Universidad Michoacana de San Nicolás de Hidalgo
Instituto de Investigaciones Químico-Biológicas

“Efecto del hierro y el diazóxido sobre el desarrollo de la fuerza muscular y el estrés oxidante en ratas con diabetes”

T E S I S

Para obtener el grado de:

Doctor en Ciencias Biológicas
Opción: Biología Experimental

PRESENTA
M.C.S. Manuel Alejandro Vargas Vargas

Directora de Tesis
D. en C. Rocío del Carmen Montoya Pérez

Co-asesor
D. en C. Alfredo Saavedra Molina

Comité tutoral
D. en C. Christian Cortés Rojo
D. en C. Alejandra Ochoa Zarzosa
D. en C. Xóchitl Angélica Rosío Trujillo Trujillo

Morelia, Michoacán. Junio 2022

AGRADECIMIENTOS Y DEDICATORIA

- **Al todopoderoso por permitirme estar y darme fortaleza.**
- **A mis padres, Dago y Mary por sus grandes enseñanzas, son y seguirán siendo mi sostén y guía.**
- **A mis hermanos, César y Carlos por ser una gran inspiración y motivación.**
- **A mi compañera de vida Dani, por estar conmigo en las buenas y en las malas apoyándome siempre.**
- **A mi amigo Dr. Ramón Camacho Delgado, por iluminar el camino de la investigación científica.**
- **A mi directora de tesis, D.C. Rocío Montoya por creer en mí, por su apoyo y guía durante mi estancia doctoral.**
- **A mi co-director de tesis, D.C. Alfredo Saavedra por sus enseñanzas**
- **Al D.C. Christian Cortes por su guía, enseñanzas y su amistad**
- **A mis compañeros de laboratorio: Eridani, Donovan, Ever, Mau, Xoch, Julián y Mariana por sus enseñanzas y los buenos momentos**
- **Al Consejo Nacional de Ciencia y Tecnología (CONACYT) becario No. 305218 y a la Coordinación de la Investigación Científica de la UMSNH.**

ÍNDICE

ABREVIATURAS

- 4 amino fenazona (4-AF)
- Adenosina trifosato (ATP)
- Adenosina difosfato (ADP)
- Cadena trasportadora de electrones (CTE)
- Canales de potasio sensibles a ATP (K_{ATP})
- Canales de potasio sensibles a ATP mitocondriales (mitoK_{ATP})
- Canales de potasio sensibles a ATP sarcolemales ($_{SARCO}K_{ATP}$)
- Citocromo b duodenal (Dcytb)
- Decilitro (dL)
- Diabetes mellitus (DM)
- Diazóxido (dzx)
- Dihidroxiacetona fosfato (DAP)
- Especies reactivas de oxígeno (ERO)
- Ferroportina (FPN)
- Fosfatidil inositol 4,5 bifosfato (PIP_2)
- Glicerol 3 fosfato (G3P)
- Glicerol fosfato deshidrogenasa (GPO)
- Glicerol quinasa (GK)
- Glutatión total (GT)
- Glutatión oxidado (GSSG)
- Glutatión reducido (GSH)
- Inositol 4,5, bifosfato (PIP_2)
- Daltones (Da)
- Lipoprotein-lipasa (LPL
- Sustancias que reaccionan con el ácido tiobarbitúrico (TBARS)
- Transportador de metales divalente (DMT1)
- Organización Mundial de la Salud (OMS)
- Peroxidasa (POD)
- Potasio (K^+)
- Guanina monofosfato cíclico (cGMP)
- Proteína Cinasa G (PKG)
- Potencial de Hidrogeno (pH)

RESUMEN

La diabetes es una enfermedad crónico-degenerativa cuya principal característica bioquímica es la hiperglucemia. El estrés oxidante provoca un aumento en las complicaciones y el mal pronóstico de esta enfermedad. Uno de los síntomas más frecuentes en personas con diabetes es la fatiga muscular, esta condición impacta no solo la calidad de vida de las personas con esta enfermedad sino que compromete el correcto autocuidado que los pacientes deben tener. El hierro juega un papel clave en el aumento del estrés oxidante mediante la producción de radical hidroxilo,por otra parte, el diazóxido (dzx) actua en los canales de potasio sensibles a ATP (mitoK_{ATP}) provocando su apertura, disminuyendo la fatiga y generando una disminución en marcadores de estrés oxidante. **Objetivo** Evaluar el desarrollo de la fuerza muscular y parámetros de estrés oxidante en los diferentes grupos experimentales **Materiales y Métodos** Se emplearon ratas machos de la cepa Wistar, el diseño experimental consta de 3 etapas: 1^{era} etapa: 1) Control, 2) Diabética, 3) Alta en hierro, 4) Diabéticas + alta en hierro 5) Baja en hierro 6) Diabética + baja en hierro. 2da etapa: 1) Control, 2) Diabética, 3) Dzx, 4) Diabéticas + dzx. 3^{era} etapa: 1) Control, 2) Diabética, 3) Alta en hierro + dzx, 4) Diabéticas + alta en hierro + dzx 5) Baja en hierro + dzx 6) Diabética + baja en hierro + dzx. Para el análisis estadístico se empleó el software Graph Pad Prism 6. **Resultados** Las ratas dabéticas con administración de diazóxido y con una dieta baja en hierro mostraron un aumento en el desarrollo de la fuerza muscular, a su vez, los tiempos de aparición de la fatiga fueron mayores en comparación con el grupo de ratas diabéticas sin tratamiento. En cuanto al perfil lipídico, el diazóxido disminuyó los niveles de colesterol total y de trigliceridos en el grupo de ratas diabéticas tratadas con daizóxido en comparación con el grupo diabético no tratado, así como los niveles de lipoproteínas de muy baja densidad. Por otro lado, el grupo de ratas diabéticas tratados con daizóxido disminuyeron sus concentraciones de peroxidación lipídica y especies reactivas de oxígeno totales, y se preservaron las concentraciones de glutatión reducido. En cuanto a las ratas diabéticas con restricción de hierro, mostraron un aumento en el desarrollo de la fuerza muscular en ambos músculos. La restricción de hierro en la dieta disminuyó las concentraciones de triglicéridos, así como los niveles de lipoproteínas de muy baja densidad.

Palabras clave: Fatiga, radicales libres, estreptozotocina, musculo y célula.

ABSTRACT

Diabetes is a chronic-degenerative disease whose main biochemical characteristic is hyperglycemia. Oxidative stress causes an increase in complications and poor prognosis of this disease. One of the most frequent symptoms in people with diabetes is muscle fatigue, this condition impacts not only the quality of life of people with this disease but also compromises the correct self-care that patients should have. Iron plays a key role in increasing oxidative stress through the production of hydroxyl radicals, on the other hand, diazoxide (dzx) acts on ATP-sensitive potassium channels (mitoKATP) causing their opening, reducing fatigue and generating a decrease in markers of oxidative stress. Objective Evaluate the development of muscle strength and oxidative stress parameters in the different experimental groups Materials and Methods Wistar strain male rats were used, the experimental design consists of 3 stages: 1st stage: 1) Control, 2) Diabetic, 3) High in iron, 4) Diabetic + high in iron 5) Low in iron 6) Diabetic + low in iron. 2nd stage: 1) Control, 2) Diabetic, 3) Dzx, 4) Diabetic + dzx. 3rd stage: 1) Control, 2) Diabetic, 3) High iron + dzx, 4) Diabetic + high iron + dzx 5) Low iron + dzx 6) Diabetic + low iron + dzx. For the statistical analysis, the Graph Pad Prism 6 software was used. Results The diabetic rats with administration of diazoxide and with a low-iron diet showed an increase in the development of muscle strength, in turn, the times of onset of fatigue. were higher compared to the group of diabetic rats without treatment. Regarding the lipid profile, diazoxide decreased the levels of total cholesterol and triglycerides in the group of diabetic rats treated with daizoxide compared to the untreated diabetic group, as well as the levels of very low density lipoproteins. On the other hand, the group of diabetic rats treated with daizoxide decreased their concentrations of lipid peroxidation and total reactive oxygen species, and the concentrations of reduced glutathione were preserved. Iron-restricted diabetic rats showed increased muscle strength development in both muscles. Dietary iron restriction decreased triglyceride concentrations, compared with untreated diabetic rats, as well as very low-density lipoprotein levels.

1. INTRODUCCIÓN

1.1. Diabetes mellitus

La diabetes mellitus es una enfermedad crónico degenerativa cuya principal característica bioquímica es la hiperglucemia, esto se puede deber a defectos en la secreción de insulina por parte de las células β pancreáticas o bien a una deficiente acción de la hormona en los tejidos periféricos, especialmente en el músculo esquelético, condición que también se le conoce como resistencia a la insulina (1).

El número de personas con diabetes ha aumentado de 108 millones en 1980 a 422 millones en 2014. La prevalencia mundial de la diabetes ha aumentado con mayor rapidez en los países de ingresos medianos y bajos, así como en adultos (mayores de 18 años) ya que está registra un aumento del 4,7% en 1980 al 8,5% en 2014 (2). El factor genético juega un papel muy importante en el desarrollo de la diabetes, sin embargo, el factor ambiental es un detonador en muchos de los casos para desencadenar dicha enfermedad (3).

1.2. Tipos de diabetes

Asignar un tipo de diabetes a una persona a menudo depende de las circunstancias presentes en el momento del diagnóstico, y muchos individuos con esta enfermedad no encajan fácilmente en una clase única, por ejemplo, una persona con diabetes mellitus gestacional (DMG) puede seguir teniendo hiperglucemia después del parto y se puede determinar que, en sí, padece diabetes tipo 2 o una persona que desarrolla hiperglucemia debido al consumo de grandes dosis de esteroides exógenos, sin embargo, una vez que se suspenden dichos fármacos sus niveles de glucosa circulante se pueden normalizar (4).

La Organización Mundial de la Salud (OMS) con datos del 2018 clasifica la diabetes en 4 grupos:

- Diabetes tipo 1: También conocida como diabetes insulino-dependiente o diabetes juvenil, este tipo de diabetes se caracteriza por una destrucción total de las células β del páncreas y como consecuencia una deficiente producción de insulina y requiere la administración diaria de esta hormona.

- Diabetes tipo 2: También conocida como no insulino-dependiente o de inicio en la edad adulta, se debe a una destrucción parcial de las células productoras de insulina acompañada de resistencia a la insulina. Hasta hace poco este tipo de diabetes, solo se observaba en adultos, pero en la actualidad también se está presentando en infantes.
- Diabetes gestacional: Caracterizada por niveles elevados de glucosa durante el embarazo, las mujeres con diabetes gestacional corren mayor riesgo de sufrir complicaciones durante el embarazo y el parto, además tanto ellas como su hijo tienen un riesgo mayor de desarrollar diabetes tipo 2.
- Otros tipos de diabetes: Se caracterizan por el uso prolongado de fármacos diabetogénicos o por algún traumatismo que dañe de forma parcial el páncreas y comprometa la secreción de insulina (1,4).

1.3. Complicaciones de la diabetes

La hiperglucemia crónica se asocia con complicaciones a largo plazo ocasionando daños micro y macro vasculares: arterioesclerosis, retinopatía, nefropatía, neuropatía, pie diabético (5) y sarcopenia, esta última hace referencia a la pérdida de masa y potencia muscular que ocurre durante el proceso del envejecimiento (6), sin embargo, esta condición es muy frecuente en enfermedades como la diabetes (7). El músculo esquelético en uno de los principales sitios de acción de la insulina y los receptores de dicha hormona en el músculo juegan un papel fundamental en la regulación de la glucosa circulante, evitando así la hiperglucemia, también es importante mencionar que dicha hormona es anabólica y participa en la síntesis de proteínas y su deficiencia está estrechamente relacionada con la disminución de la masa muscular y por ende la fatiga ya que el proceso de degradación de proteínas, así como la síntesis de las mismas se repite constantemente en el músculo esquelético, por lo que defectos en la secreción de insulina o en la señalización provoca una reducción en la síntesis de proteínas musculares (8).

1.4. Fatiga muscular y diabetes

Existen diversas maneras para definir la fatiga muscular, Asmussen en 1979 la definió como la disminución transitoria de la capacidad de trabajo del músculo esquelético durante la

actividad física; Edwards en 1981, la consideró como la incapacidad de mantener la potencia desarrollada, es decir, la intensidad del esfuerzo durante un determinado tipo de ejercicio. A pesar de las deferentes definiciones que se puedan encontrar en la literatura todas ellas reconocen, en mayor o menor medida, ciertas características fundamentales de la fatiga como la disminución de la capacidad de esfuerzo o rendimiento y la disminución de la capacidad para generar fuerza muscular máxima (9).

La fatiga muscular es uno un síntoma común en las personas que padecen diabetes. Martínez y cols. en 2014, reportaron que en las personas con diagnóstico reciente de diabetes tipo 2, el 61% la fatiga muscular como el segundo síntoma más frecuente (10), las variaciones en la glucosa (hipoglucemia o hiperglucemia) circulante son un factor importante para desencadenar episodios de fatiga aunque también se ha relacionado con factores psicológicos, como la depresión o la angustia emocional relacionada con el diagnóstico o con la intensidad de los regímenes de autocontrol de la diabetes, también puede estar relacionada con problemas de estilo de vida, como la falta de actividad física o el sobrepeso, especialmente común en las personas con diabetes tipo 2 (11).

La fatiga muscular se ha relacionado con factores fisiológicos como la apertura de los canales de potasio sensibles a ATP (K_{ATP}), el aumento en el estrés oxidante, y los efectos del óxido nítrico en el desarrollo de la fuerza muscular (12).

1.5. Músculo esquelético y sus principales fuentes de producción de especies reactivas de oxígeno

El músculo esquelético constituye el 40% de la masa corporal de un organismo y es el responsable de casi todo el consumo de oxígeno que consume un animal durante esfuerzo máximo sostenido (13),

La actividad muscular provoca aumento en la producción de ERO, se han identificado varios productores potenciales de ERO en las células musculares los cuales se activan por diferentes estímulos. Entre estos se encuentran las mitocondrias, el nicotinamida adenina dinucleótido fosfato, oxidasas, fosfolipasa A2, xantina oxidasa y lipoxigenasas (14).

1.6. Canales de potasio sensibles a ATP

Los organismos vivos han desarrollado diversos medios para monitorear el estado energético y así mantener la homeostasis. Los K_{ATP} pueden bloquear el paso de iones de potasio al aumentar niveles intracelulares de ATP para mejorar la excitabilidad de la membrana, transformando el estado metabólico celular en señales eléctricas. Estos canales se distribuyen ampliamente en muchos tejidos y participan en numerosos procesos biológicos importantes por ejemplo en células β pancreáticas, los K_{ATP} detectan indirectamente la concentración de glucosa en la sangre y provocan la liberación de insulina (15). Un aspecto muy importante de dichos canales es que las mutaciones genéticas de los genes que codifican las subunidades K_{ATP} ocasionan enfermedades como diabetes neonatal, hiperinsulinismo, síndrome de diabetes neonatal permanente (DEND) y el síndrome de Cantú, lo que indica que los K_{ATP} son dianas terapéuticas importantes. Los inhibidores del canal K_{ATP}, como las sulfonilureas, son ampliamente utilizados para tratar la diabetes tipo 2, mientras que los activadores de los K_{ATP}, se utilizan para tratar hiperinsulinismo y para la mioprotección (16).

Los canales de potasio sensibles a ATP funcionales son complejos proteicos hetero-octaméricos, compuestos por cuatro unidades rectificadoras de potasio (Kir6.1 o Kir6.2) y cuatro unidades de la familia ABC (trasportadores dependientes de ATP; ATP binding cassette) Subunidades del receptor de sulfonilurea (SUR1, SUR2A o SUR2B), con un peso molecular total de alrededor de 880 kDa (Fig. 1 y 2). El canal Las subunidades de formación de poros Kir6.x se identifican como el sensor para el ATP y su actividad está regulada por el inositol 4, 5, bifosfato (PIP_2) (17).

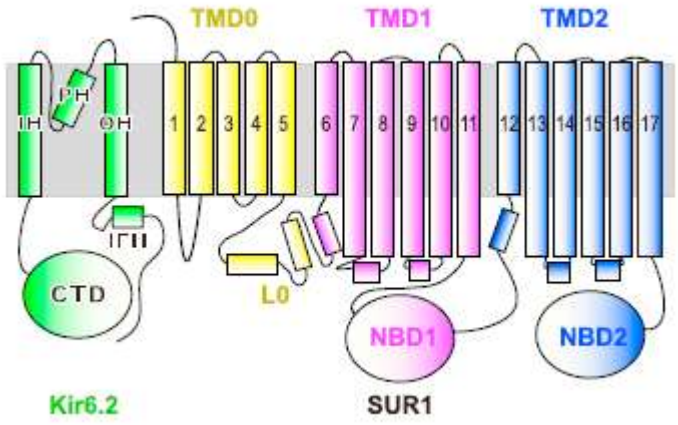


Figura 1. Representación del canal K_{ATP} con regiones Kir6.2 y SUR1. OH: hélice exterior; PH: hélice de poro; IH: hélice interior; CTD: dominio citoplásmico; TMD: dominio transmembranal; L0: bucle 0; NBD: dominio de unión a nucleótidos.

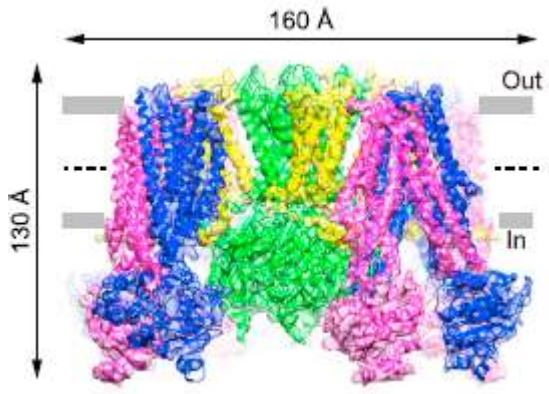


Figura 2. Vista lateral del complejo K_{ATP}. La extensión aproximada de la bicapa de fosfolípidos se muestra como líneas grises gruesas.

