METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM

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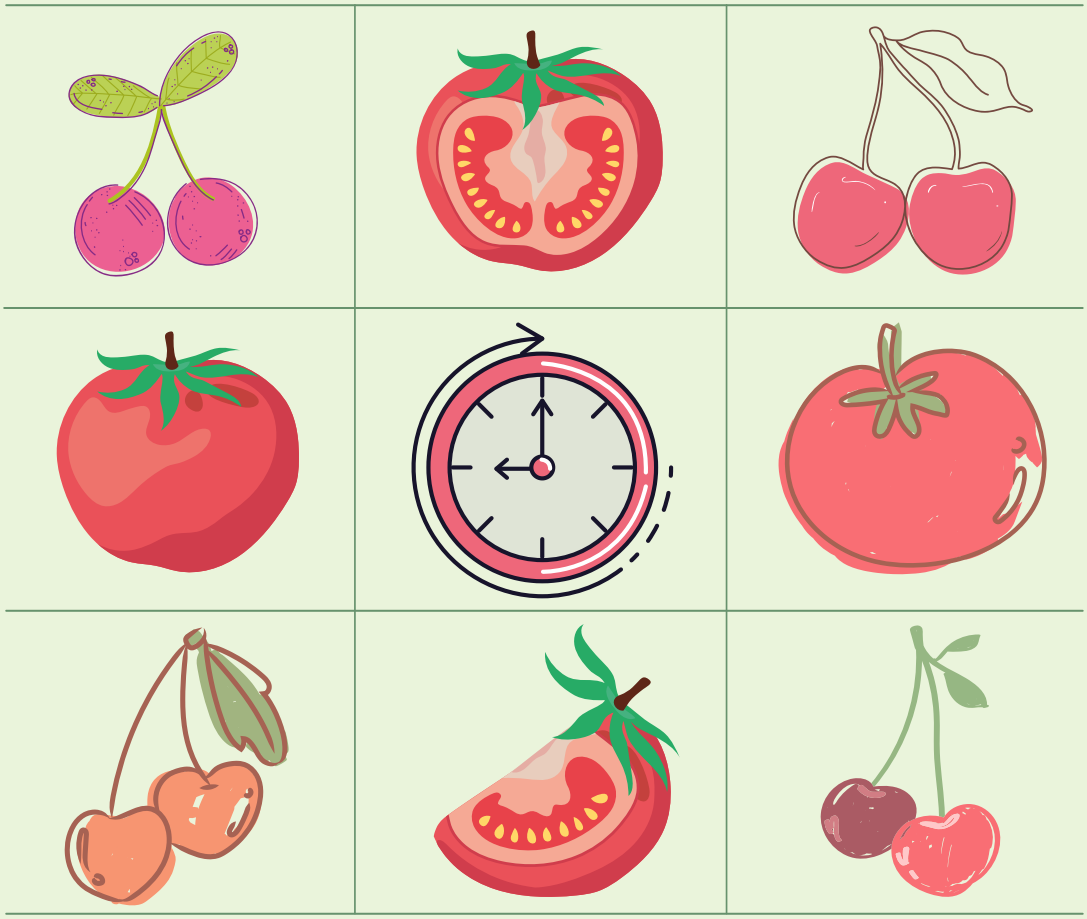

Doctoral Thesis - 2022-

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Doctoral Thesis

Directed by Dr Manuel Suárez Recio

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

NUTRIGENOMICS RESEARCH GROUP

UNIVERSITAT ROVIRA i VIRGILI

2022

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UNIVERSITAT ROVIRA i VIRGILI

DEPARTAMENT DE BIOQUÍMICA I BIOTECNOLOGIA
Campus Sescelades
C/ Marcel·lí Domingo, 1
43007 Tarragona
Tel. +34 977 55 95 21
Fax +34 977 55 82 32

FAIG CONSTAR que aquest treball, titulat "Metabolic consequences of consumption of fruits from different origins and in different photoperiods: impact on the lipidic metabolism", que presenta María Josefina Ruiz de Azua per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament Bioquímica y Biotecnologia Universitat Rovira I Virgili i que compleix els requisits per a l'obtenció de la Menció Internacional de Doctorat.

HAGO CONSTAR que el presente trabajo, titulado "Metabolic consequences of consumption of fruits from different origins and in different photoperiods: impact on the lipidic metabolism", que presenta María Josefina Ruiz de Azua para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento Bioquímica y Biotecnologia de la Universitat Rovira i Virgili y que cumple con los requisitos para la obtención de la Mención Internacional de Doctorado.

I STATE that the present study, entitled "Metabolic consequences of consumption of fruits from different origins and in different photoperiods: impact on the lipidic metabolism", presented by Maria Josefina Ruiz de Azua for the award of the degree of Doctor, has been carried out under my supervision at the Department Biochemistry and Biotechnology of Universitat Rovira i Virgili and that is eligible to apply for the International Doctoral Mention.

Tarragona, 10 de Maig 2022 / Tarragona, 10 de Mayo del 2022/ Tarragona 10th of May 2022

El director de la tesi doctoral
El director de la tesis doctoral
Doctoral Thesis Supervisor

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Esta investigación ha sido financiada por el Fondo Europeo de Desarrollo Regional (ERDF) de la Comisión Europea a través del Proyecto de Especialización y Competitividad Territorial (PECT-NUTRISALT). Esta tesis fue llevada a cabo en el Grupo de Investigación de Nutrigenómica de la Universitat Rovira i Virgili, gracias al otorgamiento de la beca predoctoral por parte de esta universidad, a través del Programa Martí-Franques, número 2018PMF-PIPF-20. La estancia de investigación internacional fue realizada en la Cátedra de Bromatología y Nutrición de la Facultad de Bioquímica y Biotecnología de la Universidad Nacional del Litoral (Santa Fe, Argentina), la cual fue financiada por la beca de Secretaría General Iberoamericana (SEGIB) y la Fundación Carolina (2020).

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A mi mamá, a mi papá

a Alejo y a Tomás.

A mis abuelas..

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Paro para pensar y me siento tan afortunada. No puedo dejar de agradecer por todas las oportunidades de crecimiento, por todos los momentos de felicidad, por todos los lugares hermosos visitados, por todas las experiencias vividas y por las personas increíbles que formaron parte de esta etapa.

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Persevera,

Y triunfarás …

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INDICE

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RESUMEN

En la actualidad, gracias a la globalización y a los sistemas de cultivo, producción y transporte, la sociedad tiene a su alcance alimentos como frutas y verduras, de diferentes partes del mundo y en cualquier época del año. Así, es posible consumir productos vegetales típicamente de verano en invierno, mientras que productos que son característicos de zonas tropicales se consiguen en países muy fríos. Esto ha generado cambios en los patrones de consumo que pueden tener algunas consecuencias metabólicas en los consumidores. En tal sentido, según la teoría de la xenohormesis, los fitoquímicos, producidos por las plantas en respuesta a determinados estímulos estresantes del ambiente, podrían actuar como moléculas señalizadoras en los heterótrofos que las consumen, permitiendo que puedan prepararse y adaptarse favorablemente a los cambios del entorno. Dado que las plantas tienen una composición de fitoquímicos característica que va a depender de la zona donde se han desarrollado y cosechado, el consumo de productos fuera de temporada o de otros lugares podría impactar el metabolismo de los consumidores.

Por otro lado, se ha descrito que los ritmos biológicos, los cuales se ven modulados entre otros factores por la cantidad de horas de luz del día, supone un estímulo de gran influencia en la transcripción de genes, tanto a nivel central (en el cerebro) como a nivel periférico en tejidos como el hígado. Al respecto, se sabe que la exposición a distintos ciclos de luz:oscuridad (fotoperiodos), influye sobre la expresión génica de enzimas y parámetros metabólicos.

Teniendo en cuenta estas premisas, el objetivo principal de la presente tesis fue investigar si el consumo de frutas y verduras de diferentes orígenes geográficos y en diferentes fotoperiodos, produciría consecuencias metabólicas en un modelo animal saludable. Para lograr dicho objetivo, primero observamos los efectos metabólicos a nivel sérico, hepático y muscular de la exposición crónica de ratas Fischer 344 sanas a distintos fotoperiodos.

Cabe destacar que los fotoperiodos se asemejaban a las distintas estaciones anuales, debido a las variaciones en la cantidad de horas al día. Seguidamente, evaluamos en ratas Fischer 344, los efectos del consumo de cereza dulce y tomate, frutas ricas en compuestos bioactivos, provenientes de distintos orígenes geográficos, a la vez que se evaluó sus ingestas en fotoperiodo corto, estándar y largo, los que simularon las estaciones de inverno, otoño/primavera y verano, respectivamente.

En primer lugar, observamos que la exposición crónica a diferentes fotoperiodos modula parámetros metabólicos séricos, el perfil de ácidos grasos en hígado y músculo esquelético, como así también la expresión génica de enzimas involucradas en dichos procesos.

En segundo lugar, observamos que la marca polifenólica del lugar de origen de las cerezas posee efectos metabólicos diferenciales, que se traducen en cambios sobre los niveles séricos de triacilglicéridos, alteración de la expresión génica de determinadas enzimas relacionadas con el metabolismo lipídico, e impacto sobre diferentes vías metabólicas. Al mismo tiempo, el fotoperiodo en el cual fueron consumidas los distintos tipos de cereza también moduló el perfil de ácidos grasos hepáticos de los animales.

Finalmente, observamos que el consumo de tomate de diferente origen y en distintos fotoperiodos también produjo efectos diferenciales a nivel metabólico, en suero y en hígado de ratas Fischer 344 sanas. En tal sentido, el consumo de tomate en temporada podría producir efectos benéficos en la salud cardiovascular.

Por lo tanto, podemos concluir que además de los efectos beneficiosos aportados por los nutrientes de las frutas, la priorización de aquellas de proximidad al lugar donde habitamos, lo cual implica su consumo en temporada, supone una modulación a nivel metabólico que otorgaría beneficios extra al bienestar del organismo.

SUMMARY

Nowadays, thanks to globalization and cultivation, production and transportation systems, society has access to foods such as fruits and vegetables from different parts of the world and at any time of the year. Thus, it is possible to consume vegetable products typically from summer during winter, while products that are characteristic of tropical areas are obtained in very cold countries. This has generated changes in consumption patterns that may have some metabolic consequences in consumers. In this sense, according to the theory of xenohormesis, phytochemicals, produced by plants in response to certain stressful stimuli from the environment, could act as signaling molecules in the heterotrophs that consume them, allowing them to prepare and adapt favorably to changes in the environment. Since plants have a characteristic composition of phytochemicals that will depend on the area where they have been grown and harvested, the consumption of products out of season or from other places could impact the metabolism of consumers.

On the other hand, it has been described that biological rhythms, which are modulated among other factors by the hours of daylight, is a stimulus that highly influences the transcription of genes, both in the central level (in the brain) and in the periphery in tissues such as the liver. In this regard, it is known that exposure to different light:dark cycles (photoperiods) influences the gene expression of enzymes and metabolic parameters.

Taking these premises into account, the main objective of this thesis was to investigate whether the consumption of fruits and vegetables from different geographical origins and in different photoperiods would produce metabolic consequences in a healthy animal model. To achieve this goal, we first evaluated the metabolic effects in the serum, liver and muscle of chronic exposure of healthy Fischer 344 rats at different photoperiods. It should be noted that the photoperiods resembled the different annual seasons, due to

variations in the number of hours per day. Next, we evaluated in Fischer 344 rats the effects of consuming sweet cherries and tomatoes, fruits rich in bioactive compounds, from different geographical origins, while their intakes were evaluated in short, standard and long photoperiods, which simulated the winter, fall/spring and summer seasons, respectively.

First, we observed that chronic exposure to different photoperiods modulates serum metabolic parameters, the profile of fatty acids in liver and skeletal muscle, as well as the gene expression of enzymes involved in these processes. Secondly, we observed that the polyphenolic profile, characteristic from the place of origin of cherries has differential metabolic effects, which translate into changes in serum levels of triacylglycerides, alteration in the gene expression of certain enzymes related to lipid metabolism, and impact on different metabolic pathways. At the same time, the photoperiod in which the different types of cherries were consumed also modulated the hepatic fatty acid profile of the animals.

Finally, we observed that the consumption of tomato from different origin and in different photoperiods also produced differential effects at the metabolic level, in serum and in the liver of healthy Fischer 344 rats. In this sense, the consumption of tomatoes in season could produce beneficial effects on cardiovascular health.

Therefore, we can conclude that in addition to the beneficial effects provided by the nutrients of the fruits, the prioritization of those of proximity to the place where we live, which implies their consumption in season, supposes a modulation at the metabolic level that would grant extra benefits to the welfare of the organism.

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ABREVIATURAS

AA: Ácido araquidónico (c5,c8,c11,c14-20:4)

Acc1: Acetil-CoA carboxilasa 1

Acc1: Enzima acetil-CoA carboxilasa

Acc2: Acetil-CoA carboxilasa 2

AdipoR1: Receptor de adiponectina 1

AdipoR2: Receptor de adiponectina 2

ADN: Ácido desoxirribonucleico

ADNc: Ácido desoxirribonucleico complementario

AE: Ácido esteárico (18:0)

AG: Ácidos grasos

AGS: Ácidos grasos saturados

AI: Índice aterogénico (Log (TAG/HDL-c)), (por su nombre en inglés: *Atherogenic Index*)

Akt: Akt serina/treonina quinasa 1

Akt: AKT serina/treonina quinasa1

AL: Ácido linoleico (c9,c12,-18:2)

ALA: Ácido Alfa linolénico (c9,c12,c15-18:3 por su nombre en inglés: Alpha-linolenic acid)

AMPK: Proteína cinasa activada por AMP, del inglés AMP-activated protein kinase

AO: Ácido oleico (c9-18:1)

AP: Ácido palmítico (16:0)

ARN: Ácido ribonucleico

At.C: Coeficiente aterogénico, ((TC-HDL-c)/HDL-c), (por su nombre en inglés: atherogenic coefficient)

ATP: Adenosin trifosfato

Bmal1: Receptor nuclear translocador de aril hidrocarburos en cerebro y músculo

Clock: Por su nombre en inglés Circadian Locomotor Output Cycles Kaput.

COX2: Prostaglandina-endoperóxido sintasa 2

Cpt1α: Enzima carnitina palmitoiltransferasa-I hepatica

Cpt1β: Enzima carnitina palmitoiltransferasa-I muscular

Cpt-II: Enzima carnitina palmitoiltransferasa- II mitocondrial

CR1: Riesgo cardiovascular 1 (TC/HDL-c), (por su nombre en inglés: Cardiovascular risk1)

CR2: Riesgo cardiovascular 2 (LDL-c/HDL-c), (por su nombre en inglés: Cardiovascular risk2)

Cry1: Gen que codifica a la proteína Cry1 (por su nombre en inglés cryptochrome 1).

CT: Colesterol total

Ct: Umbral de ciclo, (por su nombre en inglés cycle threshold)

DHA: Ácido docosahexaenoico (c4,c7,c10,c13,c16,c19-22:6, por su nombre en inglés: Docosahexaenoic acid)

DM: Dieta mediterránea

Elovl: Elongasa 6

Elovl: Familia de enzimas elongasas

EPA: Ácido eicosapentaenoico (c5,c8,c11,c14,c17-20:5, por su nombre en inglés: Eicosapentaenoic acid)

FABP: Familia de proteínas de unión a ácidos grasos, del inglés Fatty acid-binding protein.

FAD: Dinucleótido de flavina-adenina

Fads2: Enzima delta 6 desaturasa

Fads2: Δ6 desaturasa

FAMEs: Esteres metílicos de ácido graso, del inglés

FAO: Organización de Alimentos y Agricultura, (por su nombre en inglés *Food and Agriculture Organization*)

Fas1: Ácido graso sintasa

FAT/cd36: Transportador de ácidos grasos homologo a cd36.

FATP: Familia de transportadores transmembrana de ácidos grasos, (por su nombre en inglés: *Fatty Acid Transport Protein*)

FDR: del inglés: false discovery rate

G6pc: Glucosa 6 fosfatasa catalítica, (por su nombre en inglés: *glucose-6-phosphatase, catalytic*)

Had: Hidroxiacil-CoA dehidrogenasa

HCL: Ácido clorhídrico

HDAC: Histona desacetilasa

HDL-c: Lipoproteína de alta densidad (por su nombre en inglés: *high-density lipoprotein*)

HOMA-IR: Modelo homeostático de evaluación de la resistencia a la insulina (por su nombre en inglés*: Homeostatic model assessment of insulin resistance*)

IFNγ: Interferón gamma

IL-1α: Interleuquina 1α

IL-1β: Interleuquina 1β

IL-6: Interleuquina 6

IMC: Índice de masa corporal

ipRGC: Células ganglionares de la retina intrínsecamente fotoreceptoras (por su nombre en inglés: *photosensitive retinal ganglion cells*)

Irs1: Sustrato de receptor de insulina 1, (por su nombre en inglés*: Insulin receptor substrate 1*)

Irs2: Sustrato de receptor de insulina 2, (por su nombre en inglés: *Insulin receptor substrate 2*)

L12: Fotoperíodo estándar (12 horas de luz y 12 horas de oscuridad)

L18: Fotoperiodo largo (18 horas de luz y 6 horas de oscuridad)

L6: Fotoperíodo corto (6 horas de luz y 18 horas de oscuridad)

LC: Cereza de origen local (por su nombre en inglés: *Local Cherry*)

LCAT: Lecitin Colesterol Acil Transferasa

LDL-c: Lipoproteína de baja densidad (por su nombre en inglés*: low-density lipoprotein*)

LPL: Lipoprotein lipasa

LT: Tomate de origen local (por su nombre en inglés: *Local-Tomato*)

MAPK/ ERK: del inglés mitogen-activated protein kinases

MUFAs: Ácidos grasos monoinsaturados (por su nombre en inglés: *monosaturated fatty acids*)

NAD+: Dinucleótido de nicotinamida-adenina

NADPH: Nicotiamida-Adenina Dinucleotido fosfato.

NEFAs: Ácidos grasos no esterificados (por su nombre en inglés*: non-esterified fatty acid*)

nLC: Cereza de origen no local (por su nombre en inglés: non-Local Cherry)

nLT: Tomate de origen no local (por su nombre en inglés*: non-Local Tomato*)

Nrdc: Nardisilina convertasa

NSQ: Núcleo supraquiasmático hipotalámico

Pck1: Proteína serina/treonina quinasa 1, (por su nombre en inglés: *serine/threonine protein kinase*)

Per1/2: Gen que codifica a proteína de período circadiano 1/2.

PUFAs: Ácidos grasos poliinsaturados (por su nombre en inglés: *polyunsaturated fatty acids*)

Pyg: Glucógeno fosforilasa, (por su nombre en inglés: *glycogen phosphorylase*)

qPCR: Reacción en cadena de la polimerasa, cuantitativa (por su nombre en inglés: *quantitative polymerase chain reaction*)

RMN: Resonancia magnética nuclear

RT: Retrotranscripción

Scd1: Estearoil-CoA desaturasa 1

Srebp-1c: Factor 1 de transcripción de unión a elementos reguladores de esteroles, (por su nombre en inglés: *sterol regulatory element-binding protein 1c*)

TAG: Triacilglicéridos

Tbc1d1: (por su nombre en inglés: *TBC1 domain family member 1*)

TFN-α: Factor de necrosis tumoral Alpha.

TTFL: Bucles de retroalimentación de transcripcional-traduccional, (por su nombre en inglés: *transcriptional and traductional feedback loop*)

VH: Vehículo

VLDL: Lipoproteínas de muy baja densidad (por su nombre en inglés*: very low-density lipoprotein*)

1. INTRODUCCIÓN

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INTRODUCCIÓN

1.1. Dieta y estilos de vida

Se denomina patrón dietético al conjunto de alimentos y bebidas que una persona o población toma habitualmente, los cuales actúan de manera sinérgica afectando positiva o negativamente la salud. En consecuencia, los patrones dietéticos podrían predecir mejor el estado general de salud y riesgo a enfermedades que los nutrientes o alimentos de manera individual [1]. Un aporte suficiente en macronutrientes para satisfacer las necesidades fisiológicas y energéticas, junto con una adecuada hidratación y aporte de minerales, se considera un patrón dietético saludable. Los carbohidratos, proteínas y grasas, aportan elementos estructurales y energía para los procesos celulares, mientras que las vitaminas y minerales son esenciales para el desarrollo, mantenimiento, metabolismo y buen funcionamiento del organismo. Cabe destacar que las necesidades fisiológicas varían de acuerdo al sexo, actividad física, edad, contexto cultural y el estado de enfermedad de la persona, por lo que es difícil establecer un patrón saludable para todo el mundo [2].

En los últimos años, se ha evidenciado la importancia de factores dietéticos o nutricionales en la etiología, tratamiento, o prevención de determinadas patologías crónicas [3]. A modo general, se sabe que una ingesta de productos con elevado valor energético, altos en grasas saturadas, azúcares, sal, así como un bajo consumo de frutas, verduras, fibras o cereales integrales son factores claves en el desarrollo de enfermedades crónicas no trasmisibles [3]. En consecuencia, las organismos públicos y privados desarrollan y publican constantemente nuevas políticas y estrategias en materia de salud pública para concienciar y fomentar hábitos saludables en la población [4]. A pesar de ello, en las últimas décadas se ha observado un incremento a nivel mundial en ciertos parámetros como el índice de masa corporal (IMC), la glucemia en

ayunas y la presión sanguínea, los cuales son factores metabólicos de alto riesgo para el desarrollo de patologías crónicas no transmisibles [5]. De hecho, según la Organización de Agricultura y Alimentos (FAO, por su nombre en inglés Food and Agriculture Organization) y los registros de consumo, en la última década ha ido disminuyendo la ingesta y disponibilidad de frutas y vegetales, mientras se incrementa la ingesta de energía a través de productos cárnicos y azúcar principalmente [4] (**Fig. 1.1**)

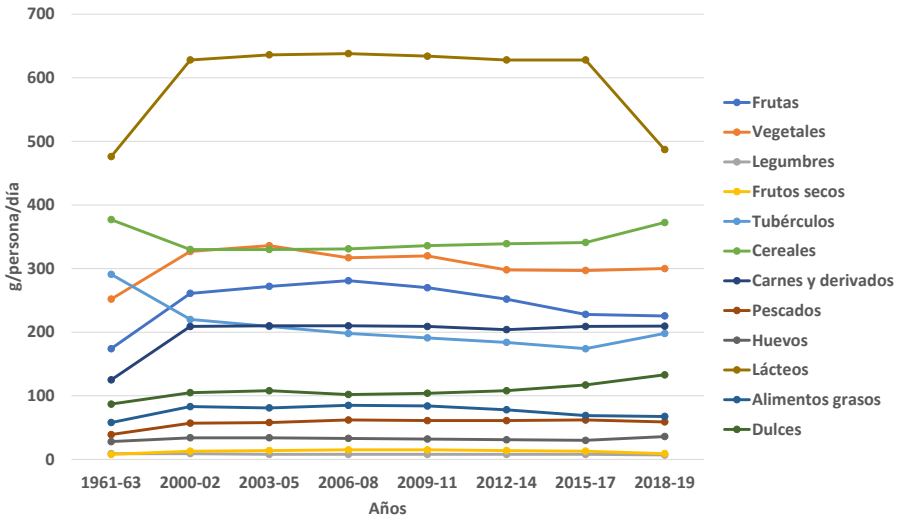

Fig. 1.1 Tendencia de los alimentos disponibles para el consumo en Europa entre 1961 y 2019 [6].

1.1.1. Dieta Mediterránea

La Dieta Mediterránea (DM) es un patrón alimentario característico de los países que bordean la costa del Mar Mediterráneo, la cual se basa en un variado y alto consumo de frutas y vegetales frescos, legumbres, cereales y granos enteros; de una ingesta relativamente alta de grasas poliinsaturadas a partir del aceite de oliva virgen, nueces y semillas; junto con una ingesta de pescados frescos y lácteos, mientras que las carnes rojas y procesados se consumen con muy baja frecuencia (**Fig. 1.2**). Además, hay una consumo ocasional de alcohol, principalmente a través del vino tinto [1,7]. En los últimos años ha crecido la

evidencia que asocia la DM con efectos protectores de enfermedades crónicas no transmisibles, como diabetes, obesidad, enfermedades cardiovasculares, alergias, varios tipos de cáncer, y enfermedades neurodegenerativas, entre otras [8–14]. Cabe destacar que, según la pirámide tradicional de la DM, no sólo la proporción de los grupos de alimentos es fundamental, sino que la selección, modo de preparación y hábitos asociados al consumo también cobran importancia [7]. En tal sentido, la DM es dinámica, debido a la variación estacional que presentan las frutas y verduras, las cuales son el núcleo de la ingesta diaria. En consecuencia, el consumo de productos vegetales frescos implica preferencia por productos locales y de temporada. Al respecto, se ha observado que la proximidad a las zonas de producción otorga a los alimentos una calidad nutricional extra, principalmente en compuestos bioactivos, que tiene consecuencias claras en los efectos beneficiosos asociados al consumo de DM [15,16].

Sin embargo, a pesar del beneficio nutricional que supone el consumo de frutas y verduras de proximidad, la globalización experimentada en las últimas décadas también ha afectado a la DM, generando una cierta desconexión entre el lugar de producción y la zona de ingesta, lo que desvincula las áreas geográficas y la dieta [17]. De hecho, algunos autores sugieren que existe un proceso de occidentalización y homogeneización de los hábitos alimentarios asociado al proceso de globalización, la modernización de la industria alimentaria y la implementación de la comida rápida [18]. En otras palabras, es posible conseguir alimentos de cualquier parte del mundo en cualquier época del año. Además, a diferencia de los sistemas alimentarios tradicionales, este hecho perjudica la rentabilidad económica local [15].

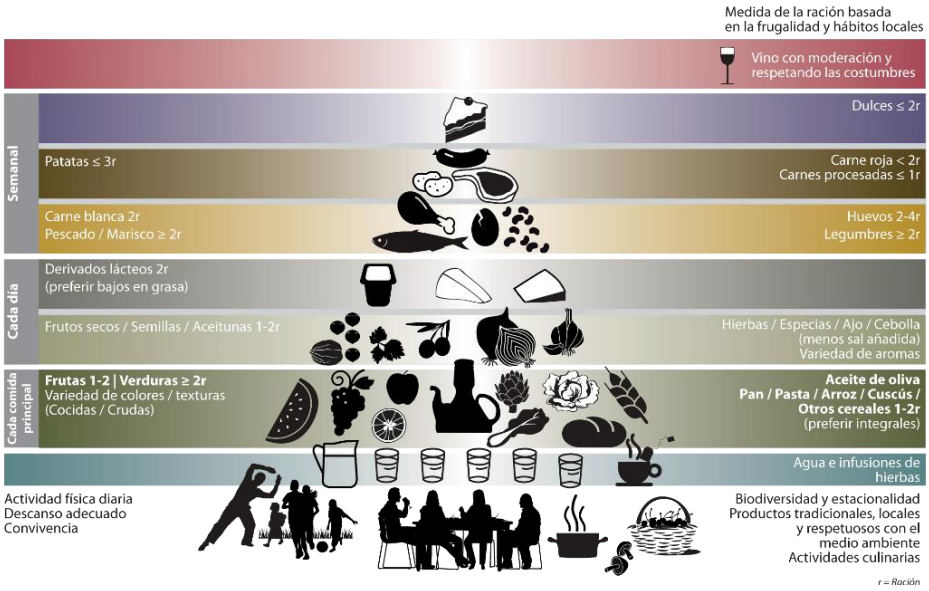

Fig.1.2 Distribución de consumo de alimentos y hábitos saludables de la dieta mediterránea [7].

Centrándonos en los productos de origen vegetal, estos cambios en los patrones de consumo han llevado a desarrollar y adoptar técnicas de cultivo y post-cosecha para mantener las frutas y vegetales en condiciones óptimas desde su origen hasta el consumidor, con consecuencias directas en las características organolépticas y nutricionales de los alimentos.

En este contexto, un desafío que tiene la agricultura actualmente es la validación de técnicas de cultivo, para lograr la producción de frutas y verduras durante todo el año. Por ejemplo, la tecnología de ambiente controlado por invernadero se desarrolló para extender el ciclo de cultivo a temporadas en las que es muy difícil producir a campo abierto, debido a los cambios estacionales evidentes. De esta forma, con el control de las condiciones ambientales, se pretende un mejor rendimiento y calidad del producto [19]. Además, con el objetivo de obtener frutos aptos y firmes para el transporte, se realiza la cosecha en etapas tempranas. De esta forma se garantiza que las frutas se mantengan durante largas distancias y por periodos de comercialización largos

[19]. El almacenamiento en atmósferas controladas y el envase en atmosferas modificadas también contribuye a alargar la vida útil y retrasar la perdida de la calidad de este tipo de productos [20]. El control de la temperatura, exposición a la luz solar, la humedad relativa o el contacto con otras frutas o vegetales, son algunos de los factores que determinan no solo la calidad nutricional, sino también las características organolépticas que busca el consumidor.

A pesar de los esfuerzos para lograr la disponibilidad de frutas y verduras de diferentes orígenes durante la mayor parte del año, las consecuencias en la cantidad y calidad de fitoquímicos deberían ser consideradas. Por ejemplo, se ha observado que el cultivo de cerezas dulces a temperaturas inferiores a 25 ºC disminuye el contenido total de fenoles y antocianinas, al igual que con un almacenamiento a 1 ºC durante un periodo igual o mayor a 15 días [19]. De igual manera, algunos autores sugieren que temperaturas de almacenamiento menores a 5 ºC reducen el contenido de licopeno de tomates, mientras que la concentración de β-caroteno disminuye en temperaturas de 21 a 26 ºC [19]. La etapa de madurez en la que son cosechados los productos vegetales también influye en la concentración de algunos ácidos aromáticos [21].

1.2. Fitoquímicos

La palabra fitoquímico es un término amplio que proviene del griego *"phyto"* (planta), y hace referencia a los metabolitos secundarios no nutritivos producidos por el reino vegetal [22]. Si bien existen varias teorías que explican su existencia y diversidad, se cree desde un punto de vista evolutivo que desempeñan funciones biológicas en la protección de las plantas contra agentes estresantes y peligrosos con el fin de adaptarse a entornos viables [23,24]. En consecuencia, también se los denomina biocompuestos. En tal sentido, las plantas han desarrollado vías metabólicas secundarias para crear diferentes fitoquímicos, los cuales actúan a diferentes niveles que ayudan a la planta a enfrentarse a condiciones climáticas desfavorables, falta de

nutrientes, presencia de patógenos o seres herbívoros, existencia de pesticidas, entre otros. Además de otorgar una función protectora y adaptativa, estos compuestos contribuyen al color, sabor y textura de los vegetales [25].

Dada la gran cantidad de metabolitos secundarios existen varias formas de clasificarlos. Estructuralmente se pueden dividir en 4 grupos principales: compuestos fenólicos (antocianinas, cumarinas, flavononas y flavonoles, entre muchos otros), terpenos (monoterpenoides, sesquiterpenoides, saponinas, fitoesteroles, carotenoides, entre otros), compuestos nitrogenados (alcaloides, betalaína, indol, isoquinolina, licopodio, quinolina, quinolizidina, entre otros) y organosulfurados (glucosinolatos) [26,27] (Fig. 1.3).

1.2.1. Compuestos fenólicos

Los compuestos fenólicos son metabolitos secundarios ampliamente distribuidos en los vegetales que se caracterizan por tener al menos un anillo aromático con uno o más grupos hidroxilo. Cuando contienen más de un anillo fenólico reciben el nombre de polifenoles. Debido a la gran variedad y complejidad que engloba esta estructuración, los compuestos fenólicos pueden dividirse a su vez en flavonoides y no flavonoides, dependiendo del número de unidades de fenol en su estructura, los grupos sustituyentes y/o el tipo de unión que presentan entre el grupo fenol [28].

Por un lado, los flavonoides se caracterizan por la presencia de una estructura básica (C6-C3-C6), formada por 2 anillos fenólicos (anillo A y anillo B) unidos por un anillo heterocíclico (anillo C). Este anillo heterocíclico, suele ser un pirano cerrado. Cabe destacar que los flavonoides se encuentran en las plantas generalmente como glucósidos [29]. Dada las variaciones en el patrón de hidroxilación y en el estado de oxidación del anillo central de pirano, se pueden encontrar una amplia gama de compuestos dentro de los flavonoides: flavanoles, antocianidinas, antocianinas, isoflavonas, flavonas, flavonoles, flavanonas y flavanonoles [30].

Fig.1.3 Clasificación de los fitoquímicos.

Por otro lado, los compuestos no flavonoides incluyen principalmente los ácidos fenólicos, los estilbenos y los lignanos. Los ácidos fenólicos se caracterizan por la presencia de un único anillo aromático y rara vez se encuentran en forma libre, encontrándose frecuentemente conjugados con otros fenoles, glucosa o componentes estructurales de la planta. Los estilbenos están caracterizados por dos restos fenilo unidos por un grupo metileno de dos carbonos. Estos compuestos pueden estar en formas conjugadas *cis* o *trans*, en forma libre o glicosilados. Los lignanos presentan una amplia gama de formas estructurales diferentes, clasificándose en ocho subgrupos. Específicamente, se caracterizan por presentar dos unidades de propilbenceno unidas entre sí por las cadenas laterales del propano.

Los polifenoles pueden encontrarse en la mayor parte de los tejidos de las plantas en distintas concentraciones. En las últimas décadas ha ido incrementando el interés en estos compuestos, específicamente en su actividad química y biológica en los humanos. No obstante, su metabolismo es rápido y poseen baja biodisponibilidad. Parte de los polifenoles de los alimentos

se absorben en el intestino delgado, pero una cantidad significativa llega al intestino grueso, donde la microbiota del colon es capaz de degradar las formas conjugadas y agliconas en moléculas de mayor absorción [30,31]. De hecho, se cree que existe una comunicación bidireccional entre polifenoles y microbioma, donde los fitoquímicos podrían modular la composición del microbioma humano [32]. En tal sentido, se ha observado que algunos polifenoles del cacao producen una mayor cantidad de butirato y promueven el crecimiento de bacterias beneficiosas [33,34] y, a su vez, que la composición individual del microbioma influye directamente sobre la capacidad de metabolizar ciertos compuestos bioactivos [32] .

Si bien no existen cantidades específicas de recomendaciones diarias, un consumo frecuente de alimentos ricos en polifenoles es asociado con efectos benéficos para la salud. En tal sentido, se ha observado que presentan efectos protectores a determinadas patologías crónicas no transmisibles como obesidad [35], diabetes mellitus [36,37], enfermedades cardiovasculares [38,39], enfermedades hepáticas [40,41], diversos tipos de cánceres [42,43], etc. En los últimos años se ha evidenciado que su ingesta podría tener efectos protectores contra enfermedades neurodegenerativas como Alzheimer o Parkinson [44]. Algunos polifenoles, como los presentes en el té verde o negro, son capaces de inhibir el crecimiento bacteriano dañino como el del *Helicobacter pylori*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes* y *Pseudomonas aeruginosa* , así como el virus de la hepatitis C, influenza, virus de inmunodeficiencia humana y *Cándida* [45]. Mecanismos bioquímicos y moleculares están implicados en dichos efectos protectores, incluyendo la modulación de vías de señalización inter e intracelulares. Específicamente, se ha observado que diversos polifenoles actúan estimulando/inhibiendo factores de transcripción nucleares, modulan la síntesis de mediadores inflamatorios (Interleuquina 6 y 1β (IL-6; IL-1β), factor de necrosis tumoral Alpha (TNF-α) y regulan el metabolismo lipídico [46–48].

Además, una reducción de apoptosis, proliferación celular, reducción de la insulino-resistencia, modulación del metabolismo glucídico, y efectos antioxidantes son otros de los mecanismos por los cuales se le atribuyen múltiples beneficios para la salud [48,49].

Cabe destacar que los alimentos contienen mezclas complejas de fitoquímicos y numerosos factores pueden afectar su concentración [50]. Por ejemplo, la cantidad de polifenoles de las cerezas va a depender de la variedad, tipo de cultivo, etapa de maduración, características de almacenamiento, etc. Alrededor de un 25% a 40% de los compuestos fenólicos totales de las cerezas *Montmorency* los constituyen los hidroxicinamatos y flavan-3-oles; sin embargo, en la variedad *Bing*, estos compuestos ocupan del 50% al 5% [51]. Además, son ricas en cianidina-3-glucósido y la cianidina-3-rutinosido, destacadas antocianinas. En la Tabla 1.1 se resumen los compuestos polifenólicos y los principales alimentos donde se encuentran.

Tabla 1.1 Contenido polifenólico presente en alimentos y bebidas a base de plantas. Adaptado de *Bravo, L.* (1998) [67].

ALIMENTO/BEBIDA	*Polifenoles Totales*	*Alimento/bebida*	*Polifenoles Totales*
Cereales (mg/100 g p.s)		**Frutas (mg/ 100 g p.f)**	
Cebada	1200-1500	Grosella negra	140-1200
Maíz	30.9	Arándano	135-280
Mijo	590-1060	cereza	60-90
Avena	8.7	Arándano rojo	128
Arroz	8.6	Grosella espinosa	22-75
Sorgo	170-10260	Toronja	50-490
Trigo	22-40	Uva	50
Legumbres (mg/100 g p.s)		Naranja	50-100
Poroto Negro	540-1200	Durazno	10-150
Garbanzos	78-230	Pera	2-25
Alubias	175-590	Ciruela	4-225
Frijoles Comunes	34-289	Frambuesa	37-429
Poroto Verde	440-800	Grosella roja	17-20
Guisantes	380-1710	Fresa	38-218
Frutos Secos (% p.s)		Tomate	85-130
Nueces De Betel	26-33	**Jugos frutales (mg/L)**	
Anacardos	33.7	Jugo de manzanas	2-16

Cacahuetes	0.04	Jugo de naranjas	370-7100
Nueces De Pecán	8-14	**Bebidas**	
Vegetales (mg/ 100 g p.f)		Hojas de té (% p.s)	
Repollo De Bruselas	6-15	• Verde	20-35
Coliflor	25	• Negro	22-33
Puerro	20-40	Taza de té (mg/200 mL)	150-210
Cebolla	100-2025	Café en granos (% p.s)	0.2-10
Perejil	55-180	Taza de café (mg/150 mL)	200-550
Apio	94	Chocolate en granos (%p.s)	12-18
		Vino (mg/L)	
Frutas (mg/ 100 g p.f)		• Blanco	200-300
Manzana	27-298	• Tinto	1000-4000
Albaricoque	30-43	Cerveza (mg/L)	60-100

p.s: peso seco; p.f: peso fresco.

1.2.2. Terpenos

La familia de los terpenos comprende a más de 30,000 compuestos liposolubles, los cuales se elaboran a partir de unidades de isopreno (C5), y se clasifican según el número de unidades que contenga. En tal sentido, existen los hemi, mono, sesqui, di, sester, tri, tetra, o politerpenos (C_5, C_{10}, C_{15}, C_{20}, C_{25}, C_{30}, C_{40} o $(C_5)_n$ n>8, respectivamente) [52]. Estos fitoquímicos se encuentran ampliamente en vegetales de color verde, frutas, granos y plantas de soja. Se cree que la capacidad de fijar carbono a través de reacciones fotosintéticas es una de las funciones claves que ejercen en las plantas [26]. También, se ha reportado que ciertos terpenos favorecen el crecimiento y desarrollo de las plantas. En tal sentido, los carotenoides otorgan colores llamativos provocando la atracción de otros organismos polinizadores [26,53]. A su vez, otros actúan como toxinas teniendo una actividad defensiva a ciertos patógenos o herbívoros [54]. Tocotrienoles, tocoferoles, retinoides, taxanos, terpenoides, fitoesteroles, fitoestrógenos, carotenoides, licopeno y limonoides, son los tipos de terpenos mayoritariamente encontrados en los alimentos vegetales [26,53].

Debido a que los terpenos se encuentran ampliamente distribuidos en la naturaleza, principalmente en las estructuras reproductivas de las plantas y en las hojas, se pueden encontrar en multitud de alimentos.

Se ha observado que tienen efectos beneficiosos para la salud ya que poseen actividad antiinflamatoria, anticancerígenas y neuroprotectoras [52]. Además de proteger contra ciertas enfermedades crónicas, estos compuestos poseen actividad hormonal y reguladora del crecimiento [26]. En tal sentido, muchos extractos de hierbas, como la raíz de valeriana, ginseng, y menta salvia se han asociado con efectos sobre la función del sistema nervioso, por contener iridoides y sequisterpenos, triterpenos y monoterpenos volátiles, respectivamente [48]. Las frutas y vegetales de color amarillo, naranja o rojo, como zanahorias, pimientos, sandias, naranjas, calabaza, son fuentes de β-caroteno, α-caroteno y ε-caroteno, son precursores de la vitamina A y por lo tanto promueven su actividad [26]. Otros carotenoides, como licopeno y luteína, encontrados en el tomate y sus productos derivados, ejercen protección frente al cáncer de útero, mama, próstata, colon-rectal, etc. [55]. Algunos autores sugieren que su papel protector reside en la capacidad de reducir el daño del ADN debido al estrés oxidativo, mientras que otros suponen una modulación de factores de transcripción, supresión de células neoplásicas e inhibición de metástasis [56]. También se le adjudican propiedades cardioprotectoras, por disminuir la acumulación lipídica e inflamación [57,58]; neuroprotectoras, ya que previene y mejora el tratamiento de Alzheimer y Parkinson [59]; antidiabéticas, debido a que mejora la sensibilidad a la insulina [60], y oséoprotectoras, por prevenir la aparición de osteoporosis [61].

Por otro lado, también se ha observado un efecto hipotensivo de diterpenos glicosilados presentes en las hojas de Stevia. Específicamente, el isosteviol inhibe la proliferación celular inducida por angiotensina-II y la secreción de endotelina-1, otorgándole efectos cardioprotectores [62].

1.2.3. Compuestos nitrogenados

Los compuestos nitrogenados se caracterizan por presentar un enlace nitrógeno-nitrógeno (N-N). Hasta la fecha se han documentado poco más de 200 productos naturales que los poseen. A pesar de encontrarse en un grupo relativamente pequeño de productos naturales conocidos (menos del 0.1%), exhiben una diversidad estructural excepcional incluyendo hidrazonas, hidrazinas, nitraminas, nitrosaminas, entre muchas otras. Además, pueden ocasionar problemas de toxicidad a muy bajas concentraciones, como por ejemplo la solanina, un alcaloide presente en la patata [63–65]. Otros compuestos nitrogenados de interés son los péptidos bioactivos presente en los alimentos, los cuales son una secuencia de 2 a 15 aminoácidos inactivas en la proteína intacta, pero que pueden ser activados a liberarse por la digestión o por procesos tecnológicos previos [65,66].

Los alcaloides son compuestos nitrogenados de bajo peso molecular, los cuales se caracterizan por presentar un anillo en su estructura donde se une, generalmente, un átomo de nitrógeno, aunque puede haber excepciones. Se han descrito más de 12,000 compuestos pertenecientes a la familia de alcaloides que se distribuyen ampliamente en la naturaleza [68]. Según su precursor pueden clasificarse en más de 20 clases diferentes, como alcaloides derivados del triptófano, de pirrolidina, de tropano, de piperidina, de piridina, de quinolizidina e indol alcaloides, entre muchos otros [69]. La presencia de estos metabolitos secundarios mejora significativamente las tasas de supervivencia de las plantas, al tener la capacidad de atraer agentes polinizadores, al mismo tiempo que presentan estrategias defensivas y de repelencia hacia depredadores por toxicidad o sabor amargo. Además, promueven la reparación del daño por su sistema antioxidante [70].

