasa metabólica basal más alta en los hombres que en las mujeres puede contribuir a un mayor nivel de estrés oxidativo en los hombres (Ide T *et al*., 2002), lo que puede reflejarse en un incremento en los niveles séricos de LDLox (João M *et al*., 2013).

En este trabajo, los niveles séricos de CD36 fueron más altos en los jóvenes con obesidad que en los jóvenes con peso normal; estos resultados concuerdan con lo publicado por otros estudios (Handberg A *et al*., 2006, Alkhatatbeh MJ *et al*., 2013). Lo que puede deberse a que los jóvenes con obesidad presentaron un mayor número de plaquetas y monocitos, ya que recientemente se demostró que la forma circulante del receptor CD36 se encuentra asociada a micropartículas originadas principalmente de plaquetas, leucocitos y células endoteliales como resultado de estimulación o apoptosis (Silverstein RL, 2009, Alkhatatbeh M, 2011), estas micropartículas se han encontrado incrementadas en pacientes con resistencia a insulina, diabetes tipo 2 y obesidad debido a la inflamación de bajo grado que presentan (Handberg A *et al*., 2009, Liani *et al*., 2012, Alkhatatbeh M, 2013).

En cuanto a los niveles de expresión del receptor CD36 en monocitos y plaquetas, se observó que los jóvenes con peso normal tuvieron mayores niveles de expresión del receptor CD36 en monocitos y plaquetas que los jóvenes con obesidad. Resultados similares fueron reportados en mujeres de origen polaco, ya que las mujeres con obesidad tuvieron una menor expresión del receptor CD36 en monocitos circulantes comparado con las mujeres con normopeso (Kuliczkowska-Płaksej *et al*., 2008). Una de las explicaciones a este hallazgo que sugirieron Kuliczkowska-Płaksej *et al*., en su trabajo es la presencia de polimorfismos en el gen CD36 en población polaca que influyen sobre la expresión del receptor CD36 asociados a obesidad, otra explicación

es que influyan factores relacionados con el género femenino, concluyendo que la expresión del receptor CD36 en monocitos circulantes no depende del grado y tipo de obesidad o de otros parámetros como las concentraciones de glucosa, requiriéndose futuras investigaciones. En otro estudio se observó que la expresión de CD36 en células sanguíneas se correlacionó de manera inversa con el IMC, atribuyéndolo a factores genéticos (Webb T *et al*., 2006). Sin embargo, estos resultados son contradictorios a los reportado por otros investigadores que han encontrado una correlación positiva de los niveles de expresión del receptor CD36 en tejido adiposo subcutáneo con el IMC (Gertow K *et al*., 2004). Esta discrepancia puede atribuirse a diferencias en la modulación de la expresión del receptor CD36 en los diferentes tipos celulares (Rać ME *et al*., 2007). Desafortunadamente, se carece de estudios en los que se reporten niveles de expresión del receptor CD36 en plaquetas, y se conoce poco sobre los factores que influyen en la regulación del gen del receptor CD36 en los megacariocitos.

En este trabajo se observó que los jóvenes con y sin obesidad que presentaban dislipidemias, glucosa alterada en ayuno e hipertensión tienen mayores niveles del receptor CD36 circulante que los jóvenes sin ninguna alteración (Anexo 1). Estos resultados son consistentes con estudios previos, donde los niveles circulantes de CD36 se han asociado con los triglicéridos, LDL-C, de manera inversa con el HDL-C, pero no con las LDLox en otras poblaciones (Glintborg *et al*., 2008; Handberg A *et al*., 2006, 2010). También se observó que tanto los jóvenes con obesidad como los jóvenes con peso normal que presentaban dislipidemias tuvieron una mayor expresión del receptor CD36 en monocitos (Anexo 2). Los jóvenes con hipercolesterolemia y HDL-C alterado tuvieron una mayor expresión del receptor CD36 en plaquetas (Anexo 3). En estudios previos se ha demostrado que tanto las LDL nativas como las LDLox pueden aumentar la expresión del receptor CD36 y contribuir a la formación de células espumosas (Han *et al*., 1997).