1.7. Canales de potasio sensibles a ATP sarcolemales

La función de los canales K_{ATP} del sarcolema ($_{SARCO}K_{ATP}$) depende de la concentración de ATP. Este tipo de canales tiene baja probabilidad de apertura en condiciones de reposo, pero incrementan su apertura cuando bajan las concentraciones de ATP, promoviendo la entrada de potasio, con lo cual aleja a las células del umbral de excitación. Así, la actividad electrofisiológica de los canales K_{ATP} acopla el estado metabólico de la célula y su función ha recibido importancia en enfermedades tales como la diabetes, la isquemia cardiaca o neuronal y en la fatiga muscular (18). En un estudio previo se observó que la recuperación de la fuerza tetánica después de la fatiga la cual fue significativamente menor en los ratones con knock-out de $K_{ir}6.2$ en comparación con los ratones control, estos datos sugieren que la función principal del canal $_{SARCO}K_{ATP}$ durante la fatiga, es reducir el desarrollo de la tensión cuando el músculo se encuentra en un estado de reposo y que por lo tanto el $_{SARCO}K_{ATP}$ juega un papel importante en la protección muscular (19, 20,21)

1.8. Canales de potasio sensibles a ATP mitocondriales

La apertura de mitoK_{ATP}, puede ocurrir por 3 mecanismos distintos:

- Directo: mediante la administración de un abridor del K_{ATP}
- Indirecta: por activación de PKCε1
- Fisiológica: mediante cinasas de señalización como la PKG (Fig. 3)

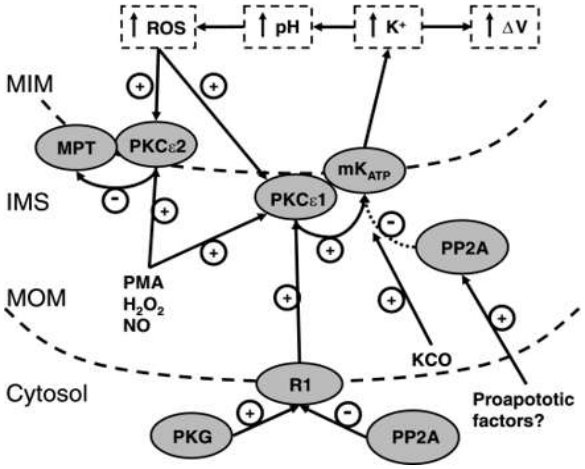


Figura 3. Formas distintas de abrir el mitoK$_{ATP}$ e iniciar la secuencia de señalización (Tomado de: Costa y Garlid, 2008)

La PKG es el último paso citosólico en la vía de señalización que provoca la apertura del mitoK$_{ATP}$ en mitocondrias aisladas en la misma medida que cromakalim y diazóxido, la PKG interacciona con las mitocondrias transmitiendo la señal a una PKCε (PKCε1) unida a la membrana interna mitocondrial (MIM), que a su vez fosforila mitoK$_{ATP}$ y hace que se abra. El aumento resultante en la afluencia de K^+ acompañada de una alcalinización de la matriz mitocondrial provoca una mayor producción de ERO por el complejo I de la cadena respiratoria. Este aumento de ERO luego activa una segunda membrana interna PKCε (PKCε2), que inhibe al poro de permeabilidad transitoria, el cual es considerado como la causa de muerte celular después de la isquemia reperfusión (22).

1.9. Diazóxido

El diazóxido es un activador del canal de potasio que aumenta la permeabilidad a los iones de potasio a través de las membranas plasmáticas e induce una relajación local en los músculos lisos. Esto provoca que los canales de calcio dependientes de voltaje se cierren, previniendo entrada de calcio a través del sarcolema y evita que se lleve a cabo la contracción muscular.

Uno de los mecanismos cardioprotectores de mitoK$_{ATP}$ sugiere que la apertura es a través de la activación de una proteína quinasa (PKCε), en los miocitos, la PKCε se dirige a varias proteínas mitocondriales, como el poro de transición de la permeabilidad mitocondrial, e inhibe su apertura, ya que cuando este se abre provoca alteración en la función mitocondrial y apoptosis (23)

Se utiliza como vasodilatador para tratar la hipertensión aguda y maligna, así como también para tratar la hipoglucemia ocasionada por un insulinoma ya que este fármaco inhibe la secreción de insulina de las células β sin embargo, estudios recientes han demostrado que el diazóxido puede prevenir y revertir la obesidad en modelos animales mediante el control de la hiperinsulinemia. La apertura directa de mitoK$_{ATP}$ por fármacos actúa sobre los receptores reguladores de sulfonilurea (SUR) de los canales K$_{ATP}$ (24).

Kowaltowski y cols. en el año 2000 demostraron que la apertura y el cierre de los mitoK$_{ATP}$ con el diazóxido provoca cambios significativos en el volumen de la matriz mitocondrial, como consecuencia de iones K^+ acompañados de agua, lo que provoca un mantenimiento correcto del volumen mitocondrial y así se conserva la arquitectura de compartimientos importantes, evitando que se alterara la estructura y función de diversos complejos proteicos los cuales forman parte de la cadena transportadora de electrones (CTE) manteniendo un equilibrio correcto de transferencia de energía entre mitocondria y citosol (25) Se ha postulado que los mitoK$_{ATP}$ desempeñan un papel fundamental en la función muscular, incluso teniendo un efecto protector sobre el músculo durante la fatiga (12).

En un estudio realizado por García y cols. en el año 2009 se evaluó el papel de la apertura de los canales mitoK$_{ATP}$ en músculo esquelético de ratones adultos, sus resultados demostraron que la administración de diazóxido (100 μM) redujo significativamente la fatiga muscular, a su vez mejoró parámetros de estrés oxidante entre ellos, la disminución de producción de ERO en mitocondrias aisladas durante el periodo de recuperación de la fatiga, los niveles de TBARS y una disminución en los niveles de GSSG y un aumento de GSH (26).

1.10. Especies reactivas de oxígeno y estrés oxidante

Las especies reactivas de oxígeno, como el anión superóxido (O_2^-), peróxido de hidrógeno (H_2O_2), y el radical hidroxilo (HO•), son moléculas de oxígeno radicales y no radicales, las cuales se forman por el reducción parcial de oxígeno, se generan endógenamente mediante procesos

fisiológicos como la cadena transportadora de electrones (Fig. 4) o mediante interacciones con fuentes exógenas como los compuestos xenobióticos (27), al desbalance entre las ERO y los sistemas de defensa antioxidantes se le conoce como estrés oxidante y su consecuencias son lesiones celulares como daño al ADN, proteínas y lípidos, que provoca una alteración en la homeostasis celular y acumulación de moléculas dañadas y está ampliamente reconocido como el responsable de las complicaciones en diferentes patologías entre ellas la diabetes (28, 29,30, 31).

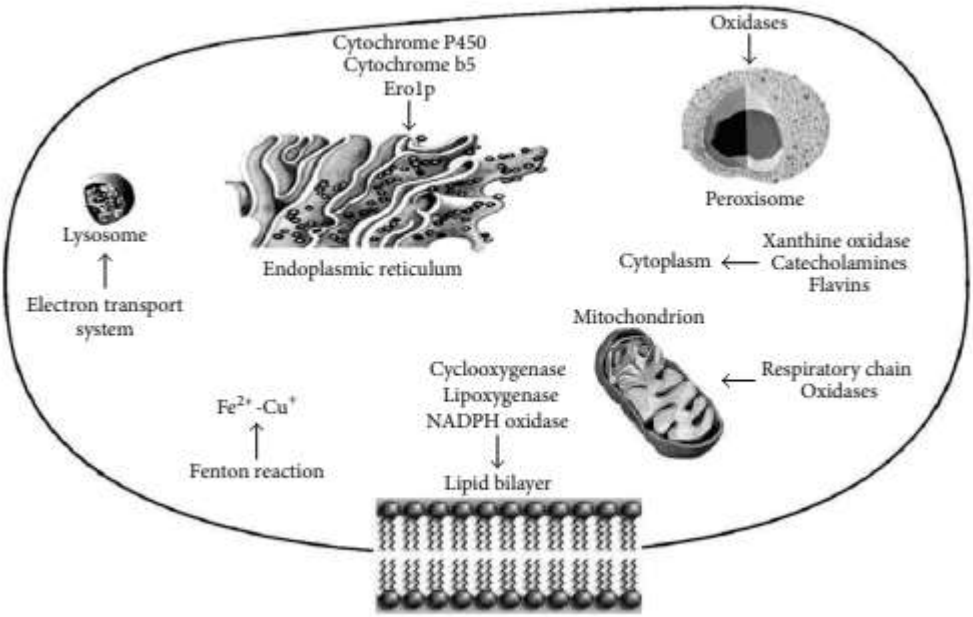


Figura 4. Fuentes celulares de producción de ERO. Los orgánulos celulares y sus componentes estructurales contribuyen a la producción de una amplia variedad de especies reactivas.

1.11. Sistemas de defensa antioxidantes

El término "antioxidante" se define como cualquier sustancia incluso en concentraciones mínimas, que inhibe o retarda la oxidación de un sustrato. Existen antioxidantes endógenos (internamente sintetizadas) y exógenos (consumidos), los cuales juegan un papel en la defensa antioxidante y pueden considerarse biomarcadores del estrés oxidativo (32). La regulación del estado redox es crítica para la viabilidad celular, la activación, la proliferación y la función del órgano. Los organismos aeróbicos tienen sistemas antioxidantes, los cuales incluyen antioxidantes enzimáticos y no enzimáticos (Fig. 5) que suelen ser eficaces para

bloquear los efectos dañinos de las ERO, sin embargo, en condiciones patológicas, los sistemas antioxidantes se ven rebasados (33).

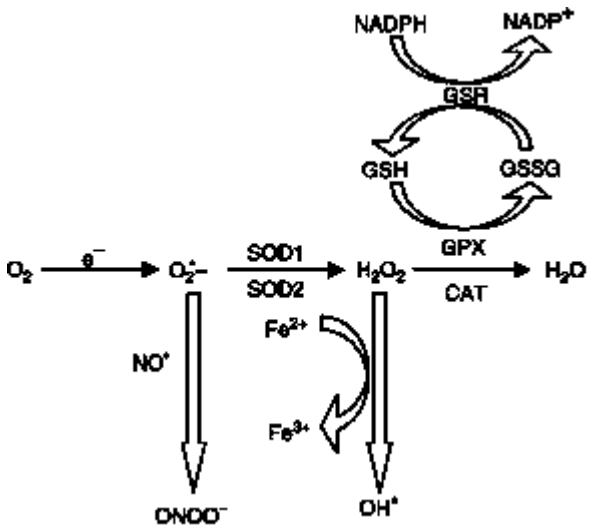


Figura 5. Sistema de defensa antioxidante primario, compuesto por cuatro enzimas: superóxido dismutasa, glutatión peroxidasa, glutatión reductasa y catalasa.

1.12. Hierro y su participación en la formación de radical hidroxilo

El hierro es un metal muy importante para procesos metabólicos ya que es cofactor de los citocromos para la transferencia de electrones en la cadena respiratoria y de algunas enzimas que participan en el ciclo de Krebs, además participa en la actividad de enzimas antioxidantes como las catalasas y peroxidasas, es también un componente esencial de la hemoglobina y de la mioglobina (34).

El hierro dietético entra al cuerpo a través de enterocitos duodenales, los cuales juegan un papel crucial en la regulación de las concentraciones de hierro en el cuerpo humano, en cuanto a la captación de hierro en su forma previamente reducida (Fe^{2+}) por la acción del ácido clorhídrico, ingresa al enterocito a través del transportador de metales di Valente (DMT1), citocromo b ferrireductasa y la citocromo b duodenal (Dcytb), posteriormente su exportación hacia el torrente sanguíneo se lleva a cabo mediante el exportador ferroportina (FPN), previa oxidación del hierro (Fe^{2+} a Fe^{3+}) mediada por la hefaestina, unaferroxidasa ligada a membrana (35) este proceso es un punto crucial en la regulación del hierro corporal; la cantidad del FPN en la membrana basolateral determina si el hierro se transporta al cuerpo y los niveles de expresión de FPN están regulados por la hormona peptídica, hepcidina. Una vez que el hierro entra al torrente sanguíneo este viaja unido a una proteína llamada transferrina. La hepcidina se encuentra

regulada y es secretada por los hepatocitos en el hígado, la hepcidina sérica se une a la FPN y estimula la internalización y posterior degradación de la FPN, lo que la convierte en un regulador negativo de la afluencia de hierro en todo el cuerpo (36).

Posteriormente el hierro es ingresado a las células por el receptor de transferrina este receptor desempeña un papel fundamental en el suministro de hierro a la célula, una vez unida la transferrina a su receptor se forma el complejo hierro-transferrina-receptor el cual es internalizado en la célula a través de un proceso de endocitosis. La liberación dentro de la célula del hierro unida a la transferrina es secuencial. La primera molécula es liberada por el pH ácido del citosol, mientras la segunda requiere ATP para su liberación. Una vez dentro de las células el hierro se deposita como ferritina, en donde cada molécula de ferritina puede contener hasta 4500 átomos de hierro, aunque normalmente tiene alrededor de 2500, almacenados como cristales de hidróxido fosfato férrico (30)

Diversos estudios muestran que la acumulación de hierro en el organismo puede aumentar el riesgo a desarrollar diabetes mellitus, y con su disminución tiene un efecto protector (37,38) ya que la toxicidad del hierro se basa en las reacciones de Fenton y Haber-Weiss, donde pequeñas cantidades del mineral son suficientes para formar radicales hidroxilo (OH•) a partir de superóxido ($O_2^{\bullet}$) y peróxido de hidrógeno (H_2O_2), conocidos como reactivos intermediarios de oxígeno, los cuales forman radicales libres (39) .

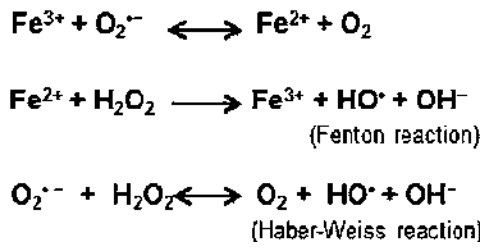


Figura 6. Reacciones de Fenton y Haber-Weiss que resultan en la generación de radicales hidroxilos en presencia de Hierro.

El hierro acelera la producción de especies reactivas de oxígeno, y a su vez dicho aumento aceleran la fatiga del músculo esquelético, los pacientes con una enfermedad de sobrecarga de hierro con frecuencia informan síntomas de debilidad y fatiga (40)

1.13. Sobre carga y restricción de hierro

Se ha reportado en ratones que la sobre carga produce resistencia a la insulina y niveles elevados de hepcidina (regulador central del metabolismo del hierro) (41). La reducción de hierro, mediante flebotomía, quelantes de hierro o una dieta baja en hierro, aumenta la sensibilidad a la insulina y su secreción por parte del páncreas (42); Por otro lado, Yasumasa y cols. en el año 2013 reportaron que la restricción dietética de hierro ejerce un efecto preventivo sobre la progresión de la nefropatía diabética en parte debido a la reducción del estrés oxidante. Diversos estudios experimentales, epidemiológicos y clínicos han demostrado la utilidad de los antioxidantes para el tratamiento la diabetes y sus complicaciones, disminuyendo el estrés oxidante (43).

2. JUSTIFICACIÓN

La diabetes es un problema de salud mundial y México ocupa actualmente el noveno lugar mundial en la prevalencia de diabetes. Esto es alarmante, y más aún cuando las proyecciones de los especialistas internacionales refieren que para el año 2025, el país ocupará el sexto o séptimo lugar, así mismo representa un problema grave para el sector salud debido a los altos costos para tratar esta enfermedad (44).

En México, el Instituto Mexicano para la Competitividad reportó que cada año, la diabetes absorbe 85 mil millones de pesos en costos sociales como tratamiento médico, ausentismo laboral y pérdidas de ingreso por mortalidad prematura (45). Actualmente no existen reportes en los cuales se haya evaluado el efecto del hierro y del diazóxido sobre diversos parámetros de estrés oxidativo y fatiga muscular, por lo que el tratamiento de una dieta baja en hierro y un abridor de los canales K_{ATP} podría mejorar tanto la condición oxidante como el mejoramiento de la fuerza reduciendo así una de las principales sintomatologías reportadas por las personas que padecen diabetes.

3. HIPÓTESIS

Una dieta baja en hierro y el diazóxido disminuyen el estrés oxidante y mejoran el desarrollo de la fuerza muscular en ratas con diabetes.

4. OBJETIVOS

4.1 Objetivo general

Evaluar el efecto del hierro y el diazóxido en el desarrollo de la fuerza muscular y estrés oxidante en ratas con diabetes.

4.2 Objetivos particulares

1. **Evaluar la fatiga muscular**

1.1. Análisis de tensión isométrica

1.2. Tiempo de establecimiento de la fatiga

2. **Medición de parámetros bioquímicos**

2.1 Determinación de perfil de lípidos

2.3 Medición del perfil de lípidos (Colesterol total, HDL, VLDL y triglicéridos)

3. **Evaluar el estrés oxidativo en los homogenados de músculo**

3.1. Peroxidación de lípidos de membrana

3.2. Evaluar la relación Glutatión reducido/Glutatión oxidado (GSH/GSSG)

3.3. Especies reactivas de oxígeno totales

5. MATERIAL Y MÈTODOS

5.1. Animales

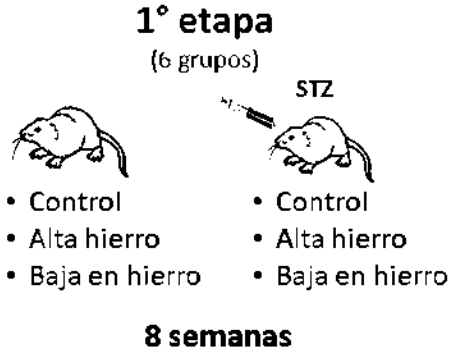
Para la presente investigación se utilizaron ratas macho Wistar con un peso de 250 - 350 g obtenidas del bioterio del IIQB de la Universidad Michoacana de San Nicolás de Hidalgo, los cuales se mantuvieron en jaulas de acrílico bajo condiciones controladas de luz-oscuridad (12h/12h) y temperatura (25 ± 2 °C), con suministro agua *ad libitum* y alimentadas con croquetas de la marca Rodent (200 mg hierro/kg).