Los alcaloides, como la cafeína, guaraná, gingerol y teobromina, están presentes en aproximadamente el 20% de las plantas. Algunos autores han

asociado los alcaloides del café, el cacao, el jengibre y el té verde, con la prevención de enfermedades neurodegenerativas como el Parkinson, con la mejora la cognición, con el incremento del rendimiento deportivo, con la disminución de la inflamación sérica y con una menor acumulación de grasa [71–73]. Además, el alcaloide piperina, presente en la pimienta negra, tiene la capacidad de intercalarse con las hebras de ADN, mejora la absorción de las vitaminas B, y sirve para el tratamiento de bronquitis y gonorrea [27]. También, existen algunos alcaloides de *Solanum* presentes en cerezas y algunos tipos de tomates, con propiedades antimicrobianas, antiflamatorias y antiestrogénicas [69].

1.2.4. Compuestos organosulfurados

Los compuestos organosulfurados son metabolitos que contienen azufre en su estructura. La capacidad de utilizar el azufre inorgánico e introducirlo en la síntesis de aminoácidos sulfurados es una capacidad de las plantas que los mamíferos no poseen, por lo que depende de su ingestión. Estos compuestos se presentan en forma de ciertos aminoácidos esenciales, por ejemplo, cisteína, cistina y metionina, que no solo son necesarios para la formación de proteínas sino también para las síntesis y buen funcionamiento de compuestos como el glutatión, determinadas enzimas, hormonas, coenzimas y vitaminas. Los compuestos organosulfurados se caracterizan por el aroma y sabor fuerte. Por ejemplo, el sabor del vino, queso, chocolate y café se debe a la presencia de estas sustancias de naturaleza volátil. A su vez, la alicina, es la responsable del olor fuerte del ajo, y junto con la aliina, ajoeno, s-alilcisteína, presentan actividad anticancerígena. Las crucíferas como el repollo y las coles de Bruselas también presentan este tipo de metabolitos secundarios, específicamente isotiocianatos de fenetilo, los cuales se asocian con efectos bloqueadores de células cancerosas [74].

Se ha observado que los glucosinolatos, presente en los vegetales crucíferos como el brócoli y la coliflor, promueven beneficios para la salud, al activar enzimas detoxificantes de fase II en el hígado [26,75]. Además, luego de la masticación de glucosinolatos, se producen otros compuestos como sulforano, el cual no solo mejora la supresión de proteínas supresoras de tumores, sino que también inhibe la actividad del marcador epigenético histona desacetilasa (HDAC), la AKT serina/treonina quinasa1 (Akt), la señalización de quinasa regulada por señales extracelulares MAPK/ERK, la prostaglandina-endoperóxido sintasa 2 (COX-2) y la expresión de la proteína ciclina D1 [76]. Efectos antioxidantes, antiinflamatorios, antiplaquetarios e imnunomoduladoras también han sido descritos para esta familia de compuestos [27].

1.3. Cerezas dulces (Prunus avium L.)

Las cerezas dulces pertenecen a la familia de las *Roseaceas* y a el género *Prunus*. Están presente en la DM, ya que su cultivo y consumo está distribuido por toda la cuenca mediterránea. Sin embargo, su ingesta es también frecuente en las zonas de clima templado, siendo su temporada de cosecha típica entre primavera y verano. Debido a su valor nutricional, tecnológico y comercial, la cereza dulce es de gran importancia económica [77–79].

Nutricionalmente, las cerezas aportan una cantidad interesante de nutrientes y compuestos bioactivos, al mismo tiempo que aportan muy pocas calorías. En tal sentido, aportan azucares simples (13 g /100 g), como fructosa, glucosa y sacarosa, junto con ácidos orgánicos como el málico, láctico y el ácido cítrico. Son ricas en fibra, carotenoides, y vitaminas hidro (C y B) y liposolubles (A, E y K). También aportan cantidades considerables de minerales como potasio (200 mg /100 g), calcio (14 mg /100 g), magnesio (10 mg /100 g) y fósforo (20 mg / 100g) [80]. Otros autores han reportado que presentan triptófano, serotonina y melatonina [81].

Si bien hay más de cien variedades de cerezas, todas destacan por su contenido en polifenoles, principalmente antocianinas, ácidos fenólicos y flavonoles [82]. Cabe destacar que los polifenoles se acumulan en la piel, incrementando su concentración durante la maduración al mismo tiempo que se degrada la clorofila y las antocianinas la tiñen de color rojo característico. Por lo tanto, el momento de cosecha y estado de maduración son claves para determinar el contenido de estos fitoquímicos [83]. La variedad, el método de cultivo, junto con las condiciones de cosecha, almacenamiento y transporte, especialmente, la temperatura y la humedad relativa, son también factores críticos que determinaran tanto las condiciones organolépticas de la fruta, como el contenido polifenólico [84]. Por ejemplo, alrededor de un 25% a 40% de los compuestos fenólicos totales de las cerezas *Montmorency* los constituyen los hidroxicinamatos y flavan-3-oles; sin embargo, en la variedad *Bing*, estos compuestos ocupan del 5% al 50% [51].

Las cerezas dulces poseen principalmente cianidina-3-*O*-rutinósido y la cianidina-3-*O*-glucósido, como antocianinas más abundantes, encontrándose aproximadamente 143.27 mg /100 g y 18.73 mg /100 g, respectivamente en fruta fresca. La peonidina-3-*O*-rutinósido y peonidina-3-*O*-glucósido, así como pelargonidina-3-*O*-rutinósido también están presentes pero en menores concentraciones. Los flavanoles, son otros flavonoides abundantes en las cerezas dulces. Al respecto, la (-)-epicatequina, (+)-catequina y la procianidina B2 se encuentran en 7.78 mg/100 g, 1.50 mg/ 100 g y 2.10 mg/ 100 g, respectivamente. Varios autores reportaron que las cerezas maduras contienen ácidos fenólicos como los derivados del ácido hidroxicinámico, siendo el ácido 3-cafeoilquínico y el 3-*p*-cumaroilquínico los que en mayor cantidad se encuentran (44.71 mg/ 100 g y 38.43 mg/ 100 g, respectivamente) [85].

Como consecuencia de la composición en fitonutrientes y sus propiedades antioxidantes, el consumo de cerezas dulces fue asociado con múltiples

beneficios en la salud. Específicamente, estudios en humanos revelaron un efecto antioxidante y antiinflamatorio, al disminuir marcadores de estrés oxidativo (isoprostano-F2, hidroxyperoxidos de lípidos, IL-6 y proteína C-reactiva) [86,87]. También se han observado efectos antidiabéticos y antilipidémicos, al disminuir la glucemia, la hemoglobina glicosidada, la proporción de lipoproteínas de muy baja densidad (VLDL) y el incremento de la lipoproteína de alta densidad (HDL-c) en sujetos con sobrepeso u obesidad y/o diabetes [88,89], y un efecto agudo hipotensor, al disminuir las presiones sistólicas y diastólicas [90]. Otros estudios en humanos demostraron efectos beneficiosos sobre la artritis, y sus factores de riesgo asociados [91], y mejoras en la calidad del sueño, estado de ánimo, funciones cognitivas [86,92] y prevención del daño muscular [93,94].

1.4. Tomate (Solanum lycopersicum)

La planta de tomate pertenece a la familia de las *Solanaceas*. Se cree que fue cultivado por primera vez en los años 700 d.C, y que en el siglo XVI llegó a Europa. Actualmente es un alimento clave de la DM, ya que no sólo se consume en su forma fresca, sino que varios productos y preparaciones derivan del tomate. En tal sentido, se consume en sopas, conservas, salsas, en ensaladas, jugos, aderezos, etc [95]. En el 2020, se produjeron 22 millones de toneladas en Europa, siendo 4 millones de toneladas de origen español [6].

Su gran consumo se debe principalmente a que, además de ser un alimento hipocalórico (34 kcal / 100 g), aporta principalmente agua, hidratos de carbono, fibra, minerales y vitaminas. En tal sentido, es una fuente de fósforo, potasio, sodio, y vitamina A, E y C [96]. Además, el tomate es una gran fuente de fitoquímicos no-nutritivos, dentro de los que se destacan carotenoides (γ- y β-carotenos, licopeno, luteína), polifenoles, (flavonoides, flavanones y flavonas) y glicoalcaloides (tomatina). El ácido cafeico, clorogénico, sinápico, *p*-cumárico y ferúlico, junto con la quercetina, rutina, kaempferol y naringenina son los

compuestos fenólicos más predominantes en la fruta. La concentración y acumulación de los compuestos bioactivos ocurre principalmente durante la maduración, durante la degradación de la clorofila y la maduración de la cáscara [68]. En consecuencia, la composición de biocompuestos va a depender de varios factores, que van desde la variedad del tomate y tipo de cultivo, hasta el estado de maduración, la exposición a rayos ultra violetas, la humedad y la temperatura [97]. Cabe aclarar, que la concentración y biodisponibilidad de determinados compuestos también va a depender de la forma en que se procesen y/o la presencia de otros nutrientes en el momento de la digestión. En tal sentido, se observó que la absorción de licopeno del tomate, se incrementa cuando se acompaña de alguna fuente de grasa, como aceite de oliva, y/o cuando está sometido a altas temperaturas [98]. En la Tabla 1.2 se resumen la composición fitoquímica de diferentes fuentes de tomate.

Tabla 1.2 *Composición fitoquímica de diferentes fuentes de tomate (mg/ 100g)*

Fitoquímico	Tomate fresco	Tomate enlatado	Salsa de tomate	Aderezos en base a tomate
Fitoeno	6.68	1.9	3.4	3.0
Fitoflueno	5.0	0.8	1.5	1.3
ζ-Caroteno	NI	0.2	0.3	0.8
Neurosporeno	NI	1.1	2.6	7.0
Licopeno	7.96	9.3	17.2	18.0
γ-Caroteno	NI	1.5	3.0	3.2
β-Caroteno	9.42	0.2	0.6	0.5

NI: No Identificado. Información obtenida de Becheer y col. (1998) [99], y Ali y col. (2021) [100].

Dado el alto contenido de compuestos bioactivos, el consumo de tomate es asociado con varios efectos beneficios sobre la salud, y sobre la prevención de

ciertas patologías. Como consecuencia a su capacidad antioxidante, reduce el estrés oxidativo molecular, la peroxidación lipídica y disminuye el riesgo a padecer ciertos tipos de cáncer (glándula mamaria, pulmón, colon hígado, entre muchos otros) [55], enfermedades cardiovasculares [101–103] y neurodegenerativas [104]. Además, también se asocia a su consumo un efecto antiaterógenico [105], antiinflamatorio [105], antiproliferativo, hipolipidémico [106,107] e hipoglucémico [105]. En tal sentido, algunos autores, han observado que la ingesta diría de 330 mL de jugo de tomate al día incrementó por 4 los niveles de HDL-c en sujetos con obesidad [108]. Otros han evidenciado que su consumo produjo un incremento en la expresión de genes relacionados con la β-oxidación en animales sanos y normopeso. A su vez, observaron efectos protectores contra esteatosis hepática en aquellos que fueron alimentados con una dieta alta en grasa [109].

1.5. Cronobiología: comprensión de los ritmos biológicos

En relación con los fitoquímicos, a finales de los 2000 surge la teoría de la xenohormesis (del griego *xenos*, extraño y *hormesis*, estimular, respectivamente), la cual propone que los heterótrofos, incluyendo los animales, han desarrollado la capacidad de detectar los fitoquímicos como moléculas de señalización, obteniendo un beneficio de ellas. Así, estos fitoquímicos informan sobre el estado del entorno de la planta y pueden servir como señal para activar una respuesta de defensa preventiva y así incrementar las posibilidades de supervivencia de los organismos que las consumen. Particularmente, se cree que los fitoquímicos provocan una interacción directa con varias vías metabólicos claves, involucrando factores de transcripción, enzimas y receptores de las vías que ejercen funciones cruciales del organismo [110,111].

Dado que la composición fitoquímica, principalmente polifenólica, de las frutas y verduras puede variar según la zona que son cultivadas y cosechadas, se

genera una firma polifenólica característica del lugar de crecimiento de la planta. Sin embargo, como consecuencia de la globalización, los estilos de vida modernos, y la facilidad de acceso a una amplia variedad de productos de diferentes orígenes, se podría pensar en una desvinculación de las señales de los polifenoles con la zona de consumo, introduciendo así señales que pueden ser malinterpretadas por el organismo y que pueden dar lugar a la aparición de alteraciones metabólicas [112].

La teoría de la xenohormesis liga con el concepto de los ritmos biológicos en los cuales se base la cronobiología. La cronobiología es el campo de la ciencia que estudia los mecanismos subyacentes a los ciclos que se dan en los sistemas biológicos como consecuencia de la adaptación a las variaciones cíclicas del ambiente debido a los movimientos de la Tierra. En tal sentido, la rotación de la Tierra sobre sí misma da como resultado las variaciones en la iluminación y temperatura en 24 horas (día y noche), mientras que la traslación alrededor del sol produce las variaciones estacionales (verano, otoño, invierno y primavera). Por lo tanto, la cronobiología abarca el estudio de los ciclos diarios (como la secreción de hormonas a lo largo del día o los ciclos de actividad y descanso), así como también los ciclos mensuales y anuales (como los ciclos reproductivos en determinadas especies) [113]. Específicamente, los ciclos diarios y anuales son conocidos también como ritmos circadianos y circanuales, ya que provienen del latín "*circa diem*" y "*circa annus*" que significa alrededor de un día y un año, respectivamente. La mayoría de los seres vivos exhiben ritmos circadianos y circanuales, como los hongos, plantas, peces, moscas y mamíferos [114,115].

En los mamíferos, los ritmos están gobernados por un sistema activo que persiste en ausencia de señales ambientales y, por lo tanto, posee oscilaciones autosostenidas (relojes endógenos centrales y periféricos). Además, los factores ambientales periódicos, denominados sincronizadores o *zeitgebers*, modulan los ritmos al inducir adaptaciones a las condiciones externas, como la

duración del día (fotoperiodo), la temperatura, el momento de alimentación, etc. En consecuencia, la interacción entre los osciladores endógenos (controlados genética y molecularmente) y los sincronizadores externos, producen la sincronización de los ritmos y el orden temporal [116,117].

Cabe destacar, que el fotoperiodo, es decir, las fases de luz/oscuridad del día, es la principal señal que utilizan los organismos para establecer el día y el momento estacional. Si bien existen otros *zeitgebers*, del entorno que pueden modular los ritmos, estos no son predecibles por no ser consistentes en el tiempo ni en magnitud [118].

1.5.1. Ritmo circadiano: reloj central

El sistema circadiano en los mamíferos incluye tres componentes: un oscilador o marcapasos central, con conexiones de entrada (aferentes) que sincronizan el oscilador con el ambiente, como así también conexiones de salida (eferentes) que permite que el oscilador module las respuestas del organismo [117]. En tal sentido, el núcleo supraquiasmático hipotalámico (NSQ) ha sido identificado como el principal marcapaso circadiano que impulsa los ritmos del comportamiento [119].

Con respecto a las vías aferentes, el NSQ recibe información fótica directa de los receptores celulares de retina denominadas "células ganglionares de la retina intrínsecamente fotoreceptoras" (ipRGC) [120]. Estas células son intrínsicamente fotosensibles a la irradiación de onda corta y se activan por cambios químicos en un fotopigmento específico: la melanopsina. Además, el NSQ también recibe información de los fotoreceptores de bastones y conos. El tracto retinohipotalámico transmite información acerca de la presencia o ausencia de luz al NSQ así como el nivel de iluminación de cada momento [116,119].

Por último, el NSQ controla la expresión de los ritmos circadianos por medio de la activación y conexión con varias areas cerebrales, como el área preóptica del

hipotálamo, el núcleo paraventricular hipotalámico, y diversas glándulas (pineal, suprarrenal, hipófisis). En consecuencia, se producen oscilaciones de la temperatura corporal, la ingesta de agua, la conducta sexual y la actividad locomotora. Además, la melatonina secretada por la glándula pineal, modula la actividad de la mayor parte de las glándulas del organismo y por lo tanto, la secreción de adrenalina, cortisol, hormonas hipofisiarias y sexuales [117,119]. Cabe destacar que la melatonina es un indicador crucial de las variaciones fotoperiódicas, ya que al ser liberada en las fases de oscuridad e inhibida durante el día, informa al cerebro de la duración del día externo [121,122].

A nivel mecanístico, el reloj molecular endógeno, es un mecanismo generado por bucles de retroalimentación de transcripcional-traduccional (TTFL, del inglés transcriptional and traductional feedback loop) positivos y negativos interconectados. Los componentes más importantes involucran los genes del reloj central: *Bmal, Clock, Per1/2, y Cry1* [123].

1.5.2. Relojes periféricos

La mayoría de los órganos y tejidos periféricos también expresan oscilaciones circadianas de forma aislada. Por lo tanto, casi todas las células del cuerpo expresan genes circadianos, llamados “genes reloj”, que responden de manera diferente a las señales externas, pero a su vez reciben y requieren información del NSQ [123]. En consecuencia, existe un sistema complejo formado por los diferentes tejidos que interactúan entre sí, con los osciladores externos circadianos, y con el NSQ, el cual garantiza una correcta alineación de fases de los relojes periféricos [124].

Por ejemplo, los ritmos circadianos tienen un papel crítico en la homeóstasis del músculo esquelético. En tal sentido, se ha observado que el músculo es modulado por el ritmo circadiano, debido a que se han observado más de 2,300 genes que muestran oscilación circadiana. Gran parte de ellos están involucrados en la transcripción de genes, en el metabolismo, en la capacidad

miogénica y en la señalización muscular [125]. La luz, el tiempo de alimentación y el tiempo y tipo de ejercicio, son fuertes moduladores del reloj molecular muscular. Cambios en el tipo de fibra, estructura sarcomérica alterada, respiración mitocondrial reducida, deterioro de la función muscular y consecuencias metabólicas como menor sensibilidad a la insulina y glucosa son algunas de los efectos de la disrupción de los ritmos circadianos [126]. Es fundamental que el reloj molecular muscular no sólo se adapte al entorno, sino que también este sincronizado con otros tejidos periféricos. Por ejemplo, se ha observado que los genes implicados en la señalización de insulina (*Irs1*, *Irs2*, *Akt2* y *Tbc1d1*) tienen una expresión reducida en la fase de luz (ayuno para roedores), mostrando que siguen un mismo patrón circadiano tanto en el músculo como en el hígado en ratones. A su vez, aquellos genes implicados en la producción de glucosa hepática (*Pck1*, *G6pc* y *Pyg*) incrementan su expresión durante esta fase [126]. Además, durante la fase de luz estos genes alcanzan su punto máximo de expresión al mismo tiempo que la sensibilidad a la insulina es baja. De manera contraria, en la fase de alimentación, donde los animales son activos, una mayor sensibilidad a la insulina es requerida por el músculo por necesitar mayor sustrato energético [126–128]. Por lo tanto, una buena coordinación entre el músculo y el hígado es necesaria para una correcta función metabólica (**Fig. 1.4**).

1.5.3. Ritmos circanuales

Al igual que los ritmos circadianos, el ritmo circanual se base en un mecanismo regulatorio, con un marcapaso a nivel cerebral, y un control jerárquico de sincronización a largo plazo en los tejidos periféricos. En casi todos los mamíferos, la glándula pineal es necesaria para generar ritmos circanuales que coincidan con el año natural o para inducir transiciones fotoperiódicas [129]. En tal sentido, la concentración de melatonina pineal endógena varia estacionalmente, creando una respuesta endócrina de la época del año [130].

Tanto las células tirotropas pineales como los tanicitos del tercer ventrículo cerebral, son células marcapasos, que regulan muchas vías hormonales y genéticas involucradas en la generación y mantenimiento de los ritmos circanuales [131]. En comparación con los relojes circadianos, los mecanismos fisiológicos de temporización circanuales son autosostenibles, más flexibles y reprogramables. Si bien la duración del periodo circanual no es afectada en gran medida por la temperatura, su ritmo se adapta a los *zeitgebers* ambientales, principalmente por el fotoperiodo, para sincronizar la fisiología con las estaciones [131,132]. En consecuencia, una coordinación hormonal compleja y altamente regulada, da como resultado mecanismos moleculares estacionales que modulan las funciones fisiológicas, conductuales y reproductivas.

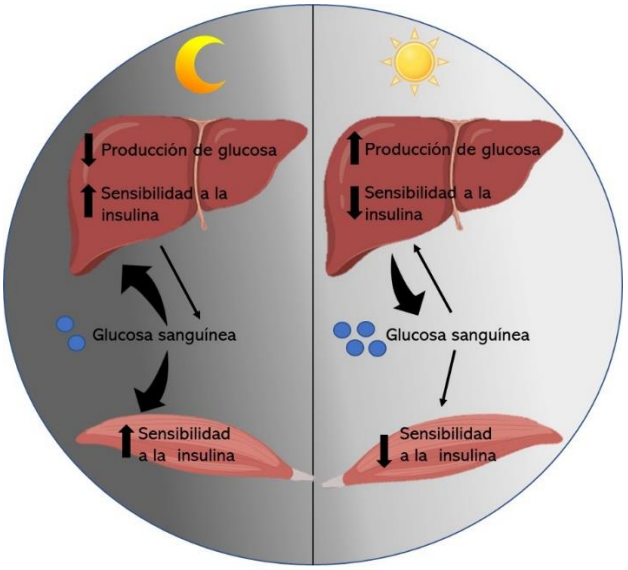

Fig. 1.4. Representación de la variación diurna en la coordinación en la homeostasis de la glucosa hepática-muscular. Adaptado de Harfmann y col. (2015)[126].

Debido a que las estaciones regulan muchos procesos fisiológicos, la estacionalidad ambiental da como resultado la programación de tejidos, generando ritmos endógenos que se aproximan a un año. En tal sentido, se ha

observado que en las especies en las que el invierno supone un mayor riesgo patológico, la exposición a días cortos produce un aumento de la función inmunitaria en previsión a mayores necesidades de defensa. Por lo tanto, la interrupción de los ritmos anuales, por ejemplo por los cambios climáticos o en la duración de las estaciones, tienen consecuencias potencialmente dramáticas para la salud de los organismos [133].

1.5.4. Disrupción de ritmos biológicos y su consecuencia metabólica

El reloj circadiano es un sistema de cronometraje biológico endógeno que sincroniza la fisiología y el comportamiento con los ciclos del día y la noche. En los últimos años, ha crecido la evidencia que asocia factores como polimorfismos genéticos de la maquinaria del reloj central y los cambios estacionales en el ciclo luz-oscuridad, con su influencia sobre determinados procesos fisiológicos. Específicamente, una amplia variedad de procesos del tracto gastrointestinal, principalmente el hígado, parecen estar bajo control circadiano. El ritmo circadiano ejerce un fuerte control sobre el sistema metabólico, debido a que la absorción, el procesamiento y desintoxicación de nutrientes, se alinean en función a las necesidades de los órganos para un correcto suministro y demanda de nutrientes [134]. En tal sentido, los pasos limitantes de la velocidad del metabolismo hepático de la glucosa, colesterol y triglicéridos están bajo regulación circadiana. Por lo tanto, disrupciones en el ritmo circadiano y en los genes reloj están relacionadas con un mayor riesgo de síndrome metabólico y/o exacerbación de estados patológicos [135]. Por ejemplo, se ha observado que ratones con pérdida de la función del gen reloj *Clock*, muestran hiperfagia, desarrollan hiperglucemia, hiperinsulinemia, esteatosis hepática, y dislipemia como resultado de la abolición de los genes relojes pancreáticos [136]. Otros estudios evidencian disminución del metabolismo glucídico dependiente de insulina en el músculo esquelético

[137], anormal secreción de ácidos grasos poliinsaturados (PUFAs, por su nombre en inglés: polyun*saturated fatty acids*)) de los adipocitos [138] y alteraciones en los centros de regulación del apetito hipotalámicos, como consecuencias de la disrupción de los relojes biológicos [112].

1.5.5. Crononutrición: efecto de compuestos bioactivos sobre los ritmos biológicos

La crononutrición es la disciplina que estudia las interacciones entre los ritmos biológicos, la nutrición y el metabolismo. Su interés surge como consecuencia de la creciente evidencia sobre el impacto del reloj biológico en el equilibrio energético, en el metabolismo, así como su influencia en la prevención o desarrollo de ciertas enfermedades [139]. La crononutrición no sólo involucra el momento de la ingesta, y cómo los ritmos biológicos afectan la salud y el metabolismo, sino también como la composición y el tamaño de la comida puede afectar nuestro sistema de reloj interno [140]. En tal sentido, se ha observado que dietas altas en grasas modulan los sistemas de relojes en todo el cuerpo, tanto en animales como en humanos. Específicamente, su consumo altera los patrones circadianos de leptina, insulina y cortisol y la señalización de AMPK quinasa, conduciendo a una mayor ingesta de alimentos, la alteración en la regulación del peso corporal y en la actividad locomotora [112,141].

Por otro lado, varios fitoquímicos han sido descriptos por afectar los ritmos circadianos, tanto a nivel central como los relojes en los tejidos periféricos. En tal sentido, se ha observado que la epigallocatequina-3-galato, un polifenol del té verde, regula la expresión rítmica de los genes reloj *Clock, Bmal1 y Cry1*, del hígado y el tejido adiposo, produciendo la mejora del corporal, reducción de colesterol y triacilglicéridos (TAG) hepáticos y plasmáticos y mejora del metabolismo glucídico en animales obesos [142]. Asimismo, el resveratrol, restauró la disincronía circadiana de genes lipogénicos en el tejido adiposo blanco, ocasionada por una dieta alta en grasa que produjo alteraciones de la

expresión rítmica de los genes reloj [143]. Un acortamiento del período circadiano del reloj molecular de células neuronales diferenciadas junto con propiedades antioxidantes y antidiabéticas, fue observado en ratones tras la administración de ácido cinámico [144]. En nuestro grupo de investigación se ha descrito la interacción de proantocianidinas y varios componentes involucrados en el sistema de sincronización circadiano. Específicamente, se observó que la proantocianidinas de la uva podrían modular significativamente los ritmos biológicos en ratas manteniendo niveles elevados de melatonina durante la fase de luz y regulando la expresión génica de *Bmal1* en el hipotálamo [145].

En relación con la crononutrición, en los últimos años ha crecido la evidencia que la temporada de consumo de las frutas y verduras también ejerce un efecto modulador en la expresión de genes implicados en los ritmos biológicos. En la Fig. 1.5 se resumen los mecanismos implicados en la interacción entre las variaciones estacionales en la composición polifenólica de las frutas, la regulación en la expresión de genes involucrados en los ritmos biológicos y sus efectos en la salud. Algunos autores han observado que la ingesta de cereza fuera de temporada modula los niveles de ARNm de nardilisina convertasa (*Nrdc)* en el músculo esquelético, y *Per2* y *Cry1* en el hígado y tejido adiposo blanco en ratas [146,147]. Asimismo, el consumo de naranjas fuera de temporada se asoció con un incremento en la síntesis de ácidos grasos (AG) en el tejido adiposo blanco y una menor captación de lípidos y β-oxidación en el tejido adiposo pardo [148].

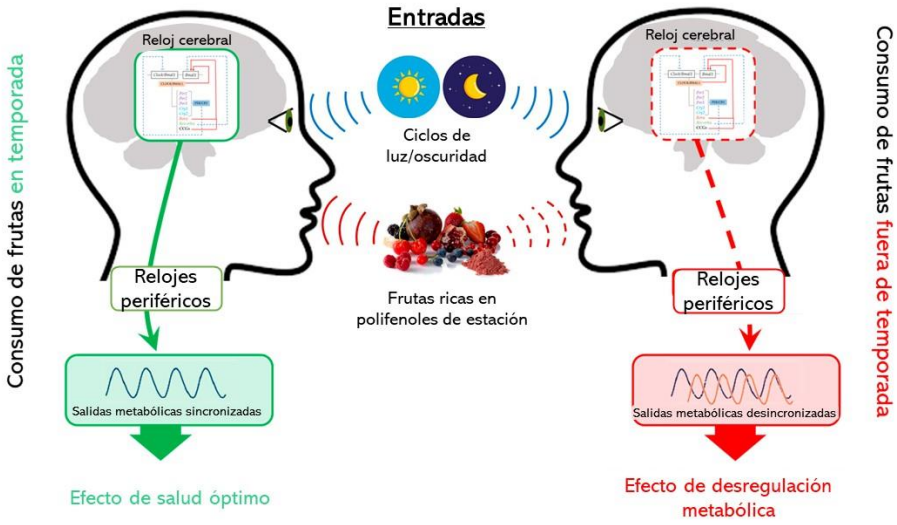

Fig. 1.5 Interacción entre la regulación génica de los ritmos biológicos, la variación estacional de la composición polifenólica de las plantas y los efectos estacionarios en la salud. Imagen adaptada de Arola-Arnal y col., (2019) [112].

1.6. Lípidos: estructuras y metabolismo

Como se ha comentado anteriormente los ritmos biológicos parecen tener una influencia directa en los niveles metabólicos de ciertas especies lipídicas como los TAG. Desde el punto de funcional, cabe destacar que los lípidos no solo son una buena fuente de energía de reserva para el organismo, sino que tienen múltiples de funciones que van desde componentes estructurales celulares, como de la membrana plasmática, participación en el transporte celular, hasta la estabilización de proteínas, señalización y transducción celular, transmisión simpática, entre otras [149]. Los TAG, AG, colesterol, fosfolípidos, glicerolípidos, son algunas de las clases de lípidos que se encuentran en el organismo.

En específico, los AG son los componentes principales de los lípidos de membrana. Estructuralmente son ácidos orgánicos con una cadena alifática con un grupo carboxilo en alguno de sus extremos. Se sintetizan a partir de acetato y por lo tanto, la gran mayoría de AG naturales tienen un número par

de átomos de carbono. La estructura química de los AG, ya sea el largo de la cadena, la presencia o no de insaturaciones, así como también el número de insaturaciones, su conjugación y el lugar donde ellas se encuentran le otorgan al AG importantes propiedades físicas, químicas y biológicas [150]. En tal sentido, los AG pueden ser: saturados (AGS, ausencia de dobles enlaces), monoinsaturados (MUFAs, con un doble enlace, por su nombre en inglés: monoun*saturated fatty acids*), o PUFAs, (con dos o más dobles enlaces). A su vez, existen los AG PUFAs esenciales, incluyendo n-6 y n-3, denominados omega [151]. El ácido linoleico (AL, *c*9,*c*12-18:2 (n-6)) y el ácido α-linolénico (ALA, *c*9,*c*12,*c*15-18:3 (n-3)), son los representantes de estas familias. Debido a que los mamíferos no poseen las enzimas necesarias para su síntesis, ambos AG son considerado esenciales, y deben ser proporcionados por la dieta. Tanto AL cómo ALA son precursores de AG de mayor longitud de cadena y grado de insaturación, como el ácido araquidónico (AA, *c*5,*c*8,*c*11,*c*14-20:4), el ácido eicosapentaenoico (EPA, *c*5,*c*8,*c*11,*c*14,*c*17-20:5) o el ácido docosahexaenoico (DHA, *c*4,*c*7,*c*10,*c*13,*c*16,*c*19-22:6), los cuales poseen potentes efectos biológicos, al ser parte de las los fosfolípidos de membrana, participar como segundos mensajeros en la transducción y ser parte de prostaglandinas e eicosanoides [152].

1.6.1. Metabolismo de AGs en hígado

Luego de una comida, la grasa dietaria es digerida y absorbida principalmente en los enterocitos del intestino delgado, donde los AG son re-sintetizados a TAG y secretados en quilomicrones. Cuando los quilomicrones llegan al hígado por circulación, elevan los ácidos grasos no esterificados (NEFAs, por su nombre en inglés: *Non-esterified fatty acids*) por acción de la lipoprotein lipasa (LPL) hepática. Los NEFAs ingresan al hepatocito principalmente a través de FAT/cd36 y proteínas transportadoras de AG, denominadas FATP (FATP2, FATP4, FATP5). La FATP5 es exclusivamente expresada en el hígado y se ha

observado que una disminución en su contenido se asocia con menor acumulación lipídica [153]. Recientemente, también se ha observado que FAT/cd36, también se encuentra en la membrana mitocondrial, permitiendo el paso de AG para su oxidación [154]. Una vez dentro del hepatocito los AG de 14 o más carbonos son activados mediante una proteína de unión a AG o mediante la acil-CoA sintasa con el fin de ser transportados a los compartimentos intracelulares o hacia el núcleo [155].

Por otro lado, los AG pueden originarse a partir de cuatro fuentes: (1) lipogénesis *de novo*, (2) reservas citoplasmáticas de TAG, (3) AG derivados de TAG de restos de lipoproteínas captados directamente por el hígado, y (4) NEFAs plasmáticos liberados por el tejido adiposo. El estado nutricional y el balance energético a corto y mediano plazo son determinantes para que una fuente u otra predomine [149]. A su vez, los AG son esterificados con glicerol-3-fosfato para sintetizar TAG, o con colesterol para formar esteres de colesterol. Estas moléculas pueden presentar diferentes destinos: acumulación en los hepatocitos, oxidación o exportación de TAG como constituyentes de VLDL hacia tejidos periféricos [156]. Los AG también pueden ser incorporados en los fosfolípidos, los cuales son componentes esenciales de las membranas celulares, formando parte de las balsas lipídicas, o en las membranas de lipoproteínas o partículas biliares [157].

1.6.2. Lipogénesis de novo

Cuando existe un exceso de carbohidratos y proteínas y no existe una demanda energética, se produce la síntesis de AG a partir de la oxidación de los sustratos excedentes que forman acetyl-CoA. El aceltyl-CoA citoplasmático, es carboxilado por la enzima acetyl-CoA carboxilasa (Acc) para formar malonyl-CoA. Cabe destacar que los mamíferos presentan dos isoformas de esta enzima, Acc1 y Acc2, situados en el citoplasma y mitocondrias, respectivamente. La formación de acetyl-CoA es el paso limitante para la

lipogénesis *de novo*, ya que es la molécula donante de grupos acetilo para la formación de la cadena esqueleto del AG. En tal sentido, la enzima ácido graso sintasa (Fas1) cataliza unas series de reacciones de condensación descarboxilativas con acetyl-CoA, malonyl-CoA y NADPH para dar como producto final el ácido palmítico (AP, 16:0) [157]. Sin embargo, para la síntesis de AG con numero impar de átomos de carbono en su cadena, se parte de una molécula de propinil-CoA como principal cebador [149]. La actividad de Fas1 está regulada hormonal y nutricionalmente, ya que la insulina y sustratos disponibles la estimulan, mientras que el glucagón, catecolaminas y alta concentración de AG citosólicos la inhiben [150,156]. A partir del AP, se pueden sintetizar los AG de cadena larga gracias a la acción de la familia de enzimas elongasas (*Elovl*) presentes en el retículo endoplasmático. Por ejemplo, la *Elovl6* cataliza la elongación de AP a ácido esteárico (AE, 18:0). A su vez, el nivel de AP y AE está determinado por la presencia enzimática de estearoyl-CoA desaturasa (*Scd1*), la cual se encarga de colocar un doble enlace en la posición 9 para formar el ácido palmitoleico y ácido oleico (AO, *c9*-18:1), respectivamente [157]. Se ha observado que animales que presentaban una disminución de *Elovl6*, estaban protegidos del desarrollo de esteatosis e inflamación hepática inducido por una dieta alta en grasa [158]. A su vez, la deleción de *Scd1* protegió contra obesidad a animales que recibían una dieta alta en carbohidratos, pero no a los que consumieron una dieta alta en grasa [159]. Por lo tanto, los niveles de AGS y MUFAs y sus efectos metabólicos, estarían influenciados por estas enzimas.

1.6.3. Síntesis de PUFAs y su importancia fisiológica

La ingesta de AL y ALA es necesaria para la síntesis de PUFAS n-6 y n-3, como se describió anteriormente. Tanto AL, ALA como AO, utilizan la misma vía metabólica para su elongación y desaturación. En tal sentido, los tres AG compiten por la Δ6 desaturasa (Fads2), enzima limitante y critica para la

biosíntesis de PUFAs, la cual participa en dos pasos metabólicos diferentes [160]. En la Fig. 1.6 se detallan los pasos sucesivos de la biosíntesis de PUFAs de las familias n-6 y n-3. La acumulación tisular de PUFAS de una u otra serie está determinada principalmente por la relación AL: ALA dietaria. En tal sentido, se ha observado que cuando la relación entre ellos es cercana a 5:1 los fosfolípidos de los tejidos son ricos en AA y pobres en EPA, en cambio, cuando la relación está por debajo de 5:1, hay un cambio de AA por EPA. Además, las dietas muy altas en PUFAs podrían inhibir la conversión de ALA a DHA, por competencia enzimática de Fads2, tanto de los precursores como del AG 24:5 n-3 [160].

La importancia de la síntesis de PUFAs de una familia u otra, radica en que estos son incorporados a los fosfolípidos de membranas celulares, y presentan un importante rol biológico al ser precursores de eicosanoides. En tal sentido, los PUFAs de ambas familias pueden competir por las enzimas fosfolipasa A2, ciclooxigensas y lipooxigenasa para su conversión a prostaglandinas, tromboxanos, leucotrienos y prostaciclinas con efectos contrarios. Por un lado, los PUFAs n-6, como el AA, promueven la síntesis de eicosanoides pro-inflamatorios, mientras que el EPA y DHA son precursores de resolvinas E y D, protectinas y maresinas, con importantes propiedades antiinflamatorias y pro-resolutivas. Estos mediadores biológicos inhiben la migración de células inmunitarias, la síntesis de metaloproteinasas de la matriz y la liberación de citocinas inflamatorias, como IL-1α, IL-1β, IL-6, IL-8, IL-12, factor de necrosis tumoral α (TNFα), interferón γ (IFNγ) y muchas más [151].

1.6.4. Exportación de TAG hepáticos hacia tejidos periféricos

El hígado tiene comunicación con tejidos extrahepáticos como el tejido adiposo o el músculo esquelético, que depende del estado de alimentación del organismo. Los AG hepáticos pueden ser oxidados o esterificados con glicerol-3 fosfato para formar TAG y ser distribuidos a tejidos extrahepáticos a través

de VLDL, ya sea para suplir demandas energéticas o para almacenamiento de energía [161]. Se ha demostrado en modelos animales que la disponibilidad de AG no es el único determinante de la tasa de producción de VLDL, sino que cree que la tasa de síntesis o el proceso de ensamblaje sea más limitante que el proceso de secreción *per se*. En tal sentido, a pesar de que la capacidad de exportación de TAG puede variar entre las especies, se ha observado que es proporcional a la capacidad de síntesis de AG *de novo* [156].

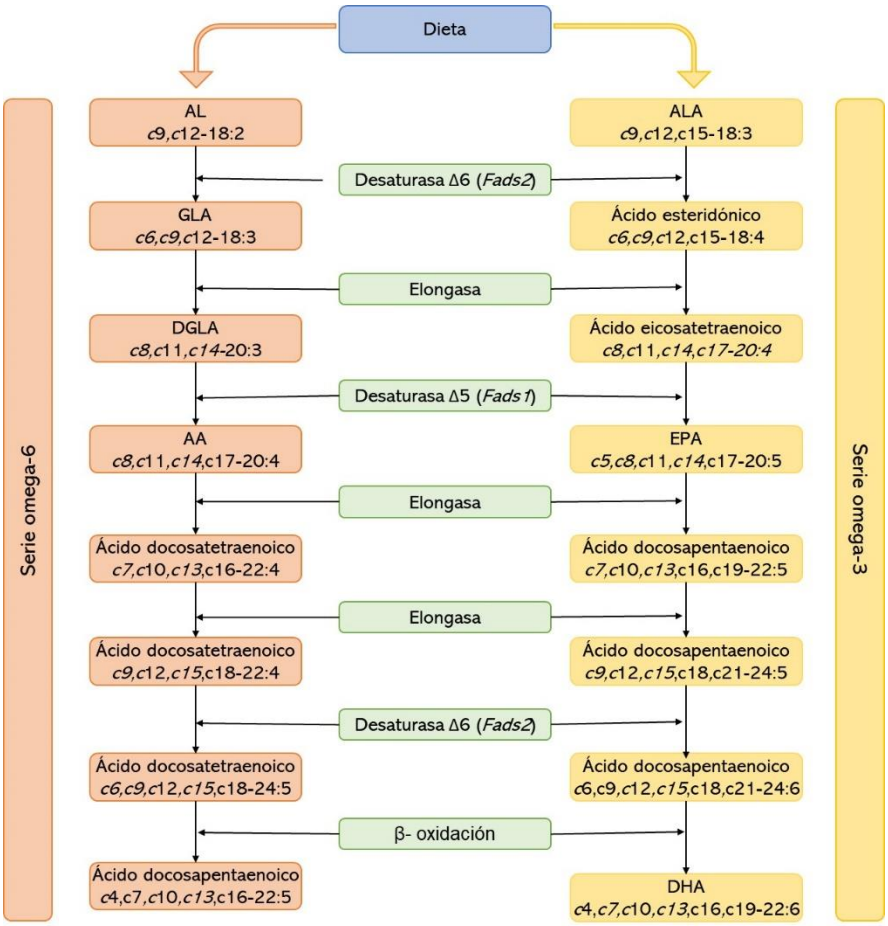

Fig. 1.6. Esquema de las vías de síntesis de ácidos grasos poliinsaturados de las series n-6 y n-3. AL: ácido linoleico; ALA: ácido α-Linolénico; GLA: ácido γ-linolénico; DGLA: ácido di-homo γ-linolénico; AA: ácido araquidónico; EPA:

ácido eicosapentaenoico; DHA: ácido docosahexaenoico. Imagen adaptada de *Wiktorowska-Owczarek* y col.(2015) [152].

1.6.5. Oxidación mitocondrial de AG en hígado

El suministro de AG al hígado a través de la lipolisis y la partición entre oxidación y esterificación microsomal, son los dos factores principales que regulan el grado de oxidación. Cabe destacar que la oxidación de AG puede ser mitocondrial, responsable de la metabolización de AG de cadena corta media y larga, o perixosomal, encargada de obtener energía a partir de AG de cadena muy larga, es decir, más de 22 carbonos. [156].

Específicamente, la β-oxidación mitocondrial de AG, es el proceso por el cual los electrones son transferidos al dinucleótido de flavina-adenina (FAD) y a la forma oxidada del dinucleótido de nicotinamida-adenina (NAD+) para formar las formas reducidas de estas coenzimas. A su vez, estas donan electrones a la cadena de transporte de electrones para impulsar la síntesis de ATP. El AG puede oxidarse completamente a dióxido de carbono en el ciclo del ácido tricarboxilico. La enzima carnitina palmitoiltransferasa-I (Cpt1α), es la enzima limitante de la velocidad de la β-oxidación de AG de 14 o más carbonos. Esta enzima cataliza la incorporación de un residuo de carnitina a un AG citoplasmático, formando acil-carnitil, para su pasaje a través de la membrana mitocondrial. Una vez en la matriz mitocondrial, la isoforma Cpt-II, cataliza la eliminación del residuo de carnitina, dejando la molécula acyl-CoA para la obtención de energía. Cabe destacar, que existe un control de la Cpt1α por el malonyl-CoA, para evitar una síntesis y oxidación de AG simultánea [152]. Los AG de menos de 14 carbonos en su cadena pasan pasivamente a través de la membrana mitocondrial. Recientemente, autores han identificado la presencia de FAT/cd36 en la membrana mitocondrial, sugiriendo su importancia en el pasaje de AG para su posterior oxidación [154].