En este trabajo se determinó la presencia de los polimorfismos -33137 A/G, -31118 G/A, -22674 T/C, 27645 Ins/Del y 30294 G/C en el gen del receptor CD36, estos

polimorfismos se han encontrado en completo desequilibrio de ligamiento en población caucásica, el haplotipo AGGIG de estos polimorfismos se relacionó con un incremento de ácidos grasos libres en hombres italianos, sugiriendo que el haplotipo AGGIG se asocia con algún grado de deficiencia de la expresión del receptor CD36 en el músculo esquelético y otros tejidos en los que su expresión es normalmente alta, por lo tanto, la disminución en la eliminación de ácidos grasos libres se asocia con una elevación de sus niveles circulantes, reorientándolos hacia el hígado donde su absorción es independiente del receptor CD36, lo que lleva a una mayor producción de triglicéridos (Ma X *et al*., 2004). Sin embargo, la distribución de las frecuencias genotípicas obtenidas en este estudio fueron diferentes a las reportadas en población caucásica, probablemente a la variabilidad genética entre poblaciones, ya que se ha reportado que nuestra población Guerrerense tiene un 66% de origen indígena, 29% europea, 4% africana y 1% asiática (Silva I *et al*, 2009).

Aunque no está claro cómo es que estos polimorfismos pueden modular la expresión del receptor CD36 así como el metabolismo de lípidos, se ha reportado que el polimorfismo -22674 T/C se localiza río arriba del promotor, a 14 bases del sito de inicio de la transcripción, que constituye el sitio de unión para el factor represor de la transcripción GFI1B, el alelo A del polimorfismo -33137 A/G se encuentra en completo desequilibrio de ligamiento con el alelo T del polimorfismo -22674 T/C y con la variante in3(TG)13; esta variante determina la expresión de un transcrito inactivo que carece de los exones 4 y 5 (Ma X *et al*., 2004). También se ha reportado que el alelo A del polimorfismo -31118 G/A se asocia con una reducción en la expresión de CD36 [Pepino MY *et al*., 2012]. Se ha sugerido que la presencia de los polimorfismos 27645 Ins/Del y 30294 G/C localizados en la región 3´UTR podrían determinar una disminución del mRNA del receptor CD36 (Ma X *et al*., 2004).

En este trabajo, el genotipo -31137GG se asoció con una reducción en los niveles de expresión del receptor CD36 en monocitos y plaquetas. Sin embargo, se carece de evidencia científica que demuestre de que manera este genotipo puede influir sobre la expresión de CD36. Los niveles circulantes del receptor CD36 así como el perfil de lípidos no se asociaron con los polimorfismos en CD36 en los jóvenes con y sin

obesidad. Sin embargo, al realizar el análisis por haplotipos en el capítulo I en 232 jóvenes con peso normal se encontró que los portadores del haplotipo AATDC (12122) tienen 3.2 veces más riesgo de tener LDL-C>100 mg/dL (p=0.02); mientras que los portadores del haplotipo AATIC (12112) tienen 2.0 veces mayor riesgo de tener CT> 200 mg/dL (p=0.02). Lo que nos sugiere que un análisis por haplotipo considerando los cinco polimorfismos estudiados en el gen de *CD36* puede explicar variaciones en el perfil de lípidos más que un sólo polimorfismo. Sin embargo, son necesarias más investigaciones en otras poblaciones, que ayuden a explicar los mecanismos biológicos que demuestren estas asociaciones. Es importante mencionar que se han realizado estudios de otros polimorfismos en regiones codificantes del gen del receptor CD36 que representan los dominios de unión para los ácidos grasos y LDLox, como los polimorfismos IVS3-6 T/C (rs3173798) y IVS4-10 G/A (rs3211892), sin embargo, su frecuencia es muy baja y no se han encontrado los genotipos polimórficos (Rać ME *et al*., 2012).

En este trabajo de investigación, se concluye que los niveles circulantes del receptor CD36 y LDLox se asociaron con factores de riesgo cardiovascular como obesidad y las dislipidemias en jóvenes, lo que nos sugiere que los niveles circulantes del receptor CD36 y LDLox pueden indicar un mayor riesgo cardiovascular en etapas tempranas de la vida. El genotipo -33137GG se asoció con niveles de expresión del receptor CD36 disminuidos en monocitos y plaquetas, lo que sugiere un efecto protector para enfermedad cardiovascular.