5.2. Inducción de diabetes experimental

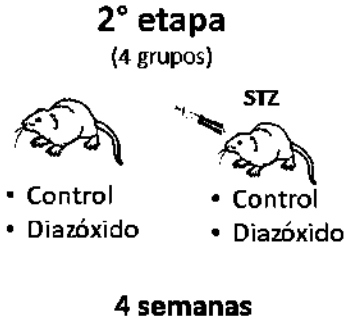
Las ratas recibieron una dosis única de streptozotocina intra-peritoneal a una dosis de 45 mg/kg de peso (STZ, Sigma-Aldrich CO. St. Louis MO, USA). Las ratas con niveles de glucosa superiores a los 200 mg/dL tras un ayuno de 8 h fueron usadas para el presente estudio y fueron consideradas diabéticas (46). Las ratas que se encontraron enfermas fueron excluidas del estudio.

5.3. Diseño experimental

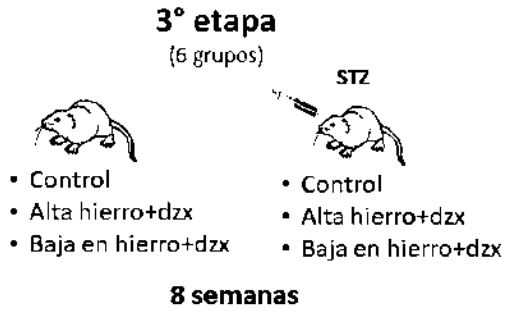
El presente estudio constó de 3 etapas. En la primera etapa se formaron 6 grupos de animales divididos de la siguiente manera:



En la segunda etapa se utilizaron de 4 grupos:



Por último, en la 3er etapa experimental se utilizaron 6 grupos de animales divididos de la siguiente manera:



5.4. Formulación de dietas y administración de diazóxido

Dieta control

La dieta control (D12050203) contenía 100 mg/kg de hierro (Research Diets, Inc.).

Dieta baja en hierro

La dieta baja en hierro (D03072501) contenía 3 mg/kg (Research Diets, Inc. New Brunswick, NJ, EE.UU.); Estas dietas serán similares a las utilizadas por previos estudios (Minamiyama et al. 2010); En donde la dieta baja en hierro contenía 3,2 mg/kg de dieta y la dieta de control, 100,9 mg/kg. Cada dieta se utilizó durante ocho semanas.

Dieta alta en hierro

Para la suplementación de dietas altas en hierro, a los animales se les administró la dieta control la cual contiene 100 mg/kg de hierro (Research Diets, Inc.) más la administración hierro

polimaltosado (Takeda, México, SA de CV, bajo licencia de Vifor International, Inc., Suiza) por administración oral a través de una sonda orogástrica (Industrial Medical Plastica Silice SA de CV, México) a una dosis de 3 mg de hierro/kg de peso corporal/día, durante cuatro semanas; Esta dosis fue previamente probada para causar una sobrecarga de hierro (47,48

Administración de diazóxido

La administración de diazóxido se realizó a través de una inyección intra peritoneal a una dosis de 45 mg/kg de peso corporal al día, este tratamiento tuvo 14 días de duración, para permitir la comparación con estudios realizados previamente (49).

A continuación, se muestra en la siguiente tabla los diferentes tratamientos:

ALIMENTACIÓN	
Control	100 mg hierro/kg
Baja en hierro	3 mg hierro / kg
Alta en hierro	Dieta control + 3 mg de hierro / kg
Diazóxido	40 mg / kg

5.5. Método de eutanasia

Las ratas fueron sacrificadas por decapitación y todos los procedimientos experimentales se realizaron de acuerdo con la Norma Oficial Mexicana (NOM 062-ZOO-1999) de especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio.

5.6. Registro de tensión y tiempos de fatiga

Una vez sacrificadas las ratas se diseccionó el músculo sóleo y EDL de ambas extremidades, un par de músculos se colocaron en una cámara con fondo resina sintética para su limpieza mientras se mantuvo contenido en solución fisiológica y se le proveyó de la mezcla de gas carbógeno, mientras que el otro par de músculos fueron colocados en tubos Eppendorf y congelados a -80 °C para su preservación. Para los análisis de tensión isométrica se utilizó solución fisiológica con las siguientes concentraciones en mM: NaCl 118, KCl 4.75, $MgSO_4$ 1.18, $NaHCO_3$ 24.8, KH_2PO4 1.18, Glucosa 10, y se ajustará el pH a 7.4.

Para realizar las mediciones de contracción isométrica los músculos se colocaron en una cámara de registro con solución fisiológica Krebs Rienger. El tendón se fijó a un transductor óptico de fuerza. Se obtuvo un campo de estimulación eléctrica con dos electrodos de platino que se colocarán dentro de la solución fisiológica, evitando el contacto con el músculo. Los músculos se sujetaron a una longitud un 30% mayor a la original. La estimulación eléctrica fue generada hasta que el músculo se fatigó (Reducción del ~70% de la fuerza inicial), y dicho tiempo se tomaron para conocer el tiempo que tarda el músculo en desarrollar fatiga. La frecuencia de estimulación empleada fue de 45 Hz para el músculo sóleo y de 50 Hz para el músculo EDL a 100 V.

5.7. Determinación del perfil de lípidos

5.7.1. Triglicéridos

Para la cuantificación de triglicéridos se utilizaron kits comerciales de la marca SPINREACT en donde los triglicéridos incubados con lipoproteinlipasa (LPL) liberan glicerol y ácidos grasos libres. El glicerol es fosforilado por glicerolfosfato deshidrogenasa (GPO) y ATP en presencia de glicerol quinasa (GK) para producir glicerol-3-fosfato (G3P) y adenosina-5-difosfato (ADP). El G3P es entonces convertido a dihidroxiacetona fosfato (DAP) y peróxido de hidrógeno (H_2O_2) por GPO. Al final, el peróxido de hidrogeno (H_2O_2) reacciona con 4-aminofenazona (4-AF) y p-clorofenol, reacción catalizada por la peroxidasa (POD) dando una coloración roja:

$$\text{Triglicéridos} + H_2O \xrightarrow{\text{LPL}} \text{Glicerol} + \text{Ácidos grasos libres}$$

$$\text{Glicerol} + \text{ATP} \xrightarrow{\text{Glicerol quinasa}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-AF} + \text{p-Clorofenol} \xrightarrow{\text{POD}} \text{Quinona} + H_2O$$

La intensidad del color formado es proporcional a la concentración de triglicéridos presentes en la muestra ensayada.

5.7.2. Colesterol total

Para la determinación cuantitativa del colesterol se utilizaron kits comerciales de la marca SPINREACT en donde el colesterol presente en la muestra origina un compuesto coloreado según la siguiente reacción:

$$\text{Ésteres colesterol} + H_2O \xrightarrow{CHE} \text{Colesterol} + \text{Ácidos grasos}$$

$$\text{Colesterol} + O_2 \xrightarrow{CHOD} \text{4-Colestenona} + H_2O_2$$

$$2\,H_2O_2 + \text{Fenol} + \text{4-Aminofenazona} \xrightarrow{POD} \text{Quinonimina} + 4H_2O$$

La intensidad del color formado es proporcional a la concentración de colesterol presente en la muestra ensayada.

5.7.3. Determinación de HDL

Las lipoproteínas de muy baja densidad (VLDL) y baja densidad (LDL) del suero o plasma, se precipitan con fosfotungstato en presencia de iones magnesio. Tras la centrifugación, el sobrenadante contiene lipoproteínas de alta densidad (HDL). La fracción de HDL colesterol se determina utilizando el reactivo enzimático de colesterol total.

5.7.4. Determinación de VLDL

Para la determinación de las lipoproteínas de baja densidad se utilizó la fórmula de Friedewald el cuál es un método indirecto que nos permite conocer la fracción VLDL a través de la siguiente fórmula:

$$LDLc = CT - (HDLc + TG/5)\ mg/dl$$

En donde: CT = colesterol total, HDL = la fracción de colesterol HDL, TG = los triglicéridos y Colesterol total (mg) / Colesterol HDL (mg).

5.8. Marcadores de estrés oxidante

5.8.1. Peroxidación de lípidos en homogenado de músculo

La determinación de sustancias reactivas al ácido tiobarbitúrico (TBARS) fue realizado como un marcador de peroxidación de lípidos en el músculo esquelético en homogenado del músculo

sóleo y EDL y en suero mediante el método descrito por Ratajczak-Wrona y cols. en 2013, con algunas modificaciones.

La concentración de proteína de los homogenados de músculos se midió para normalizar los resultados y se determinó por el método de Biuret (50) utilizando albúmina de suero bovino como proteína estándar para la construcción de una curva patrón. Posteriormente, las muestras fueron mezcladas brevemente con 1 mL de ácido tricloroacético al 50%, y se centrifugaron a 5000 *g* durante 5 min. Se tomáron 600 µL del sobrenadante y se adicionarán 600 µL de ácido tiobarbitúrico 0.375% y las muestras se incubaron en baño de agua hirviendo durante 30 min. Finalmente, el producto resultante de la reacción de ácido tiobarbitúrico con MDA se determinó a una longitud de onda de 532 nm con un espectrofotómetro Perkin Elmer Lambda 18 UV/VIS. Los resultados fueron expresados como nanomoles de TBARS producidas por mg de proteína y se calcularon con base en el coeficiente de extinción molar (ε) de malondialdehido de 1.56X105 mM^{-1} cm^{-1}.

5.8.2. Relación de glatatión reducido/oxidado (GSH/GSSG)

La concentración de glutatión en homogenados de músculo el cual se determinó mediante el método modificado por Ortiz-Ávila y cols. en 2015. Las muestras de homogenados de los músculos sóleo y EDL de los músculos de los cuartos traseros fueron tratadas inicialmente con 5% (v/v) de ácido sulfosalicílico y centrifugadas a 7800 *g* durante 10 min para eliminar las proteínas desnaturalizadas; el sobrenadante se utilizó para las mediciones. El contenido de glutatión total (GSH + GSSG) en los homogenados de los músculos sóleo/EDL y se evaluó por un método enzimático, empleando la enzima glutatión reductasa y 5,5'-ditiobis (ácido 2-nitrobenzoico) (DTNB). La cinética de la reacción fue monitoreada durante 5 minutos a una longitud de onda de 412 nm. La velocidad de formación del ácido 5'-tio-2-nitrobenzoico fue proporcional a la suma de GSH+GSSG presente. Para la determinación de glutatión oxidado (GSSG), se realizó mediante el tratamiento del sobrenadante con ácido sulfosalicílico y con 4-vinilpiridina con la finalidad de eliminar el glutatión en su forma reducida (GSH); después de 60 min, se repitió el mismo ensayo con DTNB. Los valores de GSH se calcularán como la diferencia entre el glutatión total (GSSG+GSH) y las concentraciones del GSSG.

5.8.3. Medición de especies reactivas de oxígeno

Las ERO fueron determinadas homogenados de músculo soleo/EDL. Las muestras serán analizadas en un citómetro de flujo BD AccuriTM C6, empleando el software CFlow Sampler. Durante la medición se consideró un mínimo de 10,000 eventos de la muestra de un volumen total de 200 µL. Estas fueron suspendidas en medio PBS e incubadas con glutamato/malato (10 mM) como sustrato y con una sonda fluorescente indicadora de ERO, 2´,7´diacetato de diclorodihidrofluoresceina (H_2DCFDA; Molecular Probes, Invitrogen) durante 30 min. Posteriormente se probaron l o s fármacos de interés después de 6 min de incubación. La fluorescencia de la sonda fue monitoreada en los canales de emisión de fluorescencia FL1 (530-30 nm).

5.9. Análisis estadístico

Los resultados de los datos se expresarán en promedios con desviación estándar por medio de estadística descriptiva donde se utilizaron medidas de tendencia central como media y desviación estándar. Se realizóun análisis de distribución de datos y posteriormente un análisis de varianza de una vía (ANOVA) en donde se consideró significativo una p con un valor <0.05, para el cual se empleó el programa estadístico GraphPad Prism versión 6.0.

6. RESULTADOS

Los resultados del presente trabajo se presentan a continuación en 2 capítulos. El capítulo uno corresponde a los resultados del efecto de la administración de dietas bajas en hierro sobre el desarrollo de fuerza muscular, perfil de lipídos y parámetros de estrés oxidante, con los resultados obtenidos en este primer capítulo se escribió el articulo **Dietary iron restriction improves muscle function, dyslipidemia and decreased muscle oxidative stress in streptozotocin – induced diabetic rats** (Vargas-Vargas MA, Saavedra-Molina A, Gómez-Barroso M, Peña-Montes D, Cortés-Rojo C, Miguel H, Trujillo X, Montoya-Pérez R. Dietary Iron Restriction Improves Muscle Function, Dyslipidemia, and Decreased Muscle Oxidative Stress in Streptozotocin-Induced Diabetic Rats. Antioxidants (Basel). 2022 Apr 7;11(4):731. doi: 10.3390/antiox11040731. PMID: 35453417; PMCID: PMC9030937)

En el capítulo 2 se muestran los resultados del efecto de la administración de diazóxido en los tiempos de fatiga asi como el desarrollo de fuerza muscular, perfil de lípidos y diversos parámetros de estrés oxidante y se escribió el artículo **Diazoxide improves muscle function in association with improved dyslipidemia and decreased muscle oxidative stress in streptozotocin – induced diabetic rats.**

6.1. Capítulo 1

antioxidants

MDPI

Article

Dietary Iron Restriction Improves Muscle Function, Dyslipidemia, and Decreased Muscle Oxidative Stress in Streptozotocin-Induced Diabetic Rats

Manuel Alejandro Vargas-Vargas [1], Alfredo Saavedra-Molina [1], Mariana Gómez-Barroso [1], Donovan Peña-Montes [1], Christian Cortés-Rojo [1], Huerta Miguel [2], Xochitl Trujillo [2] and Rocío Montoya-Pérez [1,*]

[1] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Múgica S/N, Col. Felicitas del Río, Morelia 58030, Mexico; 1371614e@umich.mx (M.A.V.-V.); francisco.saavedra@umich.mx (A.S.-M.); 0939531k@umich.mx (M.G.-B.); 0618853j@umich.mx (D.P.-M.); christian.cortes@umich.mx (C.C.-R.)

[2] Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Av. 25 de Julio 965, Las Víboras, Colima 24040, Mexico; huertam@ucol.mx (H.M.); rosio@ucol.mx (X.T.)

* Correspondence: rocio.montoya@umich.mx

check for updates

Citation: Vargas-Vargas, M.A.; Saavedra-Molina, A.; Gómez-Barroso, M.; Peña-Montes, D.; Cortés-Rojo, C.; Miguel, H.; Trujillo, X.; Montoya-Pérez, R. Dietary Iron Restriction Improves Muscle Function, Dyslipidemia, and Decreased Muscle Oxidative Stress in Streptozotocin-Induced Diabetic Rats. *Antioxidants* **2022**, *11*, 731. https://doi.org/10.3390/antiox11040731

Academic Editors: Sérgio Paulo Bydlowski, José Carlos Toledo Junior and Débora Levy

Received: 8 March 2022
Accepted: 5 April 2022
Published: 7 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: Diabetes mellitus is a chronic degenerative disease characterized by hyperglycemia and oxidative stress. Iron catalyzes free radical overproduction. High iron concentrations have previously been reported to promote an increase in oxidative stress; however, the effect of iron restriction in diabetes has not yet been explored, so we tested to see if iron restriction in diabetic rats reduces oxidative damage and improved muscle function. Wistar rats were assigned to 4 groups: Control; Diabetic; Diabetic rats with a high iron diet, and Diabetic with dietary iron restriction. After 8 weeks the rats were sacrificed, the muscles were extracted to prepare homogenates, and serum was obtained for biochemical measurements. Low iron diabetic rats showed an increase in the development of muscle strength in both muscles. Dietary iron restriction decreased triglyceride concentrations compared to the untreated diabetic rats and the levels of extremely low-density lipoproteins. Aggravation of lipid peroxidation was observed in the diabetic group with a high iron diet, while these levels remained low with iron restriction. Iron restriction improved muscle strength development and reduced fatigue times; this was related to better lipid profile control and decreased oxidant stress markers.

Keywords: diabetes; fatigue; iron; oxidative stress

1. Introduction

Diabetes mellitus is characterized by metabolic dysfunction resulting from changes in insulin secretion and/or resistance to its actions [1]. Chronic hyperglycemia in diabetes increases oxidative stress, causing cell damage that eventually leads to acute and chronic complications [2–4]. Muscle fatigue is an acute symptom of diabetes, causing loss of strength and mass in muscle [5]. Oxidative stress is a key factor involved in the development of muscle fatigue [6,7]. Dyslipidemia is another hallmark of diabetes associated with a higher risk of cardiovascular disease [8]; hereby, the decrease in blood lipids improves cardiovascular outcomes [9,10].

Dyslipidemia has been related to impaired muscle function in rodents [11] and humans [12]. Thus, dyslipidemia may enhance the deleterious effects of oxidative stress on muscles.

On the other hand, high intracellular iron concentrations (i.e., iron overload) enhance oxidative stress by catalyzing ROS production via the iron/H_2O_2-based formation of hydroxyl radicals [13]. Oxidative stress due to iron overload accelerates both the development and progression of diabetic complications [14,15]. Accordingly, the role of iron overload in the impaired metabolism of lipids and carbohydrates has been confirmed in diabetic rats by improving dyslipidemia and glucose homeostasis via dietary iron restriction [16]. However, it is unknown whether dietary iron restriction also improves muscle performance in diabetes. Thus we aimed to test if, besides alleviating dyslipidemia, iron restriction also improves muscle performance and oxidative stress in diabetic rats. Moreover, we fed rats with a high iron diet to induce iron overload and test if this phenomenon enhances the alterations elicited by diabetes in muscle function, blood lipids, and oxidative stress.