1.6.6. Metabolismo de AG en el músculo esquelético

El tejido muscular esquelético es uno de los tejidos extrahepáticos con un rol importante en el mantenimiento de la homeostasis energética, ya que no sólo capta glucosa y ayuda a mantener la glucemia, sino que también es un órgano oxidativo de lípidos. Al representar un gran porcentaje del peso corporal, supone una gran parte del uso de sustratos nutritivos tanto en reposo como durante el ejercicio o el incremento de actividad física [162]. Se sabe que gran parte de los quilomicrones exportados por el intestino con lípidos dietario son captados por este tejido. Además, existe una comunicación entre el músculo esquelético, el tejido adiposo y el hígado, en donde se abastece de AG a través de TAG almacenados en el tejido adiposo o presentes en VLDL y lipoproteínas de baja densidad (LDL-c, por su nombre en inglés: *low density lipoprotein*) que vienen desde el hígado [163]. Los TAG pueden ser almacenados en pequeñas cantidades intramusculares [163].

1.1.1. Oxidación de AG en el músculo

La oxidación de AG muscular tiene varios niveles de regulación. En tal sentido, la tasa de transporte de AG a través de la membrana plasmática del miocito, el transporte hacia el citosol, la tasa de captación de la membrana mitocondrial, la cantidad de mitocondrias y de enzimas encargadas en la oxidación, son algunos de los pasos que influencian en gran medida la utilización de AG como fuente de energía muscular [162].

El paso de AG hacia el interior de la célula muscular se produce gracias a la presencia de proteínas en la membrana plasmática que se unen a los AG hidrofóbicos y permiten su pasaje hacia el citosol. Estas proteínas son identificadas como las proteínas de unión de AG (FABP), FAT/cd36 y FATP. Una vez en el interior celular los AG son activados. Los AG de cadena media y larga necesitan ser transportados por la carnitina palmitoiltransfera I β (Cpt1β) hacia

la matriz mitocondrial para ser oxidados. Debido a que este paso es el limitante de la velocidad de la β-oxidación se encuentra muy bien regulado [163]. En tal sentido, la adiponectina, una adipoquina secretada por los adipocitos, juega un papel crucial en la homeostasis energética muscular. La unión de la adiponectina a sus receptores de membrana, AdipoR1 y AdipoR2, produce la sensibilización de las células musculares a la insulina y por lo tanto, incrementa la captación de glucosa sanguínea y también la β-oxidación mitocondrial de AG. Específicamente la adiponectina produce sus efectos a través de la AMP-kinasa (AMPK), la cual la fosforila e inactiva la *Acc1* muscular, disminuye la concentración de malonyl-CoA y por lo tanto, estimula a la Cpt1β para el transporte y oxidación mitocondrial de AG. Además, la adiponectina puede regular la disminución de *Acc1* a través de la modulación del factor de transcripción nuclear *Srebp-1c* [165] (Fig 1.7).

Una vez dentro de la matriz mitocondrial, los AG pueden ser β-oxidados en el ciclo del ácido tricarboxilico. Algunos autores sugieren que la disponibilidad de AG determina la tasa de oxidación de grasas en el músculo y que la oxidación de AG inhibe directamente el metabolismo del glucógeno y la glucosa [166].

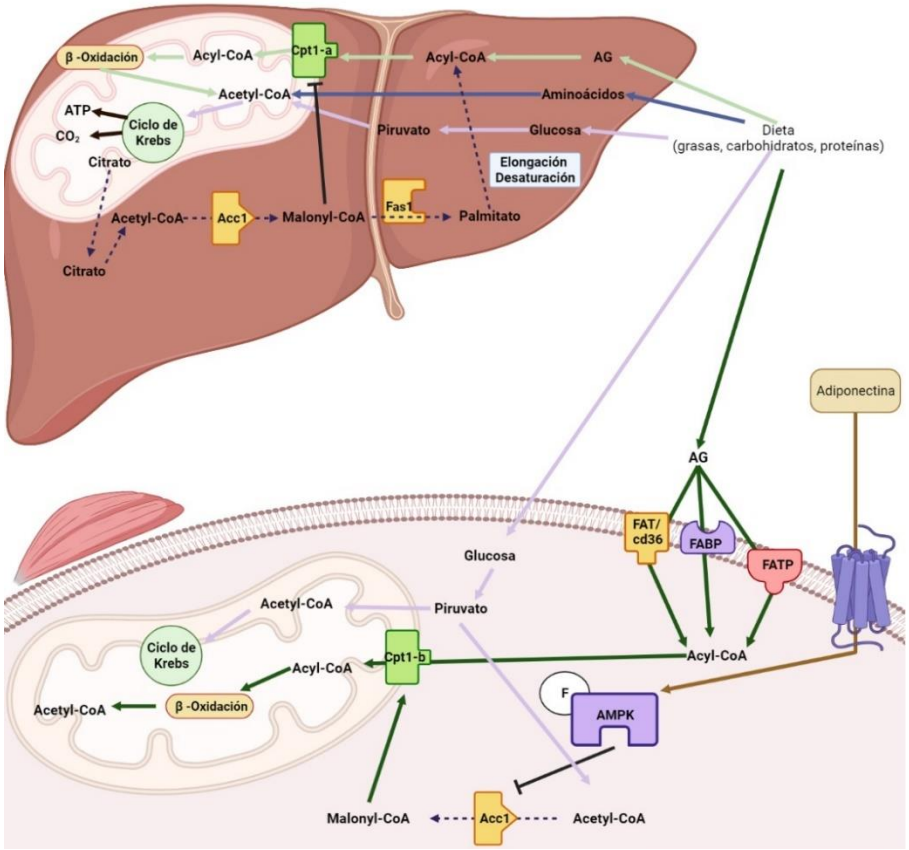

Fig. 1.7 Metabolismo lipídico y comunicación entre el hígado y músculo. Imagen adaptada de Wakil y col. (2009) [164] y Coles C.A. (2016) [165].

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UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
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UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

2.HIPÓTESIS Y OBJETIVOS

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

HIPÓTESIS Y OBJETIVOS

Las variaciones ambientales que se producen en ciclos de 24 horas y en los de 365 días anuales, generan patrones de adaptación por parte de los mamíferos, que involucran cambios en su fisiología y metabolismo con el objetivo de prolongar la supervivencia. En tal sentido, tales adaptaciones presentan una ritmicidad, denominándose ritmo circadiano (alrededor de un día) y circanual (alrededor de un año).

Específicamente, las fluctuaciones anuales se reflejan como cambios estacionales, principalmente en zonas alejadas del ecuador, en dónde se perciben notables variaciones climatológicas, en las horas de luz y oscuridad y en disponibilidad de alimentos. En consecuencia, los organismos se enfrentan a constantes cambios, regulados por mecanismos que aseguran una sincronización entre las funciones biológicas con el momento y condiciones externas adecuadas. No obstante, actualmente los ritmos biológicos se ven interrumpidos por los estilos de vida humanos modernos, que incluyen privación del sueño, estimulación lumínica durante la noche, desfase de horario o trabajos por turnos, que inducen desajustes temporales entre el medio ambiente y la ritmicidad biológica, afectando al bienestar y el estado de salud.

Por otro lado, las plantas y sus productos derivados, poseen una composición fitoquímica característica, principalmente en polifenoles, que está condicionada por los estímulos estresantes externos de donde fue plantada, desarrollada y cosechada. La teoría de la xenohormesis sugiere que esta “firma polifenólica”, serviría como señal molecular, para que los heterótrofos que consumen los productos vegetales locales, produzcan adaptaciones que les permita sobrevivir ante los mismos agentes estresantes externos que ha sufrido la planta y poder sincronizarse con las condiciones ambientales del entorno.

Sin embargo, en la actualidad, gracias a la globalización y a los avances tecnológicos, no sólo son accesibles los productos locales, sino que se pueden conseguir frutas, verduras y sus productos derivados de cualquier parte del mundo. Incluso, los alimentos de determinadas temporadas son accesibles durante todo el año. En tal sentido, considerando la teoría de *xenohormesis*, esta situación estaría provocando una ingesta de señales fitoquímicas pertenecientes a otro contexto, a otras condiciones ambientales y a otros agentes estresantes que no necesariamente se corresponden con los del área de consumo. En consecuencia, esta situación podría provocar una disrupción de la homeostasis externa e interna, pudiendo afectar procesos fisiológicos, bioquímicos y metabólicos y por lo tanto afectar en la salud de los consumidores.

Al respecto, en los últimos años, nuestro grupo de investigación ha observado que el consumo de diferentes frutas consumidas en distintas temporadas, como así también la ingesta de una misma fruta pero de diferente origen geográfico, afecta ciertas vías metabólicas, entre ellas el metabolismo lipídico. Por ejemplo, la ingesta de cerezas en diferentes fotoperiodos afecta no solo la expresión de genes relacionados con el reloj central biológico, sino que también modula la expresión de ciertas enzimas lipolíticas y lipogénicas en tejidos periféricos. Así mismo, la ingesta de naranjas de diferentes hemisferios modula diferencialmente el metabolismo en los tejidos adiposos blanco y marrón en ratas.

En base a estas premisas, es plausible pensar que el consumo de frutas de diversos orígenes geográficos, específicamente cerezas y tomates, como así también su ingesta en diferentes temporadas, provoca un efecto metabólico diferencial. Por lo tanto, **la hipótesis de la presente tesis es que el consumo de frutas de origen local, específicamente cerezas y tomates, así como su ingesta en temporada, provocan un efecto metabólico favorable extra, especialmente, en el metabolismo lipídico.**

Así, el objetivo de la tesis fue evaluar si el origen geográfico y/o la época de consumo de determinadas frutas afectan el metabolismo lipídico y parámetros metabólicos asociados en un modelo animal saludable.

En consecuencia, se propusieron los siguientes objetivos específicos:

1- *Evaluar los efectos metabólicos a nivel sérico, hepático y muscular, con énfasis en el metabolismo lipídico, que provoca la exposición a diferentes fotoperiodos.*

Para desarrollar este objetivo, hemos realizado un estudio comparativo entre grupos de animales alimentados con dieta estándar y que fueron expuestos a diferentes horas de luz diaria, a los cuales se les evalúo parámetros bioquímicos séricos, expresión génica de enzimas relacionadas con el metabolismo lipídico y perfil de AG en tejidos metabólicamente activos. También se realizó un análisis metabolómico de metabolitos séricos y hepáticos (Manuscrito 1).

2- *Evaluar las consecuencias sobre parámetros metabólicos del consumo de cereza dulce Brooks de origen local (España) y no local (Chile), así como su ingesta en diferentes fotoperiodos.*

Para conseguir este segundo objetivo se propusieron dos tareas:

2.1. Analizar si el origen geográfico de las cerezas y/o su época de consumo afectan los parámetros bioquímicos séricos y la expresión de enzimas hepáticas relacionadas con el metabolismo lipídico (Manuscrito 2).

2.2. Analizar si el origen de las cerezas y/o su época de consumo influyen sobre el metabolismo de AG y la comunicación entre los tejidos metabólicos: higado y músculo esquelético (Manuscrito 3).

3- *Evaluar las consecuencias del consumo de tomate Ekstasis de origen local (Tarragona) y no local (Almería), como así también su ingesta en diferentes temporadas, sobre parámetros metabólicos.*

Para desarrollar este objetivo, hemos conducido un estudio comparativo entre grupos de animales que recibieron tomate de diferentes orígenes geográficos (Tarragona y Almería), y que fueron expuestos a diferentes fotoperiodos, para simular las distintas estaciones. Evaluamos las consecuencias en parámetros bioquímicos séricos, y en la expresión de enzimas hepáticas claves del metabolismo de lípidos (Manuscrito 4).

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

3.PLAN DE TRABAJO

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

PLAN DE TRABAJO

Con el fin de llevar a cabo los objetivos definidos, el siguiente plan de trabajo fue propuesto:

Primer año

Con el propósito de revisar y tener información actualizada sobre el tema de la tesis doctoral, en primera instancia, se realizó una búsqueda bibliográfica basándome en libros y publicaciones científicas. Al mismo tiempo, se realizó experimentación animal. Luego, se llevó a cabo trabajo experimental en el laboratorio. Específicamente, durante el primer año, se realizó el análisis de parámetros bioquímicos séricos y parte de la determinación de las expresiones génicas hepáticas. También, durante este período de tiempo se asistió a jornadas de intercambio de conocimientos y se realizaron cursos de posgrado, para adquirir conocimientos relacionados a la publicación de artículos científicos, tratamiento y análisis estadístico de datos. Parte del primer año sucedió bajo confinamiento, que fue aprovechado para el análisis estadístico de los datos obtenidos.

Segundo año

Debido a que gran parte del segundo año el trabajo se desarrollo bajo la modalidad *home office*, el manuscrito 2 fue redactado. Además, también se realizó trabajo experimental en laboratorio, específicamente se llevó a cabo la extracción de muestras séricas y hepáticas para su posterior análisis por resonancia magnética nuclear (RMN). Durante el transcurso de este año, la estancia de investigación en la cátedra de Bromatología y Nutrición de la Universidad Nacional del Litoral, Argentina, fue realizada. Allí, se analizaron los perfiles de AG de muestras hepáticas y musculares. Se comenzó a escribir el artículo de revisión sobre el papel de los biocompuestos en la prevención y/o reversión de enfermedad de hígado graso no alcohólico, en colaboración con

dicha universidad. También se participó y expusieron resultados en webinars, seminarios y jornadas online.

Tercer año.

Durante el último año, se llevaron a cabo las escrituras de los manuscritos 1, 3 y 4 y la actual tesis. Además, se realizó trabajo experimental de laboratorio, principalmente la determinación de expresión génica de enzimas hepáticas y musculares, con el finde dilucidar los mecanismos implicados en los cambios anteriormente observados. La presentación de resultados en jornadas y congresos internacionales también fue realizada en este periodo.

Tabla 3.1. Distribución de actividades durante los tres años de doctorado (mayo 2019-mayo 2022)

	Primer año	Segundo año	Tercer año
Revisión bibliográfica y cursos			
Búsqueda bibliográfica			
Cursos de doctorado			
Conferencias y congresos			
Parte experimental in vivo			
Estudio in vivo.			
Trabajo de laboratorio			
Análisis de parámetros bioquímicos séricos			
Expresión génica de enzimas			
Extracción de muestras y RMN			
Análisis del perfil de ácidos grasos			
Estancia internacional (UNL)			

Tabla 3.1. Distribución de actividades durante los tres años de doctorado (mayo 2019-mayo 2022) (Continuación)

	Primer año	Segundo año	Tercer año
Análisis de resultados y redacción			
Análisis de resultados			
Manuscrito 1			
Manuscrito 2			
Manuscrito 3			
Manuscrito 4			
Manuscrito 5: Revisión anexada			

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

4. MATERIALES Y MÉTODOS

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

MATERIALES Y MÉTODOS

4.1. Obtención de frutas

Cerezas

Cerezas dulces (*Prunus avium*), variedad *Brooks*, fueron utilizadas en el presente experimento. Esta variedad de cerezas es un hibrido entre las variedades *Early Burlat* y *Rainier*, lo que produce un fruto dulce de maduración temprana, de muy alta calidad y con la capacidad de desarrollar frutos de gran tamaño. Fue desarrollada en la Universidad de California-Davis y en 1987 fue lanzada al mercado [1]. Cabe destacar que la época de cosecha y consumo de este tipo de fruta es en verano, específicamente, junio/julio. Para el estudio se utilizaron cerezas de diferente origen geográfico: cerezas cultivadas en Tarragona, España (LC, del inglés: Local Cherry), donadas por el agricultor en junio de 2018 y cerezas cultivadas en Cachapoal, Chile (nLC, del inglés: non-Local Cherry). Una vez descartados los huesos, las partes comestibles se congelaron en nitrógeno líquido. Posteriormente se aplastaron y se liofilizaron en el liofilizador Telstar LyoQuest (Thermo Fisher Scientific, Madrid, España) a −55 ºC por una semana. Para la obtención de los polvos se empleó un molino (Moulinette 1, 2, 3, Moulinex). Las muestras fueron almacenadas a temperatura ambiente, en frascos color ámbar para la protección lumínica, hasta su utilización.

Tomates

Los tomates utilizados en el presente trabajo, variedad *Ekstasis*, fueron cultivados bajo un sistema convencional de cultivo. Cabe destacar que la época de cosecha típica de esta variedad es otoño. En el presente experimento se utilizaron tomates de diferentes orígenes geográficos: tomates de origen local (LT: del inglés: Local Tomato) cultivados en Tarragona (41°4′29.24″ N 1°3′8.78″

E; España), y tomates de origen no local (nLT: del inglés: non-Local Tomato) procedentes de Almería (36°50′17.3″ N 2°27.584′ O; España). Ambos fueron obtenidos en el Mercado central de Tarragona, España, en noviembre de 2018. Fueron seleccionados aquellos que presentaban madurez y que carecían de marcas o zonas dañadas. Una vez obtenidas las frutas, se procedió a su congelación con nitrógeno líquido, y aplastamiento y trituración. Posteriormente, fueron liofilizados utilizando el liofilizador Telstar LyoQuest lThermo Fisher Scientific, Barcelona, España) a -55 ºC por una semana. Los polvos obtenidos de LT y nLT fueron almacenados a temperatura ambiente, protegidos de la luz y la humedad hasta su utilización.

8.2. Diseño experimental

Animales

El protocolo de experimentación animal para la realización del presente experimento fue revisado y aprobado por el Comité de Ética de Experimentación Animal de la Universidad Rovira i Virgili (número de referencia 9495).

Un total de 120 ratas Fischer 344 machos de 8 semanas de edad, de 230 gr promedio, fueron adquiridas del Laboratorio Charles River (Barcelona, España) y aleatoriamente divididas en tres grupos (n=24) dependiendo de la cantidad de horas de luz diarias a las que fueran expuestas. En tal sentido, los animales fueron expuestos a fotoperiodo corto (L6, con 6 horas de luz y 18 de oscuridad), fotoperiodo estándar (L12, con 12 horas de luz y oscuridad) y fotoperiodo largo (L18, con 18 horas de luz y 6 de oscuridad). Cabe destacar, que los fotoperiodos tratan de simular los días de las diferentes estaciones anuales. Por lo tanto, se simularía a invierno, otoño/primavera y verano, respectivamente. Luego de cuatro semanas de adaptación, los animales de cada fotoperiodo fueron nuevamente divididos aleatoriamente en subgrupos de acuerdo con el

tratamiento recibido: LC (n=8), nLC (n=8), LT (n=8), nLT (n=8) o vehículo (VH) (n=8). Todos los animales se mantuvieron en temperatura controlada a 22 ºC, con acceso a agua *ad libitum* y dieta estándar (A04, SAFE, Panlab, Barcelona, España, Composición nutricional en Anexos.). A los animales que recibieron algún tratamiento, se les administró una dosis de 100 mg de polvo de fruta liofilizada (LC, nLC, LT, o nLT) por kg de peso corporal solubilizados en agua. Los grupos VH recibieron una mezcla glucosa: fructosa (1:1) a una dosis de 21.2 mg/kg de peso corporal, para simular la cantidad de azúcares presentes tanto en la cereza como en los tomates. La suplementación de cada tratamiento fue voluntaria con jeringa una hora después de que la luz se encendiera. Luego de siete semanas de suplementación, los animales pesaron en promedio 375 gr. Tras 11 semanas de experimento fueron sacrificados por decapitación 1 hora después de haberles suministrado la dosis. La sangre del cuello fue recolectada en tubos no heparinizados, los cuales fueron incubados 1 hora a temperatura ambiente y centrifugados (2000 x g x 15 minutos a 4 ºC) para la obtención del suero. Varios órganos fueron recolectados, pesados y almacenados a -80 ºC hasta su utilización. Dentro de ellos, el tejido hepático y muscular, junto con el suero fueron los utilizados para la presente tesis. En la Figura 4.1, se detalla el diseño experimental.

Cabe destacar que para la realización del experimento se utilizaron ratas Fischer 344 debido a que es una cepa altamente fotosensible, es decir, que presentan cambios en su fenotipo fisiológico, reproductivo y de comportamiento con respecto a la cantidad de horas de luz diaria. Por el contrario, en otras cepas de laboratorio , los fotoperiodos no inducen cambios sólidos en los parámetros mencionados [2,3].

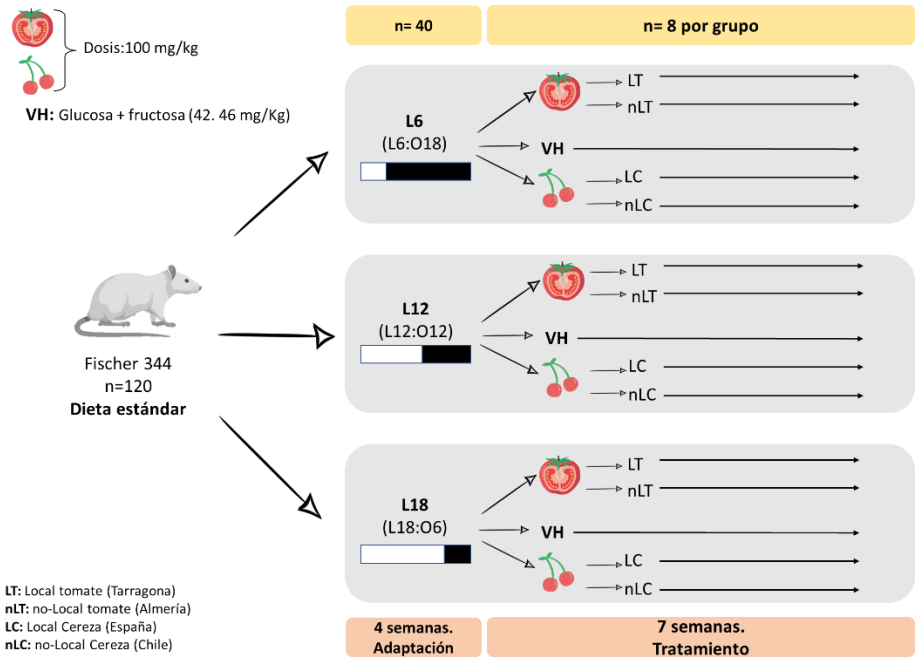

Fig.4.1. Esquema de diseño experimental.
L6: fotoperiodo corto (6 horas de luz, 18 de oscuridad); **L12**: fotoperiodo estándar (12 horas de luz y oscuridad); **L18**: Fotoperiodo largo (18 horas de luz, 6 de oscuridad); **VH**: vehículo; **LT:** Local Tomate; **nLT:** no-Local Tomate; **LC:** Local cereza; **nLC:** no-Local cereza.

Justificación de las dosis dadas

Nuestro grupo de investigación ha demostrado en los últimos años, que la dosis crónica de 100 mg/kg/día de fruta liofilizada es capaz de inducir efectos protectores sobre determinados parámetros metabólicos en animales normopeso y alimentados con dieta estándar [4–6]. Los animales fueron entrenados previamente al experimento, para que consuman voluntariamente el extracto de fruta.

Según la ecuación publicada por Reagan-Shaw y col. (2008) para la extrapolación de dosis animales a humanos, dónde se tiene en consideración la especie y el peso [7], la dosis de 100 mg fruta liofilizada/kg/día equivaldría en cada caso a 5.89 gr de cereza fresca (parte comestible) y 38 gr de tomate fresco

al día para una persona de 60 kg. Cabe destacar que para dichos cálculos se tuvo en cuenta que 100 gr de cereza fresca equivalen a 33 gr de fruta comestible liofilizada, y que los tomates frescos poseen un 94.9 % aproximado de humedad [4,5].

Como anteriormente se ha comentado, los VH recibieron una mezcla de glucosa:fructosa (1:1) de 21.2 mg/kg de peso corporal. Previamente, se calculó por grados Brix que el contenido promedio de azúcares simples entre las cerezas y tomates era de aproximadamente 47 gr sólidos solubles/100 gr de frutas liofilizadas [4,6]. Considerando que la dosis diaria de 100 mg/kg a un animal promedio de 300 gr equivaldría a 30 mg de fruta liofilizada, se les estaría dando aproximadamente 14 mg de azúcares al día. Se optó por una mezcla de glucosa y fructosa para simular los azúcares simples mayoritarios de la fruta en partes iguales. Cabe destacar que, en anteriores trabajos, el grupo de investigación ha demostrado que la cantidad de azúcares aportada por la fruta es irrelevante en el contexto total de la dieta de los animales, ya que no representa una significativa cantidad ni de energía ni de carbohidratos. En este sentido, considerando que la ingesta de energía de los animales es aproximadamente 56 kcal/día y que su peso promedio fue de 300 gr, la dosis de 21 mg/kg de glucosa: fructosa proporciona 6.36 mg al día de cada tipo de azúcar. Por lo tanto, los animales recibieron al día un total de 12.72 mg de azúcares lo que equivaldría a 0.050 kcal/día o 0.09 % de la energía ingerida diaria.

8.3. Determinación de parámetros bioquímicos clásicos

Con el fin de caracterizar el estado metabólico general de los animales se llevó a cabo la cuantificación de los principales parámetros bioquímicos clásicos en muestras de suero al finalizar el experimento. Concretamente se determinaron TAG, NEFAs, Colesterol total (CT), HDL-c, LDL-c, glucosa, insulina, así como

también del índice HOMA-IR y los diferentes coeficientes aterogénicos. Los protocolos se detallan a continuación.

Triacilglicéridos

Para la determinación de TAG circulantes en suero se utilizó un kit comercial (QCA, Amposta, España), el cual se basa en la reacción enzimática de hidrólisis de los TAG presentes en las muestras problemas en glicerol y AG libres por la acción de la lipoproteinlipasa. La glicerol quinasa fosforila el glicerol, dando lugar a glicerol-1-fosfato, que por acción de la glicerol fosfato oxidasa se oxida, formándose dihidroxi-acetona fosfato y peróxido de hidrógeno. A partir de una reacción donde participa el peróxido de hidrógeno se produce un producto que presenta absorbancia a 505 nm.

Para cada placa de ELISA de 96 pocillos utilizada para la determinación de TAG, se realizó una curva patrón de 5 puntos utilizando el estándar de concentración conocida otorgado por la casa comercial. Luego, en los pocillos restantes se pipetearon 10 µL de muestra problema, se agregó 1 mL de reactivo A (mezcla de enzimas, cofactores y estabilizantes) en todos los pocillos y se incubó durante 5 min a 37 °C. Se realizó la lectura de la absorbancia a 505 nm como indica el protocolo de la casa comercial. Todas las muestras se analizaron por triplicado. A partir de estos datos se realizaron los cálculos teniendo en cuenta cada curva patrón y considerando las fórmulas proporcionadas por la casa comercial para determinar la concentración de TAG en las muestras.

Ácidos grasos no esterificados

Se utilizaron kits comerciales a base de enzimas para determinar los NEFAs (Wako Chemicals GmbH, Neuss, Alemania). El protocolo consistió en pipetear en pocillos de una placa de ELISA un volumen de 3.5 µL de muestra o los puntos de patrón (de concentración conocida). Se añadió 150 µL del Reagent 1 (mezcla de enzimas, cofactores, ATP y estabilizantes), se mezcló e incubó durante 5 min

a 37 °C. Se añadieron 75 µL del Reagent 2 (mezcla de enzimas: acyl-CoA oxidasa y peroxidasa), se mezcló y se incubó 5 min a temperatura ambiente. Posteriormente, se llevó a cabo la lectura de la absorbancia a 546 nm en un espectrofotómetro para placas de ELISA Eon (Biotek, Vermont USA) cada 30 segundos durante 3 minutos.

Colesterol total

Para la determinación de CT en suero se empleó el kit comercial (QCA, Amposta, España), el cual se basa en unas cadenas de reacciones enzimáticas, dónde participa la enzima colesterol esterasa y la peroxidasa, que da como resultado un derivado quinónico coloreado que se puede cuantificar espectrofotométricamente a 505 nm.

Para dicha determinación se utilizaron placas ELISA de 96 pocillos, donde en cada una se realizó una curva patrón con 5 puntos de concentración conocida, utilizando el estándar de la casa comercial. Luego en los pocillos restantes se pipetearon 10 µL de muestra problema por triplicado. Luego se agregó 1 mL de reactivo A (mezcla de enzimas, fenol, cofactores y estabilizantes) en todos los pocillos y se incubó durante 5 min a 37 °C. Se realizó la lectura de la absorbancia en espectrofotómetro Eon (Biotek, Vermont USA) a 505 nm como indica el protocolo de la casa comercial. A partir de estos datos se realizaron los cálculos teniendo en cuenta cada curva patrón y considerando las fórmulas proporcionadas por la casa comercial para determinar la concentración de CT en las muestras.

Colesterol HDL

La cuantificación de HDL-c se llevó a cabo mediante la utilización de un kit comercial (QCA, Amposta, España), el cual se basa en la propiedad de un reactivo detergente que produce la liberación de la fracción HDL por solubilización. Ésta a su vez reacciona con el cromógeno, colesterol esterasa y

colesterol oxidasa, para dar color cuantificable a 600 nm. El uso de un polianión estabiliza las lipoproteínas (VLDL, LDL y quilomicrones) por adsorción, las cuales no pueden reaccionar con el complejo enzimático.

Para su determinación, se emplearon placas ELISA de 96 pocillos. Se realizó para cada placa utilizada una curva patrón con 5 puntos de concentración conocida, partiendo del estándar proporcionado por la casa comercial. Luego, en los pocillos restantes se colocó 3 µL de cada muestra o patrón y se agregó 225 µL del reactivo A (mezcla de estabilizantes, conservantes, metilalanina y polianión). Tras mezclar e incubar a 37 ºC por 5 minutos, se leyeron las absorbancias en espectrofotómetro Eon (Biotek, Vermont USA) a 600 nm. Luego se agregó 75 µL del reactivo B (mezcla de enzimas y estabilizantes), se mezcló e incubó por 5 minutos a 37 ºC nuevamente. Se realizó la lectura final de las absorbancias a 600 nm. Cabe destacar que las determinaciones se hicieron por triplicado. A partir de estos datos se realizaron los cálculos teniendo en cuenta cada curva patrón y considerando las fórmulas proporcionadas por la casa comercial para determinar la concentración de HDL-c en las muestras.

Colesterol LDL

La determinación de LDL-c en suero se realizó mediante el empleo del kit comercial (QCA, Amposta, España), el cual se basa en un primer paso de eliminación de quilomicrones, VLDL y HDL-c mediante la acción oxidativa de las enzimas colesterol esterasa y colesterol oxidasa y la degradación por parte de la enzima catalasa. De esta manera, se crea un complejo denominado colestenona y H_2O_2. Luego el LDL-c presente en las muestras se transforma en un complejo coloreado (quinona) cuantificable espectrofotométricamente, gracias a la acción de tensioactivos específicos.

Para su determinación, se emplearon placas ELISA de 96 pocillos. Se realizó para cada placa utilizada una curva patrón con 5 puntos de concentración

conocida, partiendo del estándar proporcionado por la casa comercial. Luego, en los pocillos restante se colocó 3.5 µL de cada muestra o patrón y se agregó 210 µL del reactivo A (mezcla de enzimas, estabilizantes y tampón neutro). Tras mezclar e incubar a 37ºC por 5 minutos, se leyó las absorbancias en espectrofotómetro Eon (Biotek, Vermont USA) a 600 nm. Luego se agregó 70 µL del reactivo B (mezcla de peroxidasa y estabilizantes), se mezcló e incubó por 5 minutos a 37ºC nuevamente. Se realizó la lectura final de las absorbancias a 600 nm. Cabe destacar que las determinaciones se hicieron por triplicado. A partir de estos datos se realizaron los cálculos teniendo en cuenta cada curva patrón y considerando las fórmulas proporcionadas por la casa comercial para determinar la concentración de LDL-c en las muestras.

Glucosa

La determinación de glucosa sérica se realizó mediante el uso de un kit comercial (QCA, Amposta, España). El mismo, se basa en una serie de reacciones enzimáticas de oxidación de glucosa. Específicamente, la glucosa oxidasa, reacciona con la glucosa presente en las muestras para dar ácido glucónico y peróxido de hidrogeno, el cual gracias a la peroxidasa reacciona con la 4-aminoantipirina y el ácido *p*-hidroxibenzoico, para dar como resultado un derivado quinónico, cuya coloración es proporcional a la concentración de glucosa en la muestra.

Para su determinación, se emplearon placas ELISA de 96 pocillos. Se realizó para cada placa utilizada una curva patrón con 5 puntos de concentración conocida, partiendo del estándar proporcionado por la casa comercial. Luego, en los pocillos restante se colocó 3 µL de cada muestra o patrón y se agregó 250 µL del reactivo A. Tras mezclar e incubar a 37º C por 5 minutos, se leyeron las absorbancias en espectrofotómetro Eon (Biotek, Vermont USA) a 505 nm. A partir de estos datos se realizaron los cálculos teniendo en cuenta cada curva

patrón y considerando las fórmulas proporcionadas por la casa comercial para determinar la concentración de glucosa en las muestras.

Insulina

Se utilizaron kits ELISA basados en anticuerpos frente a la insulina plasmática (Millipore, Darmstadt, Alemania). La técnica empleada se basa en un inmunoensayo enzimático tipo sándwich directo en donde dos anticuerpos monoclonales se dirigen contra determinantes antigénicos separados de la molécula de insulina. Durante la incubación, la insulina de la muestra reacciona con los anticuerpos anti-insulina conjugados en peroxidasa y anticuerpos anti-insulina ligados con pocillo de microtitración. El anticuerpo marcado con enzima no unido se elimina con un simple lavado. El anticuerpo-enzima conjugado se detecta por reacción con 3,3',5,5'-tetrametilbencidina. La actividad enzimática se mide espectrofotométricamente por el aumento de la absorbancia a 450 nm, corregido de la absorbancia a 590 nm, después de la acidificación de los productos formados. El aumento de la absorbancia es directamente proporcional a la cantidad de insulina capturada en la muestra desconocida.

Resumidamente, el protocolo consiste en lavar los pocillos de la placa ELISA 3 veces con tampón de lavado, añadir 10 µL de tampón de ensayo, tanto los pocillos de muestra como los blancos, y 10 µL de solución matriz (suero de ratón despojado de carbono). Luego, agregar los estándares de insulina de rata a los pocillos destinados para la curva patrón en concentraciones conocidas. Colocar en otros pocillos los controles de calidad (QC) I y II. Tras agregar 10 µL de las muestras en los pozos restantes, añadir 80 µL de tampón de detección de anticuerpos a todos los pocillos y cubrir la placa e incubar a temperatura ambiente durante dos horas en una placa vibratoria. Pasado el tiempo de incubación, se eliminan las soluciones y se realizan 3 nuevos lavados con 300 µL de tampón de lavado. Se añaden 100 µL de solución enzimática en todos los

pocillos y luego de cubrir la placa se incuba nuevamente durante 30 minutos en placa vibradora a temperatura ambiente. Luego de eliminar los tampones residuales, se realiza el lavado de los pocillos 6 veces con 300 µL de tampón de lavado. 100 µlLde solución de sustrato (3,3',5,5'-tetrametilbencidina en buffer) se agregan a cada pocillo, cuidando de la exposición lumínica. Luego de incubar nuevamente por 15 minutos, se añaden 100 µL de solución de finalización (HCl). Por último, se realiza la lectura en espectrofotómetro Eon (Biotek, Vermont USA) a 450 nm y 590 nm en 5 minutos.

Determinación de HOMA-IR

El índice HOMA-IR se calculó a partir de las concentraciones circulantes de insulina y glucosa, utilizando la fórmula definida previamente por Matthews y col., (1985) [8]:

$$\text{HOMA-IR} = [\text{insulina } (\mu U/L) \times \text{glucosa } (mmol/L)/ 22.5]$$

8.4. Determinación de índices aterogénicos

La determinación de parámetros clásicos del perfil lipídico, TAG, CT, HDL-c y LDL-c, son pruebas de rutina y de utilidad para la evaluación del estado aterogénico que sirven para predecir grupos de riesgo de enfermedades cardiovasculares. Sin embargo, diversos autores afirman que el uso de cálculos de cocientes específicos presentan mayor sensibilidad y especificidad para el riesgo cardiometabólico en comparación con los parámetros evaluados individualmente [9–11]. En consecuencia, en el presente estudio se empleó las siguientes relaciones:

- Índice aterogénico: AI; del inglés *atherogenic Index*: se calcula aplicando la siguiente fórmula

$$\text{Log (TAG/HDL-c)} \quad (\textit{Ecuación 1})$$

Refleja el ratio de esterificación del colesterol por la enzima Lecitin Colesterol Acil Transferasa (LCAT) y la composición de subpoblaciones de lipoproteínas que controlan el ratio. La transformación logarítmica se realiza para producir mejores correlaciones y distribución normal [12]. Valores entre -0.3 a 0-1 son asociados con riesgo cardiovascular bajo, de 0.1 a 0.24 riesgo medio y mayor a 0.24 es asociado con un alto riesgo cardiovascular [9].

- Riesgo cardiovascular 1: CR1; del inglés *cardiovascular risk 1:*

CT/HDL-c (***Ecuación 2***)

- Riesgo cardiovascular 2: CR2; del inglés *cardiovascular risk 2*:

LDL-c/HDL-c (***Ecuación 3***)

Al presentar una buena asociación con la formación de plaquetas coronarias, son considerados indicadores con mayor sensibilidad y especificidad en comparación con la determinación del CT [9,13].

Coeficiente aterogénico: At.C; del inglés *atherogenic coefficient*:

(CT-HDL-c)/HDL-c *(Ecuación 4)*

Refleja el potencial aterogénico de la fracción de lipoproteínas no HDL-c, es decir, considera LDL-c, VLDL, lipoproteínas de densidad intermedia. Según las guías de ATPIII (Adult Treatment Panel III), en individuos con hipertrigliceridemia, el colesterol no HDL-c, es considerado objetivo terapéutico [9,14].

Para su cálculo, se emplearon los valores de los parámetros bioquímicos clásicos mencionados en el punto **4.3** ("Determinación de parámetros bioquímicos clásicos"), expresados en mmol/L.

8.5. Determinación de los niveles de expresión génica en tejidos

Con el fin de evaluar la expresión génica de ciertos genes de interés en muestras de tejidos periféricos, concretamente hígado y músculo, se llevó a cabo la extracción y cuantificación del ARN y la determinación de la expresión de los genes de interés por qPCR. Seguidamente se detalla la metodología seguida en cada punto.

Extracción de ARN

El ARN total se extrajo de las muestras de hígado y del músculo gastrocnemio. Para ello se empleó entre 20-30 mg y 70 mg, de cada tejido respectivamente. Se utilizó el reactivo *TRIzol Reagent (*Thermo Fisher Scientific, Illkirch-Graffenstaden, Francia), el cual es una solución monofásica de fenol e isotiocianato de guanidina principalmente, que descompone las células, disuelve los componentes celulares y homogeniza las muestras preservando la integridad de ARN. Para tal fin, se agregaron 600 µL de Trizol por muestra y se homogeneizaron en Tissu Lyser con ayuda de bolas metálicas. Las muestras se mantuvieron en todo momento en frio con hielo. Para favorecer la separación de la materia grasa, se centrifugaron a 12000 x g durante 10 minutos a 4 ºC. El sobrenadante obtenido, que contenía ARN, ADN y proteínas, fue transferido a otro *eppendorf* para una nueva centrifugación con el fin de conseguir una limpieza eficaz de la materia grasa. El sobrenadante resultante se traspasó a otro *eppendorf* donde se le añadieron 120 µL de cloroformo y se vorteó durante 15 segundos. Tras la incubación de 10 minutos en hielo, se centrifugó durante 15 minutos a 4 ºC a 12,000 x g obteniéndose dos fases. Se traspasó la fase acuosa (superior) a otro *eppendorf*, se añadieron 300 µL de isopropanol, se agitó suavemente varias veces y se dejaron a -20 ºC durante toda la noche. Al día siguiente, las muestras se centrifugaron a 12,000 x g durante 10 minutos

a 4 ºC con el fin de precipitar el ARN. Se añadió 1 mL de etanol al 70%, se agito para lavar el sedimento y se centrifugó nuevamente a 8,000 x g durante 5 minutos a 4 ºC. Por último, el líquido fue descartado, dejando solo el pellet que se dejó secar completamente durante 10 minutos, para ser finalmente resuspendido en agua libre de ARNsas. Se añadieron 60 µL y 40 µL para hígado y músculo respectivamente, utilizando vórtex y realizando up-down para garantizar la completa homogeneización. El ARN se almacenó a -80 °C hasta realizar los posteriores análisis.

Cuantificación y comprobación de la integridad de ARN

La concentración de ARN se determinó por espectrometría, a partir de 1 µL de muestra, utilizando un espectrofotómetro NanoDrop ND-1000 UV-Vis (Thermo Fisher ScientificWilmington, USA). La lectura de las absorbancias se ajustó a 260 nm. Con el fin de evaluar la pureza del ARN y/o la presencia de contaminantes se llevó a cabo la determinación de varias proporciones de absorbancia. Concretamente se evaluaron el ratio 260/280, que como valor de referencia se fijó el valor de 2, y la proporción 260/230, que se fijó en el intervalo 1.8 y 2.2. Si las relaciones eran más bajas indicarían presencia de proteínas, fenol u otros contaminantes, y se procedía a la purificación de las muestras con acetato de sodio.

Por otro lado, la evaluación de la integridad se realizó por electroforesis de gel de agarosa. Se utilizaron 0.5 µg de ARN diluido en tampón de carga (6X azul de bromofenol y glicerol en agua). Las muestras fueron cargadas en un gel de agarosa al 1.4 %. La electroforesis se desarrolló en tampón TBE 0.5x, con tinción posterior del gel con SYBR ® *Safe DNA Gel Stain* (10000x concentrado en DMSO) (Invitrogen, Oregón, EE. UU.). Para garantizar una buena integridad del ARN se verificó la presencia de las bandas de ARNr 28S y 18S. Por el contrario, cuando las bandas no aparecían bien definidas, se consideraba indicador de degradación de ARN. En tal caso, se debe comprobar en qué punto del

protocolo se han podido degradar los ácidos nucleicos, y, en el peor de los casos, se debe volver a extraer el ARN del tejido.

Retrotranscripción

Para llevar a cabo la retrotranscripción (RT) del ARN se diluyeron 0.25 µg de ARN total en un volumen final de 5 µL de agua libre de ARNasas. El ARN se desnaturalizó a 65 °C durante 10 min en el termocicladorC1000 Touch Thermal Cycler (BioRad, USA). A continuación, se llevó a cabo la retrotranscripción del ARN a ADN complementario (ADNc). Para ello, se añadió a cada muestra 7.5 µL de una mix, preparada previamente, que contenía: 1.25 µLde Buffer 10x, 1.25 µL de MgCl2 25 mM, 2µL de desoxirribonucleótidos (dNTPs) 2.5 mM, 0.5 µL de random hexamers 50 µM, 0.5 µL de inhibidores de ARNasas (20 U/µL), 0.5 µL de transcriptasa reversa (MuLV RT; 50 U/µL) y 1.5 µL de agua libre de ARNasas. Las condiciones de la reacción fueron: 15 min a 20 °C, 30 min a 42 °C y 5 min a 95 °C. El producto final se mantuvo a 4 °C.

qPCR a tiempo real

La qPCR (del inglés, *quantitative polymerase chain reaction*) es una técnica que se basa en la reacción en cadena de la ADN polimerasa en la que se sintetiza una cadena de ADN complementaria a otra, y de esta forma se producen múltiples copias de fragmentos específicos de ADN. Para que tenga lugar la reacción, la ADN polimerasa requiere de nucleótidos y de cebadores forward y reverse. La PCR a tiempo real permite la detección de la amplificación en cada ciclo de reacción.