A continuación se propone un modelo de integración de los factores de riesgo cardiovascular identificados en este trabajo de investigación en jóvenes con y sin obesidad:

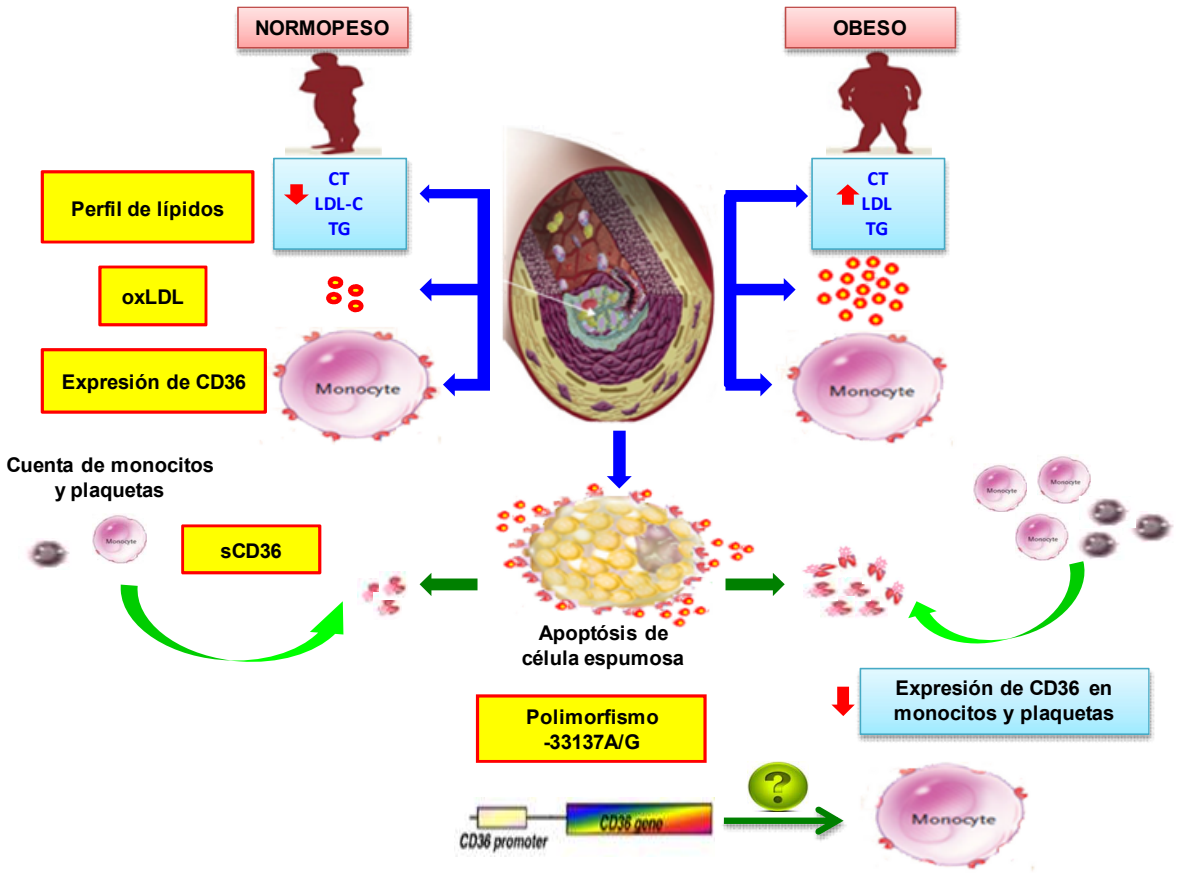

Figura 4. Modelo comparativo de factores de riesgo cardiovascular en jóvenes con normopeso y obesidad. Las concentraciones séricas del perfil de lípidos (CT,LDL-C, TG y LDLox) fueron mayores en los jóvenes con obesidad fueron mayores en los jóvenes con obesidad comparado con los de peso normal. Mientras que los niveles de expresión del receptor CD36 en monocitos fueron mayores en los jóvenes con normopeso. Sin embargo, los niveles circulantes del receptor CD36 fueron mayores en los jóvenes con obesidad, así como la cuenta de monocitos y plaquetas. Los jóvenes portadores del genotipo -33137GG tuvieron una disminución de los niveles de expresión de CD36 en monocitos y plaquetas comparado con los jóvenes portadores de los otros genotipos.

PERSPECTIVAS

Como perspectivas del trabajo se proponen los siguientes aspectos a considerar:

- Realizar las mediciones de CD36 y LDLox circulantes en personas que ya presentan una patología como la formación de placas ateroscleróticas, resistencia a insulina o diabetes mellitus tipo 2, comparándolos con los controles sanos.
- Medir los niveles de expresión del receptor CD36 en monocitos/macrófagos de muestras de placas de ateroma o en muestras de tejido adiposo por técnicas como la inmunohistoquímica y compararlos con los niveles circulantes del receptor CD36.
- Identificar las frecuencias genotípicas y alélicas de polimorfismos en el gen de CD36 que se localicen en regiones codificantes para la proteína, que representen sitios de reconocimiento del receptor CD36 con sus ligandos (ácidos grasos, LDLox) en población Guerrerense y evaluar su asociación con las concentraciones del perfil de lípidos aterogénico.
- Identificar las frecuencias genotípicas y alélicas de polimorfismos que se localicen en la región promotora del gen CD36, que puedan tener un efecto directo en los niveles de expresión del receptor CD36 y relacionarlos con sus niveles circulantes.