2. Materials and Methods

2.1. Animals and Dietary Treatments

Male Wistar rats (weight, 200–250 g) were used and preserved under standard laboratory conditions with *ad libitum* food and water. All animal procedures were conducted following the Technical specifications for the production, use, care, and handling of laboratory animals (NOM-062-ZOO-1999, Ministry of Agriculture, México) and were approved by the Institutional Committee for the Use and Care of Animals of the Instituto de Investigaciones Químico-Biológicas of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) (Number 2018-06; April 2018). The standard control diet (D12050203) contained iron at 100 mg/kg (Research Diets, Inc.). The low iron diet (D03072501, Research Diets, Inc., New Brunswick, NJ, USA) had 3 mg iron/kg. The high iron diet consisted of a standard control diet plus oral administration of polymaltosed iron (Takeda, México, S.A. de C.V., under license of Vifor International, Inc. Switzerland) through an orogastric tube at a dose of 3 mg of iron/kg body weight per day, for 8 weeks, to establish a pattern of iron overload [16]

2.2. Induction of Diabetes and Experimental Design

Experimental diabetes was induced by a single injection of streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 45 mg/kg body weight. STZ was dissolved in 0.1 M sodium citrate buffer (pH 4.5), and control rats were injected only with sodium citrate buffer. Blood glucose was measured two days after STZ injection, and rats with glucose levels >200 mg/dL were considered diabetic. Twenty four rats were divided into four groups of six animals each: (1) Control group (Control): fed only with standard rodent show diet; (2) Diabetic group (DB): diabetic rats fed only with standard rodent chow diet; (3) Diabetic plus high iron diet group (DB+IO): Diabetic rats with iron overload diet; (4) Diabetic rats with iron restriction group (DB+IR). Fresh blood samples were collected from the rat tail vein, and glucose levels were determined using a glucometer (ACCU-CHEK® Active, Roche Diagnostics, Mannheim, Germany) every week during the experimental stage.

2.3. Muscle Extraction and Tissue Preparation

Animals were sacrificed by decapitation once the experimental period was over. The soleus and extensor digitorum longus (EDL) muscles of one limb were extracted

and placed on a Petri dish with Krebs-Ringer buffer (75 mM KCl, 118 mM NaCl, 1.18 mM $MgSO_4$, 1.18 mM KH_2PO_4, 24.8 mM $NaHCO_3$, 2.08 g/L glucose, pH 7.4) for muscle tension tests. The muscles of the other limb were homogenized using Krebs Ringer buffer to assess oxidative stress biomarkers (lipid peroxidation, ROS, and glutathione), the homogenates were prepared and were preserved at a temperature of −70 °C until their subsequent use. The protein content of muscle homogenates was determined using the Biuret procedure with slight modifications using bovine serum albumin protein as the standard [17].

2.4. Hemoglobin Measurement

Hemoglobin (Hb) content was determined using the method by Drabkin and Sahli [18]. 10 µL heparinized blood was added to 2.5 mL of reactive solution (0.012 mM K_3 [Fe (CN)$_6$], 1.54 mm KCN, and 0.04 mM K_2HPO_4) and incubated for 5 min at room temperature. The optical density was recorded at 540 nm on a Perkin-Elmer Lambda 18 UV/vis spectrophotometer. Hb content was expressed in mg/dL blood and was calculated using a calibration curve with BSA.

2.5. Force Development and Fatigue Induction

The soleus and EDL muscles obtained from one of the hind legs were used for tension records. Both muscles were positioned in a Petri dish with a resin bottom (Sylgard), fixed with entomological pins immersed in Krebs-Ringer solution, and supplied with 95% CO_2 and 5% O_2. Excess fat and connective tissue were removed. Once cleaned, the muscle was mounted in a chamber for recording isometric tension, with its proximal end attached to the bottom of the chamber and the distal end to the hook of an optical transducer (World Precision Instruments, Sarasosa, FL. USA), which was connected to an amplifier (World Precision Instruments, USA) and an analogic-to-digital interface (World Precision Instruments) for the acquisition of muscle tension MDAC software (World Precision Instruments, USA) was used. Inside the recording chamber, two platinum electrodes were placed, linked to a stimulation isolating unit (Grass) to carry out the fatigue protocol. This consisted of 300 ms pulses of 100 V and 50 Hz for EDL muscle and 45 Hz for soleus muscle. Stimulation was stopped after fatigue appeared (70% of force reduction).

2.6. Lipid Profile

Serum was collected by centrifugation of blood samples at 3000× *g* for 5 min (Thermo Scientific Sorvall RC 6+ centrifuge). Next, a Fujifilm NX500i (Tokyo, Japan) system was used to analyze the total serum cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) using Spinreact (Girona, Spain) reagents following the supplier's instructions. Very low-density lipoprotein (VLDL) was calculated using the following formula: VLDL = TG/5 [19].

2.7. Determination of Lipid Peroxidation

The thiobarbituric acid (TBA) method was used to determine lipid peroxidation in muscle homogenates [20]. 0.5 mg protein was resuspended in phosphate buffer (100 mM, pH 7.4), combined with a reagent solution (0.375% thiobarbituric acid (TBA), 15% trichloroacetic acid (TCA), and 0.25 M HCl). This mixture was vortexed and incubated in a boiling water bath for 25 min. The absorbance of the supernatant was measured at 532 nm using a Shimadzu UV-2550 spectrophotometer. The results

were expressed as thiobarbituric acid reactive substances (TBARS) per milligram of protein.

2.8. ROS Measurements

The cell-permeable fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) determined ROS levels. 0.5 mg muscle protein was resuspended in 2 mL of buffer with 100 mM KCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3 mM KH_2PO_4 and 3 mM $MgCl_2$ (pH 7.4) were incubated with 5 μL of 2′,7′-dichlorofluorescein diacetate (H_2DCFDA) during 15 min at 4 °C under constant stirring. Fluorescence changes were detected in an RF-5301PC spectrofluorometer (Shimadzu Corporation, Kyoto, Japan) (λ_{ex} 485 nm; λ_{em} 520 nm) [21].

2.9. Measurement of Glutathione

Glutathione redox status was determined with the method by Rahman [22] with slight modifications. Total glutathione (GSH+GSSG) content was determined in 0.5 mg protein. Samples were suspended in 0.6% sulfosalicylic acid and 0.1% Triton X-100 in a 0.1 M potassium phosphate buffer (pH 7.5). This mixture was sonicated three times during 5 s and placed on ice for 20 s between each sonication cycle, succeeded by two freezing/thawing cycles, and centrifuged at 6500× *g*. Then, the supernatant was mixed with a potassium phosphate buffer with 0.1 units/mL glutathione reductase and 100 μM 5, 5′-Dithiobis (2-nitrobenzoic acid) (DTNB). The reaction was started by adding 50 μM β-NADPH and monitored for 5 min at 412 nm in a UV/vis spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). For determinations of oxidized glutathione (GSSG), samples were incubated for 1 h at room temperature with 0.2% 4-vinylpyridine to derivatize reduced glutathione (GSH). Then, the samples were treated as described above for determinations of GSH+GSSG. GSH concentration was calculated by subtracting the concentration of GSSG from the concentration of GSH+GSSG.

2.10. Statistical Analysis

All the data are expressed as the mean ± standard error (SEM) of at least n=6. Statistical significance was evaluated using the Student t-test or ANOVA followed by a post hoc Tukey test; statistical significance was set at $P<0.05$. The analyses were done with GraphPad Prism 6 (Prism 6.0, GraphPad Software Inc., San Diego, CA, USA).

3. Results

3.1. Effect of Dietary Iron on Blood Glucose and Body Weight in STZ-Diabetic Induced Rats

Glucose levels in all the diabetic groups (i.e., DB, DB+IO, DB+IR) remained several-fold higher than in the control group (Figure 1A). Regarding weight gain at the end of the experimental period (Figure 1B), the control group showed a weight gain of 158 g, while diabetic rats exhibited a negative balance in weight gain prevented in the DB+IR group, although weight gain was not as high as in the control group. Nevertheless, this effect was not statistically significant.

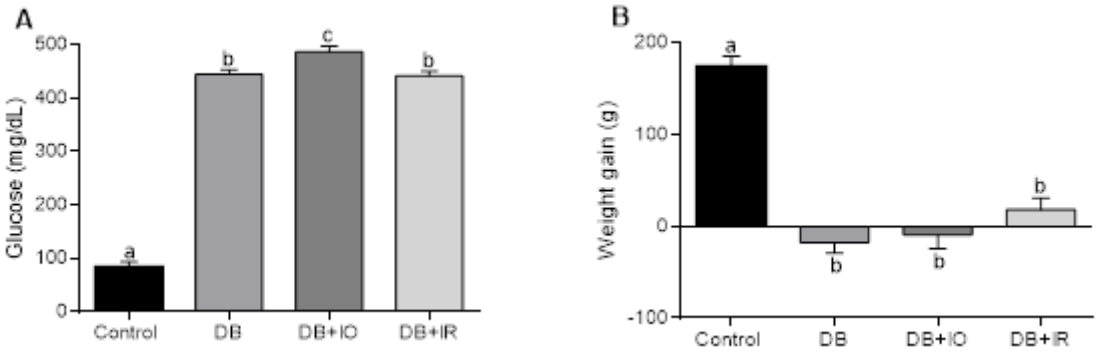


Figure 1. Fasting serum glucose (**A**) and Weight gain (**B**). Weight gain was determined by subtracting the animals' weight after eight-week treatments with the diets, determined right before sacrifice, minus the weight at the beginning of the treatments. Data are expressed as the mean ± standard error. $p < 0.05$ ($n = 6$, ANOVA plus Tukey's post hoc test). DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron—restricted diet. Different letters (a, b & c) represent statistically significant differences.

3.2. Effects of Dietary Iron on Hemoglobin

Hemoglobin content is shown in Figure 2. Higher hemoglobin levels were found in the DB+IO, while the DB+IR group exhibited lower levels, even below the levels in the control group. It should be stressed that animals from the DB+IR group did not develop anemia, as hemoglobin levels were above 10 mg/dL.

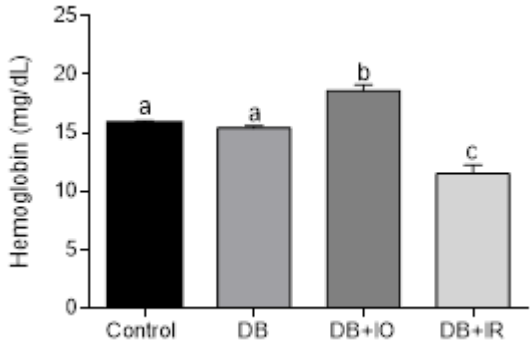


Figure 2. Effect of iron on hemoglobin levels. Data are presented as group mean ± standard error. $p < 0.05$ ($n = 6$, ANOVA plus Tukey's post hoc test). Different letters represent statistically significant differences. DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron restriction diet.

3.3. Effects of Dietary Iron on Muscle Strength Development and Fatigue

The effects of dietary iron on muscle function are depicted in Figure 3. The DB and DB+IO groups displayed a similar degree of strength loss in both EDL (Figure 3A) and soleus (Figure 3B) muscles. The iron restriction group shows an increase in the development of muscular strength in both muscles. Moreover, the time for the development of muscle fatigue was shorter in both the DB and DB+IO groups (Figure

3C). These negative effects were counteracted by iron restriction in the DB+IR group, although the fatigue time did not reach the levels of the control group.

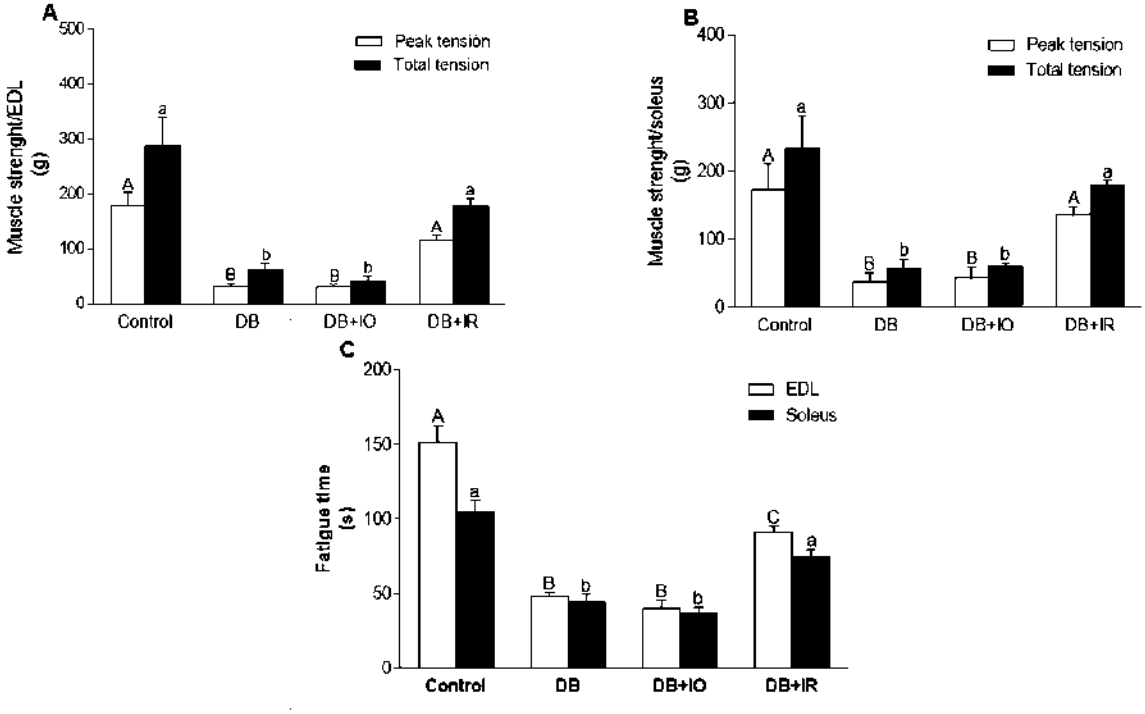


Figure 3. Effect of iron on muscle strength development in EDL (**A**), soleus muscle (**B**) peak tension (open bars) and solid bars (total tension). Effect of iron on fatigue (**C**) in EDL (open bars) and soleus muscles (solid bars). Data are presented as group mean ± standard error. $p < 0.05$ (n = 6, ANOVA plus Tukey's post hoc test). A, B & C represent the statistically significant differences between the different groups of the peak tension (open bars); a and b represent the statistically significant difference between the different groups of the total tension (solid bars); DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron restriction diet.

3.4. Effects of Iron on Serum Lipid Profile

Serum cholesterol levels are shown in Figure 4A. Compared to the control group, cholesterol levels were twofold higher in the DB group, while no significant changes were observed in the DB+IO and DB+IR groups. No differences between the groups were detected in serum HDL levels (Figure 4B). Serum triglycerides levels are shown in Figure 4C. Compared to the control group, triglycerides dramatically increased in the DB group. This was fully counteracted in the DB+IO and DB+IR groups, and similar behavior was observed for VLDL levels (Figure 4D).

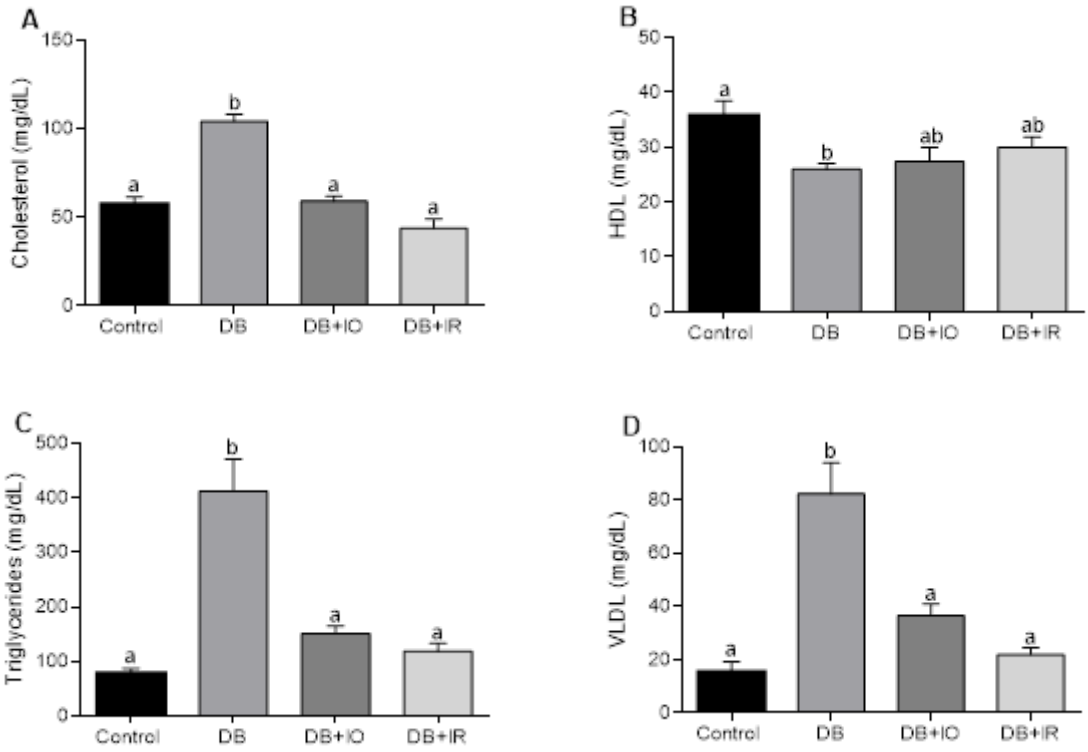


Figure 4. Serum levels of cholesterol (**A**), high-density lipoproteins (HDL) (**B**), triglycerides (**C**), and very-low-density lipoproteins (VLDL) (**D**). Data are presented as group mean ± standard error. $p < 0.05$ ($n = 6$, ANOVA plus Tukey's post hoc test). Different letters represent statistically significant differences. DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron restriction diet.

3.5. Effect of Iron on Oxidative Stress Markers

Muscle ROS levels are depicted in Figure 5. ROS levels doubled in the DB group compared to the control group, and they increased almost thrice in the DB+IO group. In contrast, dietary iron restriction decreased ROS levels in the DB+IR group almost to control levels (Figure 5).

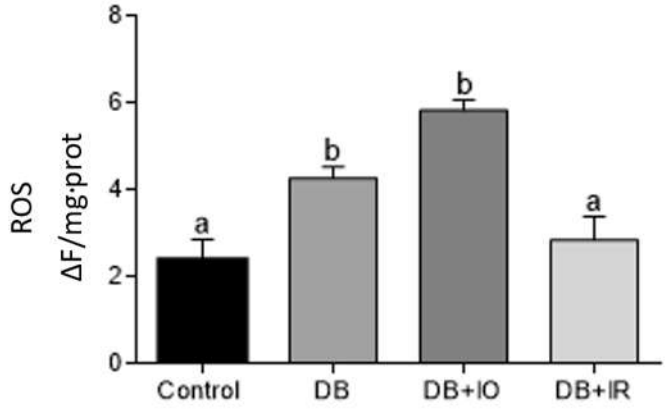


Figure 5. Effect of iron on the levels of ROS in muscle homogenates from control rats. Data are presented as group mean ± standard error. $p < 0.05$ ($n = 6$, ANOVA plus Tukey's post hoc test). Different letters represent statistically significant differences. DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron restriction diet.