Los análisis se realizaron a partir de una dilución del ADNc producto de la RT. Para el análisis de cada gen, se tomaron 2 µL de la dilución RT y se añadieron 8 µL del mix de PCR que contenía: 5 µL de Power SYBER Green PCR Master Mix (Applied Biosystems, CA, EE. UU.), 0.5 µL de cada cebador de interés (forward y reverse), y 2 µL de agua libre de ARNasas (Sigma, MO, EE. UU.). Los cebadores

fueron diseñados *ad hoc* y producidos Biomers.net (Ulm, Alemania). La PCR se realizó utilizando el sistema de PCR en tiempo real de CFx96 RealTime System (BioRad, USA) con la siguiente reacción de amplificación: desnaturalización durante 10 min a 95 °C y 40 ciclos de desnaturalización (15 s a 95 °C) y elongación (1 min a 60 °C). Una vez terminados dichos ciclos, se realizó una curva de desnaturalización para verificar la pureza del producto de PCR obtenido, que consistió en 15 s a 95 °C, 1 min a 60 °C y 15 s a 95 °C.

El umbral del ciclo (Ct, cycle threshold) se calculó mediante el *software* del instrumento (StepOne Software v2.2), y la expresión relativa de cada gen se calculó como un porcentaje utilizando el método $2^{-\Delta\Delta Ct}$ (Pfaffl 2001) y el software LinReg (Ramakers, Ruijter et al. 2003).

En la Tabla 4.1 se detallan las secuencias de nucleótidos de los primers utilizados en cada tejido.

8.6. Resonancia Magnética nuclear

Con el fin de evaluar un mayor número de cambios a consecuencia de los diferentes tratamientos y condiciones experimentales se llevaron a cabo análisis metabolómicos por resonancia magnética nuclear en muestras de suero e hígado. Previo al análisis por resonancia magnética nuclear (RMN), se realizó en un primer paso la extracción de las fases hidro y lipofílicas de las muestras.

Extracción de metabolitos polares y lipofílicos de muestras hepáticas

Luego de transferirse 30- 50 mg de hígado congelado a un *eppendorf,* se añadió 1 mL de una mezcla fría de agua: acetonitrilo (H2O:CH3CN: 1:1 v/v T= 0 ºC). Se homogeneizó en vórtex y la solución resultante se centrifugó a 19,000 x g por 15 minutos a 4 ºC. El sobrenadante que contenía la fase acuosa se transfirió un

nuevo *eppendorf*. Por otro lado, al pellet obtenido se añadió 1 mL de una mezcla fría de cloroformo: metanol (2:1 a T= 0 ºC), se homogeneizó y se dejó a temperatura ambiente durante 10 minutos. Tras la centrifugación a 19,000 x g por 30 minutos a 4 ºC, se obtuvo una fase líquida y clara, que fue cuidadosamente trasferida a un nuevo *eppendorf* para obtener la fase lipídica. Ambos tubos *eppendorf* con fase acuosa y lipídica se dejaron secar bajo corriente de N_2 hasta remover los solventes. Una vez secas las muestras se almacenaron a -80ºC hasta la determinación en RMN.

Tabla 4.1. Secuencias de los primers usados para las PCR a tiempo real en higado (H) o músculo (M).

Gen	*Forward Primer (5' to 3')*	*Reverse Primer (5' to 3')*	*Tejido*
Acc1	*TGCAGGTATCCCCACTCTTC*	*TTCTGATTCCCTTCCCTCCT*	H
Acc2	CTTTTCTAGGTCCCCGAGTGA	CTTCCGCTCCAGGGTAGAGTT	*M*
AdipoR2	CCACACAACACAAGAATCCG	CCCTTCTTCTTGGGAGAATGG	*M*
Cpt1α	GCTCGCACATTACAAGGACAT	TGGACACCACATAGAGGCAG	*H*
Cpt1ß	GCAAACTGGACCGAGAAGAG	CCTTGAAGAAGCGACCTTTG	*M*
Cs	CCGTGCTCATGGACTTGGGCCTT	CCCCTGGCCCAACGTAGATGCTC	*M*
Elovl2	TTTGGCTGTCTCATCTTCCA	GGGAAACCATTCTTCACTTC	*H*
Fads1	TACAGGCAACCTGCAACGTTC	GGTGCCACCTTGTGGTAGTTGT	*H*
Fads2	GGAACCATCGACATTTCCAG	TCTTTATGTCGGGGTCCTTG	*H*
Fas1	CTATTGTGGACGGAGGTATC	TGCTGTAGCCCAGAAGAG	*H*
FAT/cd36	GTCCTGGCTGTGTTTGGA	GCTCAAAGATGGCTCCATTG	*H, M*
Fatp5	CCTGCCAAGCTTCGTGCTAAT	GCTCATGTGATAGGATGGCTGG	*H*
Had	ATCGTGAACCGTCTCTTGGT	AGGACTGGGCTGAAATAAGG	*H*
Scd1	GAGGAACATCATTCTCATGGTCC	CGTACACGTCATTCTGGAACG	*H*
Srebp1-c	CCCACCCCCTTACACACC	GCCTGCGGTCTTCATTGT	*H*

Acc1, Acetyl-CoA carboxilasa 1; *Acc2*, Acetyl-CoA carboxilasa 2; *AdipoR2*, Receptor de Adiponectina 2; *Cpt1α*, carnitina palmitoiltransferasa 1 alfa, *Cpt1ß*, carnitina palmitoiltransferase 1 beta; *Cs*, Citrato Sintasa*; Elovl2*, enzima de elongación de ácidos grasos de cadena larga 2; *Fads1*, Δ-5 desaturasa; *Fads2*,

Δ-6 desaturasa; *Fas1*, ácido graso sintasa; *FAT/cd36*, transportador de ácidos grasos, homologo a CD36; *Fatp5*, proteína transportadora de ácidos grasos 5; *Had*, hidroxiacyl-CoA dehidrogenasa; *Scd1*, Desaturasa Δ-9; *Srebp1-c*, proteína de unión al elemento regulador del esterol.

Extracción de metabolitos polares y lipofílicos de muestras séricas

Se partió de 200 µL de suero en tubos *eppendorf* de 2 mL a 4 ºC. Luego de añadir 400 µL de metanol y 100 µL de agua bidestilada, se homogeneizó con vórtex. Se agregaron 200 µL de cloroformo y se vorteó nuevamente para conseguir una solución monofásica. Para conseguir una separación de fases, se agregaron 600 µL de cloroformo y 300 µL de agua y se vorteo nuevamente. Las muestras fueron centrifugadas a 19,000 x g por 15 minutos a 4 ºC con el fin de mejorar la separación de fases. Como resultado, la fase acuosa superior conseguida fue transferida a un nuevo tubo *eppendorf*, y la fase lipídica inferior a otro. Ambos *eppendorfs*, con el contenido acuoso y lipídico, fueron secados bajo corriente de N_2 hasta evaporar solventes. Una vez secas las muestras fueron almacenadas a -80 ºC hasta su determinación por RMN.

Análisis por resonancia magnética nuclear

Los análisis de metabolómica por RMN se llevaron a cabo en el Centro de Ciencias Ómicas (COS) que es una unidad mixta URV-Eurecat. Los extractos polares se reconstituyeron en 600 µL de tampón fosfato D_2O (PBS 0.05 mM, pH 7.4, 99.5% D_2O) que contenía ácido trisililpropiónico (TSP) 0.73 mM. Los extractos lipofílicos secos se reconstituyeron con una solución de $CDCl_3/CD_3OD$ (2:1) que contenía tetrametilsilano (TMS) 1.18 mM y luego se sometieron a agitación vorticial. Ambos extractos se transfirieron a tubos de vidrio de RMN de 5 mm para su posterior análisis. Los espectros de RMN de ^1H se registraron a 300 K en un espectrómetro Avance III 600 (Bruker, Alemania) que operaba a

una frecuencia de protones de 600.20 MHz utilizando una sonda de gradiente de banda ancha PBBO de 5 mm.

Para los extractos acuosos, se llevaron a cabo experimentos de pulsos unidimensionales de ^{1}H usando la secuencia de presaturación de la espectroscopia de efecto overhauser nuclear (NOESY) (RD–90°–t1–90°–tm–90° ACQ) para suprimir el pico de agua residual, y el tiempo de mezcla se fijó en 100 ms. Se aplicó presaturación con solvente con una potencia de irradiación de 75 Hz durante el retardo de reciclaje (RD = 5 s) y el tiempo de mezcla. La longitud del pulso de 90° se calibró para cada muestra y varió de 9.95 a 10.06 µs. El ancho espectral fue de 12 kHz (20 ppm) y se recopilaron un total de 256 transitorios en 64 k puntos de datos para cada espectro de ^{1}H.

En el caso de los extractos lipofílicos, se utilizó un pulso de 90º con secuencia de presaturación (zgpr) para suprimir la señal de agua residual del metanol. Se utilizó un RD de 5.0 s con un tiempo de adquisición de 2.94 s. La longitud del pulso de 90° se calibró para cada muestra y varió de 9.92 a 10.04 µs. Después de 4 escaneos ficticios, se recopilaron un total de 128 escaneos en 64 000 puntos de datos con un ancho espectral de 18.6 ppm.

Análisis de datos de RMN

Los espectros de RMN ^{1}H adquiridos se compararon con referencias de compuestos puros de la base de datos de espectros AMIX de perfiles metabólicos (Bruker), HMDB, el software Chenomx NMR suite 8.4 (Chenomx Inc., Edmonton, AN, Canadá) y bases de datos para la identificación de metabolitos. Además, asignamos metabolitos por ^{1}H–^{1}H homonuclear correlación (COSY y TOCSY) y experimentos de RMN 2D heteronuclear ^{1}H–^{13}C (HSQC) y por correlación con compuestos puros ejecutados internamente. Después del preprocesamiento, las regiones específicas de ^{1}H RMN identificadas en los espectros se integraron utilizando el paquete de software AMIX 3.9.

Procesamiento de datos de RMN y Análisis Multivariante.

Las concentraciones absolutas derivadas de extractos lipófilicos e hidrófilicos se organizaron juntas en una única matriz de datos. Previamente, los datos se escalaron a la varianza unitaria para dar a todos los metabolitos identificados el mismo peso en el modelo. El análisis de datos y el cálculo estadístico se llevó a cabo con el paquete estadístico IBM SPSS Statistics para Windows, versión 22.0 (SPSS Inc., Chicago, IL, USA). Para el análisis discriminante de mínimos cuadrados parciales ortogonales (OPLS-DA) y para el análisis de vías metabólicas afectadas, se utilizó el software MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Los parámetros R^2Y (interpretabilidad del modelo), Q^2 (predictibilidad del modelo) y una prueba de permutación por 1000 fueron utilizados para verificar la adecuación, fidelidad y validez del modelo. Para el análisis de vías metabólicas, el valor de p corregido (FDR, del inglés: *false discovery rate*) fue utilizado como indicador de vías metabólicas afectadas.

8.7. Determinación del perfil de ácidos grasos

Se determinaron la proporción de AG por cromatografía gaseosa (GC) con detector FID, en la Universidad Nacional del Litoral, bajo la supervisión del Dr. Claudio A. Bernal, en el marco de una estancia y colaboración internacional, financiada por la Fundación Carolina.

La identificación y cuantificación de los esteres metílicos de los AG hepático y musculares por cromatografía gaseosa, fue realizada en tres pasos: extracción de la materia lipídica, derivatización de los AG y análisis cromatográfico.

Extracción de materia lipídica del tejido hepático y muscular

La extracción lipídica se realizó por el método de Bligh and Dyer (1959) [15], el cual se basa en la homogenización de muestras con cloroformo, metanol y agua

lo que permite una rápida extracción de lípidos de tejidos con cantidad significativa de humedad. Se partió de 0.5 gr y de 0.40 gr de tejido hepático y muscular, respectivamente. Considerando que el hígado y el músculo de rata posee un 68% y 75% de humedad respectivamente, se agregó agua destilada según la siguiente fórmula, para mantener una correcta relación entre cloroformo:metanol:agua (1:2:0.8):

mL agua a agregar = 0.8 - [cantidad pesada de tejido (gr) x % de humedad (0.68 o 0.75]

Tras añadir la cantidad de agua correspondiente en cada muestra, se homogeneizó el tejido con un homogeneizador de cuchilla, limpiando entre muestra y muestra con etanol. Al homogenizado resultante se le agregó 1 mL de cloroformo y 2 mL de metanol y se agitó 2 minutos en vórtex. Tras añadir 1 mL de cloroformo y 1 mL de agua destilada, se vorteó nuevamente durante 30 segundos. Luego se realizó una filtración mediante filtro previamente enjuagado con cloroformo. El filtrado resultante se recogió en tubos de vidrio previamente enjuagados con hexano. Se incubaron durante 10 minutos a temperatura ambiente y luego se centrifugaron a 3,000 rpm por 5 minutos. La fase superior y la interfase fueron descartadas, transfiriendo la fase inferior a tubos de vidrio color ámbar de 4 cm previamente enjuagados con hexano. Los solventes se evaporaron bajo corriente de N2. Una vez secas las muestras se almacenaron hasta su metilación.

Derivatización de los ácidos grasos

La derivatización de los AG en metil ésteres se realiza con el fin de permitir su análisis por cromatografía gaseosa. En tal sentido, estructuralmente los AG se encuentran en forma de dímero unidos por puente de hidrogeno, formando moléculas de alto peso molecular y con altos puntos de ebullición, lo cual imposibilita la determinación por cromatografía gaseosa. Los ésteres metílicos

formados por la derivatización de los AG, permiten que a pesar de que tengan un peso molecular algo mayor que el respectivo AG, el punto de ebullición baje al romperse los puentes de hidrógeno, permitiendo su volatilización a las temperaturas que se realiza la cromatografía gaseosa y por lo tanto, posibilita su análisis.

Se utilizó hidróxido de potasio (KOH) metanoico para generar los metil ésteres de los AG. En primer lugar, los extractos se pasaron a tubos de vidrio de 10 cm (tapa a rosca) tras disolverlos con 1.5 mL de hexano calidad HPLC. Posteriormente se añadieron 0.5 mL de KOH metanoico y se vorteó por 1 minuto. Luego de centrifugar las muestras a 3,000 rmp por 5 minutos, la capa superior fue transferida a un vial de 2 mL. Luego de dejar secar bajo corriente de N2, se almacenaron a -20 ºC para su posterior análisis en el cromatógrafo gaseoso.

Análisis cromatográfico

El perfil de AG del tejido hepático y del músculo gastrocnemio se cuantificó mediante CG/FID, utilizando un cromatógrafo gas-líquido marca Shimadzu (GC-2014), con detector FID y columna capilar CP-Sil 88 (100 mx 0,25 mm id x 20 mm de espesor). La comparación con los tiempos de retención relativos a estándares comerciales con los metil ésteres de los AG cuantificados, permitieron su identificación. La cromatografía gaseosa es una técnica analítica en la que los componentes son arrastrados por una fase móvil que es gaseosa. De esta forma, la muestra incógnita se reparte en una fase gaseosa que corresponde al gas de arrastre o gas móvil y en otra fase liquida estacionaria. Ambas fases son no miscibles. Cabe destacar, que la elución se produce por el flujo de la fase móvil de gas inerte, cuya única función es la de transportar el analito a través de la columna. A medida que va pasando el gas portador con la muestra formada por “n” componentes, la separación de cada uno de ellos se efectúa de acuerdo con su propio coeficiente de reparto entre el gas portador

y la fase líquida. La identificación de los ésteres metílicos de los AG se basa por tanto en la comparación de las señales agudas que emergen ("picos"), con el tiempo de demora de un pico determinado y conocido correspondiente a un métil ester de un patrón estándar.

El tiempo de retención del éster metílico de un AG en particular, es característico para ese compuesto que se ha analizado en una columna con una fase líquida específica, en condiciones estandarizadas de temperatura de la columna, temperatura del detector y del inyector, flujo del gas portador e iguales condiciones de partición de flujo conocido como "splits". Este tiempo de retención total, se compara con el obtenido para el respectivo éster metílico de referencia, sometido a las mismas condiciones cromatográficas. A partir del cromatograma obtenido, los resultados se expresan como porcentaje relativo de cada área en función al total de áreas registradas de los esteres metílicos de los AG (FAMEs %).

8.8. Análisis estadístico

Los datos se expresan como la media ± SEM. En todos los casos se descartaron outliers mediante el test de Grubbs, se comprobó la normalidad y la homogeneidad mediante Shapiro-Wilk test y Levene´s test, respectivamente. Se empleó el análisis de varianza de dos factores (ANOVA de dos vías) utilizando el fotoperiodo (L6, L12, L18) y tratamiento (LC, nLC, LT, nLT, VH) como variables independientes. Luego se realizó un análisis de varianza de un factor (ANOVA de una vía), empleando los grupos de fotoperiodo y tratamiento agrupados. Posteriormente, se realizaron comparaciones post-hoc utilizando DMS test. La prueba *Student´s t-test* también fue utilizada para comparaciones individuales, es decir, entre grupos experimentales comparables.

Se utilizó el Software SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) para el análisis estadístico. La significancia estadística fue establecida en todos los casos para valores de $p < 0.05$ y se determinó tendencia cuando $0.05 < p < 0.10$.

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5.RESULTADOS

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Capítulo I

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MANUSCRITO I

Hepatic fatty acid metabolism is strongly modulated by photoperiod in Fischer 344 rats.

María Josefina Ruiz de Azua [a]; Álvaro Cruz-Carrión [a]; Anna Arola-Arnal [a]; Claudio Bernal [b]; Juliana Saín [b]; Carolina Gerstner [b]; Manuel Suarez [a].

[a] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Tarragona, Spain.

[b] Universidad Nacional del Litoral, Facultad de Bioquímica y Cs. Biológicas, Santa Fe, Argentina.

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ABSTRACT

Circadian and seasonal variations produce variations in physiological processes throughout the day and the year, respectively. In this sense, both the light and the moment of feeding are strong modulators of the central and peripheral clocks. However, little is known about its influence on certain metabolic parameters and on the composition of liver and muscle fatty acids (FA). In the present study, 24 Fischer 344 rats were exposed for 11 weeks to different photoperiods, L6, L12 and L18, with 6, 12 and 18 hours of light/day, respectively. They were fed a standard diet. Serum metabolic parameters, gene expression of liver enzymes and gastrocnemius muscle involved in the synthesis, elongation, desaturation and β-oxidation of FA were analyzed. We have found that exposure to different hours of light has a clear effect on FA composition and gene expression in the liver. Mainly, the biosynthesis of unsaturated FA was altered in the L18 animals with respect to those exposed to L12, while the L6 did not show significant changes. At the muscle level, differences were observed in the concentration of mono and polyunsaturated FA. A multivariate analysis confirmed the differences between L12 and L18 in a significant way. We conclude that exposure to long days produces changes in the composition of liver and muscle FA, as well as changes in the gene expression of oxidative enzymes compared to exposure to L12, which could be a consequence of different seasonal eating patterns.

Key words: photoperiods, fatty acids, metabolism.

Abbreviations: AA: arachidonic acid; Acc1, Acetyl-CoA carboxylase 1; Acc2; Acetyl-CoA carboxylase 2; AdipoR2, Adiponectin receptor 2; ALA: α-Linolenic acid; Cpt1α: carnitine palmitoyltransferase 1 alpha; Cpt1β: carnitine palmitoyltransferase 1 beta; CS: Citrate Synthase; DGLA: Dihomo-γ-linolenic acid; DHA: Docosahexaenoic acid; Elovl2: Elongation of very-long-chain fatty acid enzyme 2; EPA: Eicosapentaenoic acid; EPI: food pattern index; FA: Fatty acid; Fads1: Δ-5 desaturase; Fads2: Δ-6 desaturase; Fas1: Fatty acid synthase;

FAT/CD36: fatty acid translocase, homologue of CD36; Fatp5: fatty acid transport protein 5; GC: Gas Chromatography; GLA: gamma-linolenic acid; Had: hydroxyacyl-CoA dehydrogenase; HDL-c: HDL cholesterol; HOMA: homeostatic model assessment index; Kcal: kilocalories; KEGG: Kyoto encyclopedia of Genes and Genomes; L12: standard photoperiod; L18: long photoperiod; L6: short photoperiod; LA: Linoleic acid; LDL-c: LDL cholesterol; MUFAs: monounsaturated fatty acids; NEFAs: non-esterified fatty acids; NI: unidentified; OA: oleic acid; OPLS-DA: orthogonal partial least squares discriminant analysis; PA: palmitic acid; PUFAs: polyunsaturated fatty acids; REV-ERBα nuclear receptor subfamily 1 group D member 1 alpha; REV-ERBβ nuclear receptor subfamily 1 group D member 1 beta; SA: Stearic acid; Scd1: Desaturase Δ-9; SCN: suprachiasmatic nucleus; SFA: saturated fatty acids; Srebp1-c, sterol regulatory element-binding protein 1c; TAG: triacylglycerides; TC: total cholesterol.

INTRODUCTION

The rotational and translational movement of the Earth results in the variation of luminosity and temperature in either 24 hours or seasonal variations during the year, called the circadian and circannual cycle, respectively. Chronobiology studies the adaptations and physiological changes of living beings in response to these variations [1]. There are different synchronizing agents that modulate the period of the circadian rhythm, such as lighting cycles (photoperiods), feeding, social stimulation [2,3] and temperature [4]. In addition, the organisms have a core of endogenous circadian rhythms that control genetic and molecular variations through its molecular components [5,6]. In mammals these rhythms are mainly regulated by the suprachiasmatic nucleus (SCN), which exerts a complex regulation by means of a system of transcription factors, allowing the generation and maintenance of oscillations in physiological processes such as the sleep cycle, hormone secretion, body temperature and metabolism. These rhythms mainly influence the homeostatic balance of feeding, the metabolism and obtaining of energy expenditure of nutrients, and the rest/activity behavior, among others [7]. In this sense, there is a communication between the SCN and autonomic clocks located in peripheral tissues that act directly or indirectly with the cycles of fasting and feeding [8]. Such feedback between SCN and peripheral clocks is carried out through behavioral, humoral and/or neuronal factors, while peripheral oscillators receive entrainment signals through serum metabolites [6]. These processes are part of an evolutionary process that has been preserved to provide adaptation to external environmental conditions [9].

It has been observed that the circadian rhythm of peripheral metabolic organs is affected by the feeding/fasting rhythm, meaning that tissues such as the liver respond rapidly to food availability, while the SCN is more sensitive to light/dark cycles [10]. In this regards, either the circadian clock, systemic signals from

periodic food intake, or a combination of both mechanisms directly affect the expression of genes that control the peripheral clocks [5,11,12]. Specifically, in rodents it has been shown that during the illumination phase, that is, their resting period, lipogenesis is inhibited by an increase in the expression levels of REV-ERBα and REV-ERBβ. On the contrary, during darkness, their active period, the expression levels of these factors decrease, promoting the activation of lipogenic genes [13]. On the other hand, Brenner *et al*. (1981) stated that the activity of some hepatic desaturase enzymes also has a circadian activity, regulated mainly by adrenocorticotropic hormone and glucocorticoids [14]. In addition, the seasons of the year also influence these circadian rhythms, producing metabolic variations. For example, it has been observed that the rate of fatty acid (FA) desaturation is the same both in winter and in summer; however, the activity peak of the involved enzymes differs between one photoperiod and another. In this sense, it has been observed that the liver glycogen levels are strongly influenced by the time of year [15]. Mariné-Casadó *et al*. (2018), observed various metabolic consequences in rats exposed to different photoperiods (L6, L12 and L18, with 6, 12 and 18 hours of light, respectively), simulating the seasons [16]. Specifically, they observed that those animals exposed to L6 presented altered glucose metabolism and lipid oxidation, due to a higher concentration of glucose and non-esterified fatty acids (NEFAs) than those exposed to L12. In addition, changes were also observed at the level of gene expression of enzymes related to the glucose metabolic pathway and in oxidative enzymes in liver and skeletal muscle, respectively [16]. Other authors also observed that exposure to L6 produces differential effects on biometric and serum parameters and on the gene expression of key enzymes in lipolysis, β-oxidation and adipogenesis in white and brown adipose tissues compared with L18 [17].Therefore, there is clear evidence of the impact of biological rhythms on circulating metabolites, in serum, as well as in peripheral tissues such as liver, skeletal muscle or adipose

tissues. Likewise, the metabolic consequences of the same food may also be different depending on the photoperiod that is consumed. In this sense, Gilbert et al. (2020) observed that the consumption of oranges from the southern hemisphere at L6 not only increased the percentage of fat and adipocytes in white adipose tissue, but also decreased the mRNA concentration of genes related to uptake and β-oxidation in brown adipose tissue compared to VH. However, these changes were not observed when oranges were ingested at L18 [18].

Although there is evidence on the circadian rhythmicity of certain metabolic enzymes, hormones and nutrient availability both in serum and in various tissues, there is little information on how exposure to days with different hours of light and darkness affect metabolic parameters. Therefore, the purpose of this article is to analyze the effects of exposure to different photoperiods on serum markers, gene expression, and on the FA profile of the liver and skeletal muscle of Fischer 344 rats.

MATERIALS AND METHODS

Animals

Twenty-four 8-week-old male Fischer 344 (F344) rats were randomly divided in one of three photoperiods, which simulated the season of the year: short photoperiod (n=8, L6), standard (n=8, L12) and long (n=8, L18), with 6, 12 or 18 hours of light. The animals were housed in cages and in pairs at 22 ºC. They received standard diet (A04, SAFE, Panlab, Barcelona, Spain) and water *ad libitum*. As seen in **Fig.1**. the light was turned on at 8:00 am for all groups. The amount of food intake was recorded weekly. After eleven weeks, the animals were sacrificed by decapitation. The experiment lasted a total of eleven weeks because in investigations carried out by the group it was established that this number of weeks were sufficient for the animals to adapt satisfactorily, for the

metabolic changes to be established and for an refractory effect not to occur in them [19–21]. Blood was collected in non-heparinized tubes, incubated for 1 h at room temperature, and centrifuged (2000× g, 15 min, 4 ◦C) to obtain the serum. The liver and gastrocnemius muscles were rapidly weighed, frozen at liquid nitrogen and stored at -80 ºC for further analysis. The Animals Ethics Committee of the Universitat Rovira I Virgili (Tarragona, Spain) approved all the procedures (Project identification code: 9495; file number: FUE-2017-00499873).

Serum Analysis

Circulating levels of glucose, triacylglycerides (TAG), total cholesterol (TC), HDL cholesterol (HDL-c), LDL cholesterol (LDL-c) (QCA, Amposta, Spain) and NEFAs (WAKO, Neuss, Germany) were determined by enzymatic colorimetry. Serum insulin levels were determined using a rat insulin ELISA kit (Millipore, Barcelona, Spain). The homeostatic model assessment (HOMA) index was calculated, using the equation (Glycemia (mmol/L) * Insulinemia (mU/dL)/22.5).

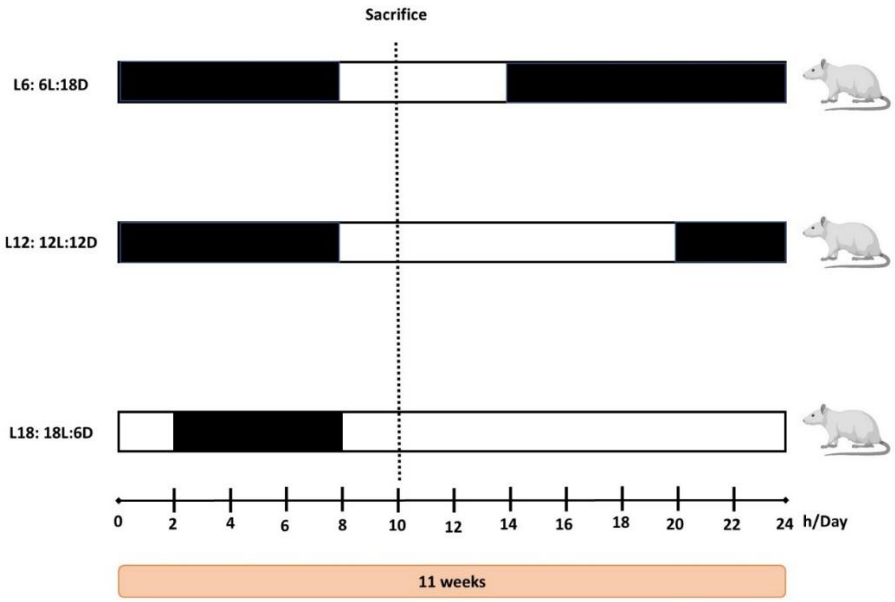

Fig 1. Experimental model: twenty-four Fischer 344 rats were exposed to 6, 12 or 18 hours of light (L6, L12 and L18, respectively) and fed ad libitum. After 11

weeks they were sacrificed 2 hours after the light was turned on. The black color of the bars in each group indicates the time of darkness; the white color indicates the hours of light that the animals had in one day [20].

Lipid extraction and GC analyses

The extraction of hepatic and muscle lipids was done following Blight and Dyer's protocol [22]. Briefly, either 400 mg or 500 mg of muscle or liver, respectively, were weighted. The samples were homogenized together with chloroform, methanol and water. After the successive steps of addition of chloroform and water, with vortex and centrifugation, the organic phase was separated and dried under stream of N_2. For the determination of lipid profile, the derivation of FA was done by methylation with methanol potassium hydroxide. The FA methyl esters (FAMEs) determination was carried out by gas chromatography (Shimadzu (GC-2014) equipped with FID detector and using the CP-Sil 88 (100 m×0.25 mm×0.2 µm, film thickness, Varian, Lake Forest, CA, USA). Four samples for group were utilized (n=4). The FAMEs were identified by comparing their retention relative times of commercial standards, and the chromatogram analysis was done with the LabSolution Shimadzu Software. Values were expressed as a percentage of the total FAMEs.

Once determined the levels of the different FAMEs, the flow of the key enzymes involved in the biosynthesis of unsaturated FA was determined following the ratios described by Sain J [23].

Gene expression

TRIzol Reagent (Thermo Fisher Scientific, Illkirch-Graffenstaden, France) was used for the extraction of liver and muscle RNA, following the guidelines indicated by the supplier. The cDNA was synthesized by reverse transcription using High-Capacity cDNA Reverse Transcription (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). The specific amplification of the cDNA was

carried out by to the polymerase chain action in real time (RT-qPCR), using iTaq Universal SYBR Green Supermix (Bio-Rad, Barcelona, Spain). It was utilized eight samples for group (n=8). Gene expression analysis was performed using primers obtained from Biomers.net (Ulm, Germany), which are detailed in Table 1. Supplementary Material. The genes of interest were those related to fatty acids metabolism in the liver and muscle: *Acc1*, Acetyl-CoA carboxylase 1; *Acc2*, Acetyl-CoA carboxylase 2; *AdipoR2*, Adiponectin receptor 2; *Cpt1α*, carnitine palmitoyltransferase 1 alpha, *Cpt1β*, carnitine palmitoyltransferase 1 beta; *CS*, Citrate Synthase; *Elovl2*, Elongation of very-long-chain fatty acid enzyme 2*; Fads1*, Δ-5 desaturase*; Fads2*, Δ-6 desaturase; *Fas1*, Fatty acid synthase; *FAT/CD36*, fatty acid translocase, homologue of CD36; *Fatp5*, fatty acid transport protein 5; Had, hydroxyacyl-CoA dehydrogenase; *Scd1*, Desaturase Δ-9; *Srebp1-c*, sterol regulatory element-binding protein 1c. The relative expression of each mRNA level was calculated as a percentage of the L12 group using the Pfaffl method [24].

Statistics analysis

The results were expressed as mean ± SEM. It was used SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA) for outliers' detection and statistics analyses. Normality and homogeneity were evaluated by using Shapiro–Wilk test and the Levene's test, respectively. For the values that met these criteria, a one-way ANOVA was utilized to determine effect of photoperiod. DMS post-hoc test was used to determine differences between the different groups. The analysis by the Student's t-test was used to compare different groups too.

Multivariate analysis

After processing the original data, an orthogonal partial least squares discriminant analysis (OPLS-DA) and pathway analysis was performed to

determine the influence of exposure to different photoperiods and the lipid profile of liver and muscle FA. (**Table 2. Supplementary material)**. All analyzes were performed after range scaling with the use of the software MetaboAnalyst (version 5.0), available online. The parameters R^2Y (interpretability of the model) and Q^2 (predictability of the model) were used to verify the suitability and reliability of the obtained OPLS-DA model. Analysis of permutations (permutation test per 1000) was applied to further test the validity of the model. In the pathway analysis, the p value and the false discovery rate (FDR) were used to verify the affected pathways.

RESULTS

Exposure to different photoperiods would produce differential eating patterns between groups

Considering the kilocalories (kcal) consumed by the experimental groups, previously found in the group [20], and given that the hour of darkness is the time of ingestion of the rats, we propose a food pattern index (EPI): kcal/h of darkness In this sense, for L6, L12 and L18 the consumption was 54.1; 54.1 and 54.9 and the hours of darkness were 18, 12 and 6, respectively. Therefore, the calculated EPI was 3.0 kcal/h; 4.5kcal/h; 15 kcal/h, respectively.

The photoperiod modulated serum parameters related to glucose metabolism. ANOVA of the results showed that photoperiod significantly affected serum glucose levels, while it tended to affect the HOMA index (p= 0.042; p= 0.095; respectively). Specifically, the animals exposed to L6 showed lower insulinemia (**Fig. 2-b**), HOMA index (**Fig. 2-c)**, and a tendency to lower blood glucose (**Fig. 2-a)**, than those exposed to a standard photoperiod (p= 0.048; p= 0.033; p= 0.055; respectively). Interestingly, the animals exposed to L12 also presented

higher glycemia (**Fig. 1-a)**, (p= 0.017) and a tendency to a higher TAG level than the L18 group (**Fig. 1-d)**, (p= 0.096; Student's t-test).

On the other hand, no statistically significant differences were observed in the levels of TC, HDL-c, LDL-C and NEFAs between the three different groups evaluated. (**Fig.1 Supplementary material).**

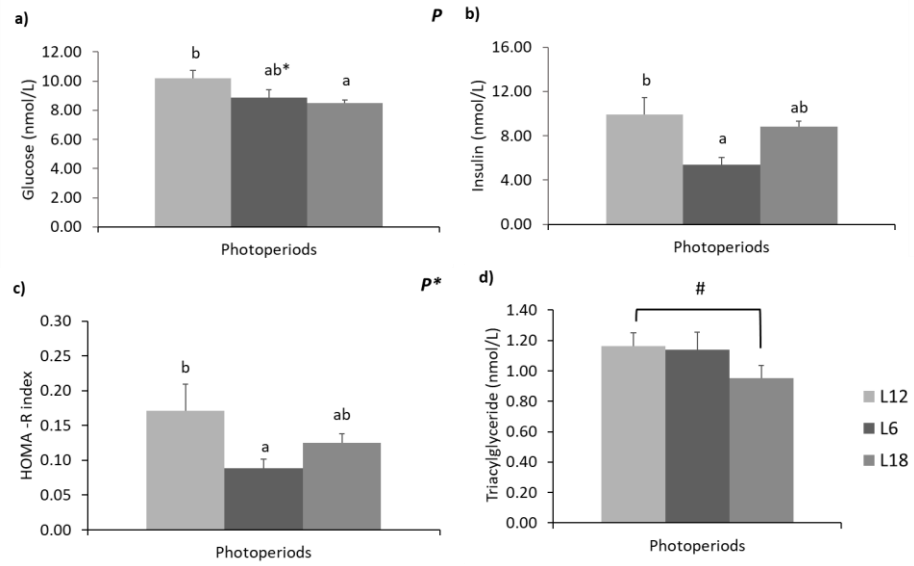

Fig. 2. Serum glucose (**a**), insulin (**b**), HOMA-R index (**c**) and triacylglycerides (**d**) in Fischer 344 rats exposed to a short, standard or long photoperiod, with 6 (L6), 12 (L12) or 18 (L18) hours of light, respectively for 11 weeks. HOMA-R, homeostatic model assessment. Data are expressed as the mean ± SEM (n = 8). One-way ANOVA analysis was used to assess the differences between groups. P, photoperiod. Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicates trend ($0.05 < p < 0.1$). # Indicates tends ($0.05 < p < 0.01$, Student's t-test).

Exposure to L18 produced a higher proportion of hepatic saturated fatty acids, but did not affect its muscle level, compared with exposure to L12

The analysis of the fatty acid profile in peripheral tissues, namely liver and muscle, showed an impact of the biological rhythms in some species of fatty

acids. Table 1 and Table 2 show the levels of fatty acids in liver and muscle of the animals exposed to different photoperiods expressed as percentage of FAMEs. In this regard, an effect of the photoperiod on the content of total hepatic saturated fatty acids (SFA) was observed (p= 0.02) (Table 1). Specifically, the animals exposed to more hours of light showed a significantly higher proportion than the rest of the animals exposed to other photoperiods (p= 0.005; p= 0.001; L18 vs L6; L18 vs L12, respectively). In addition, the photoperiod tended to affect the level of stearic acid (SA; 18.0) (p= 0.075), since the L18 group presented a significantly higher level than those L12 (p= 0.025). Similarly, the level of palmitic acid (PA; 16.0) tended to be higher in the L18 animals compared to those exposed to L12 (p= 0.090). No statistically significant differences were observed in the proportion of FA 14.0, 15.0 and 17.0, while FA 12.0 and 22.0 were not detected (Table 1).

In contrast, no effect of the photoperiod on the concentration of total SFA in muscle was observed. However, the animals exposed to L12 presented a higher level of SFA than those L18 (p= 0.029, Student's t-test). Furthermore, exposure to different hours of light significantly affected the level of FA 12:0, PA and SA in the gastrocnemius muscle (p= 0.014; p = 0.041; p= 0.03; respectively). Specifically, the L12 group showed a higher level of FA 12:0 and PA, while less SA and 22.0 than those L6 and L18 (Table 2).

Table 1. Profile of hepatic fatty acids determined by GC/FID

Fatty acid	*L12*	*L6*	*L18*	*ANOVA*
Σ SFA	41.86 ± 0.97 *a*	43.46 ± 0.28 *a**	46.27 ± 0.34 *b*	*P*
12:0	ND	ND	ND	
14:0	0.89 ± 0.11	0.86 ± 0.10	0.93 ± 0.03	
15:0	0.17 ± 0.01	0.15 ± 0.02	0.16 ± 0.01	
PA	28.98 ± 1.22*	29.40 ± 0.85	31.42 ± 0.53*	*P*
17:0	0.23 ± 0.02	0.20 ± 0.04	0.22 ± 0.01	
SA	11.57 ± 0.61 *a*	12.85 ± 0.64 *ab*	13.52 ± 0.23 *b*	*P*
20:0	ND	ND	ND	

22:0	ND	ND	ND	
Σ MUFAs	22.82 ± 1.41*	19.65 ± 2.02	19.51 ± 0.46*	
cis-9 14:1	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.00	
Palmitoleic acid	5.34 ± 0.69	5.92 ± 0.70	5.46 ± 0.35	
cis-9 17:1	0.24 ± 0.03	0.19 ± 0.03	0.24 ± 0.01	
cis-6 18:1	0.09 ± 0.01	0.09 ± 0.01	0.15 ± 0.02	
OA	11.71 ± 1.03 *a*	9.46 ± 0.89 *a*b*	8.75 ± 0.27 *b*	*P**
cis-11 18:1	5.09 ± 0.11 *a*	4.89 ± 0.05 *ab*	4.67 ± 0.16 *b*	
cis-12 18:1	0.17 ± 0.01	0.17 ± 0.03	0.16 ± 0.02	
cis-11 20:1	0.10 ± 0.01*	0.09 ± 0.01	0.08 ± 0.01*	
Σ PUFAS-cis	32.18 ± 0.46	33.84 ± 1.75	32.45 ± 0.86	
Σ PUFAS n-6	26.76 ± 0.47	27.53 ± 1.49	25.99 ± 0.76	
LA	12.32 ± 0.78 *a*	10.68 ± 0.38 *a*b*	9.61 ± 0.43 *b*	*P*
GLA	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	
cis-11, cis-14 20:2	0.19 ± 0.01 *a*	0.17 ± 0.01 *ab*	0.16 ± 0.01 *b*	*P**
DGLA	0.63 ± 0.03	0.73 ± 0.06	0.72 ± 0.00	
AA	12.88 ± 0.54*	15.39 ± 1.34*	15.22 ± 0.23	
cis-13, cis-16 22:2	0.07 ± 0.03	0.10 ± 0.03	0.05 ± 0.04	
cis-7,cis-10,cis-13,cis-16 22:4	0.40 ± 0.00	0.37 ± 0.04	0.41 ± 0.00	
Σ PUFAS n-3	5.42 ± 0.17 *a*	6.31 ± 0.28 *b*	6.40 ± 0.16 *b*	*P*
ALA (n-3)	0.25 ± 0.05 *a*	0.14 ± 0.02 *b*	0.14 ± 0.02 *b*	*P**
EPA	0.28 ± 0.02 *a*	0.37 ± 0.02 *b*	0.36 ± 0.01 *b*	*P*
cis-13,cis-16,cis-19 22:3 (n-3)	0.08 ± 0.00	0.09 ± 0.00	0.12 ± 0.01	
cis-7,cis-10,cis-13,cis-16,cis-19 22:5	0.92 ±0.07	0.91 ± 0.06	0.80 ± 0.07	
DHA	3.92 ± 0.10 *a*	4.75 ± 0.23 *b*	5.00 ± 0.20 *b*	*P*
Σ NI	0.73 ± 0.08	1.07 ± 0.15	0.99 ± 0.04	
Ratios				
Ratio n-6/n-3	4.95 ± 0.19 *a*	4.36 ± 0.08 *b*	4.06 ± 0.10 *b*	*P*
Palmitoleic Acid/PA	0.19 ± 0.00	0.20 ± 0.02	0.17 ± 0.00	
LA/ALA	40.76 ± 13.07 *a*	80.30 ± 9.34 *b*	71.17±8.86 *ab*	*P**
OA/EA	1.03 ± 0.13 *a*	0.75 ± 0.11 *a*b*	0.65 ± 0.03 b	*P**
GLA/LA	0.07 ± 0.01 *	0.09 ± 0.02	0.11 ± 0.01*	
AA/LA	1.12 ± 0.12 *a*	1.45 ± 0.13 *ab*	1.59 ± 0.06 *b*	*P**

AA/DGLA	21.08 ± 0.26	21.37 ± 2.37	21.61 ± 0.38	
EPA/ ALA	1.38 ± 0.31 *a*	2.88 ± 0.62 *b*	2.86 ± 0.58 *b*	*P**
DHA/ ALA	18.85 ± 3.36 *a*	36.52 ± 6.16 *b*	39.18 ± 7.15 *b*	*P*

Proportion of fatty acids in liver expressed as % FAME of animals exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively). Σ NI: sum of unidentified fatty acids; **ΣSFA**: saturated fatty acids **ΣMUFA**s: monounsaturated fatty acids; **ΣPUFAs**: polyunsaturated fatty acids; **AA**: arachidonic acid: cis-4,cis-8,cis-11,cis-14 20:4 (n-6); **ALA:** α-Linolenic acid: cis-9,cis-12,cis-15 18:3 (n-3); **DGLA**: Dihomo-γ-linolenic acid: cis-8,cis-11,cis-14 20:3 (n-6); **DHA**: Docosahexaenoic acid: cis-4,cis-7,cis-10,cis-12,cis16,cis-19 22:6 (n-3); **SA**: Stearic acid: 18:0; **EPA:** Eicosapentaenoic acid: cis-5,cis-8, cis-11,cis-14,cis-17 20:5 (n-3); **GLA:** gamma-linolenic acid: cis-6,cis-9,cis-12 18:3 (n-6); **LA**: Linoleic acid: cis-9,cis-12 18:2 (n-6); **OA**: Oleic Acid: cis-9 18:1; **PA**; Palmitic acid: 16:0. Values expressed as mean ± SEM (n=4). P, photoperiod effect. (One-way ANOVA, $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA).