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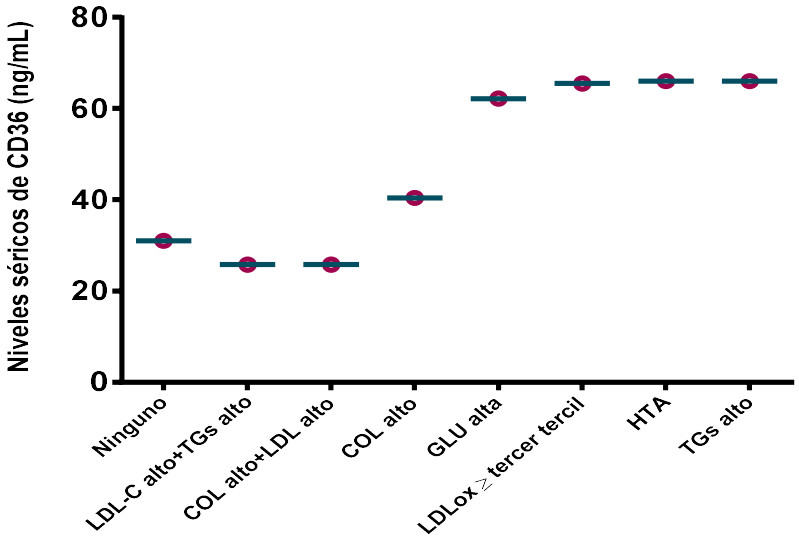
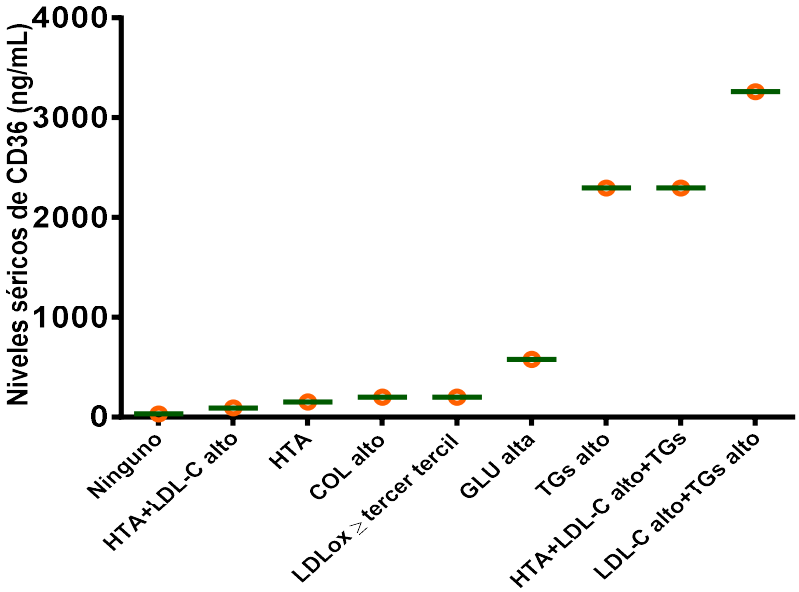
ANEXO 1

Niveles circulantes del receptor CD36 considerando algunas variables metabólicas en los grupos de estudio