A similar trend was observed in lipid peroxidation (Figure 6), as the levels of this parameter doubled in both the DB and DB+IO groups and decreased to the levels of the control group in the DB+IR group.

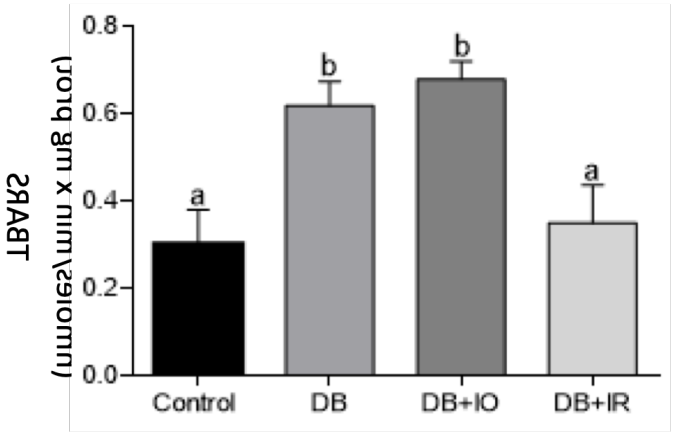
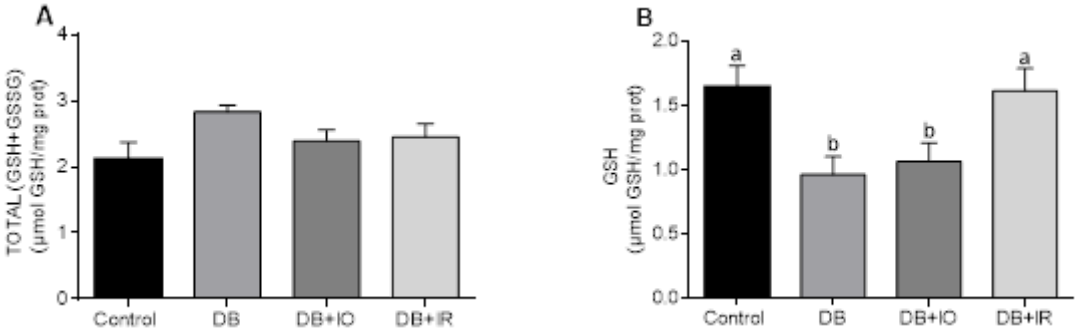


Figure 6. Effects of iron on muscle lipid peroxidation. Data are presented as group mean ± standard error. $p < 0.05$ ($n = 6$, ANOVA plus Tukey's post hoc test). Different letters indicate statistically significant differences. DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron restriction diet.

The glutathione redox status in the muscle is shown in Figure 7. No differences in total glutathione levels were observed between the different experimental groups (Figure 7A). GSH levels decreased in both the DB and DB+IO groups compared to the control group (Figure 7B). On the contrary, GSSG levels were higher in both the DB and DB+IO groups than in the control group (Figure 7C). These changes in glutathione redox state render a severe state of oxidative stress in the muscle of both the DB and DB+IO groups, as the GSH/GSSG ratio was considerably lower in these groups compared to the control group (Figure 7D). In contrast, dietary iron restriction almost normalized in the DB+IR group all the alterations in the levels of GSH (Figure 7B), GSSG (Figure 7C), and GSH/GSSG ratio (Figure 7D).



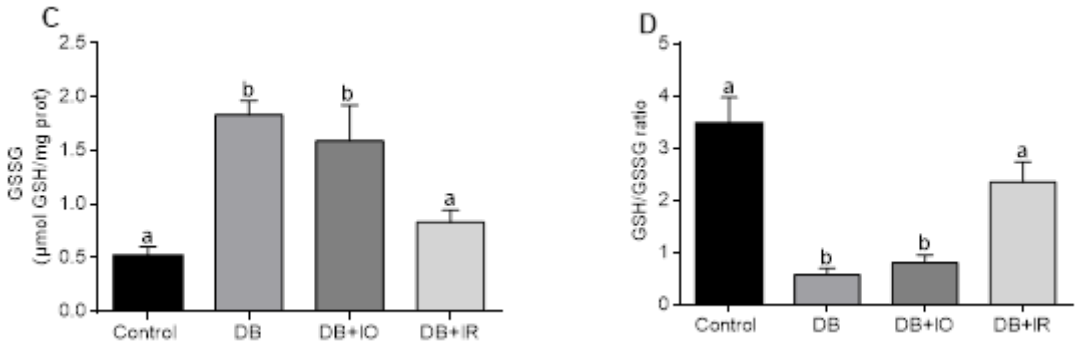


Figure 7. Effects of dietary iron on total glutathione (**A**), reduced glutathione (GSH) (**B**), oxidized glutathione (GSSG) (**C**), and GSH/GSSG ratio (**D**). Data are presented as group mean ± standard error. $p < 0.05$ ($n = 6$, ANOVA plus Tukey's post hoc test). Different letters (a, b & c) indicate statistically significant differences. DB+IO, diabetic rats with iron overload; DB+IR, diabetic rats with iron restriction diet.

4. Discussion

Dietary iron restriction improved skeletal muscle function in type 1 STZ-induced diabetic rats, accompanied by decreased muscle oxidative stress and improved dyslipidemia. Of note, the protocol of iron restriction used in this study did not induce anemia, as this condition occurs in rats when hemoglobin is lower than 10 mg hemoglobin/dL.

No weight gain was observed in the diabetic rats throughout the experimental period, consistent with the same phenotype seen in previous studies [23]. Iron restriction improves insulin sensitivity in obesity, leading to improved glucose levels [24]. STZ induces diabetes by depleting insulin production due to pancreatic β-cell destruction [25] rather than by impairing insulin sensitivity; this may explain the lack of a hypoglycemic effect of iron deprivation in this model of diabetes.

The more deleterious effects on muscular strength were observed in both the DB and DB+IO groups. This may be related to the fatigue commonly described in diabetic individuals [6], as iron overload has been observed in muscles with insulin resistance [26]. Moreover, it has also been described that iron overload leads to the development of fatigue [27] and negatively affects muscle contractility [28]. Thus, the maximum negative effect on fatigue is caused by diabetes itself, without iron overload worsening this outcome. Iron plays an essential role in the development of diabetes, and it promotes oxidative stress [15], which might be involved in the impaired ability to develop muscle strength [29]. Conversely, the iron restriction positively affected muscle strength development, both in the soleus muscle and in the EDL muscle. Little is known about the direct impact that iron has on the development of muscle strength in diabetes; however, it has been determined that iron restriction causes positive effects in various pathologies such as obesity [24].

The positive effects of iron restriction on dyslipidemia (Figure 4) agree with the beneficial effects of this approach in altered lipid and glucose metabolism in a type 2

diabetes model. It was also found that iron restriction decreased systemic oxidative stress [30].

Unexpectedly, iron overload also counteracted dyslipidemia in the DB+IR group. Iron overload has been reported to increase blood triglycerides in non-diabetic rats by enhancing hepatic oxidative stress, impairing the expression of peroxisome proliferator-activated receptor-alpha, and decreasing fatty acid beta-oxidation. Furthermore, iron overload promotes hepatic lipid secretion by increasing the mRNA expression of apoB-100, which is associated with the assembly of VLDL, and by improving the assembly of apoB-100 into VLDL by increasing the expression of microsomal triglyceride transfer protein [31]. On the other hand, iron overload induces hypercholesterolemia by enhancing the mRNA expression of hydroxymetylglutaryl CoA reductase (HMG CoA) and the activity of acyl-CoA cholesterol acyltransferase (ACAT), thus increasing cholesterol synthesis and VLDL—cholesterol secretion [32,33]. We have no data to explain why iron overload had a hypoglycemia effect in STZ—induced diabetic rats. We can speculate that this resulted from impaired hepatic lipid synthesis elicited by the combined effect of iron accumulation in the liver [34] and the damage induced by diabetes in organelles closely related to lipid metabolism like mitochondria [23], leading to a catastrophic bioenergetic collapse decreasing ATP synthesis. This, in turn, may impair biosynthetic pathways requiring ATP, such as cholesterol synthesis or biogenesis of VLDL transport vesicles [35], which may have also contributed to generalized oxidative damage in biomolecules and cellular structures [34,36,37]. Therefore, decreased dyslipidemia would result from the harmful effects of iron on bioenergetics and cellular redox state rather than an adaptive response to enhanced iron concentrations and oxidative stress.

The diabetic rats exhibited higher VLDL levels, which are closely associated with coronary artery calcification in diabetes, and this association becomes more substantial when triglyceride levels are above normal levels [38]. Individuals with type 2 diabetes frequently exhibit macrovascular complications of atherosclerotic cardiovascular disease. It has been shown that HDL is protective against atherosclerosis [39]. The decrease in HDL observed in the diabetic rats and the null effect of iron restriction on impaired HDL levels (Figure 5B) suggests that the beneficial effects of iron restriction might not be enough to counteract atherosclerotic disease in the microvasculature, as HDL may attenuate inflammation by decreasing the activation of mediators of this process such as the nuclear factor kappa B (NF-κB), which, in turn, suppresses the expression of HIF-1α, VEGFA, and VEGFR2 [40].

There is a positive correlation between the amount of intake of iron and ROS levels in skeletal muscle (Figure 5). In agreement, decreased ROS levels were accompanied by lower oxidative stress, as supported by the higher GSH/GSSG ratio observed with iron restriction in the DB+IR group (Figure 7). Diabetes set higher levels of oxidative stress even in the absence of excessive iron intake, as oxidative stress markers were not higher in the DB+IO group than in the DB group. On the other hand, iron restriction improved muscle strength and significantly delayed the onset of fatigue (Figure 3). In this regard, it is known that increased levels of ROS have many deleterious effects on skeletal muscle, such as reducing force generation and atrophy [30], which is consistent with the data in Figures 3 and 4. Our findings show that iron restriction also lowers lipid peroxidation and improves glutathione redox status (Figures 6 and 7D), suggesting that iron restriction in the diet decreases

radical hydroxyl production, reducing the damage to membrane lipids and preserving the glutathione redox status.

5. Conclusions

The addition of iron in the diet does not exacerbate the damage caused by diabetes. However, the restriction of iron promotes positive effects. It enhances muscle strength, delays fatigue, improves lipid homeostasis in diabetes, and also decreases oxidative stress markers such as ROS levels and TBARS levels, and decreases the GSH/GSSG ratio.

Author Contributions: Conceptualization, M.A.V.-V. and R.M.-P.; methodology, M.A.V.-V., M.G.-B., and D.P.-M.; formal analysis, M.A.V.-V., C.C.-R. and R.M.-P.; project administration, R.M.-P.; investigation, M.A.V.-V., A.S.-M.; resources, A.S.-M., H.M., X.T. and R.M.-P.; funding acquisition, R.M.-P.; writing—original draft preparation, M.A.V.-V., C.C.-R., A.S.-M., D.P.-M., and R.M.-P.; writing—review and editing, M.A.V.-V., C.C.-R. and R.M.-P.; supervision, R.M.-P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially financed by Coordinación de la Investigación Científica—UMSNH, R.M.P. CIC-UMSNH2019.

Institutional Review Board Statement: All animal procedures were approved by the Institutional Committee for the Use and Care of Animals of the Instituto de Investigaciones Químico-Biológicas of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) (Number 2018-06; April 2018).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors have no conflict of interest to declare.

References

1. Silva, J.; Souza, E.; Echazú Böschemeier, A.G.; Costa, C.; Bezerra, H.S. Diagnosis of diabetes mellitus and living with a chronic condition: Participatory study. *BMC Public Health* **2018**, 18, 699.
2. Papatheodorou, K.; Banach, M.; Bekiari, E.; Rizzo, M.; Edmonds, M. Complications of Diabetes. *J. Diabetes Res.* **2018**, 2018, 3086167.
3. Elisabetta, B.; Laura, L. Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. *Oxid. Med. Cell. Longev.* **2019**, 2019, 5953685.
4. Fakhruddin, S.; Alanazi, W.; Jackson, K. Diabetes-induced reactive oxygen species: Mechanism of their generation and role in renal injury. *J Diabetes Res*. **2017**, 2017, 8379327.
5. Kalra. S.; Sahay, R. Diabetes fatigue syndrome. *Diabetes Ther.* **2018**, 9, 1421–1429.
6. Leigh, C.; Gerald, S. Hyperglycemia-induced diaphragm weakness is mediated by oxidative stress. *Crit. Care* **2014**, *18*, 88.
7. Devulapally, Y.; Singh, N.D.; Bai, P.K. A comparative study of skeletal muscle fatigue in diabetic and non-diabetic human beings. *Natl. J. Physiol. Pharm. Pharmacol.* **2018**, *8*, 1529–1532.
8. Jia, A.; Zeng, W.; Yu, L.; Zheng, H.; Lu, Z.; Song, Y. Very low-density lipoprotein cholesterol is associated with extent and severity of coronary artery disease in patients with type 2 diabetes mellitus. *SAGE Open Med*. **2019**, 7, 2050312119871786.
9. Schofield, J.D.; Liu, Y.; Rao-Balakrishna, P. Diabetes dyslipidemia. *Diabetes Ther.* **2016**, *7*, 203-219.
10. Warraich, H.J.; Rana, J.S. Dyslipidemia in diabetes mellitus and cardiovascular disease. *Cardiovasc. Endocrinol*. **2017**, *6*, 27–32.
11. Andrich, D.; Ou, Y.; Melbouci, L. Altered lipid metabolism impairs skeletal muscle force in young rats submitted to a short-term high-fat diet. *Front. Physiol.* **2018**, *26*, 1327.
12. Blakeley, C.E.; Van Rompay, M.I.; Schultz, N.S. Relationship between muscle strength and dyslipidemia, serum 25(OH) D, and weight status among diverse schoolchildren: A cross-sectional analysis. *BMC Pediatr.* **2018**, *18*, 23.
13. Bresgen, N.; Eckl, P.M. Oxidative stress and the hemodynamics of iron metabolism. *Biomolecules* **2015**, *5*, 808–847.

14. Simcox, J.A.; McClain, D.A. Iron and diabetes risk. *Cell Metab*. **2013**, *17*, 329–41.
15. Swaminathan, S.; Fonseca, V.A.; Alam, M.G.; Shah, S.V. The role of iron in diabetes and its complications. *Diabetes Care* **2007**, *30*, 1926–1933.
16. Márquez-Ibarra, A.; Huerta, M.; Villalpando-Hernández, S.; Ríos-Silva, M.; Díaz-Reval, M.I.; Cruzblanca, H.; Mancilla, E.; Trujillo, X. The effects of dietary iron and capsaicin on hemoglobin, blood glucose, insulin tolerance, cholesterol, and triglycerides, in healthy and diabetic wistar rats. *PLoS ONE* **2016**, *11*, e0152625.
17. Gornall, A.G.; Bardawill, C.J.; David, M.M. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751–66.
18. Balasubramaniam, P.; Malathi, A. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. *J. Postgrad. Med.* **1992**, *38*, 8–9.
19. Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **1972**, *18*, 499–502.
20. Buege, J.A.; Aust, S.D. Microsomal lipid peroxidation. *Meth. Enzymol.* **1978**, *52*, 302–310.
21. Huerta-Cervantes, M.; Peña-Montes, D.J.; Montoya-Pérez, R.; Trujillo, X.; Huerta, M.; López-Vázquez, M.Á.; Olvera-Cortés, M.E.; Saavedra-Molina, A. Gestational diabetes triggers oxidative stress in hippocampus and cerebral cortex and cognitive behavior modifications in rat offspring: Age- and sex-dependent effects. *Nutrients* **2020**, *12*, 376.
22. Rahman, I.; Kode, A.; Biswas, S. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat. Protoc.* **2006**, *1*, 3159–3165.
23. Ortiz-Ávila, O.; Gallegos-Corona, M.A.; Sánchez-Briones, L.A. Protective effects of dietary avocado oil on impaired electron transport chain function and exacerbated oxidative stress in liver mitochondria from diabetic rats. *J. Bioenergetics Biomembr.* **2015**, *47*, 337–53.
24. Cooksey, R.C.; Jones, D.; Gabrielsen, S.; Huang, J.; Simcox, J.A.; Luo, B.; Soesanto, Y.; Rienhoff, H.; Abel, E.D.; McClain, D.A. Dietary iron restriction or iron chelation protects from diabetes and loss of beta-cell function in the obese (ob/ob lep-/-) mouse. *Am. J. Physiol. Endocrinol*. **2010**, *298*, 1236–1243.
25. Szkudelski, T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol. Res*. **2001**, *50*, 537–46.
26. Lee, K.; Cui, R.; Kim, D.J.; Choi, S.; Lee, W.J.; Kang, Y.; Kim, T.H.; Moon, H.U.; Jeon, J.Y.; Han, S.J. and Kim S.J. Iron overload plays an important role in er stress-induced insulin resistance in human skeletal muscle cells. *Diabetes* **2018**, *67*, 1912.
27. Makker, J.; Hanif, A.; Bajantri, B.; Chilimuri, S. Dysmetabolic hyperferritinemia: All iron overload is not hemochromatosis. *Case Rep. Gastroenterol.* **2015**, *9*, 7–14.
28. Halon-Golabek, M.; Borkowska, A.; Herman-Antosiewicz, A..; Antosiewicz, J. Iron metabolism of the skeletal muscle and neurodegeneration. *Front. Neurosci.* **2019**, *13*, 165.
29. Steinbacher, P.; Eckl, P. Impact of oxidative stress on exercising skeletal muscle. *Biomolecules* **2015**, *5*, 356–377.
30. Minamiyama, Y.; Takemura, S.; Kodai, S.; Shinkawa, H.; Tsukioka, T.; Ichikawa, H.; Naito, Y.; Yoshikawa, T.; Okada, S. Iron restriction improves type 2 diabetes mellitus in Otsuka Long-Evans Tokushima fatty rats. *Am. J. Physiol. Endocrinol.* **2010**, *298*, 1140-9.
31. Silva, M.; da Costa Guerra, J.F.; Sampaio, A.F.; de Lima, W.G.; Silva, M.E.; Pedrosa, M.L. Iron dextran increases hepatic oxidative stress and alters expression of genes related to lipid metabolism contributing to hyperlipidaemia in murine model. *Biomed. Res. Int.* **2015**, *2015*, 272617.
32. Brunet, S.; Thibault, L.; Delvin, E.; Yotov, W.; Bendayan, M.; Levy, E. Dietary iron overload and induced lipid peroxidation are associated with impaired plasma lipid transport and hepatic sterol metabolism in rats. *Hepatology* **1999**, 29, 1809–1817.
33. Graham, R.M.; Chua, A.C.; Carter, K.W.; Delima, R.D.; Johnstone, D.; Herbison, C.E.; Firth, M.J.; O'Leary, R.; Milward, E.A.; Olynyk, J.K.; et al. Hepatic iron loading in mice increases cholesterol biosynthesis. *Hepatology* **2010**, *52*, 462–471.
34. Ahmed, U.; Latham, P.S.; Oates, P.S. Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. *World J. Gastroenterol.* **2012**, *18*, 4651–4658.
35. Tiwari, S.; Siddiqi, S.A. Intracellular trafficking and secretion of VLDL. *Arterioscler. Thromb. Vasc. Biol.* **2012**, *5*, 1079–1086.
36. Asmat, U.; Abad, K.; Ismail, K. Diabetes mellitus and oxidative stress-A concise review. *Saudi Pharm. J.* **2016**, *24*, 547–553.
37. Milic, S.; Mikolasevic, I.; Orlic, L.; Devcic, E.; Starcevic-Cizmarevic, N.; Stimac, D.; Kapovic, M.; Ristic, S. The role of iron and iron overload in chronic liver disease. *Med. Sci Mon.* **2016**, *22*, 2144–2151.