Table 2. Profile of fatty acids in gastrocnemius muscle tissue determined by GC/FID

Fatty acid	*L12*	*L6*	*L18*	*ANOVA*
Σ SFA	40.05 ± 0.16	39.83 ± 0.10	39.22 ± 0.24	
12:0	0.10 ± 0.01 *a*	0.58 ± 0.00 *b*	0.07 ± 0.00 *b*	*P*
14:0	1.09 ± 0.19	0.83 ± 0.06	0.81 ± 0.00	
15:0	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	
PA	30.51 ± 0.14 *b*	28.08 ± 0.74 *a*	28.47 ± 0.46 *a*	P
17:0	0.18 ± 0.01	0.18 ± 0.02	0.22 ± 0.00	
SA	7.22 ± 0.27 *b*	9.30 ± 0.50 *a*	9.83 ± 0.17 *a*	P
22:0	0.01 ± 0.00 *	0.03 ± 0.01 *	0.02 ± 0.00	
Σ MUFAs	24.39 ± 1.80 *a*	18.15 ± 1.18 *b*	16.65 ± 0.77 *b*	P
cis-9 14:1	0.11 ± 0.03	0.04 ± 0.01	0.05 ± 0.00	
cis-7 16:1	0.27 ± 0.01	0.26 ± 0.02	0.27 ± 0.00	
Palmitoleic acid	5.63 ± 0.72 *a*	3.15 ± 0.26 *b*	3.08 ± 0.18 *b*	P
cis-9 17:1	0.16 ± 0.02	0.12 ± 0.02	0.15 ± 0.01	
cis-6 18:1	0.09 ± 0.01	0.08 ± 0.01	0.11 ± 0.02	
OA	13.39 ± 1.46 *a*	10.10 ± 1.04 *a*b*	8.49 ± 0.53 *b*	P
cis-11 18:1	4.44 ± 0.11	4.25 ± 0.08	4.34 ± 0.07	

cis 12 18:1	0.07 ± 0.00	0.07 ± 0.01	0.09 ± 0.01	
cis-11 20:1	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	
Σ PUFAS-cis	34.67 ± 1.64 *a*	40.13 ± 1.74 *b*	42.19 ± 0.52 *b*	*P*
PUFAS n-6	25.15 ± 0.95 *a*	27.66 ± 0.64 *b*	28.46 ± 0.17 *b*	*P*
LA	16.21 ± 0.39	16.66 ± 0.19	16.86 ± 0.47	
GLA	0.04 ± 0.00*	0.03 ± 0.01*	0.04 ± 0.01	
cis-11, cis14 20:2	0.12 ± 0.01	0.11 ± 0.01	0.13 ± 0.00	
DGLA	0.30 ± 0.04	0.33 ± 0.03	0.35 ± 0.02	
AA	7.46 ± 0.80 *a*	9.53 ± 0.67 *b*	10.03 ± 0.32 *b*	*P*
cis-7,cis-10,cis-13,cis-16 22:4	0.43 ± 0.04 *a*	0.53 ± 0.02 *b*	0.51 ± 0.01 *b*	*P*
cis-4,cis-7,cis-10,cis-13,cis-16 22:5	0.47 ± 0.07	0.48 ± 0.04	0.54 ± 0.03	
PUFAS n-3	9.52 ± 0.70 *a*	12.46 ± 1.10 *b*	13.73 ± 0.46 *b*	*P*
ALA	0.33 ± 0.05	0.29 ± 0.04	0.27 ± 0.04	
EPA	0.11 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	
cis-7,cis-10,cis-13,cis16,cis19 22:5	1.89 ± 0.27*	2.35 ± 0.20	2.49 ± 0.08*	
DHA	7.15 ± 0.47 *a*	9.78 ± 0.84 *b*	10.85 ± 0.44 *b*	*P*
Σ NI	1.00 ± 0.17	1.13 ± 0.21	1.08 ± 0.09	

Proportion of fatty acids in muscle expressed as % FAME of animals exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively). **Σ NI**: sum of unidentified fatty acids; **ΣSFA:** saturated fatty acids; **ΣMUFAs**: monounsaturated fatty acids; **Σ PUFAs**: polyunsaturated fatty acids; **AA**: arachidonic acid: cis-4,cis-8,cis-11,cis-14 20:4 n-6; **ALA**: α-Linolenic acid: cis-9,cis-12,cis-15 18:3 (n-3); **DGLA:** Dihomo-γ-linolenic acid: cis-8,cis-11,cis-14 20:3 (n-6); **DHA**: Docosahexaenoic acid: cis-4,cis-7,cis-10,cis-12,cis16,cis-19 22:6 (n-3); **SA:** Stearic acid: 18:0; **EPA**: Eicosapentaenoic acid: cis-5,cis-8,cis-11,cis-14,cis-17 20:5 (n-3); **GLA:** gamma-linolenic acid: cis-6,cis-9,cis-12 18:3 (n-6); **LA**: Linoleic acid: cis-9,cis-12 18:2 (n-6); **OA**: Oleic Acid: cis-9 18:1; **PA**: Palmitic acid: 16:0. Values expressed as mean ± SEM (n=4). P, photoperiod effect. (One-way ANOVA, $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA).

Photoperiod affected the level of certain liver and muscle MUFAs

Although the concentration of total hepatic MUFAs was not significantly affected by exposure to different hours of light, a trend towards a higher content of MUFAs was observed in L12 animals compared to L18 (p= 0.08). This trend was evidenced in the level of hepatic oleic acid (OA, cis-9 18:1), since the animals exposed to L12 presented 2.95% of this FA compared to those exposed to L18 (p= 0.028), while the difference of 2.25% with the L6 animals did not reach statistical significance (p= 0.078) (Table 1). Similarly, it was observed that the L12 group presented a higher proportion of FA cis-11 18:1 and a tendency to have a higher level of FA cis-11 20.1 than the L18 group (p= 0.038; p = 0.082, respectively). It should be noted that no differences were found between palmitoleic acid (cis-9 16:1) levels due to variability between groups.

In the gastrocnemius muscle, the photoperiod significantly affected the concentration of total MUFAs, palmitoleic acid and OA (p= 0.006; p= 0.003; p= 0.029, respectively). In this sense, the animals exposed to L12 presented 7.74% and 6.23% more muscle MUFAs and 2.5% palmitoleic acid than those exposed to L18 or L6 (MUFAs; p= 0.007; 0.028; palmitoleic acid; p= 0.015; p= 0.011, respectively). It should be noted that the proportion of OA was higher in those animals exposed to L12 compared to L18 (p= 0.010), while this difference did not reach statistical significance in relation to those exposed to fewer hours of light (p= 0.054). It should be noted that the FA level of cis-11 18.1 and cis-11 20.1 did not differ between groups (Table 2).

The photoperiod modulated the levels of some essential FA on liver and muscle

Although exposure to different hours of light did not affect the proportion of total hepatic PUFAs, it did statistically affect the level of n-3 PUFAs. As seen in Table 1, the animals exposed to L6 or L18 showed a higher content of PUFAs of

the n-3 series than those L12. However, animals exposed to L12 had higher ALA, but lower amounts of EPA and DHA compared to those exposed to L6 or L18 (ALA: p= 0.036, p= 0.041; EPA: p= 0.013, p= 0.024; DHA: p= 0.021, p= 0.006, respectively). In this sense, the flow of biosynthesis of n-3 PUFAs reflected by the DHA/ALA ratio, showed the differential effect of exposure to different photoperiods (p= 0.029), being greater in those animals exposed to long days or short days, compared to the group L12 (p = 0.019; p = 0.022, respectively). With regard to total hepatic n-6 PUFAs, no differences were found between the experimental groups. However, the biosynthesis of n-6 PUFAs reflected by the AA/LA ratio tended to be affected by the number of hours of light to which the animals were exposed (p = 0.063), with those exposed to L18 showing a higher flux than the L12 group (p= 0.023). Likewise, photoperiod significantly affected the level of essential FA LA (p= 0.021), while it tended to do so with cis-11, cis-14 20:2 (p= 0.065). Specifically, the L12 group showed a higher level of both FAs than the L18 group (p= 0.007; p= 0.031, respectively). It should be noted that it also presented a higher proportion of LA than L6, but this difference did not reach statistical significance (p= 0.065).

Similarly, as shown in **Table 2**, in the gastrocnemius muscle photoperiod significantly affected both the concentration of total PUFAs, as well as the level of those from the n-3 and n-6 series (p= 0.002; p= 0.003; p= 0.001). Mainly, the animals that were exposed to L6 or L18, presented a higher concentration of these sums of PUFAs compared to the L12 animals. However, when analyzing each FA, only a significant effect of exposure to different hours of light was observed, on the level of DHA, with the L12 animals showing the lowest level (L12 vs L6: p= 0.023; L12 vs L18: p= 0.004). In addition, the L18 animals tended to present a higher concentration of cis-7, cis-10, cis-13, cis-16, cis-19 22:5 than the L12 group (p= 0.060). On the contrary, no significant differences were observed in the concentration of either ALA or EPA. In relation to the n-6 PUFAs, the photoperiod affected the level of AA and FA cis-7, cis-10, cis-13, cis-

16 22:4, where the L12 animals presented a significantly lower proportion of both FA than the rest of the groups.

L18 affected the expression of hepatic genes related to lipid metabolism compared to L12

Exposure to different photoperiods mainly affected the gene expression of *Fas1* and *Srebp-1c*, after performing a one-way ANOVA (p= 0.04; p=0.037, respectively). As can be seen in **Fig.3**, the animals exposed to L18 presented a higher expression of *Fas1* compared to those animals exposed to L12 and L6 (p= 0.036; p= 0.022, respectively); while tending to a higher expression of *Srebp-1c* than those L6 (p=0.024). It should be noted that the difference in the level of *Srebp-1c* RNA between L6 and L12 did not reach statistical significance (p= 0.083). Contrarily, the L12 group showed a tendency to higher gene expression of *Cpt1α* and *FAT/Cd36* in relation to the L18 group (p= 0.076; p= 0.075, respectively). On the other hand, the concentration of mARN of *FATP5* transporter tended to be lower in animals exposed to the L6 photoperiod compared to the rest of the groups.

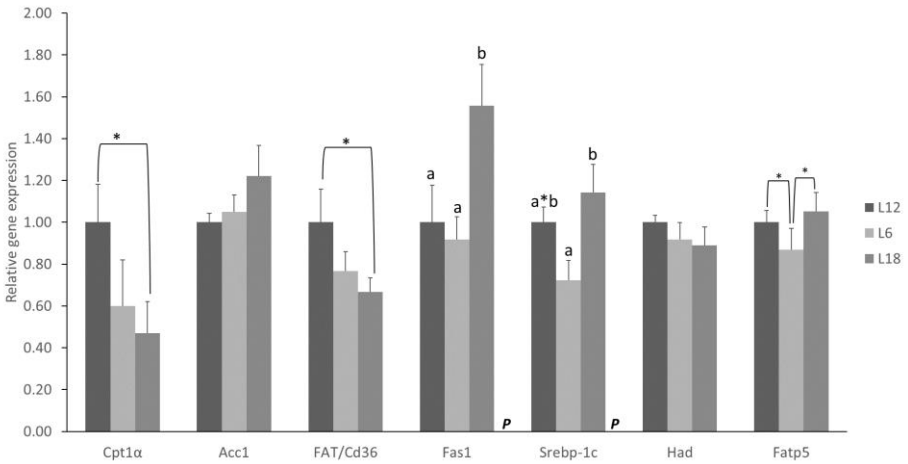

Fig.3. Gene expression of lipogenic enzymes in liver in Fischer 344 rats exposure to three photoperiods (L6, L12, L18) during 11 weeks. Cpt1α, Carnitine palmitoyltransferase 1-α; Acc1, Acetyil-coenzyme A carboxylase; FAT/Cd36,

fatty acid translocase homolog of CD36; Fas1, fatty acid synthase; Srebp-1c, sterol regulatory element-binding protein 1; Had, hydroxyacyl-CoA dehydrogenase; Fatp5, fatty acid transporter 5. Values expressed as mean ± SEM (n = 8). The values were normalized by the L12 group. P, photoperiod effect (one-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicate trend ($0.05 < p < 0.1$).

The gene expression of the enzyme Scd1 was modulated by exposure to different photoperiods

As seen in **Fig. 4**, when analyzing expression of hepatic enzymes related to the onset of FA, only the expression of *Scd1* was statistically altered by exposure to different photoperiods (p= 0.029). Specifically, both L6 and L18 animals showed 34% and 33% lower mRNA concentration than those exposed to L12 (p= 0.019; p= 0.021, respectively). In this sense, the OA/EA ratio showed that those animals exposed to L12 presented a higher enzymatic flux of Scd1 than the rest of the L18 group, while in comparison with the L6, the difference did not reach statistical significance (L12 vsL18 p= 0.024; L12 vs L6 p= 0.080) (**Table 1**). In addition, it was also found that the L6 group tended to have higher *Fads1* expression than the L18 group, although this difference did not reach statistical significance (p= 0.099). The analysis of the gene expression of the *Fads2* and *Elovl2* enzymes was not altered by exposure to different hours of light. However, L18 animals showed an increased EPA/ALA ratio, which reflects a higher Fads2 enzyme flux than L12 animals (p= 0.050). Similarly, the GLA/LA ratio also tended to be higher in the L18 group (p= 0.070), confirming the effect of the number of hours of light on this enzyme (**Table 1**).

Exposure to different photoperiods did not modulate the expression of muscle oxidative enzymes, but it did affect that of the FA transporter FAT/cd36

Exposure to different hours of daily light tended to affect the mRNA concentration of the FA transporter *FAT/cd36* in muscle (p= 0.058). As shown in **Fig.5** in animals exposed to L18, the gene expression of this transporter was only 36% of what was expressed in animals exposed to L6 (p= 0.018). Similarly, we found that the L18 group also tended to have a lower level of *AdipoR2* mRNA compared to the L6 group (p= 0.059). The gene expression of the rest of the muscle enzymes involved in FA oxidation did not show changes with the different photoperiods.

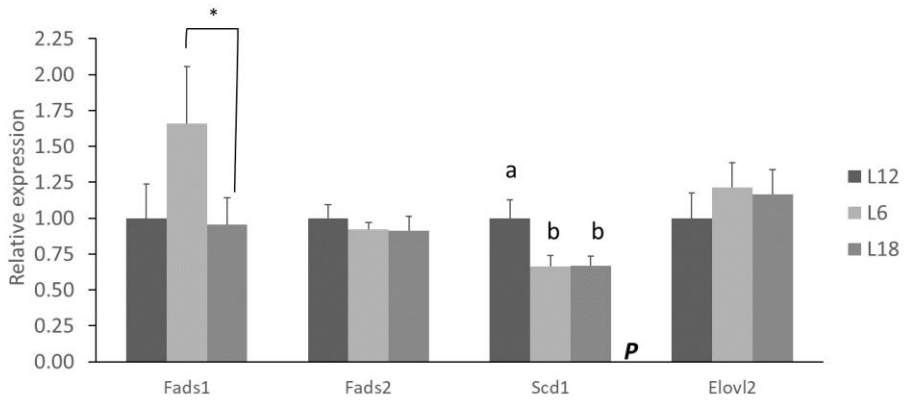

Fig.4. Genetic expression of enzymes related to the biosynthesis of unsaturated FA in liver. The mRNA levels of Fads1, Δ-5 desaturase; Fads2, Δ-6 desaturase; Scd1, Desaturase Δ-9; Elovl2, Elongation of very-long-chain fatty acid enzyme 2; of male Fischer 344 rats exposed for 11 weeks to different photoperiods (short; L6, standard; L12 or long; L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L12 group. P, photoperiod effect (One-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicate trend ($0.05 < p < 0.1$).

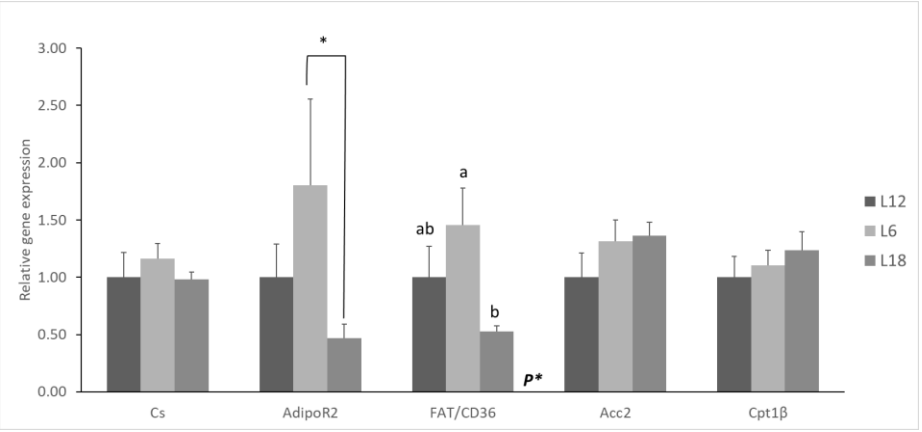

Fig. 5. Genetic expression of enzymes related to the metabolism of FA in gastrocnemius muscle. The mRNA levels of Cs, Citrate Synthase; AdipoR2, Adiponectin receptor 2; FAT/CD36, fatty acid translocase, homologue of CD36; Acc2, Acetyl-CoA carboxylase 2; Cpt1β, carnitine palmitoyl transferase 1 beta of male Fischer 344 rats exposed for 11 weeks to different photoperiods (short; L6, standard; L12 or long; L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L12 group. P, photoperiod effect (One-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicate trend ($0.05 < p < 0.1$).

Multivariate analysis determined two FA metabolic pathways affected between L12 and L18 animals

The GC data of twenty-eight FA hepatic and twenty-one FA of muscle, as well as the sum of AGS, MUFAs, PUFAs, PUFAs n-3 and n-6 were used to establish OPLS-DA (Fig. 6). This type of analysis allowed to obtain more reliable information about the intergroup differences in FA and the degree of correlation between the experimental groups. Three comparisons between the photoperiods were established for a better analysis. Table 3 summarizes the quality of the parameters of the OPLS-DA model. As it can be seen in Fig.6 -a; -b; -c, when comparing animal liver FAs L12 vs L18, L18 vs L6 and L12 vs L6, the explained variability of the axes was 54.7%, 47.3% and 37%, respectively, thus highlighting the potential of these analyses. On the other hand, when

performing the multivariate analysis of muscle FA, the explained variability of the axes was 64.6%, 47.3%, and 49.4%, respectively (**Fig.6 -d; -e; -f)**.

On the other hand, as shown in **Fig 7-a**, when performing the pathway analysis, both the metabolism of LA (**Fig.7-b**) and the biosynthesis of unsaturated FA (**Fig.8**) were the main metabolic pathways affected by exposure to L18 compared to L12. **Table 4** shows the results obtained by the Metaboanalyst, as well as the different parameters for the validation of said routes. Specifically, the metabolism of unsaturated fatty acids was statistically affected (FDR: 0.040), while the metabolism of LA tended to be affected by the number of hours of light (FDR: 0.065). Considering this metabolic pathway, the differences between the proportion of LA between the L12 and L18 animals could be involving phosphatidylcholine. However, when making comparisons between L12 vs L6 or L18 vs L6, there were no significant differences in these metabolic pathways.

Table 3. Parameters for assessment of the quality of the opls-da models in rats Fischer 344.

Tissue	OPLS-DA comparison	R^2X	r^2y	q^2y	p Permutation Q^2
LIVER	*L12 vs L18*	0.291	0.864	0.683	0.026
	L12 vs L6	0.177	0.687	0.239	0.343
	L6 vs L18	0.184	0.939	0.338	0.125
MUSCLE	*L12 vs L18*	0.221	0.754	0.29	0.041
	L12 vs L6	0.177	0.687	0.239	0.365
	L6 vs L18	0.134	0.513	-0.039	0.581

R^2X and R^2Y are the variance explained by each comparison, in the descriptor matrix (X) and response matrix (Y), respectively. Q^2Y is the predicted variation in the class response.

Table 4. Analysis of metabolic pathways with hepatic FA.

Pathway name	Total metabolites	Hits	Statistical comparison	p value	FDR	Impact
Biosynthesis Of Unsaturated Fatty Acids	36	10	L12 vs L18	0.006	0.040 *	0
			L12 vs L6	0.110	0.386	0
			L6 vs L18	0.375	0.438	0
Linoleic Acid Metabolism	5	1	L12 vs L18	0.018	0.065 **	1
			L12 vs L6	0.103	0.386	1
			L6 vs L18	0.112	0.263	1

Liver fatty acids from animals exposed to 6, 12 and 18 hours of light (L6, L12, L18, respectively) were exposed to pathway analysis using Metaboanalyst. Main metabolic pathways significantly affected by exposure to different photoperiods, the total amount of metabolites involved in the pathway, the amount of metabolites that have information, the p value, false discovery rate (FDR), and impact, are shown. P value < 0.05 and FDR < 0.05 were considered significant (*); while a 0.05 < p value and/or FDR < 0.100 were considered a trend (**). Kyoto encyclopedia of Genes and Genomes (KEGG) was database used.

DISCUSSION

It is now well documented that the circadian rhythm of physiological processes in mammals is mainly modulated by the amount of daily light [3,4,6,25]. In this sense, melanopsin activates neuronal pathways to establish the master clock of the hypothalamic SCN, depending on the wavelength of light captured by the retina [25,26]. Therefore, depending on the cyclical environmental changes, the central nervous system will orchestrate the clocks in the peripheral tissues and consequently their biological functions, such as the availability and metabolism of nutrients [5,8].

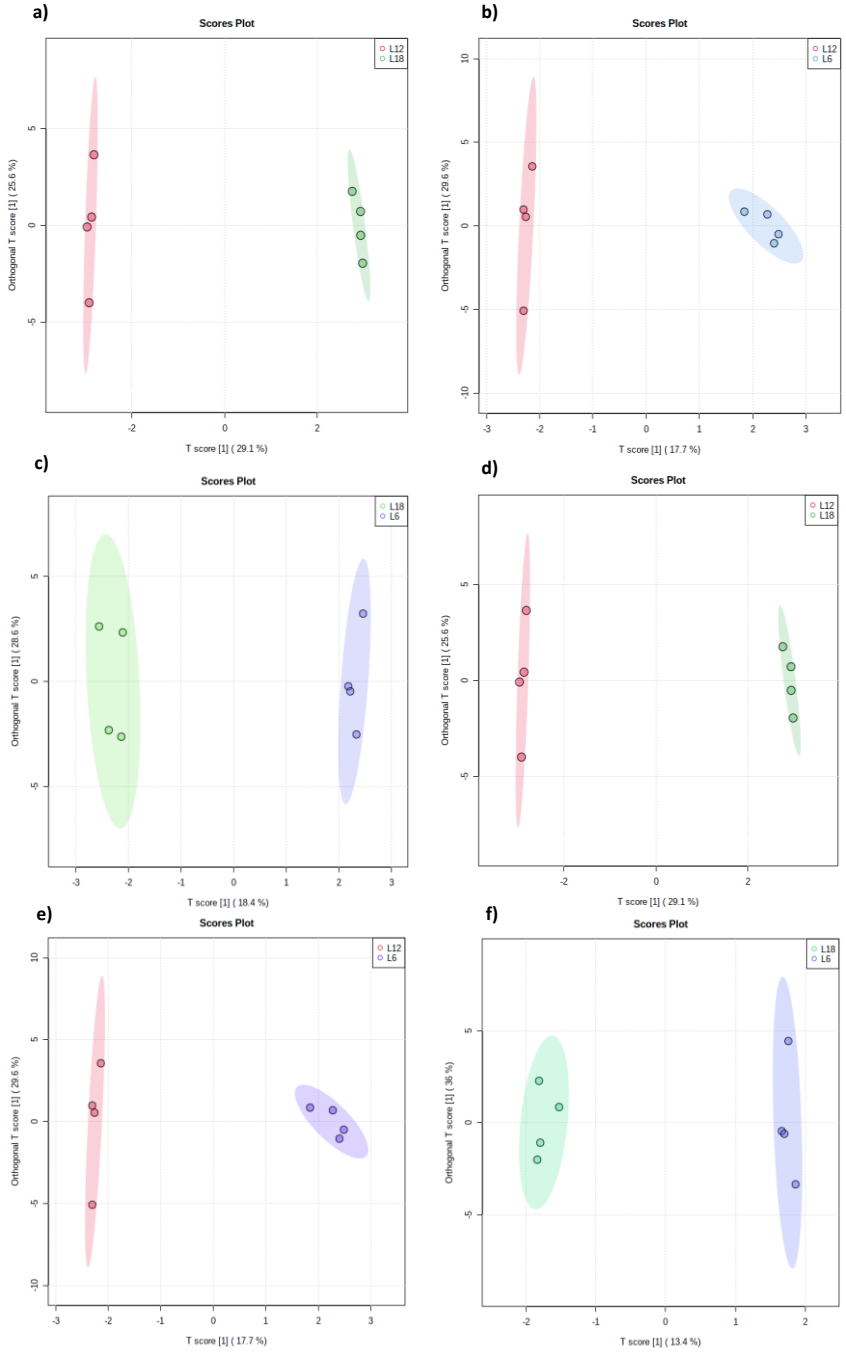

Fig 6. OPLS-DA based on liver (**a b c**) and muscle (**d, e, f**) FA of Fischer 344 rats exposed to 6, 12 and 18 hours of light (L6, L12, L18, respectively). Statistical comparisons by pairs of photoperiods were made: L12 vs L18; L12 vs. L6 and L6 vs. L18.

On the other hand, there is evidence in different animal models showing that through independent pathways, light can synchronize the peripheral clocks of tissues such as the liver, kidneys, hearts, adrenal glands and muscles, among others [5,27,28]. However, there is little evidence on the impact of seasonal rhythms and their consequences on the tissue lipid profile [15].

In the present study we have evaluated the effects of exposure to different photoperiods on various plasma metabolic parameters, gene expression of liver and muscle enzymes, as well as the content of the FA profile of these tissues in Fischer 344 rats. Overall, exposure to different hours of daily light modulated the lipid profile of the animals.

We have previously documented that those animals exposed to different photoperiods do not show differences in body weight or in the amount of food consumed per day [20]. Therefore, analyzing the experimental design, and considering that the rats feed in the dark, these animals had different hours to eat the same amount of food, which is expressed by the EPI of each group. In this sense, we can observe different eating patterns. It should be noted that the timing of food intake functions as an activating signal for peripheral clocks in metabolic tissues, helping to maintain robust circadian rhythms [29–31].

Exposure to different hours of light modulates lipid metabolism in liver and muscle, mainly in those exposed to long days compared to standard days.

After analyzing the liver tissue of animals exposed to L12, we have observed a trend towards greater gene expression of the enzyme *Cpt1α* and *FAT/cd36* together with a lower expression of *Fas1* and a lower proportion of SFA with respect to those exposed to L18. In this sense, the enzyme *Cpt1α* is the limiting enzyme for the passage of long-chain FA Co-A through the mitochondrial wall for its β-oxidation [32]. In turn, the FAT/cd36 transporter translocate to the plasma membrane of the hepatocyte, allowing the uptake of FA from the

circulation [33]. However, it has recently been observed that FAT/cd36 is also found in the mitochondrial membrane, actively participating in the passage of FA for oxidation [34]. While increased expression of *FAT/Cd36* has been associated with metabolic diseases such as obesity, insulin resistance, and diabetes, increased *FAT/cd36* gene expression has also been shown to be associated with increased oxidation of long-chain FA [34,35]. In addition, the lipogenic enzyme Fas1 is responsible for the synthesis of palmitoyl-Coa from acetyl-Co units in the liver [33].

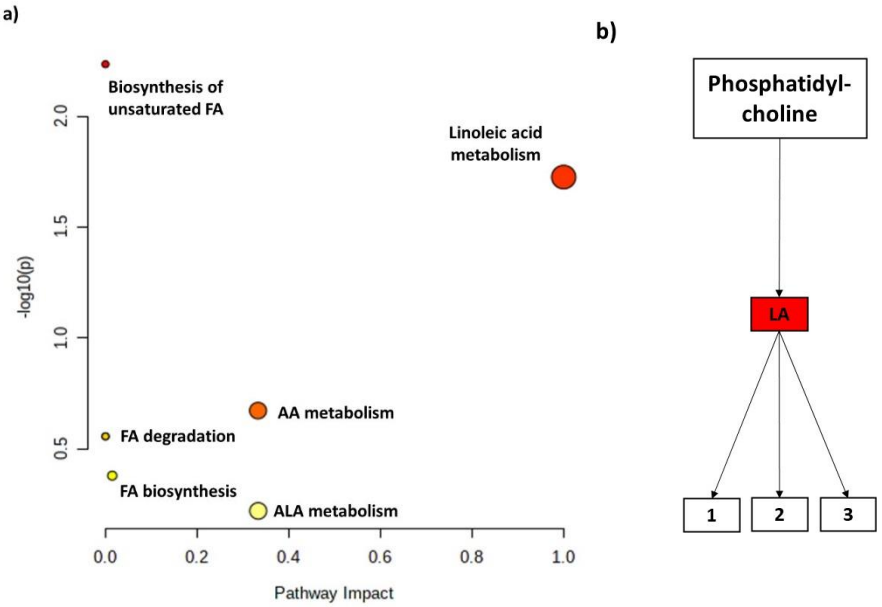

Fig.7. Pathway analysis based on data from lipidic metabolites of liver samples of animals exposure to L18 compared to L12. Overview of pathway analysis (**a**) and pathway of linoleic acid metabolism (**b**) from Kyoto encyclopedia of Genes and Genomes (KEGG) database. Red colored box indicates increased of proportion of FA. **1**, 9,10-Epoxyoctadecenoic acid; **2**, 12,13-Epoxyoctadecenoic acid; **3**, 13S-Hydroperoxy-9Z,11E-octadecadienoic acid; **LA**: Linoleic acid.

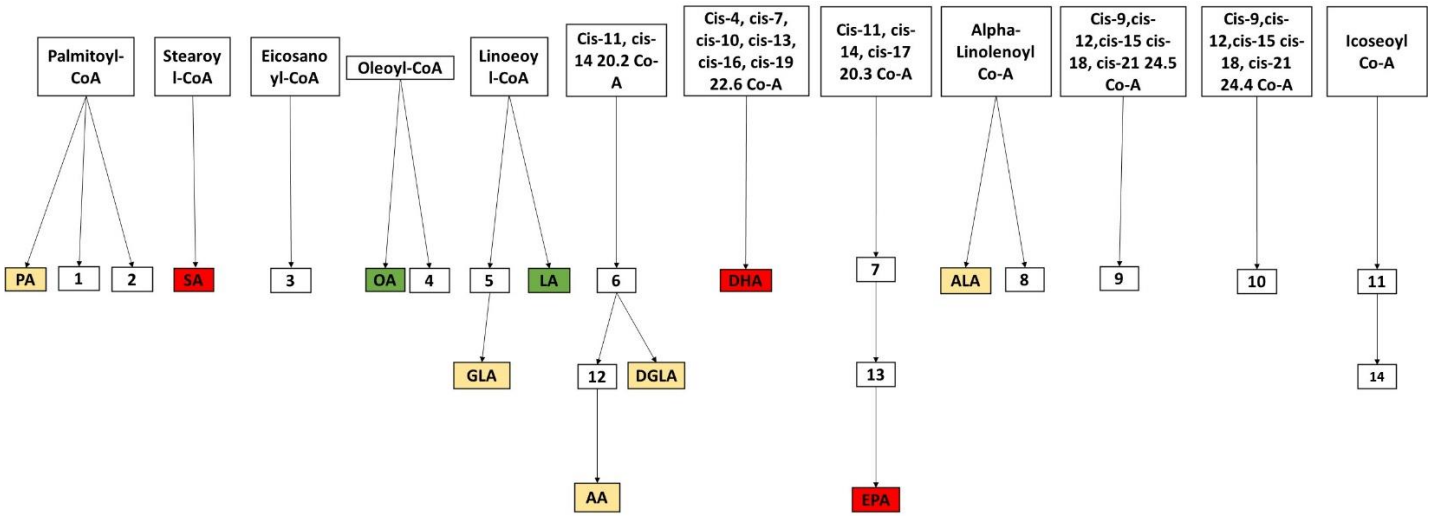

Fig.8. Pathway analysis based on data from lipidic metabolites of liver samples of animals exposure to L18 compared to L12. Pathway of select biosynthesis of unsaturated fatty acid (FA) from Kyoto encyclopedia of Genes and Genomes (KEGG) database. Red and green colored boxes indicate increased and decreased proportion of FA; yellow boxes indicate small variation; white boxes indicate no changes of lipid classes. **1**, Sapienoyl-CoA;**2**, Palmitoleoyl-CoA;**3**, Icosanoic acid; **4**, (6Z,9Z)-Octadecadienoyl-CoA; **5**, gamma-Linolenoyl-CoA; **6**, (8Z,11Z,14Z)-Icosatrienoyl-CoA; **7**, (8Z,11Z,14Z,17Z)-Icosatetraenoyl-CoA; **8**, (6Z,9Z,12Z,15Z)-Octadecatetraenoyl-CoA; **9**, (6Z,9Z,12Z,15Z,18Z,21Z)-Tetracosahexaenoyl-CoA; **10**, (6Z,9Z,12Z,15Z,18Z)-Tetracosapentaenoyl-CoA; **11**, (8Z,11Z)-Icosadienoyl-CoA; **12**, Arachidonoyl-CoA; **13**, (5Z,8Z,11Z,14Z,17Z)-Icosapentaenoyl-CoA; **14**, (5Z,8Z,11Z)-Icosatrienoyl-CoA; **AA**: Arachidonic acid; **ALA**: alpha-Linolenic acid; **DGLA**: Dihomo-ɣ-linolenic acid; **DHA**: docosahexanoic acid; **EPA:** Eicosapentaenoic acid; **GLA**: gamma-linolenic acid; **LA**: Linoleic acid; **OA**: Oleic acid; **PA:** Palmitic acid; **SA**: Stearic acid.

Therefore, these results would explain the lower concentration of hepatic SFA, as a consequence of greater mitochondrial oxidation and/or less synthesis of FA in L12 animals compared to L18 animals. It should be noted that these differences were not found between the L12 and L6 animals, indicating the importance of the light stimulus. Other authors also found in L12 animals a higher expression of *FAT/Cd36* and the enzymes phosphoenolpyruvate carboxykinase 1 and fructose 1-6 biphosphatase 1, and higher concentration of liver glycogen, together with a lower concentration of glucophophatidylcholine than in L18, despite not having analyzed hepatic FA profile [16]. In addition, hepatic lipogenesis, as well as other metabolic enzymatic processes, have a circadian regulation through several factors. In this sense, the state of feeding/fasting, certain hormones such as insulin, glucagon, glucocorticoids, or dietary composition directly influence the gene expression of transcription factors and key enzymes in lipid metabolism [14,36]. Although the concentration of *Srebp-1c* mRNA does not differ between groups, it is known that this transcription factor is under circadian regulation, and that its oscillations also determine fluctuations in the expression of its target genes such as *Fas1*, *Scd1* and *Acc1*, throughout a day [37]. Considering that the treated animals were exposed to days with different amounts of light hours, it can be expected that the moment of greater gene expression of key enzymes in lipid metabolism will be displaced, as well as differ in the moment of ingestion, which would grant seasonal variability to digestive processes.

On the other hand, in the present study, we have observed greater differences in the hepatic FA profile between the L12 and L18 animals and to a lesser extent between the L12 and L6 groups. In this sense, the pathway analysis has shown that the biosynthesis of unsaturated FA is significantly affected by the L18 photoperiod compared to exposure to L12. As shown in **Fig.2** of the **Supplementary material**, *Scd1* is a key enzyme at the beginning of the synthesis of unsaturated FA, since it introduces the first cis bond in its substrates, PA or

SA, for the formation of palmitoleic acid and OA [38]. Since these FA are the major constituents of the plasma membrane, their proportion would give it fluidity and/or rigidity [38]. Therefore, a decreased ratio of OA/SA and/or palmitoleic acid/PA would indicate a greater presence of SFA, which is associated with certain pathologies such as cancer, diabetes, obesity, hypertension, among other chronic metabolic diseases [39]. Specifically, the L18 group presented a higher proportion of the SFA PA and SA, together with a lower gene expression of *Scd1* and its desaturation product OA, compared to the L12 group, suggesting that exposure to long days would not only reduce the oxidation of FA, but also decreases the establishment of SFA, causing greater hepatic accumulation and providing greater rigidity to the plasma membrane. On the other hand, L6 animals also showed lower *Scd1* gene expression compared to L12 animals. However, this difference was not reflected in the proportion of SFA, palmitoleic acid or OA.

Leyton *et al*. (1987) stated that n-3 PUFAs have a higher rate of oxidation in 24 h than n-6 PUFAs or MUFAs [40]. We have found that although there are no differences between the amount of total PUFAs or those of the n-6 series, the L12 group has a lower amount of hepatic n-3 PUFAs than those exposed to L18 and L6. Gibson *et al*. (2011), proposed a biosynthetic model of PUFAs, where OA, LA and ALA use the same metabolic pathway for their elongation and desaturation [41]. In this process, *Fads2* is considered the key enzyme for the biosynthesis of PUFAs, since it is involved in the first step and is where the three FAs compete for it. Furthermore, by introducing a double bond in the FA cis-9,cis-12,cis-15,cis-18 24:4 and cis-9,cis-12,cis-15,cis-18,cis-21 24:5, also there is competition with them. The metabolic pathway of unsaturated FA biosynthesis is summarized in **Fig.2** of the **Supplementary material.** Therefore, since no differences were found in the proportion of PUFAs n-6 between the groups, we can establish that the higher level of PUFAs n-3 found in the L18 and L6 animals was not due to enzymatic competition between FA.

Consequently, even though there are no differences in the gene expression of *Fads1, Fads2* and *Elovl2*, key in their synthesis, there are differences in the hepatic EPA/ALA, DHA/ALA ratios, indicating a greater flow towards the biosynthesis of PUFAs n- 3 in animals exposed to L18 and L6. In this sense, it is important to highlight that there are differences between the FA of the n-3 series between the experimental groups. Mainly, the L12 group has a higher content of hepatic ALA and a lower proportion of its elongation and desaturation products, EPA and DHA. Therefore, this difference in the proportion of n-3 PUFAs would indicate either an increase in the rate of oxidation of the L12 group, which is accompanied by a tendency to greater expression of the enzymes involved in β-oxidation (compared to with the L18 photoperiod), or to a lower hepatic synthesis of PUFAs-n3. It should be noted that the oxidation of FA depends mainly on the ability to capture FA from the blood into the interior of the cell, as well as the intrinsic capacity of the tissue for oxidation [42]. In this sense, the differences between the L12 and L18 groups in the expression of the *Cpt1α* and the *FAT/cd36* transporter may support this theory.

Despite no differences in the proportion of hepatic n-6 PUFAs, we found a higher content of LA, cis-11, cis-14 20:2 and a lower level of AA in animals exposed to L12 compared to L18. Therefore, even though we did not find differences in the gene expression of key enzymes for their synthesis between both groups, we found a greater flow of synthesis towards these FA in the animals exposed to long days, given by the AA/LA and AA/GLA ratios. In this sense, the pathway analyzes showed a trend in a differential effect of the L18 photoperiod on the metabolism of LA. Although only one metabolite was involved in this pathway, more studies involving phosphatidyl choline and its metabolic cascade are suggested to understand its seasonal variation.

It has been shown that the dietary n-6/n-3 ratio is an important determinant in the state of inflammation, maintenance of homeostasis, and the development

of chronic diseases such as cancer, rheumatoid arthritis, coronary heart disease, obesity, diabetes, etc[43]. However, although all the experimental groups consumed the same type and amount of food daily, the L18 animals ingested double or triple the number of kcal per hour than those exposed to L12 or L6, respectively. Therefore, the different distribution of consumption times between the groups could determine the differences in the composition of hepatic FA.

On the other hand, skeletal muscle also plays an important role in FA uptake for oxidation and energy production. In this sense, the L12 group has shown ahigher concentration of muscular SFA, together with a tendency to an amount of plasmatic TAG compared to L18 group, indicating that there could be a greater export of SFA from the liver to the gastrocnemius muscle. Mariné-Casadó *et al*. (2018) did not observe changes in serum TAG in animals exposed to different photoperiods [16], however, Xie *et al*. (2017) stated that exposure to long days slightly increases this parameter [44]. Due to the discrepancy between the results, investigations in this regard are required. A higher proportion of muscle SFA, mainly PA, is associated with insulin resistance and higher blood glucose [45]. In this sense, the slight increase in SFA together with a higher proportion of PA found in the L12 animals could also explain their higher glycemia compared to the L18 animals. In addition, the L12 animals showed a lower proportion of both total PUFAs and those of the n-6 and n-3 series in muscle.. A higher level of 22:5 n-3 and DHA would also explain the lower amount of SFA and glycemia in L18 animals, since these FAs, mainly DHA, are associated with greater oxidation of glucose and lipids, and with anti-inflammatory effects, through AMPK phosphorylation [46]. A greater muscle β-oxidation of PUFAs over MUFAs or SFA could be hypothesized, however, the expression of key enzymes in muscle β-oxidation, *Cpt1β* and *FAT/cd36*, did not show differences between groups. As reinforced by the OPLS-DA model of liver and muscle FA, there is a clear separation between the L12 and L18

populations, however, these differences are not found between the L12 and L6 animals.

Exposure to L18 stimulates lipogenesis through Srebp-1c gene expression and key enzymes in de novo synthesis.

The SREBP family are master regulators of lipid metabolism [33]. Specifically, hepatic Srebp1-c activates genes related to TAG synthesis, such as *Acc1* and *Fas1*, as well as enzyme genes responsible for FA elongation and desaturation, such as *Scd1* and elongase complexes [47]. In agreement with our findings, the animals exposed to L18 presented a tendency to a higher gene expression of *Srebp-1c*, together with a higher concentration of *Fas1* mRNA and a tendency to a higher expression of the FA transporter *FATP5, compared to animals exposed to L6*. These results suggest a stimulation of lipogenesis, which is accompanied by a higher proportion of hepatic SFA in L18 animals. Specifically, this difference in total SFA content could be the result of a higher proportion of PA and SA in L18 animals. However, they do not reach statistical significance. Interestingly, the composition of hepatic FA between L18 and L6 animals did not show differences. Although there are limited studies on the lipid profile and exposure to photoperiods, we previously observed that animals exposed to L18 have a higher % body fat than those exposed to L6 [20]. As rats are nocturnal animals, it makes sense that those animals that are active for more hours a day have a lower percentage of lipids. However, differences in the gene expression of hepatic oxidative enzymes were not found. In turn, the entry of adiponectin through its receptor produces the phosphorylation of Acc2, with the consequent decrease in malonyl-CoA and the activation of cpt1β, stimulating muscle β-oxidation [48,49]. In this sense, we have found a tendency to a greater expression of *AdipoR2*, accompanied by a greater concentration of mRNA of *FAT/cd36*, which would indicate a greater oxidation of muscle FA, even though the expression of *Cpt1b* was not modified. In addition to the

number of active or inactive hours of the animals, it is also important to note that those L18 animals have an EPI three times higher than the L6. Consumption of the same kcal, but in much less time could be responsible for these changes at the level of accumulation of SFA.