Con peso normal

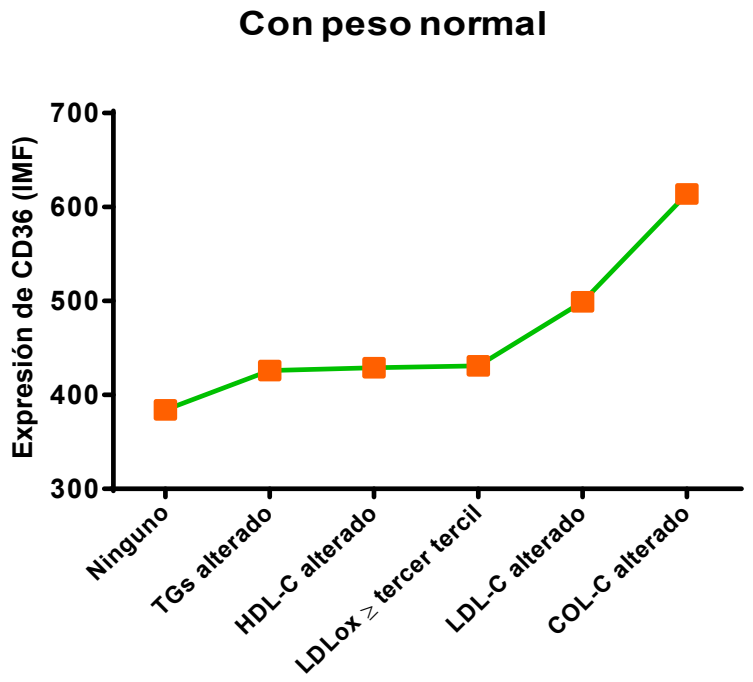


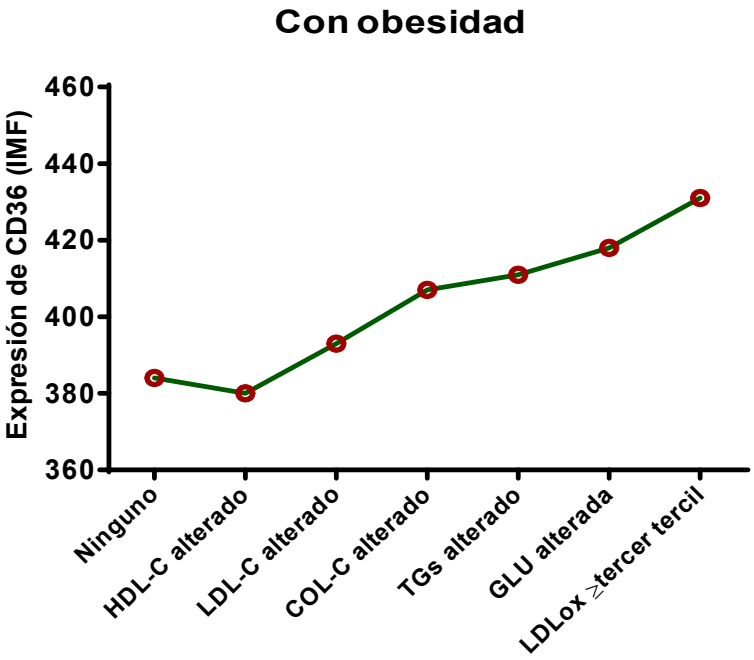
Con obesidad



ANEXO 2

Niveles de expresión del receptor CD36 en monocitos considerando las alteraciones del perfil de lípidos en los grupos de estudio

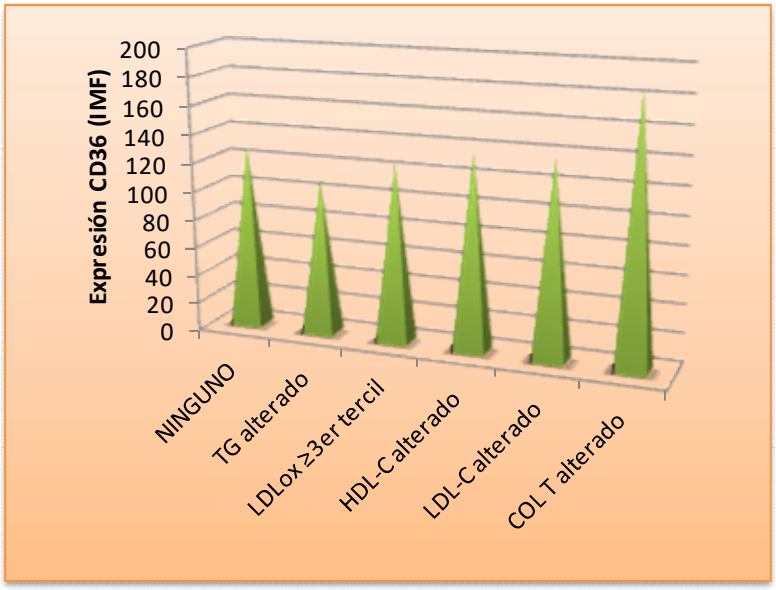




ANEXO 3

Niveles de expresión del receptor CD36 en plaquetas considerando las alteraciones del perfil de lípidos en los grupos de estudio

Con peso normal



Con obesidad