38. Prenner, S.B.; Mulvey, C.K.; Ferguson, J.F.; Rickels, M.R.; Bhatt, A.B.; Reilly, M.P. Very low-density lipoprotein cholesterol associates with coronary artery calcification in type 2 diabetes beyond circulating levels of triglycerides. *Atherosclerosis* **2014**, *236*, 244-50.
39. Kostapanos, M.S.; Elisaf, M.S. High density lipoproteins and type 2 diabetes: Emerging concepts in their relationship. *World J. Exp. Med.* **2014**, *4*, 1–6.
40. Wong. N.K.P.; Nicholls. S.J.; Tan. J.T.M.; Bursill. C.A. The role of high-density lipoproteins in diabetes and its vascular complications. *Int. J. Mol. Sci.* **2018**, *19*, 1680.

6.2. Capítulo 2

Diazoxide improves muscle function in association with improved dyslipidemia and decreased muscle oxidative stress in streptozotocin – induced diabetic rats

Manuel Alejandro Vargas-Vargas[1], Alfredo Saavedra-Molina[1], Mariana Gómez-Barroso[1], Donovan Peña-Montes[1], Christian Cortés-Rojo [1], Huerta Miguel[2], Trujillo Xochitl[2], Montoya-Pérez Rocío[1]*

1. *Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo. Francisco J. Múgica S/N, Col. Felicitas del Río, Morelia, Michoacán, México. 58030.*
2. *Centro Universitario de Investigaciones Biomédicas, Universidad de Colima. Av. 25 de Julio 965, Las Víboras, Colima, Col. 24040.*

***Corresponding author:**
Rocío Montoya-Pérez, PhD.
Universidad Michoacana de San Nicolás de Hidalgo
Instituto de Investigaciones Químico-Biológicas
Francisco J. Múgica S/N
Col. Felicitas del Río,
Morelia, México. CP 58030.
Tel. +52 1 (443) 3223200 Ext. 4217
E-mail: rmontoya@umich.mx

Abstract

Aim/Introduction: Diabetes is a chronic degenerative disease whose main biochemical characteristic is hyperglycemia, this may be due to a decrease in insulin secretion in the pancreas or a bad action of the hormone in insulin-dependent tissues. Hyperglycemia leads to dyslipidemia and an increase in oxidative damage, which leads to the appearance of diabetic complications. We tested effect of diazoxide in streptozotocin diabetic rats in muscle function, lipid profile and oxidative stress biomarkers. **Materials and Methods:** Wistar rats were divided into 4 groups of six animals each: 1) Control group, 2) diabetes group, 3) Control group + diazoxide and 4) Diabetic + diazoxide (DB + DZX). 4 weeks after, the rats were sacrificed, the Soleus and Extensor Digitorum Longus muscles were extracted to prepare homogenates and serum was obtained for biochemical measurements. Oxidative damage was evaluated by the thiobarbituric acid method and the fluorescent for reactive oxygen species (ROS) probe 2,4-H_2DCFDA, respectively **Results:** Dabetic rats with diazoxide administration showed an increase in the development of muscle strength in both muscles, in turn, the times of onset of fatigue were longer compared to the group of diabetic rats without treatment. Regarding the lipid profile, diazoxide decreased total cholesterol levels in the group of diabetic rats treated with daizoxide ($\bar{x}$46.2mg/dL) compared to the untreated diabetic group ($\bar{x}$=104.4 mg/dL), secondly diazoxide decreased triglyceride concentrations ($\bar{x}$=105.3 mg/dL) compared to the untreated diabetic rats ($\bar{x}$=412.2mg/dL) as well as the levels of very low-density lipoproteins ($\bar{x}$=20.4 mg/dL vs $\bar{x}$=82.44 mg/dL). Regarding the various markers of oxidative stress, the diabetic group treated with daizoxide was able to reduce the concentrations of TBARS and total reactive oxygen species as well as preserve the concentrations of reduced glutathione **Conclusion:** Diazoxide causes an increase in the development of strength in the EDL and soleus muscle, it also causes the onset of fatigue to be delayed, it reduces cholesterol and triglyceride concentrations and improves oxidative stress parameters such as TBARS, ROS and glutathione status.

Keywords Diabetes, diazoxide, oxidative stress

Introduction

Hyperglycemia resulting from uncontrolled glucose regulation is widely recognized as the causal link between diabetes and diabetic complications, Hyperglycemia-induced oxidative stress as the causal link between high glucose and the pathways responsible for hyperglycemic damage (1) including muscle fatigue as one of the most common acute symptom of diabetes, causing loss of muscle strength and mass (2).

Increased oxidative stress also is one of the main factors involved in the development of muscle fatigue (3, 4). Dyslipidemia is another hallmark of diabetes that is associated with a higher risk of cardiovascular disease (5); conversely, the decrease in blood lipids improves cardiovascular outcomes (6, 7). On the other hand, a negative relationship has been stablished between dyslipidemia and muscle function in rodents (8) and humans (9). Thus, in diabetes, increased oxidative stress and dyslipidemia may have additive deleterious effects in muscle function.

The ATP-sensitive K+-channels (KATP) are widely distributed in the tissues including neurons, vascular, pancreatic beta cells, cardiac, and skeletal muscles (10), fatigue is significantly decreased in healthy mouse by mitoKATP channel openers (11). The effect of diazoxide on muscle fatigue in diabetes is still unknown, thus, to address these issues, we studied the effect of diazoxide on diabetic rats in muscle streght development, lipid profile and oxidative stress biomarkers.

Materials and Methods

Animals and treatments

Male Wistar rats weighing 200-250 g were used and maintained under standard laboratory conditions with free access to food and water. All animal procedures were conducted following the Mexican Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, México) and were approved by the Institutional Committee of the Universidad Michoacán de San Nicolás de Hidalgo for the Use and Care of Animals. Diazoxide was administered for 14 days intraperitoneally at a dose of 35 mg/kg

Induction of diabetes and experimental design

Diabetes was induced by a single intraperitoneal injection of 45mg/kg BW of STZ (Sigma-Aldrich, St. Louis, MO, USA). STZ was dissolved in 0.1 M sodium citrate buffer (pH 4.5) and control rats were injected with the same vehicle. Two days after STZ injection, blood glucose levels were measured, and rats with levels >200 mg/dL were considered diabetic. 24 rats were divided into 4 groups of six animals each: 1) Control group (CT): fed only with standard rodent chow; 2) diabetes group (DB): fed with standandard rodent chow diet; 3) Control group + diazoxide (CT + DZX): fed with estándar rodent chow diet plus diazoxide administration; 4) Diabetic + diazoxide (DB + DZX): fed with standard rodent chow diet plus diazoxide administration. Fresh blood samples were collected from the rat tail vein, and glucose levels were determined using a glucometer (ACCU-CHEK® Active, Roche Diagnostics, Mannheim, Germany).

Muscle extraction and tissue preparation

The animals were sacrificed once the experimental period was over. The soleus and extensor digitorum longus (EDL) muscles of one limb were extracted and placed on a Petri dish with Krebs-Ringer buffer (118 mM NaCl, 75 mM KCl, 1.18 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 2.08 g/L glucose, pH 7.4) for muscle tension tests. The muscles of the other limb were homogenized to assay oxidative stress biomarkers. The protein content of muscle homogenates was assayed by a medication of the Biuret procedure using BSA as the standard (12).

Force development and fatigue induction

The soleus and EDL muscles obtained from one of the hind legs were used for tension records, which were placed in a Petri dish covered with a transparent resin bottom (Sylgard), fixed with entomological pins immersed in Krebs-Ringer solution and supplied with 95% CO_2 and 5% O_2. Excess fat and connective tissue were removed under a stereoscopic microscope. Once cleaned, the muscle was mounted in a chamber for recording isometric tension, with its proximal end attached to the bottom of the chamber and the distal end to the hook of an optical transducer (World Precision Instruments, USA), which was connected to an amplifier (World Precision Instruments, USA) and an analogic-to-digital interface (World Precision Instruments) for data acquisition of the tension generated by the muscle, using the MDAC software (World Precision Instruments, USA). Inside the recording chamber, two platinum electrodes were placed, which were connected to a stimulation isolating unit (Grass, USA) to carry out the fatigue protocol. This consisted of pulses of 100 V, 300 ms of duration and frequency of 45 Hz for soleus muscle and 50 Hz for EDL muscle. The stimulation was stopped after fatigue appeared (70% of force reduction).

Lipid profile

At the end of the experimental protocol, the animals were sacrificed by decapitation. The serum was collected by centrifugation of whole blood samples at 3000 *g* for 5 min (Thermo Scientific Sorvall RC 6+ centrifuge). Next, a Fujifilm NX500i (Tokyo, Japan) system was used to analyze the total serum cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) using Spinreact reagents, following the supplier's instructions. Very low-density lipoprotein (VLDL) was calculated using the following formula: VLDL=TG/5 (13).

Determination of lipid peroxidation

Lipid peroxidation was evaluated in homogenates of muscle with the thiobarbituric acid (TBA) method (14) with slight modifications. 0.5 mg of protein was suspended in phosphate buffer (100 mM, pH 7.4), mixed with the reagent solution containing 0.375% thiobarbituric acid (TBA), 15% trichloroacetic acid (TCA), and 0.25 M HCl, and incubated for 25 min in a boiling water bath. Next, the mixtures were vortexed and incubated in a boiling water bath for 25 min. Finally, the colored product was determined by measuring the absorbance at 532 nm using a Shimadzu UV-2550 spectrophotometer. Lipid peroxidation levels were reported as thiobarbituric acid reactive substances (TBARS) per milligram of protein.

ROS measurements

To determine the concentration of ROS, cell-permeable fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) was used. 0.5 mg of muscle protein was resuspended in 2 mL of a buffer with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 100 mM KCl, 3 mM $MgCl_2$, and 3 mM KH_2PO_4 (pH 7.4) Fluorescence changes were detected in a RF-5301PC spectrofluorophotometer (Shimadzu Corporation, Kyoto, Japan) (λex 485 nm; λem 520 nm) (15).

Measurement of glutathione

Glutathione redox status was determined by the method reported by Rahman (16) with slight modifications. Total glutathione content was determined in 0.5 mg of protein. Samples were suspended in 0.1% Triton-X and 0.6% sulfosalicylic acid in a 0.1 M potassium phosphate buffer (pH 7.5). Subsequently, the mixture was sonicated 3 times and placed on ice for 20 seconds between sonicates, followed by two freezing/thawing cycles, and centrifuged at 6500 *g*. Then, the supernatant was placed in the potassium phosphate buffer, with 100 µM 5, 5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and 0.1 units/mL of the enzime

glutathione reductase (GR). Once the above was done to start the reaction, 50 µM of β-NADPH was added and monitored for 5 min at 412 nm in a UV/vis spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). 0.2% 4-vinylpyridine was used to obtain oxidized glutathione (GSSG), once samples were incubated with this reagent for 1 h at room temperature. GSH concentration was calculated by subtracting the concentration of GSSG from the concentration of total glutathione.

Statistical analysis

All the data are expressed as the mean ± standard error of the mean (SEM). Statistical significance was evaluated using Student t-test and ANOVA, followed by multiple comparisons analysis performed with a post hoc Tukey test; statistical significance was set at $P<0.05$. The analysis was done with GraphPad Prism 6 (Prism 6.0, GraphPad Software Inc., San Diego, CA, USA).

Results

Effect diazoxide on blood glucose and body weight in STZ-diabetic induced rats

Circulating glucose levels were measured after leaving all groups on an 8-hour fast, as shown in Fig. 1A. it can be appreciated that the effect of diazoxide on circulating glucose levels in rats with diabetes had no significant impact. On the other hand, in terms of body weight, diazoxide in the group of diabetic rats, despite the fact that it did not have a significant effect compared to the group without treatment, it is observed that the treatment with diazoxide manages to gain slightly weight instead of losing weight as happened with the group of diabetic rats without treatment (Fig. 1B).

Figure 1. Fasting serum glucose (1A) and eight gain (1B). Weight gain was determined by subtracting the animals weight after eight-week treatments with the diets, determined right before sacrifice, minus the weight at the beginning of the treatments. Data are expressed as the mean ± standard error. $P<0.05$ (n = 6, ANOVA plus Tukey's post hoc test). Different letters represent statistically significant differences. C, control group; DB, diabetic rats; C+DZX, con group with diazoxide administration; DB+DZX, diabetic with diazoxide administration.

Effects of diazoxide on muscle strength development and fatigue

To determine the effect of diazoxide on the development of muscle strength and fatigue times, the muscle tension protocol was carried out and these results are shown below. The effect of diazoxide in animals with diabetes was shown to increase the development of muscle strength both in maximum tension and in total tension in the EDL muscle (Fig. 2A) and soleus muscle (Fig. 2B).

On the other hand, the times of muscle fatigue are shown in Fig. 2C, and it is observed that fatigue times agree with the results of muscular tension, where it is observed that diabetic group treated with diazoxide have higher fatigue times in both muscles, which indicates that diazoxide delays the times of onset of muscle fatigue in diabetic rats.

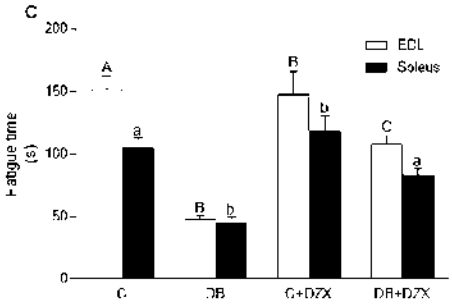


Figure 2. Diazoxide effect on muscle strength development in EDL (2A), soleus muscle (2B) and effect of diazoxide on fatigue times in EDL (open bars) and soleus muscle (solid bars) (2C). Data are expressed as the mean ± standard error. P <0.05 (n = 6, ANOVA plus Tukey's post hoc test). **A** and **B** represents the statistically significant differences between the different groups of the peak tension (open bars); **a** and **b** represent the statistically significant difference between the different groups of the total tension (solid bars). C, control group; DB, diabetic rats; C+DZX, con group with diazoxide administration; DB+DZX, diabetic with diazoxide administration.

Effects of diazoxide on serum lipid profile

At the end of the experimental stage, serum lipid levels were measured, total cholesterol levels can be seen in the figure 3A, and it is observed that the group of diabetic rats without treatment had more than double the cholesterol concentrations compared to the other groups, in turn, diazoxide was shown to decrease total cholesterol concentrations in the group of diabetic rats. Regarding HDL, there was a significant difference between the control group and the other groups (Fig. 3B).

Then, the triglyceride levels are shown in the different groups (Fig. 3C), it is observed that the group of diabetic rats without treatment had the highest levels with a significant difference with the other groups, it should be noted that the group of diabetic rats treated with diazoxide decreased concentrations to normal levels. Finally, the VLDL levels in the group of diabetic rats with diazoxide normalized their levels to normal concentrations, contrary to what happened in the group of diabetic rats without treatment, which had the highest levels (Fig. 3D.