CONCLUSION

In summary, this study provides evidence on the metabolic consequences of exposure to different hours of light. Specifically, the photoperiod modulates the lipid metabolism. A long photoperiod could be associated with less lipid oxidation, greater FA synthesis, and greater accumulation of SFA and PUFAs n-3 in the liver with respect to exposure to an L12 photoperiod. At the muscular level, the effects were less evident. However, a higher proportion of total MUFAs and PUFAs was also observed. A multivariate analysis confirms the differential effects of photoperiods. The consequences of exposure to L6 were less precise. However, animals exposed to L18 showed a higher gene expression of lipogenic enzymes together with a higher proportion of SFA with respect to those L6. These effects could be due to the different eating patterns that are established with the number of active hours of the animals, which produce the same energy consumption, but in different time intervals.

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SUPPLEMENTARY MATERIAL

Table 1. Nucleotide sequences of primers used for real time quantitative PCR.

Gene	*Forward Primer (5' to 3')*	*Reverse Primer (5' to 3')*	*Tissue*
Acc1	*TGCAGGTATCCCCACTCTTC*	*TTCTGATTCCCTTCCCTCCT*	L
Acc2	CTTTTCTAGGTCCCCGAGTGA	CTTCCGCTCCAGGGTAGAGTT	*M*
AdipoR2	CCACACAACACAAGAATCCG	CCCTTCTTCTTGGGAGAATGG	*M*
Cpt1α	GCTCGCACATTACAAGGACAT	TGGACACCACATAGAGGCAG	*L*
Cpt1ß	GCAAACTGGACCGAGAAGAG	CCTTGAAGAAGCGACCTTTG	*M*
Cs	CCGTGCTCATGGACTTGGGCCTT	CCCCTGGCCCAACGTAGATGCTC	*M*
Elovl2	TTTGGCTGTCTCATCTTCCA	GGGAAACCATTCTTCACTTC	*L*
Fads1	TACAGGCAACCTGCAACGTTC	GGTGCCACCTTGTGGTAGTTGT	*L*
Fads2	GGAACCATCGACATTTCCAG	TCTTTATGTCGGGGTCCTTG	*L*
Fas1	CTATTGTGGACGGAGGTATC	TGCTGTAGCCCAGAAGAG	*L*
FAT/Cd36	GTCCTGGCTGTGTTTGGA	GCTCAAAGATGGCTCCATTG	*M*
Fatp5	CCTGCCAAGCTTCGTGCTAAT	GCTCATGTGATAGGATGGCTGG	*L*
Had	ATCGTGAACCGTCTCTTGGT	AGGACTGGGCTGAAATAAGG	*L*
Scd1	GAGGAACATCATTCTCATGGTCC	CGTACACGTCATTCTGGAACG	*L*
Srebp1-c	CCCACCCCCTTACACACC	GCCTGCGGTCTTCATTGT	*L*

The table shows the nucleotide sequences of primers used for PCR amplification in liver (L) and muscle (M) tissues. *Acc1*, Acetyl-CoA carboxylase 1; *Acc2*, Acetyl-CoA carboxylase 2; *AdipoR2*, Adiponectin receptor 2; *Cpt1α*, carnitine palmitoyltransferase 1 alpha, *Cpt1ß*, carnitine palmitoyltransferase 1 beta; *CS*, Citrate Synthase*; Elovl2*, Elongation of very-long-chain fatty acid enzyme 2; *Fads1*, Δ-5 desaturase; *Fads2*, Δ-6 desaturase; *Fas1*, Fatty acid synthase; *FAT/CD36*, fatty acid translocase, homologue of CD36; *Fatp5*, fatty acid transport protein 5; *Had*, hydroxyacyl-CoA dehydrogenase; *Scd1*, Desaturase Δ-9; *Srebp1-c*, sterol regulatory element-binding protein 1c.

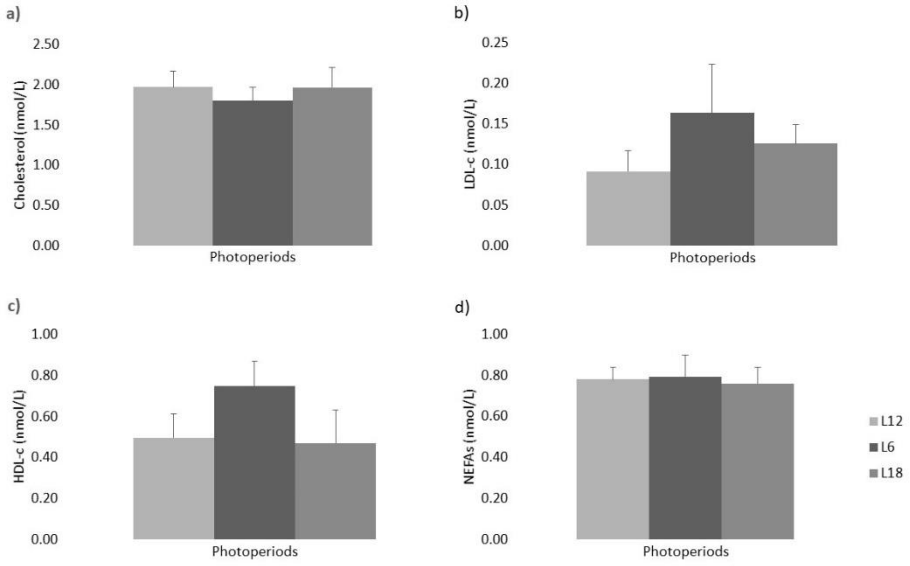

Fig. 1. Plasma cholesterol (**a**); *LDL-c*, low density lipoprotein cholesterol (**b**); HDL-c, high-density lipoprotein cholesterol (**c**) and NEFAs, non-esterified fatty acids (**d**) in Fisher 344 rats exposed to a short, standard or long photoperiod, with 6 (L6), 12 (L12) or 18 (L18) hours of light, respectively for 11 weeks. Data are expressed as the mean ± SEM (n = 8). One-way ANOVA analysis was used to assess the differences between groups.

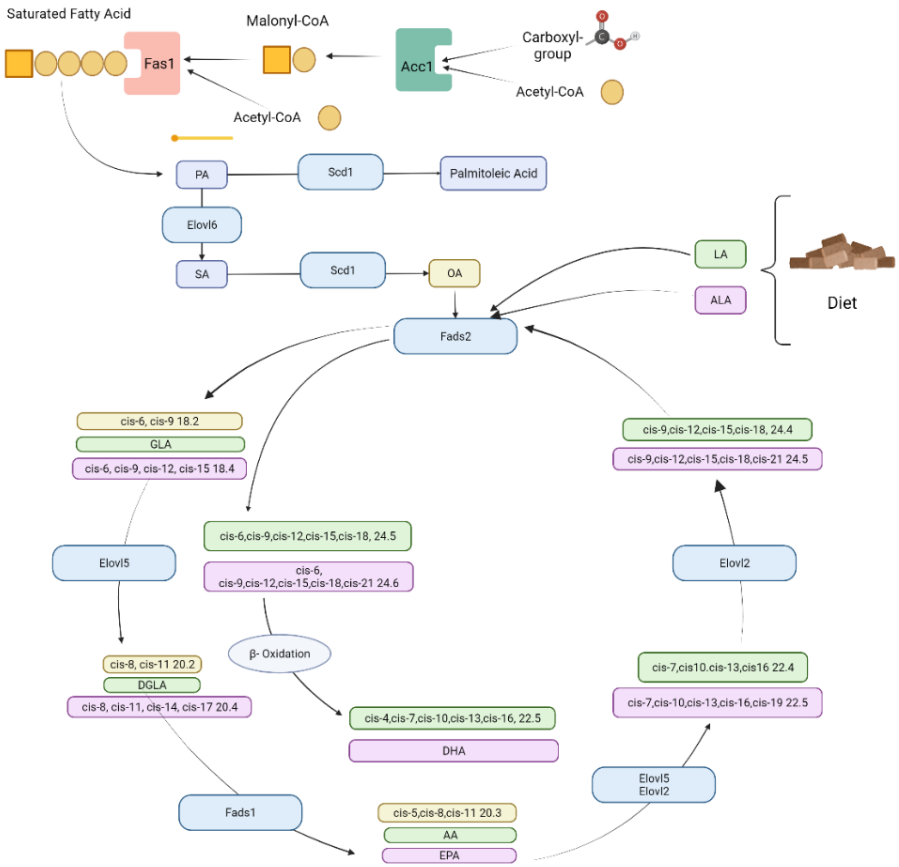

Fig.2. Metabolic pathway of FA lipogenesis and biosynthesis of unsaturated FA. Abbreviations: Enzymes: Fas1, Fatty acid synthase; Acc1, Acetyl-CoA carboxylase 1*; Fads1*, Δ-5 desaturase; *Fads2*, Δ-6 desaturase; Elovl2, Elongation of very-long-chain fatty acid enzyme 2; Elovl5, Elongation of very-long-chain fatty acid enzyme 5; *Scd1*, Desaturase Δ-9. Fatty acids: AA: arachidonic acid: cis-4, cis-8, cis-11, cis-14 20.4 n-6; ALA: α-Linolenic acid: cis-9, cis-12, cis-15 18.3 n-3; DGLA: Dihomo-γ-linolenic acid: Cis-8, cis-11, cis-14 20.3 n-6; DHA: Docosahexaenoic acid: Cis-4,cis-7,cis-10,cis-12,cis16,cis-19 22.6 n-3; SA: Stearic acid: 18.0; EPA: Eicosapentaenoic acid: cis-5, cis-8, cis-11, cis-14, cis-17 20.5 n-3; GLA: gamma-linolenic acid: Cis-6, cis-9, cis-12 18.3 n-6; LA: Linoleic acid: cis-9, cis-12 18.2 n-6; OA: Oleic Acid: cis-9 18.1; PA; Palmitic acid: 16.0. Adapted from Gibson et al [1].

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Capítulo II

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

MANUSCRITO 2

Seasonal Consumption of Cherries from Different Origins Affects Metabolic Markers and Gene Expression of Lipogenic Enzymes in Rat Liver: A Preliminary Study

Ma. Josefina Ruiz de Azua, Álvaro Cruz-Carrión, Begoña Muguerza, Anna Arola-Arnal and Manuel Suarez.

Nutrigenomics Research Group, Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, 43007 Tarragona, Spain

mariajosefina.ruiz@urv.cat (.M.J.R.d.A); alvarojavier.cruz@urv.cat (Á .C.-C.); begona.muguerza@urv.cat (B.M.); anna.arola@urv.cat (A.A.-A.)

* Correspondence: manuel.suarez@urv.cat

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ABSTRACT

The phytochemical composition of fruits, especially polyphenols, depends on the environmental conditions under which these fruits are cultivated and the agronomic practices followed. Therefore, the consumption of fruits from different origins, with different polyphenol signatures, could have differential effects on health. In addition, recent studies have shown that variation in the biological rhythms due to changes in the photoperiod in the different seasons differentially affect the metabolism in animal models, thus conditioning their response to food consumption. Considering all, this article evaluates the effects of consumption of sweet cherry from different sources, local (LC) and non-local (nLC), on plasma metabolic parameters and the gene expression of key enzymes of lipid metabolism in Fischer 344 rats under photoperiods simulating different seasons. Animals were classified into three photoperiods (L6, L12 and L18) and three treatments (LC, nLC and VH). Both the photoperiod and the treatments significantly affected the evaluated parameters. An effect of the photoperiod on triacylglycerides, non-esterified fatty acids and the mRNA concentration of crucial enzymes from the hepatic lipid metabolism was observed. Furthermore, the consumption of fruit in L12 lowered blood glucose, while the different treatments affected the hepatic expression of genes related with lipidic enzymes.

Keywords: xenohormesis; polyphenols; photoperiods; seasonality; liver lipogenic enzymes.

Abbreviations: Acc1: Acetyl-CoA carboxylase 1; AI: atherogenic index; At.C: atherogenic coefficient; Cat: catechin; cd36: fatty acid translocase, homologue of CD36; Cpt1α: carnitine palmitoyltransferase 1 alpha; CR1: cardiovascular risk 1; CR2: cardiovascular risk 2; Cy3R: cyanidin-3-O-rutinoside; F344: Fischer 344; Fas1, Fatty acid synthase; Fatp5: fatty acid transport protein 5; GAE: equivalents of gallic acid; Had, hydroxyacyl-CoA dehydrogenase; HDL-c: HDL

cholesterol; HOMA: homeostatic model assessment index; L12: standard photoperiod; L18: long photoperiod; L6: short photoperiod; LC: Local Cherry; LDL-c: LDL cholesterol; MWAT: Mesenteric white adipose tissue; NEFAs: non-esterified fatty acids; nLC: non-Local Cherry; P: Photoperiod effect; Ppia: Peptidylprolyl Isomerase A; Quer: Quercetin; Srebp1-c: sterol regulatory element-binding protein 1c; STD: standard diet; T: Treatments effect; TAC: anthocyanins; TAG: triacylglycerides; TC: total cholesterol; TFaC: flavanol; TFoC: flavonols; TPC: total content of polyphenols,VH: Vehicle; VLDL-c: very low density lipoproteins.

INTRODUCTION

Polyphenols are secondary metabolites synthesized by plants, which, thanks to their characteristic phenolic structure, have certain protective effects against chronic diseases, such as cardiovascular [1,2], neurodegenerative [3], metabolic pathologies [4,5] and even against some types of cancer [6,7]. These phytochemicals are present in fruits, vegetables, tea, chocolate, coffee, oils, among others. Therefore, a regular consumption of these foods would provide health benefits [8,9]. Cherries, for their part, have a low caloric content and not only provide sugars, fiber, vitamin C, potassium, tryptophan, serotonin and melatonin, but also guarantee a significant contribution of bioactive phytochemicals [10]. Although the concentration and composition of these phytocompounds will depend on various factors, both sweet cherries (Prunus avium L) and sour cherries (Prunus cerasus L) are rich in polyphenols, mainly anthocyanins, hydroxycinnamic acid and flavonoids [11]. In particular, their consumption has been as-sociated with anti-inflammatory, anti-oxidative and antifungal properties, in both animal and human models [12–14].

The day/night rhythm, as well as the wake/sleep rhythm, regulate many physiological processes in mammals, including body temperature, hormonal secretion, food intake, and lipid metabolism. Further seasonal changes and the duration of daylight exposure modulate the circadian rhythm, thus influencing the clock genes and resulting in direct and indirect effects on metabolic parameters. Consequently, changes in the photoperiod can produce alterations that predispose to obesity and increase the risk of metabolic syndrome [15].

Hormesis is the concept that defines the process by which living beings adapt to low-dose exposure of external harmful stimuli to achieve resistance to future exposures and thus enhance the survival of the species. The development of signaling pathways, including transcription and regulatory factors in the gene

expression of cytoprotective proteins, are some of these adaptive responses [16]. For example, plants develop a distinctive phytochemical composition, primarily polyphenols, that will depend on the environment and the stressful stimuli that they receive [17]. Within this context, xenohormesis is defined as inter-species hormesis, where organisms are able to detect these signaling molecules synthesized by other species and thus adapt and adequately prepare themselves for adverse situations [18]. In this sense, the consumption of fruits and vegetables (and their phytochemicals) by heterotrophs would serve as an alert signal, allowing their adaptation, stimulation and cellular strengthening in the face of adversity and stress [17]. However, recently it has been postulated [19] that out-of-season consumption could introduce misleading signals, thus altering the metabolism. For example, an increase in the gene expression of enzymes related to the transport and β-oxidation of fatty acids was observed in the soleus and gastrocnemius muscles of normal weight rats that ingested cherry out-of-season, while in obese rats their consumption out-of-season produced an increase in the glycemia and insulinemia, accompanied by a better use of lipids [20]. Furthermore, it has been observed that the antioxidant effect of cherries from different origins depend on the photoperiod of consumption, mainly preventing oxidative stress when they are ingested in the season of consumption [21]. These differential effects could be attributed to differences in the post-harvest treatments, transport and storage conditions, including time, temperature, and exposure to light, which significantly affect the polyphenol content [22]. Therefore, it could be thought that the differential effect of fruit consumption will depend not only on the photoperiod to which the animals are exposed, but also on the origin of the fruit, which would cause a characteristic polyphenolic composition, and that it can be modified by various post-harvest factors.

For all the aforementioned, the objective of the present study was to investigate the consequences of cherry consumption from different sources,

both local and non-local, to male Fisher 344 rats exposed to different photoperiods, on metabolic parameters.

MATERIALS AND METHODS

Fruits, preparation and characterization

For the experiment, sweet Brooks cherries that were cultivated and harvested in different countries, Spain (LC: local cherry) and Chile (nLC: non-local cherry), were used. After discarding the pips, they were frozen in liquid nitrogen, ground and lyophilized at -55 °C using the Telstar LyoQuest lyophilizer (Thermo Fisher Scientific, Barcelona, Spain). The different lyophilized fruits presented the following nutritional composition expressed as a function of their dry weight (dw). On the one hand, LC contained 64.1 mg/g of protein, 10.1 mg/g of total lipids, 795 mg/g of carbohydrates, of which 433 mg/g are sugars and 109.5 mg/g was dietary fiber. On the other hand, the nLC had 55.1 mg/g of protein, 7 mg/g of total lipids and 807.8 mg/g of carbohydrates, of which 500 mg/g are sugars and 107.4 mg/g were dietary fiber [21]. The total content of polyphenols (TPC), anthocyanins (TAC), flavanol (TFaC) and flavonols (TFoC) in the LC and nLC extracts were quantified according to the procedures described by Iglesias-Carres [23,24]. Such compounds were expressed in mg equivalents of gallic acid (GAE), cyanidin-3-O-rutinoside (Cy3R), catechin (Cat), Quercetin (Quer), respectively. The LC extracts presented 8.17 ± 0.20 (mg GAE/g dw), 1,31 ± 0.02 (mg Cy3R eq/g dw), 0.44 ± 0.002 (mg Cat eq/g dw), 0.55 ± 0.00 (mg Quer eq/g dw) and the nLC 7.64 ± 0.41 (mg GAE/g dw), 1.23 ± 0.03 (mgCy3R eq/g dw), 0.38 ± 0.03 (mg Cat eq/g dw) and 0.63 ± 0.05 (mg Quer eq/g dw), respectively [21].

Animals

The Animal Ethics Committee of the University Rovira i Virgili (Tarragona, Spain) approved all of the procedures (Project identification code: 9495; file number:

FUE-2017-00499873). Seventy-two 8-week-old Fischer 344 (F344) rats were acclimatized for 4 days. (Charles River Laboratories, Barcelona, Spain). We used F344 rats due to their high sensitivity to the amount of daily light hours, having consequences on metabolic, physiological and behavioral parameters [25–27]. The animals were housed in pairs in cages at 22 ° C, and, to simulate the differences in the length of the day in the different seasons of the year, were divided into three groups: short-day photoperiod (n=24, L6, 6 hours of light and 18 hours of darkness); standard photoperiod (n=24, L12, 12 hours of light and 12 hours of darkness); long-day photoperiod (n=24, L18, 18 hours of light and 6 hours of darkness). Animals underwent 4 weeks of adaptation where they were fed ad libitum with a standard diet (STD) (A04 SAFE, Panlab, Barcelona, Spain). After acclimatization, within each photoperiod animals were divided into 3 groups according to the treatment: vehicle (VH, n=8), LC (n=8) or nLC (n=8). The VH-treated group was supplemented with a sugar solution (glucose: fructose 1:1, 21.2 mg per kg of body weight), to equal the amount of carbohydrates that were administered to the animals treated with the fruits. It should be noted that considering the weight of the rats and the amount of food consumed, the contribution of the vehicle only represented a contribution of 0.063 kcal/day, which would be equivalent to 0.11% of their daily energy intake [21]. Therefore, the metabolic impact of this sugar load was not relevant in the context of the whole diet. Treated animals were administered a dose of 100 mg of freeze-dried fruit per kg body weight (diluted in water) one hour after the lights were switched on. The 3 treatments were administered by voluntary licking through a syringe to guarantee the consumption of the entire dose. After 7 weeks of treatment, the animals were sacrificed by decapitation, having their last dose one hour before being guillotined. The blood was collected from the neck, in non-heparinized tubes, incubated for 1 h at room temperature, and immediately centrifuged at 1200 g for 15 min to collect the serum. The liver and serum obtained were stored at -80 °C.

Serum Analysis

Circulating levels of glucose, triacylglycerides (TAG), total cholesterol (TC), HDL cholesterol (HDL-c), LDL cholesterol (LDL-c) (QCA, Amposta, Spain), non-esterified fatty acids (NEFAs) (WAKO, Neuss, Germany) were determined by enzymatic colorimetry. Serum insulin levels were determined using rat insulin ELISA kit (Millipore, Barcelona, Spain). The Homeostatic model assessment (HOMA) index was calculated, using the equation:

(Glycemia (mmol/L) * Insulinemia (mU/dL)/22.5).

Cardiovascular risk indices

Although traditionally the quantification of LDL-c, HDL-c and TC is used to diagnose cardiovascular diseases and disorders, today there is evidence that their values separately are not as effective as the relationships between them. Consequently, different ratios are used to assess the existence of serum lipid alteration [28–31]. Therefore, the following relationships were calculated in the present study: atherogenic index [AI: Log (TAG/HDL-c)], cardiovascular risk 1 (CR1: (TC/HDL-c)), cardiovascular risk 2 (CR2: (LDL-c/HDL-c)), atherogenic coefficient (At.C: [TC-HDL-c]/HDL-c). For its calculation, the values of the biomarkers expressed in mmol/L were used, which were quantified as detailed in section 2.3 “Serum Analysis”.

Hepatic Gene Expression Analysis

Total liver RNA was extracted using TRIzol Reagent (Thermo Fisher Scientific, Ill-kirch-Graffenstaden, France) following the supplier's instructions. cDNA was synthesized by reverse transcription using High-Capacity cDNA Reverse Transcription (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). Specific cDNA amplification was performed by real-time polymerase chain reaction (RT-qPCR) using iTaq Universal SYBR Green Supermix (Bio-Rad, Barcelona, Spain).

The primers used for the different genes were obtained from Biomers.net (Ulm, Germany) and are described in Supplementary Table 1. The genes of interest were those related to lipid metabolism: Carnitine palmitoyltransferase 1-α (*Cpt1α*), Acetyil-coenzyme A carboxylase (*Acc1*), fatty acid translocase homolog of CD36 (*Cd36*), fatty acid synthase (*Fas1*), sterol regulatory element-binding protein 1 (*Srebp-1c*), hydroxyacyl-CoA dehydrogenase (*Had*), and fatty acid transporter 5 (*Fatp5*). The relative expression for each gene was calculated as a percentage of the L18-VH group, considering that cherry season consumption is in summer. The Pfaffl [32] method was used considering the efficiency of each particular primer and using *Ppia* as the endogenous control gene.

Statistical Analysis

Data are shown as mean ± SEM. Statistical analyses were carried out with SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA). Outliers were discarded and normality was evaluated using the Shapiro-Wilk test and homogeneity with the Levene´s test. For those values that met these criteria, a two-way analysis of variance (ANOVA) was performed (Photoperiod X Treatment). In cases where there was a significant effect or trend of any of the variables, a one-way ANOVA was continued using a DMS post-hoc test to determine the differences between the different groups. The analysis by Student´s test was used to compare pairs of groups within the same photoperiod or the same treatment in different photoperiods. Kruskal-Wallis and Mann-Whitney U tests were performed, as appropriate. The threshold of statistical significance was established at $p < 0.05$; and the trend one at $0.05 < p < 1.0$.

RESULTS

Sweet cherry is a typical spring/summer fruit, which consumption in season corresponds to long days (L18). It should be noted that in our previous

manuscript, carried out with the same group of animals, we did not observe significant differences neither in caloric consumption nor in the weight of the animals. Nevertheless, the animals exposed to L12 presented a higher amount of MWAT than those animals exposed to L6 or L18. However, the rats exposed to L6 had the lowest % of body fat and muscle compared to the rest of the photoperiods [21]. Therefore, this evidence would reflect the importance of the amount of light hours to which they are exposed on the body composition of animals, which is accompanied by changes in metabolic parameters observed in the present study.

Exposure to different photoperiods significantly affects triglycerides and blood NEFAs.

There is a significant effect of exposure to different photoperiods on plasma TAG levels ($p = 0.03$, two-way ANOVA) (Table 1). Although no differences were found between the VH of each photoperiod group, it was observed that in L6 the animals treated with nLC presented significantly higher levels of TAG than those treated with LC ($p = 0.01$). In turn, consuming nLC in L6 also tended to increase TAG levels compared to VH ($p = 0.06$). Furthermore, nLC in different photoperiods had a differential effect, since in L6 it was associated with higher plasma TAG levels compared to L12 or L18 ($p = 0.04$; $p = 0.006$ respectively).
Moreover, NEFAs levels were also affected depending on the photoperiod ($p = 0.01$, two-way ANOVA), while exposure to different amounts of light along with the administration of different treatments tended to have a significant effect ($p = 0.05$, one-way ANOVA) (Table 1). Specifically, we have observed a dramatic decrease in serum levels of NEFAs from those animals that consumed both types of cherries in the L18 photoperiod compared to their respective VH, and also in relation to the other L6 and L12 photoperiods. Similar behavior was observed in animals in group L6, although the differences between treatments were not statistically significant.

TC, HLD-c and LDL-c levels were not significantly affected by the photoperiod or by the consumption of any type of fruit (Table 1). However, it is observed that the animals treated with LC in L6 and L18, tended to present a higher concentration of LDL-c than the L12-LC group (p = 0.078, p = 0.066, respectively, one-way ANOVA). Nevertheless, similar conduct is also observed in relation to HDL-c in these groups, where L18-LC animals tended to have a higher level than those L12-LC (P = 0.055). Furthermore, cherry consumption in L18 seems to have a beneficial effect on this biomarker, when higher levels in LC and nLC are observed compared to their respective VH (p = 0.036, p = 0.062, respectively).

Exposure to L12 increases blood glucose, while cherry consumption normalizes it.

As observed in Table 1, cherry consumption tends to have an effect on plasma glucose levels (p=0.087, two-way ANOVA). Specifically, its intake regardless of its origin, decreased glucose levels with respect to VH consumption in L12 (L12-LC vs L12-VH p=0.021; L12-nLC vs L12-VH p=0.046). However, in the other photoperiods this differential effect between fruit consumption and their respective VH was not observed.

On the other hand, there is a differential effect between the VH of different photoperiods, since the animals belonging to the L12-VH group presented higher levels of blood glucose in relation to those L18-VH (p=0.04, Student´s test) (Table 1).

Insulin levels and HOMA index tend to be affected by exposure to different photoperiods and treatments, concomitantly.

By applying a two-way ANOVA, it can be seen that the interaction between exposure to different photoperiods and the treatment tends to affect plasma

insulin levels in animals (p=0.064) (Table 1). Specifically, the L6-VH group had lower insulin levels than those VH exposed to L12 or L18 (p=0.08; p=0.03, respectively, Student´s t-test). In addition, in L6 the intake of any type of cherry increased the plasma insulin levels compared to its respective VH (L6-LC vs L6-VH p=0.01; L6-nLC vs L6-VH p=0.03, Student´s t-test). Also, it was observed that the intake of LC in L18 tended to decrease insulinemia compared to its consumption in L6 (p=0.058, Student´s t-test). In relation to these results, the HOMA index is closely linked to the insulin and blood glucose values and therefore, a similar behavior can be observed. In this sense, the animals that were exposed to short days and that consumed any type of cherry, presented a higher HOMA index compared to their VH (L6-LC vs L6-VH p=0.03; L6-nLC vs L6-VH p=0.05, one-way ANOVA). Furthermore, those groups treated with VH in L12 showed a higher HOMA index than L6-VH (p=0.03), while compared with those exposed to a long photoperiod this difference did not reach statistical significance (p=0.06, Student´s test).

The cardiovascular risk indices are affected by both photoperiod and treatment.

Although neither the rat exposure to different photoperiods nor the treatment affects the content of TC, HDL-c and LDL-c, relevant effects on their derived indices, namely the relationship CR1 and the At.C (Table 1), are observed. Specifically, the L12-LC group presents higher CR1 and At.C compared to nLC and VH animals within the same photoperiod (CR1: p = 0.01, p = 0.01, and At.C: p=0.01, p= 0.01, respectively). Furthermore, L12-LC animals also have these same higher rates compared to those groups that received the same type of fruit in L6 or L18 (CR1 and At.C: p=0.02, p =0.00, respectively). Furthermore, exposure to different hours of daylight also affects AI. Particularly, rats that consumed LC in the L12 photoperiod presented higher AI compared to L18-LC animals (p=0.061). It should be noted that in L18, that simulates the fruit

season, the treated animals had lower AI than their respective VH, although these differences are not significant. Therefore, taking into consideration all the indices, a chronic exposure to more hours of light, added to cherry consumption in its season, seems to decrease cardiovascular risk and less atherogenic power.

Gene expression of Acc1 and Fas1 lipogenic enzymes is increased by chronic exposure to the L18 photoperiod.

Due to the fact that photoperiod and treatment mainly influence the plasmatic lipid fraction, its impact on the gene expression of various hepatic enzymes involved in lipid oxidation and biosynthesis was evaluated to elucidate the involved metabolic pathways. mRNA levels are expressed as relative units, normalized with the L18-VH group. As it can be seen in **Figure 1a**, there is a significant effect of the photoperiod and a trend due to the treatment on the mRNA levels of the *Acc1* lipogenic enzyme (two-way ANOVA).

Occasionally, animals treated with cherries and exposed to the L18 photoperiod tended to present higher levels of the *Acc1* than their control VH (L18-nLC $p=0.098$; L18-LC $p=0.060$, Student´s test). Furthermore, when comparing the effect of cherry consumption between different groups exposed to different hours of daily light, it can be seen that LC consumption in L12 was associated with a lower gene expression of this enzyme compared to L18-LC animals ($p=0.031$, one-way ANOVA). A similar effect occurs with animals exposed to the L6 photoperiod, which tended to have lower mRNA levels relative to the L18 group, regardless of the origin of the ingested fruit (L18-nLC vs L6-nLC $p=0.051$, Student´s test; L18-LC vs L6-LC $p=0.092$, one-way ANOVA). However, there were no significant differences between the VH.

Table 1. Plasma parameters and atherogenic indices in Fischer 344 rats exposed to different photoperiods and supplemented with local or non-local cherry or vehicle for 7 weeks.

Serum Parameters	L6			L12			L18			2w A
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
TAG (mmol/L)	1.14 ± 0.11 *a*b*	1.03 ± 0.14 *b*	1.42 ± 0.12 *a*	1.16 ± 0.08 *a*b*	1.11 ± 0.10 *b*	1.11 ± 0.08 *b*	0.95 ± 0.08 *b*	0.96 ± 0.10 *b*	1.00 ± 0.10 *b*	*P*
TC (mmol/L)	1.80 ± 0.16	1.94 ± 0.21	2.07 ± 0.22	1.97±0.20	2.21 ± 0.14	2.21 ± 0.15	1.97 ± 0.25	1.84 ±0.21	1.98± 0.23	
HDL-c (mmol/L)	0.75 ± 0.11 *ab*	0.72 ± 0.17 *ab*	0.82 ± 0.17 *ab*	0.65 ± 0.18 *ab*	0.45 ± 0.08 *ab**	0.78 ± 0.17 *ab*	0.38 ± 0.16 *a*	0.94 ± 0.29 *b*	0.89 ± 0.19 *a*b*	
LDL-c (mmol/L)	0.16 ± 0.06	0.20 ± 0.05	0.18 ± 0.05	0.09 ± 0.02	0.08 ± 0.02	0.16 ± 0.06	0.11 ± 0.03	0.21 ± 0.04	0.18 ± 0.06	
NEFAs (mmol/L)	0.79 ± 0.10 *a*	0.69 ± 0.09 *ab*	0.79 ± 0.05 *a*	0.78 ± 0.06 *a*	0.76 ± 0.07 *a*	0.79 ± 0.06 *a*	0.76 ± 0.08 *a*	0.56 ± 0.06 *b*	0.55 ± 0.05 *b*	*P*
Glucose (mmol/L)	9.38 ± 0.69 *ab*	8.46 ± 0.46 *ab**	8.69 ± 0.43 *ab*	9.91 ± 0.55 *b*	8.06 ± 0.52 *a*	8.32 ± 0.92 *a*	8.50 ± 0.19 *ab**	8.49 ± 0.19 *ab**	8.24 ± 0.57 *a*	*T**
Insulin (ng/mL)	5.37 ± 0.63 *b*	10.65 ± 1.56 *a*	9.86 ± 1.69 *a*	8.95 ±1.65 *ab**	9.07 ± 1.45 *ab*	9.22 ± 1.71 *a*	9.14 ± 1.32 *ab**	7.07 ± 0.77 *a*b*	8.12 ± 1.03 *ab*	*PxT **
HOMA	0.09 ± 0.01 *b*	0.17 ± 0.03 *a*	0.16 ± 0.03 *a*	0.18 ± 0.04 a	0.13 ± 0.02 *ab*	0.15 ± 0.04 *ab*	0.13 ± 0.01 *ab*	0.11 ± 0.01 *ab*	0.12 ± 0.02 *ab*	*PxT **

(continuation)

Atherogenic ratios										
AI	0.24 ± 0.09	0.24 ± 0.12	0.14 ± 0.13	0.21 ± 0.09	0.44 ± 0.11 *	0.38 ± 0.13	0.03 ± 0.12	0.13 ± 0.13 *	0.33 ± 0.20	P*
CR1	2.13 ± 0.33 *a*	3.90 ± 0.86 *a*	1.97 ± 0.32 *a*	3.69 ± 0.62*a*	6.38 ± 1.30 *b*	3.63 ± 0.51 *a*	2.00 ± 0.39 *a*	3.19 ± 0.74 *a*	2.80 ± 0.45 *a*	*P, T*
CR2	0.20 ± 0.05	0.34 ± 0.07	0.27 ± 0.13	0.30 ± 0.10	0.23 ± 0.06	0.10 ± 0.03	0.16 ± 0.05	0.30 ± 0.05	0.24 ± 0.12	
At.C	1.13 ± 0.33 *a*	2.90 ± 0.86 a	0.97 ± 0.32 *a*	2.69 ± 0.62*a*	5.38 ± 1.30 *b*	2.63 ± 0.51 *a*	1.00 ± 0.39 *a*	2.19 ± 0.74 *a*	1.80 ± 0.45 *a*	*P, T*

Animals were exposed to a short, standard or long photoperiod, with 6 (L6), 12 (L12) or 18 (L18) hours of light, respectively, and supplemented with vehicle (VH) or with local cherry (LC) or non-local cherry (nLC). Data are expressed as the mean ± SEM. (n = 8). Two-way ANOVA analysis (2x2 photoperiod factorial design (L6, L12 or L18) x treatment (VH, LC or nLC), was used to assess the differences between groups. P: photoperiod; T: treatment effect; PXT: effect of Interaction. TAG: triacylglyceride, TC: total cholesterol, HDL-c: high-density lipoprotein, LDL-c: low density lipoprotein, NEFAs: non-esterified fatty acids, HOMA: Homeostatic model assessment, AI: atherogenic index (Log (TAG/HDL-c), CR1: cardiovascular risk 1 (TC/HDL-c), CR2: cardiovascular risk 2 (LDL-c/HDL-c), At.C: atherogenic coefficient ([TC-HDL-c]/HDL-c). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicates trend ($0.05 < p < 0.1$).

On the other hand, exposure to different photoperiods significantly affects the levels of the hepatic Fas1 mRNA (**Figure 1b**). Furthermore, their expression tends to be influenced by the interaction of photoperiod and treatment. Particularly remarkable, the group treated with nLC in L12 had lower mRNA levels than those who consumed the same fruit but in L18 or L6 (p=0.00, p=0.005, respectively, one-way ANOVA). In addition, the animals that were exposed to higher amount of light hours significantly increased the expression of this lipogenic enzyme, when comparing the different treatments with those of the L12 and L6 photoperiods. Specifically, the L18-VH group showed a higher mRNA concentration than L12-VH and L6-VH (p =0.038; p =0.047, respectively). Although no significant effect of the treatment alone was observed, it can be seen that within the L18 group, the animals that received LC presented a higher expression of *Fas1* than their respective VH, while compared with those that ingested nLC this difference is not significant (p=0.060). A similar effect occurs in the L12 photoperiod, where nLC consumption tends to decrease its expression with respect to LC (p=0.096, Student´s test). However, when the animals were exposed to a few hours of light, a higher gene expression was observed when they received nLC instead of LC, despite not being significant.

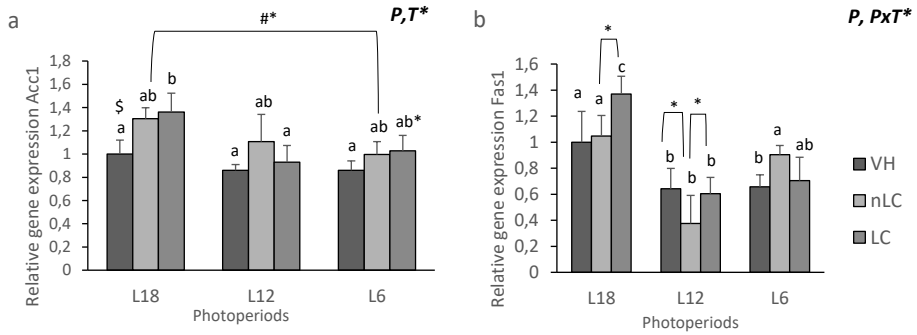

Figure 1. Gene expression of liver lipogenic enzymes. The mRNA levels of Acetyl-coenzyme A carboxylase (*Acc1*) (**a**) and fatty acid synthase (*Fas1*) (**b**) of male Fisher 344 rats treated for 7 weeks with vehicle (VH), non-Local cherries (nLC) or Local (LC), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were

normalized by the L18-VH group. P, photoperiod effect; T, treatment effect; P x T, photoperiod effect and treatment. (two-way ANOVA, p <0.05). Different letters above the bars indicate significant differences (p <0.05) (post-hoc DMS, one-way ANOVA). $ significant effect of treatment in the same photoperiod (Student´s test). * Indicate trend (0.05 <p <0.1).

The gene expression of Srebp-1c and Cpt1α tends to be affected by exposure to different photoperiods.

Exposure to different photoperiods tends to affect the mRNA levels of the Srebp-1c and Cpt1α enzyme in the different groups, when applying the two-way ANOVA analysis. On the one hand, as seen in **Figure 2a**, exposure to long days was associated with a higher expression of the *Srebp-1c* enzyme than on shorter days, both L12 and L6. (L18-VH vs L12-VH p = 0.034; L18-VH vs L6-VH p=0.008). Regarding the expression of Cpt1α, it was observed that the VH exposed to L12 and L6 showed a higher expression compared to those exposed to L18, although these differences were not significant (p = 0.053, p = 0.368, respectively, Student´s test) (**Figure 2b**). Likewise, the animals that ingested LC and were exposed to short days showed a higher gene expression than those that consumed it on long days (p = 0.02, Student´s test).

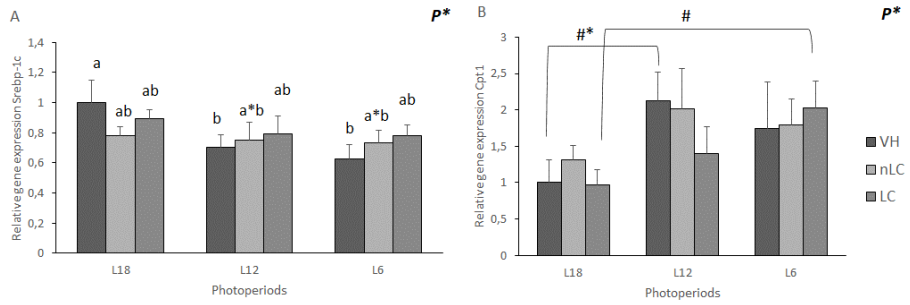

Figure 2. Gene expression of liver protein and enzyme. The mRNA levels of sterol regulatory element-binding protein 1 (*srebp-1c*) (**a**) and Carnitine palmitoyltransferase 1 α (*cpt1α*) (**b**), of male Fisher 344 rats treated for 7 weeks with vehicle (VH), non-Local cherries (nLC) or Local (LC), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L18-VH group. P,

photoperiod effect (two-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). # Effect of the same treatment compared between different photoperiods (Student´s test). * Indicate trend ($0.05 < p < 0.1$).

The gene expression of enzymes related to lipid oxidation is not significantly affected by the photoperiod or by the treatment.

In addition to understanding the metabolic pathways related to hepatic lipid biosynthesis, we have also analyzed the expression of those enzymes that play a key role on fatty acid oxidation. On the one hand, neither the amount of daily light nor the different treatments affected the expression of Had and Fatp5, since no significant differences were observed between the groups (Figure 3a, 3b). However, in the mRNA concentration of the translocase Cd36, although it did not reach the significant difference, it seems that the consumption of cherry in L18 would decrease its expression compared to its respective VH (Figure 3c). Furthermore, it appears that exposure to short days would also decrease the expression of Cd36 compared to long days, although when cherry is consumed the gene expression remains the same in both photoperiods.

DISCUSSION

The development of the metabolic syndrome is multifactorial, where not only lifestyle and genetic predisposition influence, but environmental stimuli such as the number of hours of daylight to which individuals are exposed also affect the circadian rhythm and therefore to circulating biomarkers. In this sense, both the quantity and the quality of the food consumed play a fundamental role in the genesis of this disease [15,25,33]. Likewise, the polyphenols present in fruits and vegetables have a protective function against certain metabolic disorders [13].

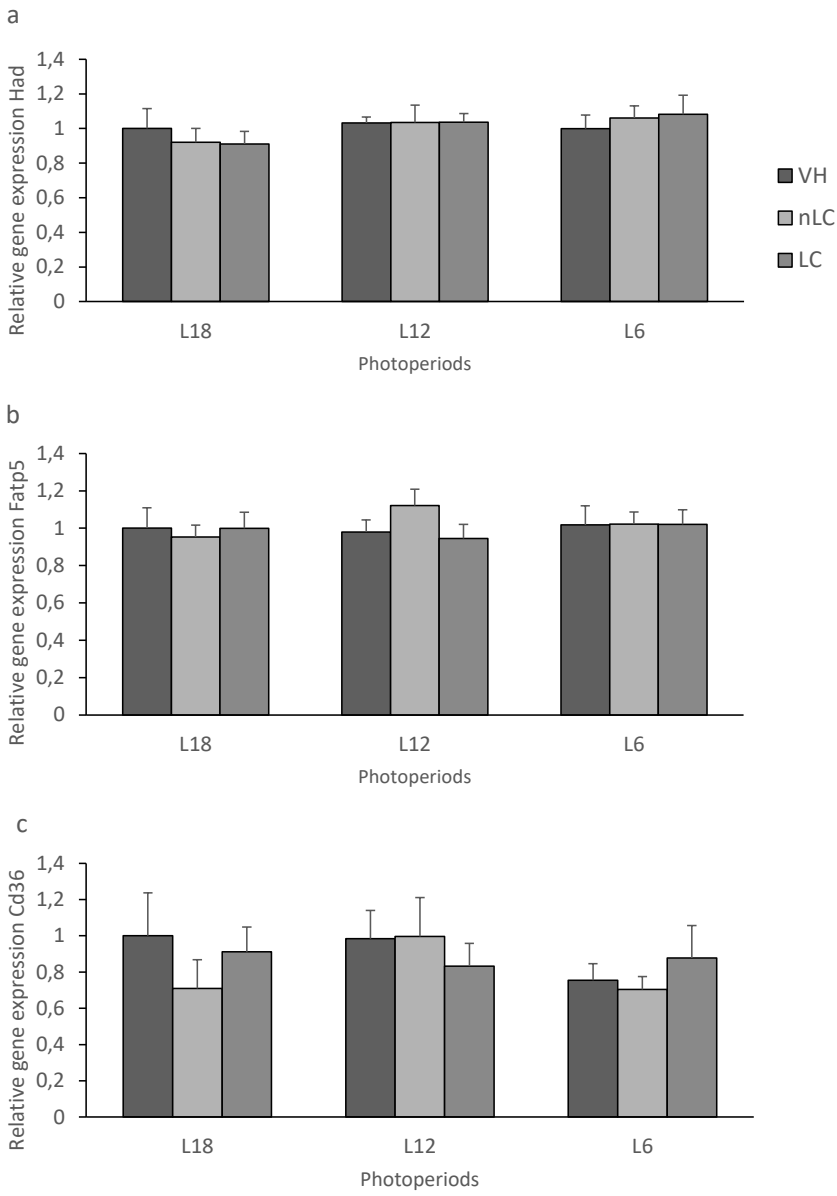

Figure 3. Gene expression of liver enzymes related to lipid metabolism. The mRNA levels of hydroxyacyl-CoA dehydrogenase (*Had*) (**a**), fatty acid transporter 5 (*Fatp5*) (**b**) and fatty acid translocase homolog of CD36 (*Cd36*) (**c**), of male Fischer 344 rats treated for 7 weeks with vehicle (VH), non-Local cherries (nLC) or Local (LC), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L18-VH group.

In the present study, the animals received a daily dose of 100 mg freeze-dried fruit/kg body weight of sweet cherry. Considering the humidity of the fruit and applying the corresponding formula to translocate this dose to humans [34], this treatment is equivalent to a human equivalent dose of 6.46 g of fresh sweet cherry in a 70 kg person per day. The results demonstrate that the consumption of cherry in its natural season, L18, could stimulate the gene expression of lipogenic enzymes and a decrease in the serum concentration of NEFAs, at the same time that it improves insulin sensitivity. Consequently, we noted that the exposure to different photoperiods affects lipid metabolism, specifically the plasma concentration of TAG, NEFAs and the expression of lipogenic genes. Glycemia and insulinemia are influenced both by the photoperiod and by the consumption of cherries from different sources.

In relation to animals exposed to L18, it has been observed that there are significant differences in the circulating levels of NEFAs. Specifically, those treated with VH presented higher levels of NEFAs, which would reflect a higher oxidation of TAG by peripheral tissues than those treated with LC and nLC. Therefore, in the animals that consumed cherry, as it was expected, we observed the highest activity of the key enzymes for the de novo synthesis of hepatic fatty acids, since Acc1 catalyzes the carboxylation of Acetyl-CoA to form malonyl-CoA, which serves as an inhibitor of mitochondrial β-oxidation and a precursor for the synthesis of Palmitoyl-CoA by Fas1 [35]. In this sense, the increased gene expression of Acc1 and Fas1 in season (L18-LC and L18-nLC), confirms the fact that its consumption could be stimulating lipogenesis. However, no significant differences were observed in the amounts of TAG between the groups of animals in L18, which could be due to a compensatory mechanism in the increase of gene expression of mitochondrial Acc2 as a consequence of a decrease or suppression of Acc1 cytosolic [35]. It should be noted that despite the fact that the TC levels have not been modified by any of the treatments, the concentrations of LDL-c and HDL-c changed. Although the

differences are not statistically significant, a beneficial effect is observed in animals that have consumed cherry in L18. Various studies demonstrate the inverse correlation between HDL-c concentration and cardiovascular events thanks to its role in the mobilization of cholesterol from peripheral cells to the liver, where it is catabolized and eliminated [36]. Furthermore, there is evidence on the cardioprotective properties of polyphenols, mainly anthocyanidins and their metabolites, because they increase the reverse transport of cholesterol and the concentration of HDL-c through the activation of the liver X receptor (LXR) and/or the regulation of lipid transporters [37–39]. Therefore, it is expected that in the present study, the animals fed with cherry in L18 show a higher average concentration of HDL-c, which even reaches twice that observed in their respective VH. However, these beneficial effects are not seen in L6 or L12, demonstrating once again the importance of consuming the fruits in their natural season. Animals treated with VH also tended to have less LDL-c compared to those consuming LC and nLC. Although we did not analyze the gene expression of enzymes related to LDL-c and HDL-c metabolism, we could assume that cherry consumption in its season interferes with the activity of enzymes related to lipoprotein metabolism, either in its synthesis as in its excretion. For all the previously explained, we can understand that the consumption of cherry in its season, that is, L18, could be sending signals to decrease the use of fatty acids by peripheral tissues, at the same time that it stimulates the expression of lipogenic enzymes and the cholesterol synthesis by some kind of positive regulation.

On the other hand, no significant differences were observed in the levels of TAG, NEFAs or TC in the animals exposed to the L12 photoperiod. It can be seen that those animals that consumed nLC had higher HDL-c and LDL-c concentrations than the other treatments, but this change did not reach statistical significance. Our results highlight that although the L12-nLC group had the highest HDL-c levels, their cardiovascular risk was increased, since the

ratio CR1 and the At.C were significantly higher than the rest. Surprisingly, they tended to have a lower concentration of Fas1 mRNA compared to those treated with VH or LC. As for the gene expression of the enzymes related to the lipolytic activity *Had*, and that of the transporters *Cd36, Cpt1α, and Fatp5*, they were similar between the groups exposed to L12. Similarly, the mRNA levels of the *Acc1* and *Srebp-1c* lipogenic enzymes showed no significant difference. It has also been observed that animals treated with VH significantly had higher glycemia than those treated with cherries, regardless of origin. However, insulin levels between groups were similar. This could be due to the fiber content present in the administered lyophilizate, allowing a delay in gastric emptying, and a decreased response to glycemic load [36,40]. Furthermore, the content of polyphenols, mainly anthocyanins, also exerts hypoglycemic effects. In this sense, there is evidence that the consumption of cherry juice inhibits the activity of the enzyme α-glucosidase and dipeptilpeptidase 4, improving glycemia [41].

The L6-nLC animals showed significantly higher gene expression of the hepatic lipogenic enzyme *Fas1*, which would cause higher plasma TAG concentration than the rest of the treated animals. Surprisingly, when the two types of cherry were ingested out of season, only the animals that consumed the nLC showed this elevation of the TAG, while those fed with LC were similar to their VH. This situation could be showing that the nLC polyphenol phytochemical signature could be causing an alteration in the lipid metabolism when consumed in a season opposite to that of its harvest, which is not observed with the local fruit. It should be noted that despite the fact that the harvest season of the fruits was the same, the transport conditions are crucial in the preservation of the polyphenolic content [22,42,43]. Specifically, temperature, relative humidity, exposure to light and transfer time are the factors that have the greatest influence on the useful life of biocompounds. Cold storage would produce an increase in some phenolic compounds, while that of anthocyanidins would

decrease by up to 52% in 15 days [44]. Actually, in our previous manuscript we observed that LC had a significantly higher content of TPC, TAC, TFaC and TFoC than nLC [21]. In addition, cherry intake, regardless of its origin, significantly increased the circulating insulin levels compared to its respective VH in L6. However, this situation did not affect the blood glucose of the animals. It should be noted that rats, being nocturnal animals, have more hours of movement in the short photoperiod. Therefore, it could be assumed that increased activity may be a secondary mechanism by which glucose is consumed and hence, potentially responsible for the absence of statistically significant differences in their concentration between the different groups. On the other hand, it is important to note, that the insulin levels observed in the present study are higher compared to similar experiences [45]. In this sense, this difference could be explained by the fact that the treatment dose or VH that entails a carbohydrate supply is carried out one hour before sacrifice. Besides, it has been seen that exposure to different photoperiods caused significant differences in the gene expression of lipogenic enzymes. Specifically, a clear effect has been observed on the VH-treated groups, where exposure to L18 photoperiod significantly increased *Srebp-1c* and *Fas1* mRNA levels, compared to L12 or L6. It should be clarified that Srebp-1c improves the transcription of genes necessary for the synthesis of fatty acids, such as citrate lyase ATP, Acc1 and Fas1, while limiting the gene expression of enzymes involved in the synthesis of TAG [46]. Therefore, this increased *Fas1* response can be expected in the L18-VH group, although no significant differences in *Acc1* expression were found between the VHs. Shimano et al. (1997) stated that an overexpression of hepatic Srebp-1c in transgenic mice increases up to four times the amount of mRNA of enzymes involved in the synthesis of fatty acids, including *Fas1*, while plasma TAG levels decrease [47]. The transcription of SREBP-1C is mainly regulated by 3 factors: LXRs and insulin promote its activity while glucagon inhibits it [46]. Although glucagon values and LXRs expression

have not been analyzed in the present study, plasma insulin values showed that the L18-VH group has the highest levels compared to the rest of the photoperiods. Therefore, a higher concentration of this anabolic hormone might be stimulating the expression of *Srebp-1c*, which in turn stimulates the expression of the *Acc1* and *Fas1* despite not being reflected this fact in the actual concentration of plasma lipids. In this sense, there is evidence showing that mice with high levels of lipogenic enzymes can present a decrease in the rates of fatty acid synthesis, due to post-translational regulation through changes in phosphorylation and activation of the activity of key enzymes for the process [46].

By observing the effects of cherry consumption from different origins in the 3 photoperiods under study, we have shown that the groups fed with cherry in L18 expressed more *Acc1* and *Fas1* mRNAs than the animals that consumed it out of season, either in L6 or in L12. However, this difference was only significant with LC. The precursor malonyl-CoA, synthesized by Acc1, causes the inhibition of Cpt1α, key gene for the oxidation of fatty acids in the mitochondria [48]. As expected, we could confirm this, showing that animals fed with LC in L18 have a lower expression of Cpt1α and at the same time that Acc1 is increased with respect to the consumption of LC in L6. In this sense, it has been recently observed that animals exposed to L18, regardless of the one received, presented a higher % of body fat than those exposed to L6, which is why the higher expression of lipogenic enzymes would be explained [21]. Furthermore, although a lower expression of lipogenic enzymes was observed, it was not reflected in the concentration of plasma lipids, since the L6-nLC animals presented significantly higher levels of TAG compared to those that received nLC in L12 or in L18. Although this effect is only observed on TAG in L6-nLC, other authors have suggested that an increase in TAG in L6 in animals fed with STD could be due to a lower uptake of TAG by the white adipose tissue (WAT) compared with those exposed to L12 or L18 [27]. Although there is little

evidence on the differential effects of consuming the same fruit but from different sources on metabolic parameters, Iglesias-Carres et al., observed that the phenolic composition of oranges from the southern and northern hemispheres were similar, although they differed in the type of compounds that predominated in each [49]. Furthermore, hepatic metabolism is regulated by the synchronization or desynchronization of the nuclear receptors related to clock genes, which are modulated not only by the circadian and circannual rhythm but also by the polyphenols and melatonin present in the fruits [20,25,26]. Likewise, the L6-nLC animals, in addition to presenting the highest levels of TAG, also had a higher concentration of plasma NEFAs compared to the L18-nLC group. A possible mechanism could be that the consumption of cherry in season, L18, stimulates a greater uptake of NEFAs by some fat deposit or oxidative tissue compared with L6. In this sense, there is evidence to suggest that fruits with a high content of polyphenols, such as grapes and cherries, would increase lipid catabolism by increasing the absorption and oxidation of fatty acids in muscle compared to VH [25,50]. A higher concentration of circulating NEFAs is strongly associated with faulty insulin signaling [51]. These findings are consistent with our results, since animals that consumed cherry during the off-season showed higher insulin levels than those that ingested them in L18. Furthermore, both groups showed similar blood glucose levels, which may indicate that consuming cherry in L18 could improve insulin sensitivity. It should be noted that the plasma insulin levels in the groups treated with VH, nLC and LC in L18 show a completely opposite pattern to that observed in L6.

In the present study, we observed that there is an effect of the photoperiod and the treatment on the CR1 and on the At.C. Due to the detrimental effect of a high content of circulating LDL-c and TAG and its correlation with very low-density lipoproteins (VLDL-c), the plasma concentrations of these lipids have been used for years as predictors of coronary heart disease. However, currently

there are other measures of dyslipidemia that are predictive for this type of pathology [52]. Specifically, a high CR1 is positively related to obesity and early insulin resistance, being a more efficient parameter for diagnosing cardiovascular diseases obstructive and atherosclerotic plaque, even when normal LDL-c values are present [53]. Particularly, we observe that the animals fed with LC in L12 presented a CR1 and an At.C significantly higher than the animals belonging to the same photoperiod, and even compared to those that ingested LC in L6 and L18. Similarly, this situation is repeated in the other photoperiods, where the LC group show these elevated parameters in relation to the others, although they do not become significant. While it could be understood that off-season consumption of cherry causes undesirable effects on these atherogenic markers, providing erroneous signals according to the xenhormesis theory, it is not explained why in L18, which resembles the growing season of the fruit, the LC produces this increase in the same way. However, the animals that consume cherry within L18, present the lowest AI values compared to their VH and with the other groups, although the differences are not significant. It should be noted that higher AI is directly associated with high body weight, BMI, higher blood pressure levels, and higher risk of insulin resistance [54]. In addition, it is also related to smaller and denser cholesterol particles, associated with higher long-term mortality in patients with cardiovascular risk factors [55]. Therefore, despite the fact that animals that consume fruit in L18 have a CR1 and At.C higher than the other animals, their cardiovascular risk is offset by the protective effect that a lower AI gives them.

CONCLUSIONS

We report that there is a clear effect of exposure to different photoperiods on the plasma concentration of blood lipids and on the expression of liver lipogenic

enzymes, while the treatment also tends to affect the glucidic metabolism and the gene expression of hepatic enzymes in Fischer 344 normal weight rats. We have observed a significant effect of the photoperiod in the levels of TAGs and NEFAs, thus modulating the values of the atherogenic index, the cardiovascular risk factor 1 and the atherogenic coefficient. On the other hand, the different treatments evaluated (VH, LC and nLC) significantly affected the cardiovascular risk factor 1 and the atherogenic coefficient. An interaction of both parameters (photoperiod and treatment) was observed in the levels of insulin and the HOMA index. We conclude that, considering the xenohormesis theory, the differential effect on the metabolic response could be affected by the origin and the time of consumption of this fruit, possibly due to the different content of bioactive compounds given by the polyphenolic signature of each of the cherries.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of University Rovira i Virgili (Tarragona, Spain), (Project identification code: 9495; file number: FUE-2017-00499873).

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SUPPLEMENTARY MATERIAL

Table 1. Nucleotide sequences of primers used for real time quantitative PCR.

Gene	*Forward primer (5' to 3')*	*Reverse primer (5' to 3')*
Acc1	TGCAGGTATCCCCACTCTTC	TTCTGATTCCCTTCCCTCCT
Cd36	GTCCTGGCTGTGTTTGGA	GCTCAAAGATGGCTCCATTG
Cpt1α	GCTCGCACATTACAAGGACAT	TGGACACCACATAGAGGCAG
Fas1	CTATTGTGGACGGAGGTATC	TGCTGTAGCCCAGAAGAG
Fatp5	CCTGCCAAGCTTCGTGCTAAT	GCTCATGTGATAGGATGGCTGG
Had	ATCGTGAACCGTCTCTTGGT	AGGACTGGGCTGAAATAAGG
Srebp-1c	CCCACCCCCTTACACACC	GCCTGCGGTCTTCATTGT

The table shows the nucleotide sequences of primers used for PCR amplification. *Acc1*, acetyl CoA carboxylase; *Cd36*, fatty acid translocase, homologue of CD36; Cpt1α, carnitine palmitoyltransferase 1 alpha; Fas1, sterol regulatory element-binding protein 1; *Fatp5*, fatty acid transport protein 5; *Had*, hydroxyacyl-CoA dehydrogenase; *Srebp1c*, sterol regulatory element-binding protein 1c.

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Capítulo III

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MANUSCRITO 3

Comprehensive analysis of the impact of the consumption of cherries from different geographical origins and different photoperiods in the fatty acids profile in healthy Fischer rats.

Ruiz de Azua, Ma.Josefina1; Cruz-Carrión, Álvaro1; Manoccio, Francesca; Muguerza, Begoña1; Arola-Arnal, Anna1; Bernal, Claudio2; Suarez, Manuel 1*

[1] Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Tarragona, Spain.

[2] Cátedra Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, C.C. 242, 3000, Santa Fe, Argentina.

*Corresponding author: manuel.suarez@urv.cat

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ABSTRACT

Consumption of fruits from different origins with particular polyphenolic marks modulates the gene expression of certain metabolic enzymes, as well as certain serum parameters that are dependent on the photoperiod of consumption. However, there is little information about how these changes affect the profile of hepatic and muscular fatty acids (FA) and how they interfere in different metabolic pathways. Seventy-two Fischer 344 rats, fed a standard diet and either vehicle (VH), local cherry (LC) or non-Local cherry (nLC), were exposed to different hours of light to simulate the photoperiods for 7 weeks. The FA profile of liver and muscle determined by gas chromatography, as well as the gene expression of key enzymes for FA metabolism were evaluated. In addition, the composition of hydro and lipophilic metabolites in serum and liver was also analyzed by nuclear magnetic resonance (NMR). Consumption of cherry in season decreased saturated FA in the liver, mainly palmitic acid compared to their respectively VH; interestingly in the other photoperiods this effect was not observed. Muscle PUFAs decreased, possibly due to increased oxidation. nLC-L18 varied mainly in serum hydrophilic metabolites with respect to its VH. In conclusion, consumption of cherries in season improves the hepatic lipid profile and would increase oxidation at the muscular level.

Key Words: Fatty acid profile; photoperiod; polyphenols, seasonality.

Abbreviations: Acc2: Acetyl-CoA carboxylase 2; AdipoR2: Adiponectin receptor 2; AMPK: AMP-activated protein kinase; CD3OD: deuterated methanol; CDCl 3: deuterated chloroform; Cpt1β: carnitine palmitoyltransferase 1 beta; Cs: Citrate Synthase; D2O: deuterium oxide; Elovl2: Elongation of very-long-chain fatty acid enzyme 2; F344: Fischer 344; FA: Fatty acid; Fads1: Δ-5 desaturase; Fads2: Δ-6 desaturase; FAMEs: fatty acids methyl esters; FAT/CD36: fatty acid translocase, homologue of CD36; FDR: false discovery rate; GLUT4: glucose transporter type 4; L12: standard photoperiod; L18: long photoperiod; L6: short

photoperiod; LC: Local Cherry; MUFAs: monounsaturated fatty acids; NEFAs: non-esterified fatty acids; nLC: non-Local Cherry; NMR: nuclear magnetic resonance; PA: Palmitic acid; PLS-DA: partial least squares discriminant analysis; PPAR: Peroxisome Proliferator Activated Receptor; PUFAs: polyunsaturated fatty acids; RT-qPCR: real-time polymerase chain reaction; SA: Stearic acid; Scd1, Desaturase Δ-9; SFA: saturated fatty acids; TAG: triacylglycerides; TMS: tetramethylsilane; TSP: trisilylpropionic acid; VH: Vehicle.

INTRODUCTION

Fatty acids (FA) have different physicochemical and biological properties, which depend on the length of the carbon chain, as well as the number and configuration of their double bounds, if they have [1,2]. In this sense, there are three main classes of FA: saturated fatty acids (SFA), mono-unsaturated (MUFAs), and polyunsaturated (PUFAs), including n-3 PUFAs and n-6 PUFAs depending on the position of the first double bond [1,3]. The importance of FA lies not only on their role as energy substrate, but also because they exert other important functions such as being substrates for lipid mediators of multiple processes, actin cell signaling, facilitate the absorption of certain vitamins and modulate the expression of genes involved in metabolism, inflammation or blood pressure, among many other [2,4]. In this sense, to achieve these functions FA can be part of different types of glycerolipids, glycerophospholipids and sphingolipids. **Fig.1** summarizes the main lipid mediators that include FA in their structure.

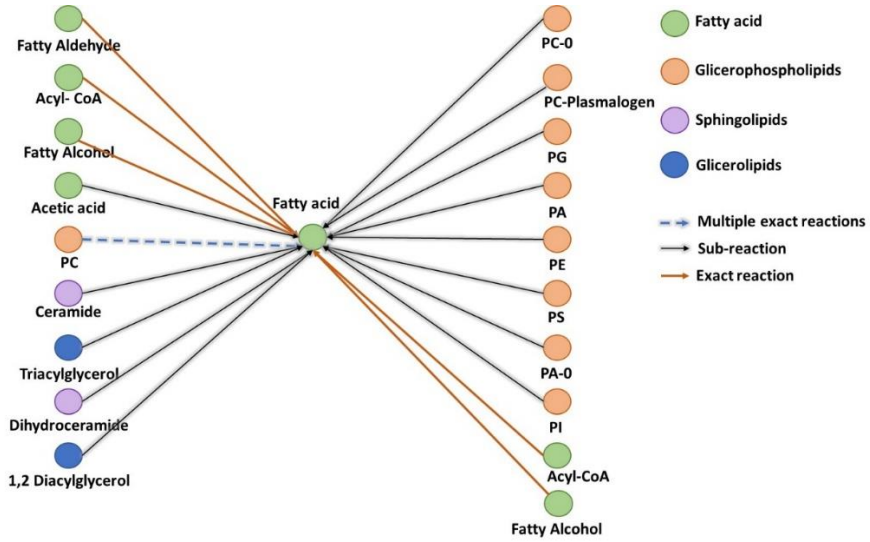

Fig.1. Main lipid mediators that include FA in their structure. PC, phosphatidylcholine; **PC-0,** 1-alkyl-2-acyl-sn-glycero-3-phosphocholine; **PC-**

plasmalogen, 1-(Z)-alk-1-enyl-2-acyl-sn-glycero-3-phosphocholine; **PG,** 1,2-diacyl-sn-glycero-3-phospho-(1'-sn-glycerol); **PA,** 1,2-diacyl-sn-glycero-3-phosphate; **PE,** 1,2-diacyl-sn-glycero-3-phosphoethanolamine; **PS,** 1,2-diacyl-sn-glycero-3-phosphoserine; **PA-0,** 1-alkyl-2-acyl-sn-glycero-3-phosphate; **PI,** 1,2-diacyl-sn-glycero-3-phospho-(1'-myo-inositol). Image adapted from Lipids maps ®.

One of the main tissues involved in the metabolism of FA is the liver. The liver is an organ with multiple functions, including metabolic, energetic, detoxifying, immunological, conjugation, synthesis and excretion of both endogenous and external substances, among others. Metabolically, it is a key organ, where the metabolization and conjugation of bioactive compounds takes place and they are transported to other peripheral tissues through the circulation [5,6]. With regard to the lipid metabolism, in the liver, both the oxidation of fats and the synthesis, elongation and desaturation of FA take place [6]. In this sense, the enzymes Δ6 and Δ5 desaturases (*Fads2* and *Fads1*, respectively), play a key role in the synthesis of PUFAs. In addition to the liver, other tissues are involved in the metabolism of FA. For example, there is crosstalk between the liver and skeletal muscle. In this regard, muscle cells not only take up glucose from the circulation and help to maintain blood glucose, but are also an important site for lipid oxidation. [7]. It has been shown that several factors have a clear impact in the hepatic lipid metabolism. For example *Fads1* and *Fads2* have been shown to be regulated by nutritional, hormonal, and genetic factors. Thus, diets with high protein content or deficient in essential FA stimulates *Fads2* activity, while a high intake of glucose, fructose or poor in protein suppresses it [8]. Other authors have observed that (poly)phenols, which are phytochemicals found in fruits, vegetables, grains, and plant products [9], also influence its activity. In this sense, depending on the nature of the (poly)phenol, the activity of the enzyme was inhibited or stimulated. For example, quercetin induced the expression of *Fads1*, while it was inhibited by apigenin and fistein

[10]. A stimulatory effect of resveratrol on *Fads1* and *Fads2* mRNA concentration has also been observed [11].

Consumption of (poly)phenols is associated with multiple benefits for the body, including anti-inflammatory, anti-oxidant, anti-carcinogenic, anti-diabetic, protective effects of cardiovascular diseases, and lipid-lowering properties, among others [12–14]. Such consequences are due to the modulation of key metabolic pathways, through inhibition or stimulation of gene expression of enzymes and transcription factors in different metabolic tissues. Therefore, diets rich in products with high (poly)phenolic content such as the Mediterranean diet provide beneficial effects to their consumers [15,16]. Among these products, cherries are rich in (poly)phenols, mainly anthocyanidins, hydroxycinnamic acid and flavonoids [17,18]. Its intake has been observed to be associated with a decreased risk of chronic diseases, such as arthritis, cardiovascular disease, metabolic syndrome and cancer [17,19,20]. An improvement in sports performance and recovery from muscle damage were also effects associated with its intake [21,22].

In relation with the beneficial effects of (poly)phenols, these could be dependent on the origin of the fruits. In this regard, the xenohormesis theory suggests that the composition of phytochemicals in fruits and vegetables, which depends on several factors such as agricultural practices, environment and stress conditions, could act as signals those heterotrophs that consume them could use to adapt to the environment and increase survival. Consequently, the consumption of plants from different origins may give erroneous signals that would impact our health [23–25]. In addition, considering the chrononutrition and the influence of circadian and circannual rhythms on the modulation of metabolism, the number of hours of daylight also influence the effects of consuming these foods. In this regard, we have recently observed that cherry consumption differentially influences the serum concentration of triacylglycerides (TAG) and non-esterified FA (NEFAs), as well

as the oxidative state and the gene expression of certain liver enzymes depending on the origin and the photoperiod [26,27].

Little is known about how photoperiods and polyphenols interact in the modulation of gene expression of metabolic enzymes and in the biochemical and inflammatory parameters. In addition, there is limited information on how these variables influence the FA composition of metabolically active tissues, such as the liver and muscle. In the present study, we aim to characterize the hepatic and muscular FA profile in Fischer 344 rats exposed to different hours of daily light and supplemented with cherries of different origin. In addition, we also provide evidence on changes in key hepatic and serum hydro and lipophilic metabolites.

MATERIALS AND METHODS

Fruits preparation

For the present study, *Brooks* sweet cherries that were grown and harvested in different geographic origins were used: cherries from Tarragona, Spain (LC, Local-Cherry) were donated by the farmer in June (in-season consumption), and cherries from Cachapoal, Chile, (nLC non-Local Cherry) were purchased in local market in December (out-of-season consumption). After discarding the pips, they were frozen in liquid nitrogen, ground and lyophilized at −55 ◦C using the Telstar LyoQuest lyophilizer (Thermo Fisher Scientific, Barcelona, Spain). The nutritional composition of both fruits has been previously characterized [27]. Briefly, LC had a significantly higher content of protein, total lipids, dietary fiber, total polyphenols, anthocyanins, and flavanols; while nLC presented a higher content of carbohydrates, sugars and flavonols [27].

Animals and experimental design

Seventy-two 8-week-old male Fischer 344 (F344) rats were housed in pairs in boxes at 22 °C and acclimatized for 4 days. In order to simulate the days of the different annual seasons, the animals were divided into: short-day photoperiod (n = 24, L6, 6 h of light and 18 h of darkness); standard photoperiod (n = 24, L12, 12 h of light and 12 h of darkness); long-day photoperiod (n = 24, L18, 18 h of light and 6 h of darkness). After 4 weeks of adaptation with standard diet (STD) (A04 SAFE; Panlab, Barcelona, Spain) and water *ad libitum*, within each photoperiod the animals were again randomly divided according to the treatment received: vehicle (VH, n = 8), LC (n = 8) or nLC (n = 8). The VH-treated group was supplemented with a sugar solution (glucose: fructose 1:1, 21.2 mg per kg of body weight), to equal the amount of carbohydrates that were administered to the animals treated with the fruits. The amount of kcal provided by the VH represents only 0.11% of daily energy, so the effect of the sugar load is not considered to generate a relevant impact [27]. One hour after the light was turned on, the treated animals were administered VH or a dose of 100 mg per kg of body weight of freeze-dried fruit (diluted in water). To guarantee a complete intake of the dose, the 3 treatments were given by voluntary licking through a syringe. After 7 weeks of treatment, the animals were sacrificed by decapitation, having their last dose one hour before being guillotined. The blood was collected from the neck, in non-heparinized tubes, incubated for 1 h at room temperature and immediately centrifuged at 1200×g for 15 min to collect the serum. The liver, muscle and serum obtained were stored at −80 ◦C.

Hepatic and muscular gene expression analysis.

Liver and gastrocnemius muscle total RNA was extracted using TriPure reagent (Roche Diagnostic, Sant Cugat del Vallès, Barcelona, Spain) according to the manufacturer's protocol. cDNA was synthesized by reverse transcription using High-Capacity cDNA Reverse Transcription (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). Specific cDNA amplification was performed by real-time polymerase chain reaction (RT-qPCR) using iTaq Universal SYBR Green Supermix (Bio-Rad, Barcelona, Spain). Eight samples per group were used (n=8). Different primers related to hepatic metabolism of FA and muscle lipid metabolism, obtained from Biomers.net (Ulm, Germany), (Supplementary Table 1): *Acc2*, Acetyl-CoA carboxylase 2; *AdipoR2*, Adiponectin receptor 2; *Cpt1ß*, carnitine palmitoyltransferase 1 beta; *CS*, Citrate Synthase; *Elovl2*, Elongation of very-long-chain fatty acid enzyme 2; *Fads1*, Δ-5 desaturase; *Fads2*, Δ-6 desaturase; *FAT/CD36*, fatty acid translocase, homologue of CD36; *Scd1*, Desaturase Δ-9. The relative expression of each mRNA level was calculated as a percentage of the L18 group using the Pfaffl method [28].

Hepatic and muscle lipid extraction and GC analysis.

The extraction of hepatic and muscle lipids was done following by Blight and Dyer protocol [19]. Briefly, 400 mg or 500 mg of either muscle or liver, respectively, were weighted. The samples were homogenized together with chloroform, methanol and water maintaining the ratio 1:2:0.8. After another addition of addition of 1 mL of chloroform and 1 mL of water (ratio 2:2:1.8), with vortex and centrifugation, the organic phase was separated and dried under stream of N2. For the determination of lipid profile, the derivation of FA was done by the methylation of methanol potassium hydroxide. The FA methyl esters (FAMEs) determination was carried out by gas chromatography

(Shimadzu (GC-2014) equipped with FID detector and using the CP-Sil 88 (100 m×0.25 mm×0.2 µm, film thickness, Varian, Lake Forest, CA, USA). Four samples per group were used (n=4). FAMEs were identified by comparing their retention relative times of commercial standards, and the chromatogram analysis was done with the LabSolution Shimadzu Software. The values were expressed as a percentage of the total FAMEs.

Evaluation of the enzymatic flux of unsaturated FA biosynthesis

The enzymatic flow of key enzymes in the biosynthesis of unsaturated FA was estimated by determining the relationship between product and substrate of the results of the FAMEs obtained, following the ratios described for Sain, J. [29] .

Extraction of serum and liver metabolites for NMR analysis.

The extraction of the hydro and lipophilic phases of the livers was carried out following the protocol previously described by Palacios *et al*. (2020), with some modifications [30]. Briefly, in the liver, 50 mg of tissue was used and 1 mL of water:acetonitrile mixture (H2O:CH3CN: 1:1 v/v) was added for subsequent homogenization. The resulting solution was centrifuged (19000 g, 15 min, T= 4 °C) and the aqueous supernatant was carefully transferred to a fresh eppendorf. To obtain the lipophilic phase, 1 mL of chloroform:methanol solution (CHCl 3 /CH 3OH 2:1 T=0 ºC) was added. Then, it was vortexed, homogenized and after 10 minutes of incubation at room temperature, it was centrifuged. The aqueous residues were removed under N_2 stream and the hepatic lipid supernatant was obtained. Eight samples per group were used (n=8).

On the other hand, for the extraction of the polar and lipophilic metabolites of the serum, 200 mL of sample was used. 400 µL of methanol and 100 µL of water

were added, and after stirring with a vortex, 200 µL of chloroform was added. After obtaining a monophasic solution, 600 µL chloroform and 300 µL water were added again, centrifuged (19000 x g, 4 ºC, 15 min) to obtain the aqueous supernatant (upper phase) and the lipid supernatant (bottom phase). Both extracts were subjected to a N_2 stream to dryness and stored at -20 ºC up to further analysis.

NMR analysis and data processing.

The dried hydrophilic and lipophilic extracts were reconstituted in 600 µL of deuterium oxide (D2O) phosphate buffer (0.05 mM PBS, pH 7.4, 99.5% D2O) with 0.73 mM trisilylpropionic acid (TSP) and in a deuterated chloroform (CDCl 3) / deuterated methanol (CD3OD) (2:1) solution with 1.18 mM tetramethylsilane (TMS), respectively for NMR measurement. Extracts were transferred to 5 mm glass NMR tubes for analysis. The detailed technical characteristics of the equipment can be consulted in the procedure carried out by Palacio *et al.* [30].

The acquired ^{1}H NMR spectra were compared to references of pure compounds from the metabolic profiling AMIX spectra database (Bruker), HMDB, Chenomx NMR suite 8.4 software (Chenomx Inc., Edmonton, AN, Canada) and databases for metabolite identification. In addition, we assigned metabolites by ^{1}H–1H homonuclear correlation (COSY and TOCSY) and ^{1}H–^{13}C heteronuclear (HSQC) 2D NMR experiments and by correlation with pure compounds run in-house. After pre-processing, specific ^{1}H NMR regions identified in the spectra were integrated using the AMIX 3.9 software package.

Statistical and multivariate analysis.

Homogeneity was evaluated by Levene's test, as well as the normality of the data obtained.First, the data was evaluated by one-way analysis of variance (ANOVA) to determine the effect of photoperiod, *P*, treatment, *T*; and/or photoperiod and treatment, *PxT*. Then, a two-way ANOVA and post-hoc test was performed to determine the differences between the different experimental groups. The data were evaluated by Student's t-test too. Statistical Product and Service Solutions (SPSS) software (SPSS Inc., Chicago, IL, USA) was used. Values of ($p < 0.05$) were considered statistically significant and values of ($0.05 < p < 0.1$) were considered trend.

Regarding multivariate analysis, after processing the original data, partial least squares discriminant analysis (PLS-DA) and pathway analysis was performed to determine the influence of exposure to different photoperiods and the lipid profile of liver and muscle FA. All analyzes were performed after range scaling with the use of the software MetaboAnalyst (version 5.0) available online. R2Y (the interpretability of the model) and Q2 (the predictability of the model) were used to verify the suitability and reliability of the PLS-DA model. The permutation test per 1000 was applied to further test the validity of the model. In the pathway analysis, the p value and the false discovery rate (FDR) were used to verify the affected pathways.

RESULTS

Consumption of cherry in season decreased the content of total hepatic SFA, but did not influence muscle content compared to their respective VH.

Table 1 shows the % FAMEs of the main hepatic FA, while Supplementary Table 2 shows all the FAs analyzed. Results show that both photoperiod and its interaction with the treatment significantly affected the proportion of hepatic SFA, while treatment alone tended to do so (*P*, p = 0.041; *PxT*, p = 0.001; *T*, p = 0.094) (Table 1). Specifically, cherry consumption in season, regardless of the origin, decreased SFA levels compared to the VH (L18-VH vs L18-LC, p = 0.002; L18-VH vs L18-nLC, p = 0.00). This difference could be due to a 6.03% and 9.51% less content in the levels of palmitic acid (PA) in LC and nLC, respectively. In addition, the L18-nLC group also tended to have a lower level of Stearic acid (SA) in relation to their respective VH (p = 0.062). Interestingly, nLC intake at L12 tended to have the opposite effect on SFA (p= 0.077). Likewise, both groups treated at L12 showed a higher level of SA than their respective VH (L12-VH vs L12-LC, p = 0.004; L12-VH vs L12-nLC; p = 0.007).

At the muscular level, the treatment significantly affected the proportion of SFA, while the interaction of photoperiod with the treatment tended to do so (*T*, p = 0.023; *PxT*, p = 0.086). Table 2 shows the % FAMEs of the main muscle FA, while Supplementary Table 3 shows all the FAs analyzed. Specifically, the L12-nLC and L6-LC groups presented a higher proportion of SFA than their respective VH (p = 0.016; p = 0.007, respectively). Particularly, the L12-nLC presented more SA and less of the fatty acid 14:0 (p = 0.004; p = 0.041; respectively), while those L6-LC tended to have a greater amount of PA than their VH (p = 0.058). In addition, the animals exposed to L18, despite not having presented significant changes in SFA between treatments, those that

consumed LC presented less amount of SA (p = 0.007) and tended to have a greater proportion of muscle 15:00 than those that received VH (p = 0.098).

Seasonal cherry consumption did not affect hepatic MUFAs, but muscle MUFAs tended to increase with LC.

Results showed a trend towards the interaction of photoperiod with treatment affecting the ratio of hepatic MUFAs (*PxT*, p = 0.078). In this sense, cherry consumption, both LC and nLC, produced a decrease in MUFAs in those animals exposed to L12 (p = 0.018; p = 0.020, respectively), mainly due to a decrease in oleic acid (OA) (p = 0.013; p = 0.023, respectively). Likewise, the consumption of LC in L6 also tended to decrease the MUFAs with respect to its VH (p = 0.085), with palmitoleic acid being the FA significantly affected (p = 0.042). Although the treatments did not alter the MUFAs in the animals exposed to L18, in both the LC and nLC treated groups, the proportion of c11-18:1 increased significantly, by 13.38% and 17.06%, respectively. It was also observed that the L18-nLC group increased the level of c11-20:1 with respect to its VH (p = 0.031) (Table 1).

Table 2 shows that the photoperiod significantly affected the proportion of muscle MUFAs (*P*, p= 0.042, two-way ANOVA). Specifically, animals that consumed LC in season tended to increase them with respect to their VH (p = 0.064: Student's t-test), while those exposed to L12 decreased it (p = 0.020, Student's t-test). Particularly, the L18-LC group showed a significant increase in c11-18:1 (p = 0.044) and a tendency to higher palmitoleic acid (p = 0.087, Student's t-test), while those exposed to L12 showed a totally opposite effect.

The consumption of LC in season produced a decrease in total PUFAs in the muscle, while at the liver level it did not produce changes.

The treatment significantly affected the levels of total, n-6 and n-3 hepatic PUFAs, the latter being also affected by the interaction of photoperiod and the treatment (*T*, p = 0.012; p = 0.041; p = 0.005; respectively; *PxT*, p = 0.087) (Table 1). Specifically, the L18-nLC animals showed an increase in total PUFAs, and n-6 PUFAs. Moreover, the L18-nLC showed a higher level of c11,c14-20:2, LA, ALA and c7,c10,c13,c16,c19-22:5 (n-3) and a lower proportion of Eicosapentaenoic acid (EPA) (L18- VH vs L18-nLC, p = 0.004, p = 0.007, p = 0.044, p = 0.076, p = 0.048, respectively). However, those animals from the L18-LC group did not have differences with respect to their VH regarding this parameter.

The groups that received cherry, independently of its origin, and were exposed to L12, showed a higher level of hepatic total PUFAs and n-3 PUFAs compared to the control group (L12-VH vs L12-nLC, p = 0.051; p = 0.002, respectively; L12-VH vs L12 -LC, p = 0.020, p = 0.001, respectively). In detail, the treated animals showed a higher proportion of Docosahexaenoic acid (DHA) and Arachidonic acid (AA) than their respective VH. In addition, those animals that received nLC had 36.14% less ALA while, on the contrary, they have 35.95% more Gamma-Linolenic acid (GLA). Such an effect was not observed in the L12-LC animals. It should be noted that the animals exposed to L6 did not show significant changes in the sums of the different PUFAs, or in hepatic FA.

As seen in Table 2, at the muscular level, total PUFAs decreased in animals treated with cherry at L18 (L18-VH vs L18-nLC; L18-VH vs L18-LC, p = 0.036; p = 0.026; respectively). Interestingly, the L18-LC group also had a lower level of PUFAs n-3 and n-6 (L18-VH vs L18-LC: p = 0.017; p = 0.043; Student's t-test). In contrast, those L12-nLC and L12-LC, not only had a higher proportion of total PUFAs (p = 0.002; p = 0.001, respectively), but also had an increase in n-6 (p =

0.028; p = 0.032; respectively). Furthermore, the L12-LC group also had 30.95% more n-3 PUFAs than its respective VH (p = 0.017). Regarding the animals that were exposed to L6, no significant changes were observed in the total PUFAs, nor in those of the n-3 or in n-6 series.

Specifically, the L18-LC animals tended to have a lower level of DHA compared to their VH (p = 0.076). In the case of the L18-nLC, they also had a lower proportion of EPA (p = 0.069, Student's t-test). In contrast, the L12-LC group not only had a higher level of DHA than the VH (p = 0.008, Student's t-test), but also tended to show more AA (p = 0.089) and FA c7, c10,c13,c16-22:4 (p = 0.081). In addition, the L12-nLC presented a higher level of LA than their VH (p = 0.019).

Seasonal LC consumption increased the unsaturation flux through Scd1.

The Table 3 shows the flux of hepatic enzymes related to the biosynthesis of unsaturated FA, expressed by product/substrate ratios. The L18-LC animals tended to have higher values compared to the VH of the Scd1 enzyme flux, which can be estimated from the ratio between palmitoleic acid and PA (p = 0.079, Student's t-test). In contrast, no such effect was observed in the animals that ingested nLC. Interestingly, the groups that received LC and that were exposed to L6 and L12, showed an opposite flux, being their respective VH the ones that showed a higher flux (p = 0.052; p = 0.020; respectively). Regarding the flux through this enzyme, expressed by the ratio between oleic acid and stearic acid, the interaction of photoperiod and the treatment significantly affected it (*PxT*, p = 0.041, two-way ANOVA). Although no changes were observed in the animals exposed to L18 or L6, those that received cherry in L12 showed statistically lower values of this parameter with respect to the VH (L-12-VH vs L12-nLC; L12-VH vs L12-LC, p = 0.009, p = 0.006, respectively).

Table 1. Liver fatty acid profile of Fischer 344 rats fed a standard diet determined by Gas Chromatography.