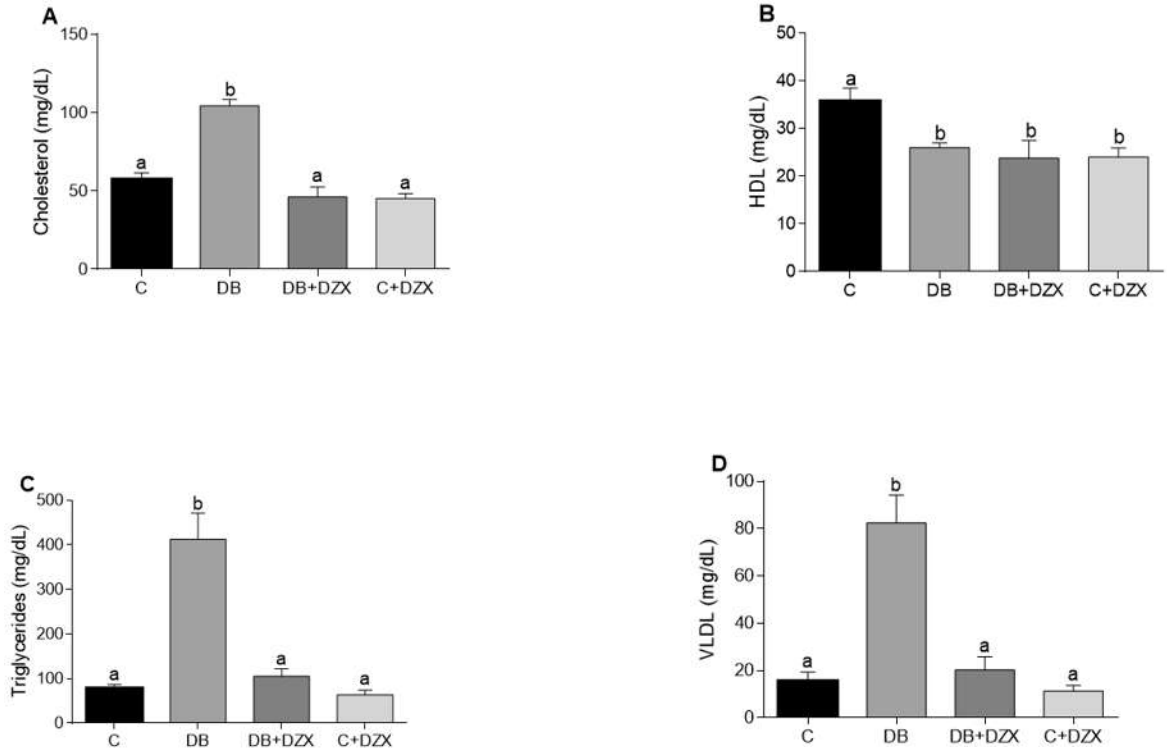


Figure 3. Serum levels of cholesterol (A), high-density lipoproteins (B), triglycerides (C), and very-low-density lipoproteins (D). Data are expressed as the mean ± standard error. P <0.05 (n = 6, ANOVA plus Tukey's post hoc test). Different letters represent statistically significant differences. C, control group; DB, diabetic rats; C+DZX, con group with diazoxide administration; DB+DZX, diabetic with diazoxide administration.

Effect of diazoxide on oxidative stress markers

Subsequently, we evaluated the concentration of ROS in muscle homogenate, and we observed a decrease in said concentrations in the group of rats treated with diazoxide, not so, for the group of diabetic rats without treatment (Fig. 4). Regarding the group of healthy rats with diazoxide treatment, no modification was observed in this parameter.

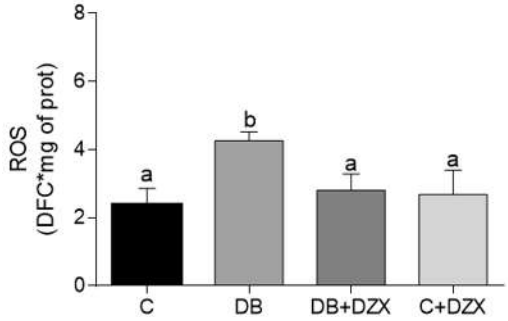


Figure 4. Effect of diazoxide administration in levels of ROS in muscle homogenates from control rats. Data are expressed as the mean ± standard error. *P* <0.05 (n = 6, ANOVA plus Tukey's post hoc test). Different letters represent statistically significant differences. C, control group; DB, diabetic rats; C+DZX, con group with diazoxide administration; DB+DZX, diabetic with diazoxide administration.

In regard to lipid peroxidation (Fig. 5), the diabetic group without doubled in the diabetic group with respect to the control group without treatment it had the highest levels but without showing any significant difference between the control group and the group of diabetic rats with diazoxide administration, but with the group of healthy rats with diazoxide administration.

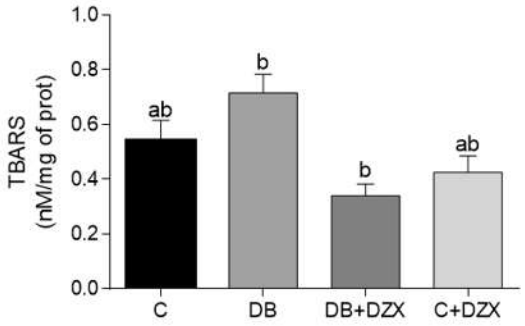
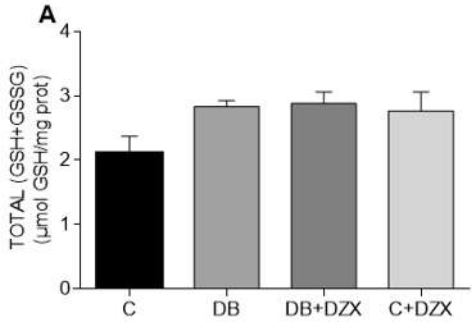
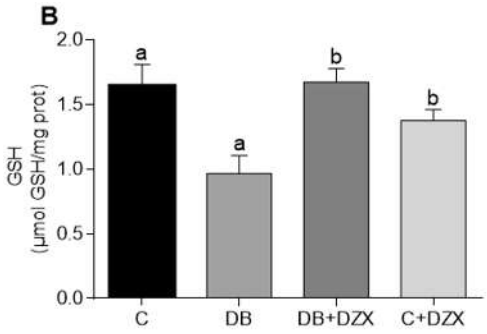


Figure 5. Effect diazoxide administration in lipid peroxidation of muscle. Data are expressed as the mean ± standard error. *P* <0.05 (n = 6, ANOVA plus Tukey's post hoc test). Different letters indicate statistically significant differences. C, control group; DB, diabetic rats; C+DZX, con group with diazoxide administration; DB+DZX, diabetic with diazoxide administration.

The glutathione redox status in the muscle is shown in the Fig 6. No significant difference was observed in total glutathione concentrations between the different groups (Fig. 6A). However there is a clear decrease in the concentrations of reduced glutathione in the group of diabetic rats without treatment, contrary to what was observed when diabetic rats were administered with diazoxide (Fig. 6B). The levels of oxidized glutathione are shown in Fig. 6C, where it is observed that in all the groups there was no significant difference except for the group of diabetic rats without treatment, said group obtained almost twice the concentration of oxidized glutathione than the rest of the groups. Regarding the GSH/GSSG ratio, it is observed that the GSH/GSSG ratio was higher in the group of diabetic rats with administration of dioxide compared to the group of diabetic rats without treatment (Fig. 6D).





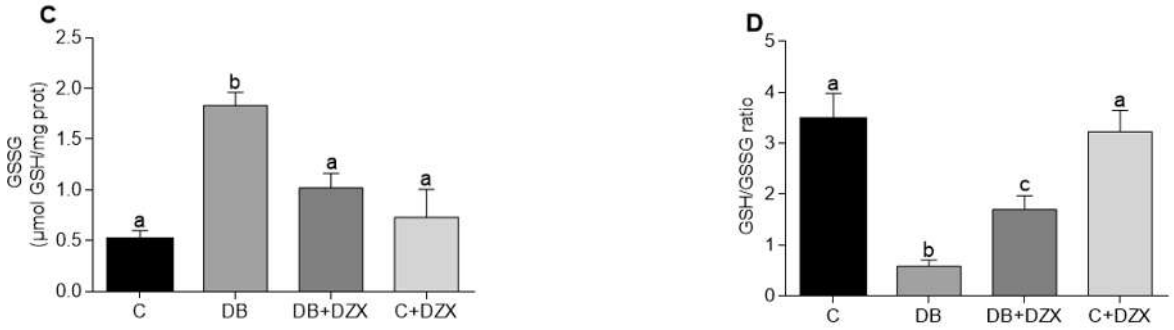


Figure 6. Effect of diazoxide in total glutathione (A), reduced glutathione (GSH) (B), oxidized glutathione (GSSG) (C), and GSH/GSSG ratio (D). Data are expressed as the mean ± standard error. P <0.05 (n = 6, ANOVA plus Tukey's post hoc test). Different letters indicate statistically significant differences. C, control group; DB, diabetic rats; C+DZX, con group with diazoxide administration; DB+DZX, diabetic with diazoxide administration.

Discussion

In the present study we undertook the task of examining the effect of the drug diazoxide on different parameters such as muscle strength, lipid profile and different markers of oxidative stress in an experimental diabetes model.
The effect of diazoxide on the development of muscle strength and some oxidative stress markers had previously been studied in healthy mice (17),
However, it is the first time that the effect of diazoxide has been analyzed in an experimental diabetes model on one of the most common symptoms of this disease, such as muscle fatigue (18). Another of the biochemical alterations commonly found in diabetes is the lipid profile, which is related to the risk of developing cardiovascular events (19), that together with the increase in oxidative stress leads to the appearance of the different complications typical of diabetes (20,21).
Diabetes is characterized by an alteration in glucose levels and involuntary weight loss peso (22). In our results (Fig 1A), we can see that diazoxide had no effect on glucose levels, previous studies have reported that diazoxide improves glucose levels in Zucker diabetic fatty (23), however in the present study an experimental diabetes model was used that simulates type 1 diabetes where there is a total absence of insulin, it is for this reason that daizoxide in the present model it had no effect on circulating glucose. As shown in Fig. 1B, body weight was not modified by the action of diazoxide, however the group of rats with diabetes treated with diazoxide had a slight increase in weight without significant difference compared to the group of diabetic rats. no treatment during the experimental stage.
Regarding the muscle streght development, DB + DZX group showed an increase in this parameter, both in peak tension and the total tension in the EDL muscle (Fig. 2A) and soleus (Fig. 2B), as well as a delay in the times of onset of fatigue (Fig. 2C). Previously diverse studies have reported that the opening of ATP-sensitive potassium channels with both diazoxide and nicolandil improves ATP production and reduces muscle fatigue (17, 24). Diazoxide causes significant changes in the volume of the mitochondrial matrix, as a consequence of the entry of K^+ ions accompanied by water, which causes a correct maintenance of the mitochondrial volume and thus the architecture of important compartments is preserved, avoiding the alteration of the structure and function of various protein complexes which are part of the electron transport chain maintaining a correct balance of energy transfer between mitochondria and cytosol (25)

In patients with type 1 diabetes there is a high frequency of dyslipidemia, mainly hypercholesterolemia and hypertriglyceridemia (26,27). In the results obtained in the present investigation, it was shown that diazoxide was able to reduce total cholesterol levels in rats with diabetes Fig. 3A and normalize triglyceride levels (Fig 3C), we also found that the levels of TBARS and total ROS decreased in the group of diabetic rats treated with daizoxide, these results may be related since dyslipidemia is a common risk factor for developing atherosclerosis and this is related to increased ROS production, on the other hand clinical studies have documented strong evidence between plasma levels of oxidative stress parameters and atherogenic lipoproteins in patients with cardiovascular disease (28)
In this sense, the group of diabetic rats treated with diazoxide obtained normal levels of VLDL, contrary to the group of diabetic rats that did not receive any treatment, the elevated levels of this lipoprotein are related to cardiovascular disease in patients with diabetes mellitus with a two to four fold increase in the risk of cardiovascular disease compared to non-diabetic individuals (27,29).

Glutathione (GSH), the most abundant low molecular weight thiol compound synthesized in cells, it plays critical roles in protecting cells from oxidative damage and the toxicity of xenobiotic electrophiles, and maintaining redox homeostasis (30), several investigators have reported that erythrocyte glutathione (GSH) concentration is decreased in patients with type 2 diabetes (31).

Glutathione is present in both reduced (glutathione) and oxidized (glutathione disulfide [GSSG]), the precise relative amounts depend on the redox status of the cell, but in healthy cells, glutathione predominates over GSSG by as much as 100-fold (32). Lutchmansingh et al in 2018 discovered that people with type 2 diabetes mellitus had decreased levels of reduced glutathione, however they found that the group of patients with diabetes who already had certain complications of the disease obtained lower levels of reduced glutathione, in this investigation the levels were not related of glutathione reduced with the levels of glicemis or HbA1c but with the appearance of complications of the disease (31).

Low levels of antioxidant defenses and high concentrations of end-products of oxidation such as TBARS can lead to higher levels of total cholesterol, which could be observed in the study carried out by Valente T. et al. 2021 (33).
In our study that found that serum LDL cholesterol and triglycerides were inversely correlated with GSH / GSSG, which agrees with the research carried out by Valente T et al in 2021, in turn these results coincide with what was found in the levels of lipid peroxidation since the group of rats with diabetes without treatment had the highest levels of TBARS as can be seen in figure 5, contrary to what happens when diazoxide was administered to diabetic rats since they managed to decrease the concentrations of TBARS significantly. Having said all the above, treatment with diazoxide manages to improve various oxidative stress biomarkers, witch are related to the increase in cholesterol and triglycerides. It is important to emphasize that high levels of cholesterol and triglycerides are closely related to fatigue (34), and once these parameters are controlled, this contributes to less fatigue and the muscle to generate greater force.

In conclusion, diazoxide causes an increase in the development of strength in the EDL and soleus muscle, it also causes the onset of fatigue to be delayed, it reduces cholesterol and triglyceride concentrations and improves oxidative stress parameters such as TBARS, ROS and glutathione status.

ETHICAL CONSIDERATIONS
All animal procedures were conducted following the Mexican Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, México) and were approved by the Institutional Committee of the Instituto de Investigaciones Químico-Biológicas o the Universidad Michoacana de San Nicolás de Hidalgo for the Use and Care of Animals (Number 2018-06; april, 2018).

DISCLOSURE
The authors appreciate the partial economic support of Coordinación de la Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo. CIC-UMSNH-2020-RMP; CIC-UMSNH-2020-ASM.

The authors declare no conflict of interest.

References

1. Rolo AP, Palmeira CM. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol.* 2006 Apr 15; 212(2):167-78. doi: 10.1016/j.taap.2006.01.003. Epub 2006 Feb 20. PMID: 16490224.
2. Sanjay Kalra and Rakesh Sahay. Diabetes fatigue syndrome. *Diabetes Ther* 2018 ; 9(4):1421–1429.
3. Callahan Leigh and Supinski Gerald. Hyperglycemia-induced diaphragm weakness is mediated by oxidative stress. *Crit Care* 2014; 18(3):88
4. Devulapally Yamini, Singh Negi Devender, Bai Pasula Kasturi. A comparative study of skeletal muscle fatigue in diabetic and non-diabetic human beings. *Natl J Physiol Pharm Pharmacol* 2018; 8(11): 1529-1532
5. Jia A, Zeng W, Yu L. et al. Very low-density lipoprotein cholesterol is associated with extent and severity of coronary artery disease in patients with type 2 diabetes mellitus. *SAGE Open Med,* 2019. doi: 10.1177/2050312119871786.
6. Schofield JD, Liu Y, Rao-Balakrishna P et al. Diabetes Dyslipidemia. *Diabetes Ther* 2016; 7:203-19.

7. Warraich, Haider J, and Jamal S Rana. Dyslipidemia in diabetes mellitus and cardiovascular disease. *Cardiovasc. Endocrinol.* 2017; 6 (1): 27-32.
8. Andrich DE, Ou Y, Melbouci L, et al. Altered Lipid Metabolism Impairs Skeletal Muscle Force in Young Rats Submitted to a Short-Term High-Fat Diet. *Front. Physiol.* 2018; 26 (9): 1327
9. Blakeley CE, Van Rompay MI, Schultz NS, et al. Relationship between muscle strength and dyslipidemia, serum 25(OH) D, and weight status among diverse schoolchildren: a cross-sectional analysis. *BMC Pediatr* 2018; 18(1):23.
10. Tricarico, D., Selvaggi, M., Passantino, G., De Palo, P., Dario, C., Centoducati, P., Tateo, A., Curci, A., Maqoud, F., Mele, A., Camerino, G. M., Liantonio, A., Imbrici, P., & Zizzo, N. (2016). ATP Sensitive Potassium Channels in the Skeletal Muscle Function: Involvement of the KCNJ11(Kir6.2) Gene in the Determination of Mechanical Warner Bratzer Shear Force. *Frontiers in physiology*, *7*, 167. https://doi.org/10.3389/fphys.2016.00167
11. Maria C. García, Ascención Hernández, Jorge A. Sánchez, Role of mitochondrial ATP-sensitive potassium channels on fatigue in mouse muscle fibers, Biochem. Biophys. Res. Commun. Volume 385, Issue 1, 2009, Pages 28-32, ISSN 0006-291X,
12. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 1949; 177(2):751-66.
13. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 1972; 18(6):499-502.
14. Buege JA, Aust SD. Microsomal lipid peroxidation. *Meth. Enzymol.* 1978; 52:302-10.
15. Huerta-Cervantes M, Peña-Montes DJ, Montoya-Pérez R, et al. Gestational Diabetes Triggers Oxidative Stress in Hippocampus and Cerebral Cortex and Cognitive Behavior Modifications in Rat Offspring: Age- and Sex-Dependent Effects. *Nutrients* 2020; 12(2): 376.
16. Rahman, I., Kode, A. & Biswas, S. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat. Protoc.* 2006; 1: 3159–3165.
17. García MC, Hernández A and Sánchez JA. 2009. Role of mitochondrial ATP-sensitive potassium channels on fatigue in mouse muscle fibers. *Biochem Biophys Res Commun*. 385: 28-32.
18. Fritschi, C., & Quinn, L. (2010). Fatigue in patients with diabetes: a review. *Journal of psychosomatic research*, *69*(1), 33–41.
19. Arrieta, F., Iglesias, P., Pedro-Botet, J., Tébar, F. J., Ortega, E., Nubiola, A., Pardo, J. L., Maldonado, G. F., Obaya, J. C., Matute, P., Petrecca, R., Alonso, N., Sarabia, E., Sánchez-Margalet, V., Alemán, J. J., Navarro, J., Becerra, A., Duran, S., Aguilar, M., Escobar-Jiménez, F., … Grupo de Trabajo Diabetes y Enfermedad Cardiovascular de la Sociedad Española de Diabetes (SED) (2016). Diabetes mellitus y riesgo cardiovascular: recomendaciones del Grupo de Trabajo Diabetes y Enfermedad Cardiovascular de la Sociedad Española de Diabetes (SED, 2015) [Diabetes mellitus and cardiovascular risk: Working group recommendations of Diabetes and Cardiovascular Disease of the Spanish Society of Diabetes (SED, 2015)]. *Atencion primaria*, *48*(5), 325–336.
20. Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation research*, *107*(9), 1058–1070.
21. Visweswara Rao Pasupuleti, Chandra Sekhar Arigela, Siew Hua Gan, Sirajudeen Kuttulebbai Nainamohamed Salam, Kumara Thevan Krishnan, Nurhanan Abdul Rahman, Mohammad Saffree Jeffree, "A Review on Oxidative Stress, Diabetic Complications, and the Roles of Honey Polyphenols", Oxidative Medicine and Cellular Longevity, vol. 2020, Article ID 8878172, 16 pages, 2020.
22. American Diabetes Association (2013). Diagnosis and classification of diabetes mellitus. *Diabetes care*, *36 Suppl 1*(Suppl 1), S67–S74.
23. Alemzadeh, R.; Tushaus, K.M. Modulation of Adipoinsular Axis in Prediabetic Zucker Diabetic Fatty Rats by Diazoxide. Endocrinology 2004, 145, 5476–5484.
24. Sánchez-Duarte, E.; Trujillo, X.; Cortés-Rojo, C.; Saavedra-Molina, A.; Camargo, G.; Hernández, L.; Huerta, M.; Montoya-Pérez, R. Nicorandil improves post-fatigue tension in slow skeletal muscle fibers by modulating glutathione redox state. *J. Bioenerg. Biomembr.* **2017**, *49*, 159–170.
25. Kowaltowski A.J. Seetharaman S. Paucek P. Garlid K.D. Bioenergetic consequences of opening the ATP-sensitive K+ channel of heart mitochondria Am J Physiol 2001 280 H649 H657
26. Bruno Vergès, Dyslipidemia in Type 1 Diabetes: A Masked Danger, Trends in Endocrinology & Metabolism, Volume 31, Issue 6, 2020, Pages 422-434, ISSN 1043-2760,
27. Tomic, S., Brkic, S., Maric, D., & Mikic, A. N. (2012). Lipid and protein oxidation in female patients with chronic fatigue syndrome. *Archives of medical science : AMS*, *8*(5), 886–891. https://doi.org/10.5114/aoms.2012.31620
28. Vergès B. (2015). Pathophysiology of diabetic dyslipidaemia: where are we?. *Diabetologia*, *58*(5), 886–899. https://doi.org/10.1007/s00125-015-3525-8

29. Manfredi Rizzo, Jelena Kotur-Stevuljevic, Kaspar Berneis, Giatgen Spinas, Giovam Battista Rini, Zorana Jelic-Ivanovic, Vesna Spasojevic-Kalimanovska, Jelena Vekic, Atherogenic dyslipidemia and oxidative stress: a new look, Translational Research, Volume 153, Issue 5, 2009, Pages 217-223, ISSN 1931-5244, https://doi.org/10.1016/j.trsl.2009.01.008.
30. Basa, A. L., & Garber, A. J. (2001). Cardiovascular disease and diabetes: modifying risk factors other than glucose control. *The Ochsner journal*, *3*(3), 132–137.
31. Forman, H. J., Zhang, H., & Rinna, A. (2009). Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular aspects of medicine*, *30*(1-2), 1–12. https://doi.org/10.1016/j.mam.2008.08.006
32. Lutchmansingh, F. K., Hsu, J. W., Bennett, F. I., Badaloo, A. V., McFarlane-Anderson, N., Gordon-Strachan, G. M., Wright-Pascoe, R. A., Jahoor, F., & Boyne, M. S. (2018). Glutathione metabolism in type 2 diabetes and its relationship with microvascular complications and glycemia. *PloS one*, *13*(6), e0198626. https://doi.org/10.1371/journal.pone.0198626
33. Blondet, N. M., Messner, D. J., Kowdley, K. V., & Murray, K. F. (2018). *Mechanisms of Hepatocyte Detoxification. Physiology of the Gastrointestinal Tract, 981–1001.* doi:10.1016/b978-0-12-809954-4.00043-8
34. Valente T, Valente F, Lucchesi MBB, Punaro GR, Mouro MG, Gabbay MAL, Higa EMS, Dib SA. Relationship between short and long-term glycemic variability and oxidative stress in type 1 diabetes mellitus under daily life insulin treatment. Arch Endocrinol Metab. 2021 Mar 19:2359-3997000000338. doi: 10.20945/2359-3997000000338. Epub ahead of print. PMID: 33740334.

7. DISCUSIÓN GENERAL

En el presente trabajo de investigación se evaluó el efecto del hierro y el fármaco diazóxido en el desarrollo de fuerza muscular, perfil de lípidos y diversos marcadores de estrés oxidante en ratas Wistar con diabetes mellitus. Previamente se ha reportado que la sobrecarga de hierro provoca efectos deletéreos en la enfermedad, provocando una alteración en los niveles de colesterol, triglicéridos y aumento en el estrés oxidante (50,51). En el mismo sentido, diversos estudios han demostrado que una restricción de hierro con animales con diabetes mejora marcadores perjudiciales en la enfermedad como la sensibilidad a la insulina y el perfil de lípidos (48), además, existe evidencia que la sobrecarga de hierro provoca un aumento en diversos marcadores de estrés oxidante y así con la aparición de complicaciones de esta enfermedad (52).

Por otro lado, la disminución del ATP juega un papel importante en el desarrollo de la fatiga muscular y cualquier evento relacionado con la alteración de la producción de energía, esto determina la tendencia de la fatiga muscular.

Se ha demostrado que la apertura de los mitoK_{ATP} participa en la protección del músculo durante la fatiga en un modelo de ratones sanos, a su vez que disminuyen la producción de especies reactivas de oxígeno (26).

En el presente estudio, se reporta por primera vez que la restricción de hierro y el diazóxido en animales con diabetes provoca un aumento en el desarrollo de la fuerza muscular y disminuye los tiempos en la cual los músculos desarrollan fatiga, por otro lado, modulan las concentraciones de triglicéridos en suero y de lipoproteínas de muy baja densidad, así como diversos marcadores de estrés oxidante.

8. CONCLUSIÓNES

La administración de diazóxido y las dietas bajas en hierro promueven una disminución en los niveles de diferentes parámetros de estrés oxidante, esto contribuye a que el desarrollo de la fuerza muscular prevalezca y los tiempos de aparición de fatiga sean mayor

9. REFERENCIAS BIBLIOGRÁFICAS

1. Abdulfatai B. Olokoba, Olusegun A. Obateru, Lateefat B. Olokoba. 2012. Type 2 Diabetes Mellitus: A Review of Current Trends. Oman Medical Journal. 27 (4): 269-273
2. Mathers CD, Loncar D (2006) Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3(11): e442. doi:10. 1371/journal.pmed.0030442
3. Carrillo Carmen, Panduro Cerda Arturo. 2001. Genética de la diabetes mellitus tipo 2. Investigación en Salud. 3 (99): 27-34
4. American Diabetes Association, Diabetes care, Vol 31, Suppl 1, Jan 2008
5. Rodríguez Jiménez L y Del Rio García M. 2013. Actualización y puesta al día en diabetes mellitus tipo 2. Curso de Experto Universitario en Urgencias y Emergencias. Ed. Universidad Internacional de Andalucía.España: pp: 51
6. Serra Rexach. 2006. Consecuencias clínicas de la sarcopenia. Nutr. Hosp. 21 (Supl. 3) 46-50
7. Cetrone M, Mele A, Tricarico D. Effects of the antidiabetic drugs on the age- related atrophy and sarcopenia associated with diabetes type II. Curr Diabetes Rev. 2014; 10(4):231-7.
8. Umegaki Hiroyuki. 2015. Sarcopenia and diabetes: Hyperglycemia is a risk factor for age-associated muscle mass and functional reduction. J Diabetes Investig Vol. 6 No. 6
9. López CH. J., Fernández V.A. (2006) Fisiología del ejercicio. 3ra edición. Buenos Aires, Argentina. Editorial medica panamericana.
10. Martinez M. Goedendorp, Cees J. Tack, Elles Steggink, Lotte Bloot, Ellen Bazelmans, y Hans Knoop.2014. Chronic Fatigue in Type 1Diabetes: Highly Prevalent but Not Explained by Hyperglycemia or Glucose Variability. Diabetes Care Volume 37: 73-80
11. Fritschi C y Quinn L, .2010. Fatigue in patients with diabetes: A review. Journal of Psychosomatic Research 69 (2010) 33 41
12. Sánchez-Duarte, X. Trujillo, C. Cortés-Rojo, A. Saavedra-Molina, G. Camargo, L. Hernández, M. Huerta, R. Montoya-Pérez. Nicorandil improves post-fatigue tension in slow skeletal muscle fibers by modulating glutathione redox state. J Bioenerg Biomembr
13. Lindstedt SL. Skeletal muscle tissue in movement and health: positives and negatives. The J Exp Biol. 2016;219:183–8.
14. Steinbacher, P., & Eckl, P. (2015). Impact of oxidative stress on exercising skeletal muscle. *Biomolecules*, *5*(2), 356–377. https://doi.org/10.3390/biom5020356
15. Seino, S. (1999). ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. Annu. Rev. Physiol. 61, 337–362.
16. Flagg, T.P., Enkvetchakul, D., Koster, J.C., and Nichols, C.G. (2010). Muscle KATP channels: recent insights to energy sensing and myoprotection. Physiol. Rev. 90, 799–829.
17. Li Ningning, Jing-Xiang Wu, Dian Ding, Jiaxuan Cheng, Ning Gao, Lei Chen. 2017. Structure of a Pancreatic ATP-Sensitive Potassium Channel Cell 168, 101–110
18. Inagaki N., Gonoi T., Clement J.P.T., Namba N., Inazawa J., Gonzalez G., Aguilar-Bryan L., Seino S., Bryan J. (1995b) Reconstitution of K_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270: 1166– 1170.

19. Gong B., Miki T., Seino S., Renaud J.M. (2000). A K_{ATP} channel deficiency affects resting tension, not contractile force, during fatigue in skeletal muscle. Am. J. Physiol. Cell Physiol. 279 (5): C1351–1358
20. Kane G.C., Behfar A., Yamada S., Perez-Terzic C., O'Cochlain F., Reyes S., Dzeja P.P., Miki T., Seino S., Terzic A. (2004) ATP-sensitive K^+ channel knockout compromises the metabolic benefit of exercise training, resulting in cardiac deficits. *Diabetes* 53: S169–S175.
21. Thebet M., Miki T., Seino S., Renaud J.M. (2005). Treadmill running causes significant damage in skeletal of muscle KATP channel deficient mice. *Physiol Genomics* 22: 204–212.
22. Costa A, Garlid K, West I, Lincoln T, Downey J, Cohen M, Critz S. 2005. Protein Kinase G Transmits the Cardioprotective Signal From Cytosol to Mitochondria. Circulation Research. . 329 -336
23. Sarja H, Anttila T, Mustonen H, Heravi J, Haapanen H, Tuominen H, Miinalanainen I, Juvonen T, Anttila V. 2017. Diazoxide Attenuates Ischemic Myocardial Injury in a Porcine Model. Heart Surgery Forum. 20 (4); 153-161.
24. Friciu M, Zaraa S, Roullin VG, Leclair G (2016) Stability of Diazoxide in Extemporaneously Compounded Oral Suspensions. PLoS ONE 11(10): e0164577.
25. Kowaltowsky A, Seetharaman S, Paucker P y Garlid K. 2000. Bioenergetic Consequences of opening the ATP-sensitive K^+ channel of heart mitochondrial. *Am J Physiol Heart Circ Physiol*. 280: H649-H657.
26. García MC, Hernández A, Sánchez JA. Role of mitochondrial ATP-sensitive potassium channels on fatigue in mouse muscle fibers. Biochem Biophys Res Commun. 2009 Jul 17;385(1):28-32. doi: 10.1016/j.bbrc.2009.05.019. Epub 2009 May 8. PMID: 19427835.
27. Turrens J. F. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of physiology*, *552*(Pt 2), 335–344. doi:10.1113/jphysiol.2003.049478
28. Rosado-Pérez J y Mendoza-Núñez V. 2007. Inflamación crónica y estrés oxidativo en la diabetes mellitus. Bioquímica. 32(2): 58-69
29. Ray P, Huang B, Tsuji Y. 2012. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal. 2012 May ; 24(5): 981–990. doi:10.1016/j.cellsig.2012.01.008.
30. Ullah Asmat, Khan Abad y Khan Ismail. 2015. Diabetes mellitus and oxidative stress-A concise review. Saudi Pharmaceutical Journal. 3 (1): 1-7
31. Di Meo S. Tanea T. Reed,2 Paola Venditti,1 and Victor Manuel Victor. 2016. Role of ROS and RNS Sources in Physiological and Pathological Conditions. http://dx.doi.org/10.1155/2016/1245049
32. Asmat Ullah, Abad Khan a y Ismail Khan. 2016. Diabetes mellitus and oxidative stress: A concise review. Saudi Pharmaceutical Journal (24): 547–553
33. Birben E Sahiner M, Sackesen C, Erzurum S, Kalayci o. 2012. Oxidative Stress and Antioxidant Defense. WAO Journal
34. Gómez-Gutierrez A, Parra-Rosa B, Bueno-Sánchez J. 2013. Papel del receptor 1 de transferrina en la captación del hierro y su relación con la deficiencia gestacional de hierro y la preeclampsia. Revista Cubana de Obstetricia y Ginecología. 39(1)33-42

35. Sheftel A, Masson A, Ponka P. 2012. The Long History of Iron in the Universe and in Health and Disease. Biochim Biophys Acta. 2012 March ; 1820(3): 161–187. doi:10.1016/j.bbagen.2011.08.002.
36. Worthen C, Enns C. The role of hepatic transferrin receptor 2 in the regulation of iron homeostasis in the body. Front. Pharmacol., 06 March 2014 https://doi.org/10.3389/fphar.2014.00034
37. Fernández-Real JM, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes. 2002; 51(8): 2348–2354. pmid:12145144
38. Zhao Z, Li S, Liu G, Yan F, Ma X, Huang Z. Body iron stores and heme-iron intake in relation to risk of type 2 diabetes: a systematic review and meta-analysis. PLOS ONE. 2012;7(7): e41641. doi: 10.1371/journal.pone.0041641. pmid:22848554
39. Geissler C, Singh M. Iron, meat and health. Nutrients 3: 283-316.
40. Reardon TF, Allen DG. Iron injections in mice increase skeletal muscle iron content, induce oxidative stress and reduce exercise performance. *Exp Physiol*. 2009;94(6):720-730. doi:10.1113/expphysiol.2008.046045
41. Dongiovanni P, Ruscica M, Rametta R, Recalcati S, Steffani L, Gatti S. 2014. Dietary iron overload induces visceral adipose tissue insulin resistance. Am J Pathol (182):2254–2263
42. Cooksey RC, Jones D, Gabrielsen S, Huang J, Simcox JA, Luo B, et al. Dietary iron restriction or iron chelation protects from diabetes and loss of beta-cell function in the obese (ob/ob Lep-/-) mouse. Am J Physiol Endocrinol Metab. 2010; 298(6): 1236–1243.
43. Fatmah A Matough, Siti B Budin, Zariyantey A Hamid, Nasar Alwahaibi, Jamaludin Mohamed. 2011. The Role of Oxidative Stress and Antioxidants in Diabetic Complications. SQU Med J. (12): 1; 5-18
44. Arredondo A. y De Icaza E. 2011. Costos de la Diabetes en América Latina: Evidencias del Caso Mexicano. Value in health 14 (2011) S85–S88
45. Federación Mexicana de Diabetes. 2015. La diabetes e hipertensión en México. Disponible en: http://fmdiabetes.org/diabetes-e-hipertension-en- mexico/
46. Ríos-Silva M, Trujillo X, Trujillo-Hernández B, Sánchez-Pastor E, Urzúa Z, Mancilla E, et al. Effect of chronic administration of forskolin on glycemia and oxidative stress in rats with and without experimental diabetes. Int J Med Sci. 2014;11(5):448–452. doi: 10.7150/ijms.8034. pmid:24688307
47. Maaroufi K, Had-Aissouni L, Melon C, Sakly M, Abdelmelek H, Poucet B. 2009. Effects of prolonged iron overload and low frequency electromagnetic exposure on spatial learning and memory in the young rat. Neurobiol Learn Mem.;92(3): 345–355. doi: 10.1016/j.nlm.2009.04.002. pmid:19394433
48. Márquez- Ibarra A, Miguel Huerta, Salvador Villalpando-Hernández, Mónica Ríos-Silva, María I. Díaz-Reval, Humberto Cruzblanca, Evelyn Mancilla y Xóchitl Trujillo. 2016. The Effects of Dietary Iron and Capsaicin on Hemoglobin, Blood Glucose, Insulin Tolerance, Cholesterol, and Triglycerides, in Healthy and Diabetic Wistar Rats. PLoS ONE 11(4): e0152625. doi:10.1371/journal.pone.0152625
49. Thompson K, Molina R, Donaghey T, Brain JD, Wessling-Resnick M. The influence of high-iron diet on rat lung manganese absorption. Toxicol Appl Pharmacol. 2006;210(1–2):17–23. pmid:15993455

50. Ahmed, U., Latham, P. S., & Oates, P. S. (2012). Interactions between hepatic iron and lipid metabolism with possible relevance to steatohepatitis. *World journal of gastroenterology*, *18*(34), 4651–4658. https://doi.org/10.3748/wjg.v18.i34.4651
51. Yang, J., Zhang, G., Dong, D., & Shang, P. (2018). Effects of Iron Overload and Oxidative Damage on the Musculoskeletal System in the Space Environment: Data from Spaceflights and Ground-Based Simulation Models. *International journal of molecular sciences*, *19*(9), 2608. https://doi.org/10.3390/ijms19092608
52. Giacco, F., & Brownlee, M. (2010). Oxidative stress and diabetic complications. *Circulation research*, *107*(9), 1058–1070. https://doi.org/10.1161/CIRCRESAHA.110.223545

10. ANEXOS

1. Artículo de divulgación publicado en la revista “Saber más”, revista de divulgadion científica de la Universidad Michoacana de San Nicolás de Hidalgo.

https://www.sabermas.umich.mx/archivo/articulos/343-numero-40/620-fatiga-muscular-y-diabetes.html#:~:text=La%20diabetes%20mellitus%20es%20una,la%20glucosa%20en%20la%20sangre).

ARTÍCULO

Fatiga muscular y diabetes

Manuel Alejandro Vargas Vargas y Rocío del Carmen Montoya Pérez

2. Artículo de divulgación publicado en la revista “Saber más”, revista de divulgadion científica de la Universidad Michoacana de San Nicolás de Hidalgo.

https://www.sabermas.umich.mx/archivo/articulos/343-numero-40/621-funcion-de-antioxidantes-en-la-diabetes-mellitus.html