	L6			L12			L18			2xA
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
Σ SFA	43.46 ± 0.28 a*	43.10 ± 0.55 a	43.32 ± 0.55 a	41.87 ± 0.96 a*	42.59 ± 0.74 a	43.38 ± 0.36 a *	46.27 ± 0.34 b	43.48 ± 0.72 a	41.89 ± 0.30 a	P, T*, PxT
14:0	0.86 ± 0.10 ab	0.74 ± 0.04 a*b	0.87 ± 0.02 ab	1.00 ± 0.02 a	0.74 ± 0.07 b	0.76 ± 0.04 b	0.93 ± 0.03 ab	0.92 ± 0.09 a	0.88 ± 0.07 ab	T
15:0	0.15 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	
PA	29.40 ± 0.85 ab*	27.89 ± 0.72 a	29.08 ± 0.58 ab	29.00 ± 0.38 a	27.58 ± 0.89 a	28.48 ± 0.27 a	31.42 ± 0.53 b	29.52 ± 0.92 ab*	28.43 ± 0.27 a	P*, T
17:0	0.20 ± 0.04	0.24 ± 0.01	0.23 ± 0.01	0.23 ± 0.02	0.25 ± 0.02	0.25 ± 0.01	0.22 ± 0.01	0.22 ± 0.03	0.25 ± 0.01	
SA	12.85 ± 0.64 ab*c	14.07 ± 0.53 a	12.95 ± 0.18 ab	11.57 ± 0.61 b	13.84 ± 0.20 a	13.69 ± 0.47 a	13.52 ± 0.23 c	12.60 ± 0.81a*bc	12.11 ± 0.55 bc*	PxT
Σ MUFAS	19.65 ± 2.02 ab **	18.47 ± 1.16 a **	19.91 ± 0.42 ab	22.82 ± 1.41 b	19.10 ± 0.98 a	19.18 ± 0.69 a	19.51 ± 0.46 a	21.33 ± 1.42 ab	20.45 ± 0.77 a	PxT*
Palmitoleic Acid	5.92 ± 0.70 b	4.44 ± 0.64 a	5.15 ± 0.07 ab	5.34 ± 0.69 ab	4.17 ± 0.40 a	4.29 ± 0.33 a	5.46 ± 0.35 ab	5.97 ± 0.45 b	4.70 ± 0.36ab*	T*
OA	9.46 ± 0.89 a	8.40 ± 0.36 a	8.81 ± 0.53 a	11.71 ± 1.03 b	9.04 ± 0.60 a	9.29 ± 0.29 a	8.75 ± 0.27 a	9.39 ± 1.05 a	9.59 ± 0.90 a	
c11-18:1	4.89 ± 0.05 ab	5.00 ± 0.22ab	5.28 ± 0.23 ab	5.11 ± 0.10 ab	5.25 ± 0.11 ab	4.98 ± 0.19 b	4.67 ± 0.16 b	5.29 ± 0.21 a	5.47 ± 0.17 a	T*, PxT*
c11-20:1	0.09 ± 0.01 ab	0.08 ± 0.00 a	0.09 ± 0.00 a*b	0.10 ± 0.01 b	0.10 ± 0.01 b	0.09 ± 0.01 ab	0.08 ± 0.01 a	0.08 ± 0.00 a	0.10 ± 0.01 b	P*, PxT
Σ PUFAS-cis	33.84 ± 1.75 ab	36.91 ± 1.31 a	35.05 ± 0.96 ab	32.08 ± 0.51 b	36.87 ± 1.70 a	36.04 ± 0.90 ab*	32.39 ± 0.89 b	33.52 ± 1.66 ab	36.28 ± 0.68 a	T

(continuation)

Σ PUFAS n-6	27.53 ± 1.49 ab	30.08 ± 1.20 a	28.47 ± 0.85 ab	26.76 ± 0.48ab	30.06 ± 1.53 a	29.44 ± 0.88 a	25.99 ± 0.79 b	26.99 ± 1.28 a*b	29.72 ± 0.61 a	T
LA	10.68 ± 0.38 ab*	11.21 ± 0.95 ab	10.83 ± 0.28 ab	12.32 ± 0.78 b	11.52 ± 0.53ab	11.10 ± 0.67 ab	9.61 ± 0.43 a	10.27 ± 0.49 a	12.30 ± 0.78 b	PxT*
GLA	0.09 ± 0.01 a	0.10 ± 0.01 ab	0.11 ± 0.00 ab	0.09 ± 0.01 a	0.10 ± 0.01ab*	0.12 ± 0.00 b	0.11 ± 0.01 ab	0.11 ± 0.00 ab	0.10 ± 0.01 ab	
20:2	0.17 ± 0.01 a	0.20 ± 0.01 a	0.20 ± 0.01 a*b	0.19 ± 0.01a*b	0.20 ± 0.02 b	0.19 ± 0.02 ab	0.16 ± 0.01 a	0.18 ± 0.01 a	0.21 ± 0.01 b	T
DGLA	0.73 ± 0.06	0.71 ± 0.13	0.69 ± 0.06	0.63 ± 0.03	0.71 ± 0.05	0.68 ± 0.05	0.72 ± 0.00	0.78 ± 0.12	0.62 ± 0.09	
AA	15.39 ± 1.34 ab	17.33 ± 0.47 ab	16.28 ± 0.60 ab	12.88 ± 0.54 b	16.99 ± 1.01 a	16.79 ± 0.19 a	15.22 ± 0.23 ab	15.16 ± 1.36 ab	15.89 ± 0.82ab	T*
Σ PUFAS n-3	6.31 ± 0.28 a	6.84 ± 0.16 a	6.58 ± 0.27 a	5.42 ± 0.17 b	6.81 ± 0.18 a	6.60 ± 0.07 a	6.40 ± 0.16 a	6.53 ± 0.40 a	6.55 ± 0.28 a	T, PxT*
ALA	0.14 ± 0.02 a	0.16 ± 0.04 a	0.17 ± 0,01 a	0.25 ± 0.05 b	0.19 ± 0.02 ab	0.16 ± 0.03 a	0.14 ± 0.02 a	0.15 ± 0.03 ab*	0.23 ± 0.03 b	PxT*
EPA	0.37 ± 0.02 ac	0.38 ± 0.06 a	0.32 ± 0.01 ab	0.28 ± 0.02 bc*	0.32 ± 0.02 ab	0.30 ± 0.02 b	0.36 ± 0.01 c	0.37 ± 0.02 ac	0.28 ± 0.02 b	P*, T
22:5	0.91 ± 0.06	0.99 ± 0.04	1.00 ± 0.11	0.92 ± 0.07	0.98 ± 0.05	0.95 ± 0.08	0.80 ± 0.07	0.97 ± 0.13	1.01 ± 0.07	
DHA	4.75 ± 0.23 a	5.16 ± 0.10 a	4.94 ± 0.23 a	3.92 ± 0.10 b	5.13 ± 0.19 a	4.99 ± 0.06 a	5.00 ± 0.20 a	4.87 ± 0.34 a	4.91 ± 0.23 a	T, PxT

Proportion of fatty acids in liver expressed as % FAMEs of animals exposed to different photoperiods (short, L6; standard, L12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). ΣSFA: saturated fatty acids; ΣMUFAs: monounsaturated fatty acids; ΣPUFAs: polyunsaturated fatty acids; Δ9D: Delta 9 desaturase: Palmitoleic acid/PA; Δ6D: Delta 6 Desaturase: GLA/AA; Δ5D: Delta 5 Desaturase: AA/DGLA; 20:2, C11,c14- 20:2; 22:5, C7,c10,c13,c16,c19- 22:5; AA: arachidonic acid: c4,c8,c11,c14-20:4 n-6; ALA: α-Linolenic acid: c9,c12,c15-18:3 n-3; DGLA: Dihomo-γ-

linolenic acid: C8,c11,c14-20:3 n-6; DHA: Docosahexaenoic acid: C4,c7,c10,c12,c16,c19-22:6 n-3; SA: Stearic acid: 18:0; EPA: Eicosapentaenoic acid: c5,c8,c11,c14,c17- 20:5 n-3; GLA: gamma-linolenic acid: c6, cis-9,c12-18:3 n-6; LA: Linoleic acid: c9,c12-18:2 n-6; OA: Oleic Acid: c9-18:1; PA; Palmitic acid: 16:0. Values expressed as mean ± SEM (n=4). P, photoperiod; *T,* treatment; *PxT*, interaction of photoperiod and treatment. (Two-way ANOVA (2xA), $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA). ** Indicates trend ($0.05 < p < 0.1$) which pars in the same photoperiod.

Table 2. Muscle fatty acid profile of Fischer 344 rats fed a standard diet determined by Gas Chromatography.

	L6			L12			L18			2xA
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
ΣSFA	39.39 ± 0.44 b	41.30 ± 0.56 a	40.32 ± 0.37 ab*	40.05 ± 0.16 b	40.51 ± 0.29 ab **	41.31 ± 0.92 a **	39.22±0.24a b	40.83 ±0.07 ab #	39.89 ± 0.45 b	T, PxT*
14:0	0.83 ± 0.06 a	0.78 ± 0.04 a	0.70 ± 0.02a	1.09 ± 0.19 b	0.90 ± 0.08 ab	0.80 ± 0.02 a	0.81 ± 0.00 a	0.96 ± 0.09 ab	0.91 ± 0.06 ab	
15:0	0.13 ± 0.01	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.01*	0.17 ± 0.01*	0.16 ± 0.01	
PA	28.08 ± 0.74 a	29.64 ± 0.60 a*b	29.38 ± 0.59 ab	29.91 ± 0.61 c	29.22 ± 0.42 ac **	30.63 ± 0.53 ac **	28.47±0.46 abc*	29.36 ± 0.53 ab	28.57 ± 0.45b	P
17:0	0.18 ± 0.02ab*	0.20 ± 0.01 ab	0.21 ± 0.02 ab	0.18 ± 0.01 a	0.19 ± 0.01 ab	0.19 ± 0.02 ab	0.22 ± 0.00 b	0.21 ± 0.01 ab	0.22 ± 0.01 ab	P
SA	9.30 ± 0.50 a	9.35 ± 0.41 a	8.91 ± 0.55 ab	8.04 ± 0.84 b	9.15 ± 0.21 a	8.42 ± 0.91 a	9.83 ± 0.17 a	8.70 ± 0.64 b	9.17 ± 0.39 ab*	PxT
20:0	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.03	
22:0	0.03 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.02 ab	0.01 ± 0.00 a**	0.02 ± 0.00 a	0.05 ± 0.02 ab **	0.02 ± 0.00 a	0.03 ± 0.02 ab*	0.06 ± 0.03 b	T

(continuation)

Σ MUFAS	18.15 ± 1.18 ab	16.23 ± 0.54 a	14.62 ± 0.43 a	20.01 ± 4.56 b	18.68 ± 0.67ab #	18.29 ± 1.66 b ##	16.65 ± 0.77 a	19.40 ± 0.94ab ##	19.67 ± 1.53 ab	P
Palmitoleic Acid	3.15 ± 0.26 a	2.79 ± 0.15 a	3.05 ± 0.50a	4.76 ± 1.02 b	3.68 ± 0.36 ab #	2.96 ± 0.27 a#	3.08 ± 0.18 a	3.91 ± 0.37 ab ##	3.62 ± 0.40 ab	
OA	10.10 ± 1.04 ab*	8.53 ± 0.39 ab	7.22 ± 0.28b	13.39 ± 1.46 c	9.79 ± 0.38 ab	10.98 ± 1.93 ac	8.49 ± 0.53 ab	9.96 ± 0.60 a	10.68 ± 1.25 ab	
c11-18:1	4.25 ± 0.08 ab	4.23 ± 0.09 a	4.20 ± 0.06a	4.44 ± 0.11 ab	4.42 ± 0.05 ab	4.33 ± 0.15 ab	4.34 ± 0.07 ab	4.65 ± 0.06 c	4.56 ± 0.18 bc	P
c11-20:1	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	
Σ PUFAS-cis	40.13 ± 1.74 a	38.81 ± 3.26 ab	43.45 ± 0.21 a	34.67 ± 1.64 c	39.63 ± 0.83 b	39.60 ± 0.40 b	42.19 ± 0.52 a	38.73 ± 0.88 b	39.24 ± 1.26 b	P, PxT
ΣPUFAS n-6	27.66 ± 0.64 a	25.79 ± 2.62 a	27.65 ± 1.43 a	25.15 ± 0.95 b	27.16 ± 0.39 a	27.36 ± 1.21 a	28.46 ± 0.17 a	27.18 ± 0.47a #	27.20 ± 0.85 a	P, PxT*
LA	16.66 ± 0.19 a	15.53 ± 0.32 a*b	15.48 ± 0.53 a*b	16.21 ± 0.39 a	16.23 ± 0.24 a	14.54 ± 0.51 b	16.86 ± 0.47 a	16.48 ± 0.66 a	16.14 ± 0.49 a	P*. T
GLA	0.03 ± 0.01 b	0.04 ± 0.01 ab	0.05 ± 0.01 a	0.04 ± 0.00 ab*	0.04 ± 0.00 ab	0.03 ± 0.00 a*b	0.04 ± 0.01 ab	0.04 ± 0.01 ab	0.04 ± 0.01 ab	
DGLA	0.33 ± 0.03	0.37 ± 0.02	0.39 ± 0.00	0.30 ± 0.04	0.35 ± 0.01	0.33 ± 0.03	0.35 ± 0.02	0.34 ± 0.03	0.33 ± 0.02	
c11,c14-20:2	0.11 ± 0.01 b	0.13 ± 0.01 ab	0.14 ± 0.02 a	0.12 ± 0.01 ab	0.12 ± 0.01 ab	0.12 ± 0.00 ab	0.13 ± 0.00 ab	0.13 ± 0.00 a #	0.13 ± 0.01 a	T*
AA	9.53 ± 0.67 a	11.36 ± 0.02 a	10.54 ± 1.37 ab*	7.46 ± 0.80 b	9.35 ± 0.38 ab*	10.50 ± 0.15 a	10.03 ± 0.32 a	9.22 ± 0.83 ab	9.63 ± 0.57 ab	
c7,c10,c13, c16-22:4	0.53 ± 0.02	0.60 ± 0.03	0.57 ± 0.06	0.47 ± 0.05	0.52 ± 0.02	0.54 ± 0.06	0.51 ± 0.01	0.53 ± 0.03	0.55 ± 0.00	

(continuation)

c4,c7,c10,c13,c16-22:5	0.48 ± 0.04*	0.60 ± 0.04	0.62 ± 0.08 *	0.47 ± 0.07	0.54 ± 0.02	0.48 ± 0.08	0.54 ± 0.03	0.47 ± 0.03	0.51 ± 0.04	
ΣPUFAS n-3	12.46 ± 1.10 a	13.02 ± 0.82 a	14.38 ± 0.32 a	11.14 ± 1.70 b	12.47 ± 0.45 a	10.82 ± 1.53 b	13.73 ± 0.46 a	11.55 ± 0.49ab #	12.04 ± 0.52 ab	P, PxT
ALA	0.29 ± 0.04	0.21 ± 0.03	0.21 ± 0.01	0.33 ± 0.05	0.26 ± 0.01	0.27 ± 0.05	0.27 ± 0.04	0.32 ± 0.06	0.31 ± 0.05	
EPA	0.12 ± 0.01 ab	0.13 ± 0.01 ab	0.14 ± 0.00 a	0.11 ± 0.02 ab	0.13 ± 0.01 ab	0.10 ± 0.01 b	0.13 ± 0.01 ab	0.12 ± 0.01 ab	0.11 ± 0.00a*b ##	
c7,c10,c13,c16,c19-22:5	2.35 ± 0.20	2.81 ± 0.14	2.70 ± 0.41	2.21 ± 0.37	2.42 ± 0.12	2.26 ± 0.33	2.49 ± 0.08	2.39 ± 0.25	2.32 ± 0.14	
DHA	9.78 ± 0.84 ab	9.87 ± 0.76 ab	10.95 ± 0.11 a	8.50 ± 1.38 b	9.65 ± 0.36 ab	8.19 ± 1.22 b	10.85 ± 0.44 a	8.80 ± 0.28 a*b	9.30 ± 0.44 ab	

Proportion of fatty acids in muscle expressed as % FAMEs of animals exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). ΣSFA: saturated fatty acids ΣMUFAs: monounsaturated fatty acids; ΣPUFAs: polyunsaturated fatty acids; AA: arachidonic acid: c4,c8,c11,c14-20:4 n-6; ALA: α-Linolenic acid: c9,c12,c15-18:3 n-3; DGLA: Dihomo-γ-linolenic acid: C8,c11,c14-20:3 n-6; DHA: Docosahexaenoic acid: C4,c7,c10,c12,c16,c19-22:6 n-3; SA: Stearic acid: 18:0; EPA: Eicosapentaenoic acid: c5,c8,c11,c14,c17- 20:5 n-3; GLA: gamma-linolenic acid: c6, cis-9,c12-18:3 n-6; LA: Linoleic acid: c9,c12-18:2 n-6; OA: Oleic Acid: c9-18:1; PA; Palmitic acid: 16:0. Values expressed as mean ± SEM (n=4). P, photoperiod; *T,* treatment; *PxT*, photoperiod and treatment interaction. (Two-way ANOVA (**2xA**), $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA). ** Indicates trend ($0.05 < p < 0.1$) which pars in the same photoperiod. # Indicates significant statistical differences $p < 0.05$ and ## indicates trend $0.05 < p < 0.1$ (Student´s t-test) with respective VH.

Table 3. Liver enzymatic flux of FA biosynthesis calculated by product/substrate ratios of % FAMEs.

	L6			L12			L18			2xA
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
Scd1	0.20±0.02a*b ##	0.16±0.02a ##	0.18±0.00 a	0.19 ± 0.01 ab	0.15±0.01 a#	0.15±0.01ab #	0.17 ± 0.01 ab	0.20 ± 0.01 b ##	0.17 ± 0.01 ab*	
Scd1 OA/SA	0.75±0.11a	0.60±0.04a	0.68±0.05 a	1.03 ± 0.13 b	0.66±0.05 a	0.68±0.02a	0.65 ± 0.03 a	0.77 ± 0.14 a	0.81 ± 0.11 ab	PxT
Δ6D	0.01±0.00ab	0.01±0.00ab	0.01±0.00 a*b	0.01 ± 0.00 b	0.01±0.00 a*b	0.01±0.00a	0.01 ± 0.00 ab	0.01 ± 0.00 ab	0.01 ± 0.00 a*b	PxT*
Δ5D	21.37±2.37	26.56±4.36	24.02±2.10	21.08 ± 0.26	24.43±2.38*	25.32±2.24	21.62 ± 0.38	20.23 ± 2.08 *	27.10 ± 3.47 *	
DHA/ALA	36.52±6.17a	37.78±8.61a	30.08±2.68ab	18.85 ± 3.36 b	27.30±2.86 ab	34.96±6.97a	39.19 ± 7.15 a	37.05 ± 8.45 a	23.58 ±4.59 ab	PxT*
EPA/ALA	2.88±0.62b	3.04±1.06ab	1.96±0.15 ab	1.34 ± 0.31 b	1.71±0.16 ab	2.09±0.44ab	2.86 ± 0.58 a	2.79 ± 0.57 a	1.35 ± 0.31 a*b	
AA/LA	1.45±0.13ab*	1.58±0.12a	1.50±0.05 ab	1.12 ± 0.12 b	1.47±0.05 ab*	1.53±0.07a	1.59 ± 0.06 a **	1.50 ± 0.17 a	1.32±0.14ab **	PxT*
Elongase	0.91±0.12 ab	1.18±0.13ab	1.02±0.03 ab	1.02±0.16ab **	1.30±0.14 a	1.18±0.10a**	0.87±0.07 ab**	0.91 ± 0.10 b	1.19±0.12ab **	T*

Scd1, Stearoyl-CoA desaturase, Palmitoleic acid/ Palmitic acid; *Scd1* OA/SA, Stearoyl-CoA desaturase, oleic acid/ stearic acid; *Δ6D*, delta-6 desaturase, gamma-linolenic acid/Linoleic acid; *Δ5D*, delta-5 desaturase, arachidonic acid/ Dihomo-γ-linolenic acid; DHA/ALA, Docosahexaenoic acid /α-Linolenic acid; EPA/ALA, Eicosapentaenoic acid / α-Linolenic acid; AA/LA, Arachidonic acid/Linoleic acid; Elongase, c11-18:1/palmitoleic acid. Values expressed as mean ± SEM (n=4). P, photoperiod; T, treatment; PxT, photoperiod and treatment interaction effect. (Two-way ANOVA (**2xA**), $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA). ** Indicates trend ($0.05 < p < 0.1$) which pars in the same photoperiod. # Indicates significant statistical differences $p < 0.05$ and ## indicates trend $0.05 < p < 0.1$ (Student´s t-test).

On the other hand, the interaction of photoperiod with the treatment tended to affect the DHA/ALA and AA/LA ratios, which are indicators of the biosynthesis of PUFAs n-3 and n-6, respectively *(PxT*, p = 0.055; p = 0.087, two-way ANOVA, respectively). In this sense, the L12-nLC animals showed statistically higher values than their respective VH in both parameters (p = 0.019; p = 0.026, correspondingly). On the contrary, the L12-nLC group showed a tendency to the opposite behavior, that is, to a lower the synthesis of PUFAs n-6, by presenting lower values than their respective VH in this parameter (p = 0.095).

Table 4. Pathway analysis of liver FA profile of nLC vs VH in L18 determined by to Metaboanalyst software.

Pathway Name	Match Status	p	-Log(p)	FDR	Impact
FA elongation	1/39	0.002	2.654	0.007*	0.0
FA degradation	1/39	0.002	2.654	0.007*	0.0
LA metabolism	1/5	0.020	1.686	0.041*	1.0
FA biosynthesis	2/47	0.025	1.594	0.041*	0.014
Biosynthesis of unsaturated FA	10/36	0.029	1.528	0.041*	0.0
ALA metabolism	1/13	0.102	0.987	0.120	0.333
AA metabolism	1/36	0.506	0.295	0.506	0.332

The levels of twenty-seven hepatic fatty acids (FA) from animals exposed 18 hours of light (L18) and treated with nLC or VH were exposed to pathway analysis using Metaboanalyst. Main metabolic pathways significantly affected, the total amount of metabolites involved in the pathway, the amount of metabolites that have information, the p value, false discovery rate (FDR), and impact, are shown. ALA: alpha-Linoleic acid; LA: Linolenic acid; AA: Arachidonic acid. p value < 0.05 and FDR < 0.05 were considered significant (*). Kyoto encyclopedia of Genes and Genomes (KEGG) was database utilized.

The treatments evaluated tended also to significantly affect the flux through the enzyme elongase, estimated from the ratio c11-18:1/PA, indicator of *de novo* biosynthesis of FA (*T*, p = 0.055, two-way ANOVA). Specifically, the L18-nLC animals showed statistically higher values than their respective VH. Also, the consumption of LC produced such effects in the animals (p = 0.062).

The consumption of nLC in season mainly affected the metabolism of LA, while the intake of LC did not produce changes.

Because of the significant changes in the FA profile in the liver and muscle of the treated animals, we performed a pathway analysis to clarify the affected pathways in the usual season of cherry consumption, L18. Table 4 shows the main metabolic pathways that had significant changes when comparing animals treated with nLC and VH. Although several pathways seem to be affected, only LA metabolism has a significant FDR and an impact of 1. In Fig.2-a and Fig.2-b, an overview of the pathway analysis and detailed pathway of LA metabolism are shown, respectively. However, when performing the pathway analysis between the L18-LC and L18-VH groups, no significant alterations were observed in any metabolic pathway (Supplementary Table 3 and Supplementary Fig.1).

The consumption of LC in season increased the expression of hepatic Scd1 and that of AdipoR2 in muscle

Due to the changes observed in the lipid profile, enzymes flux and affected pathways in relation to FA, we performed a gene expression analysis of hepatic enzymes of interest. As shown in Fig.3-a, the hepatic expression of the *Scd1* enzyme tends to be affected by the treatment (*T*, p = 0.057, two-way ANOVA), since the consumption of LC in season has increased it significantly with respect to their VH (p = 0.028). However, this effect was not observed when the animals ingested it at L12 or L6, or even when they consumed nLC at L18. Therefore,

these results points to a differential effect of the consumption of locally produced cherries in season over their consumption out of season compared to their respective VH or even the consumption of cherries from different geographical origins.

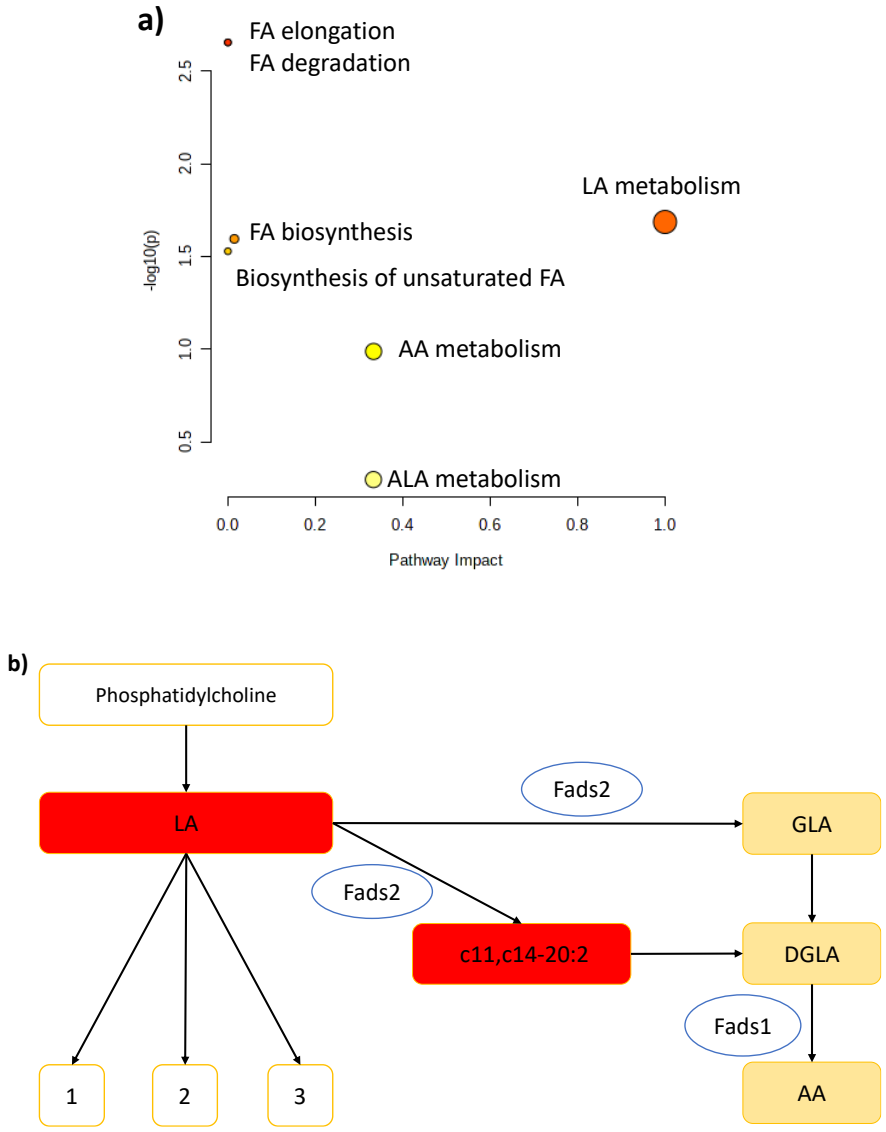

Fig.2. Pathway analysis based on data from twenty-seven hepatic fatty acid (FA) of the L18-nLC vs L18-VH groups. Overview of pathway analysis (**a**) and pathway of linoleic acid metabolism (**b**) from Kyoto encyclopedia of Genes and Genomes

(KEGG) database. Red boxes indicate increase of FA in L18-nLC animals; white boxes indicate no changes of lipid classes. **1,** (9R,10S)-(12Z)-9,10-Epoxyoctadecenoic acid; **2**, (12R,13S)-(9Z)-12,13-Epoxyoctadecenoic acid; **3**, (9Z,11E)-(13S)-13-Hydroperoxyoctadeca-9,11-dienoic acid; **AA**, arachidonic acid: c4,c8,c11,c14-20:4 n-6; LA, Linolenic acid: c9,c12-18:2; **ALA:** α-linoleic acid: c9,c12,c15-18:3 n-3; **DGLA**, Dihomo-γ-linolenic acid: C8,c11,c14-20:3; **GLA**, gamma-linolenic acid: c6, cis-9,c12-18:3 n-6; ***Fads1***; Δ-5 desaturase; ***Fads2***, Δ-6 desaturase.

On the other hand, hepatic *Fads2* mRNA concentration was significantly affected by both photoperiod and treatment (*P*, p = 0.009; *T*, p = 0.027; two-way ANOVA), since L18-nLC animals showed lower enzyme expression than those L18-LC and tended to express lower than their respective VH (p = 0.027; p = 0.072, respectively) (**Fig.3-b**). However, out of season this effect has not been observed. In contrast, at L6 and L12, LC consumption tended to increase the gene expression of this desaturase compared to their respective VH (p = 0.067; p = 0.086, respectively).

As shown in **Fig.4-a**, the gene expression of *Fads1* was decreased with cherry consumption, regardless of its origin, in L6 (L6-VH vs L6-LC; L6-VH vs L6-nLC, p = 0.023). However, no statistically significant effect of treatment, photoperiod, or intersection of both was observed by two-way ANOVA. The expression of *Elovl2* was not observed to be modified in any of the experimental groups (**Fig.4-b**).

As previously said the gastrocnemius muscle is one of the main catabolic tissues of FA, so we decided to analyze the gene expression of transporters as well as those key genes involved in oxidation processes. Gene expressions of gastrocnemius muscle enzymes and transporters were differently affected. On the one hand, the mRNA concentration of *AdipoR2* was significantly increased in the animals that consumed LC compared to those that ingested VH (p = 0.011), while it remained in trend with those L18-nLC (p = 0.099) (**Fig.5-a**). These differential effects of the treatment were not observed in the other

photoperiods, even in L12 the behavior of the animals was completely the opposite, although it did not reach statistical significance.

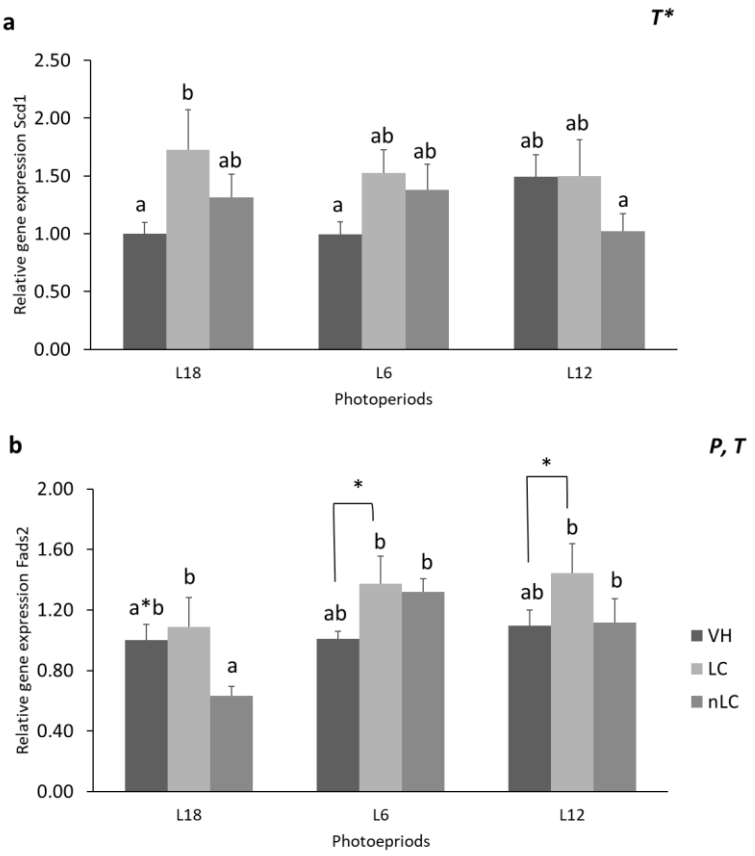

Fig 3. Genetic expression of liver enzymes related to biosynthesis of unsaturated fatty acids. The mRNA levels of Δ-9 desaturase (Scd1) (a), Δ-6 desaturase (Fads2) (b) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), Local (LC) or non-Local cherries (nLC), and exposed to different photoperiods (short; L6, standard; L12 or long; L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L18-VH group. P, photoperiod effect; T, treatment effect (two-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicates trend ($0.05 < p < 0.1$).

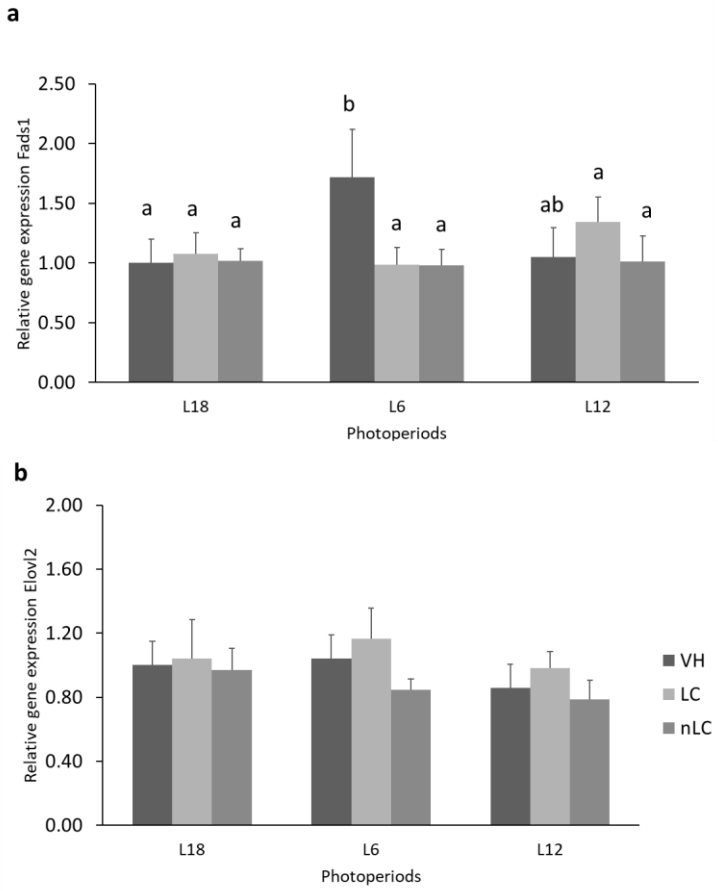

Fig 4. Genetic expression of liver enzymes related to biosynthesis of unsaturated fatty acids. The mRNA levels of Δ-5 desaturase (Fads1) (**a**) and Elongation of very-long-chain fatty acid enzyme 2 (Elovl2) (**b**) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), Local (LC) or non-Local cherries (nLC), and exposed to different photoperiods (short; L6, standard; L12 or long; L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L18-VH group. P, photoperiod effect; T, treatment effect (two-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicates trend ($0.05 < p < 0.1$).

Similarly, animals that consumed LC in season also tended to have increased gene expression of the muscle FA transporter, *FAT/cd36*, compared to their respective VH ($p = 0.098$). Likewise, the L18-nLC animals also showed such an effect ($p = 0.039$, Student's t-test). In contrast, those exposed to L6 and

ingested VH showed a higher concentration of mRNA than those who received nLC (p = 0.078) (**Fig.5-b**).

Photoperiod significantly affected muscle gene expression of *Acc2* (*P*, p = 0.004, two-way ANOVA). As observed in **Fig.6-a**, the animals exposed to L18 and consumed cherry, showed a higher concentration of mRNA of the enzyme than those exposed to L6 or L12. It should be noted that the L6-LC group tended to present a lower gene expression than its respective VH (p = 0.065).

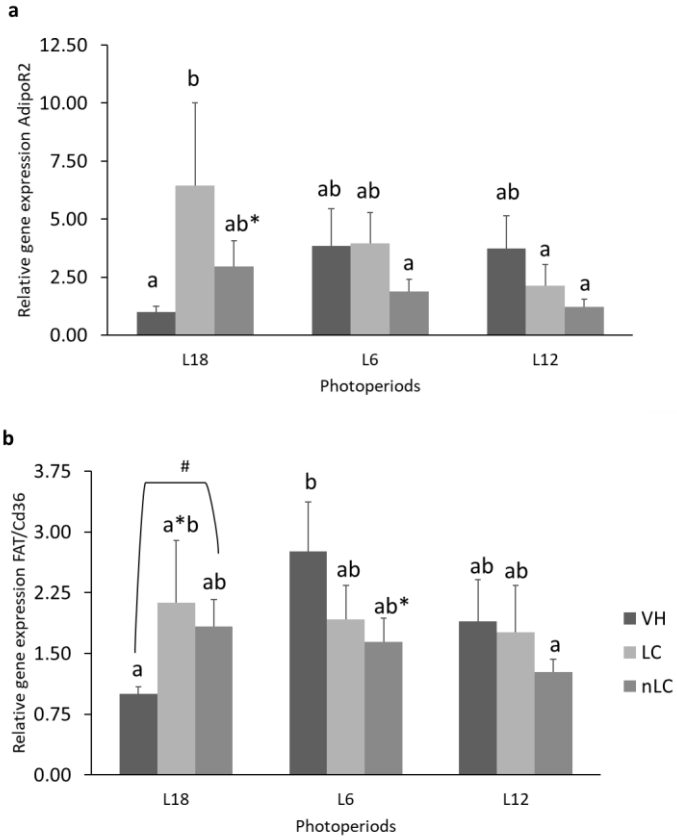

Fig.5. Genetic expression of muscle enzymes related to lipid metabolism. The mRNA levels of Adiponectin receptor 2 (*AdipoR2*) (**a**), Fatty acid translocase, homologue of CD36 (FAT/cd36) (**b**) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), Local (LC) or non-Local cherries (nLC), and exposed to different photoperiods (short; L6, standard; L12 or long; L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L18-VH group. *P*, photoperiod effect (two-way ANOVA, p < 0.05). Different letters above the bars

indicate significant differences ($p < 0.05$, post-hoc DMS, one-way ANOVA). * Indicates trend ($0.05 < p < 0.1$). # Indicates significant differences ($p < 0.05$ Student´s t-test). ## Indicates trend ($0.05 < p < 0.1$, Student´s t-test).

On the other hand, the mRNA concentration of Citrate Synthase (*Cs)* increased significantly in the animals that received nLC compared to those that received LC ($p = 0.044$, Student's t-test) (Fig.6-b). In addition, they tended to have a higher expression in relation to their respective VH ($p= 0.067$, Student's t-test).

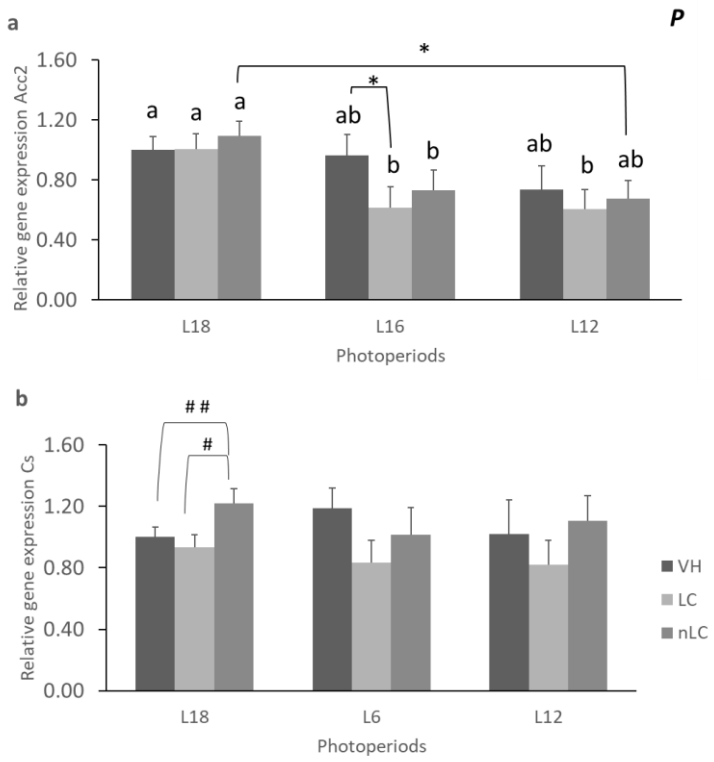

Fig.6. Genetic expression of muscle enzymes related to lipid metabolism. The mRNA levels of Acetyl-CoA carboxylase 2 (*Acc2*) (a) and Citrate Synthase (*Cs*) (b) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), Local (LC) or non-Local cherries (nLC), and exposed to different photoperiods (short; L6, standard; L12 or long; L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L18-VH group. *P*, photoperiod effect (two-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p <$

0.05, post-hoc DMS, one-way ANOVA). * Indicates trend (0.05 < p < 0.1). # Indicates significant differences (p < 0.05 Student´s t-test). ## Indicates trend (0.05 < p < 0.1, Student´s t-test).

Multivariate Chemometric Analysis of NMR Data

Due to the observed changes in hepatic and muscle FA profile, in the gene expression of enzymes and metabolic pathways, we decided to study the hydrophilic and lipophilic metabolites in both serum and liver to try to unravel the mechanisms involved in the observed effects. From the list of metabolites evaluated in the NMR method, after alignment and normalization of the spectra, a total of 49 and 54 serum and liver metabolites were found in the samples, respectively. In the serum aqueous phase, 34 metabolites were identified and integrated, and 15 metabolites in the lipid phase. (**Supplementary Table 4 and Table 5, respectively).** In the liver aqueous phase, 44 metabolites were identified and integrated, and 10 metabolites in the lipid phase (**Supplementary Table 6 and Table 7).** Specifically, amino acids and intermediate metabolites of energetic processes have been identified, such as pyruvate, succinate or citrate in the aqueous phase and mainly lipid molecules such as cholesterol, total phospholipids, sphingomyelin, total FA, among others, were determined in the hydrophobic phase. Interestingly, total phospholipids or sphingomyelin have been identified, without determining the specific compounds, which would have provided clearer information on the enzymes and pathways affected.

Multivariate analysis showed a differential effect on serum metabolite homeostasis between treatments at different photoperiods.

Aiming to determine how the type of cherry influenced the homeostatic balance of the metabolites depending on the photoperiod, first, we analyzed

by PLS-DA the hydrophilic and lipophilic metabolites, both in serum (**Fig.7**) and in liver (**Fig.8**).

Notably, when analyzing the serum metabolites, a clear grouping of the animals that consumed cherry, both LC and nLC, can be observed, in L6 and also in L18. The explained variability was 23.1% and 32.1%, **(Fig.7-a** and **Fig.7-c**, respectively). In the constructed PLS-DA model, for de L6 group, the R2 and Q2 values were 0.654 and 0.238 employing the 1sh principal component, and for de L18 group the values were 0.977 and 0.434, employing the 5th principal component. However, at L12 the experimental groups did not show a clear differentiation.

In the liver, although the model was not significant, a clear grouping and separation of the animals that have consumed LC in L6 and L18 with respect to their respective VH was observed **(Fig.8-a; Fig.8-c**). In the case of animals exposed to L12, both the LC and nLC groups seem to differ from the VH (**Fig.8-b**). The explained variability in L6, L12 and L18 was 27%, 15.9% and 20.1%, respectively.

DISCUSSION

In the present study we have evaluated the effect of the consumption of cherries from different origins, as well as the consequences of their intake in and outside of their usual consumption season, on the FA profile, on the gene expression of key enzymes and transporters in the lipid metabolism in muscle and liver and on hepatic and serum metabolites.

We have observed that the consumption of cherries in season, L18, regardless of the origin, decreases the proportion of SFA in the liver, mainly due to a decrease in palmitic acid (PA) while animals that consume them presented a higher content of c11-18:1. It is important to highlight that PA is synthesized *de novo* by the enzyme fatty acid synthase (Fas1), to be later modified by

successive steps of elongation and/or desaturation. In this sense, the enzyme Scd1 is key in introducing the first *cis* bond at position 9 of PA, to form palmitoleic acid [31,32]. It is believed that due to its autocrine and/or paracrine characteristics and its physiological effects on cellular homeostasis, palmitoleic acid remains in low concentrations in hepatocytes, as a consequence of its rapid β-oxidation or formation to c11-18 by elongation [32,33]. It is likely that for this reason, in the present study we could not observe differences in the proportion of palmitoleic acid, while there was a higher level of c11-18:1 in those who cherries at L18. Therefore, we could infer that the lower amount of PA in the treated animals is due to the fact the metabolism is driven to a greater establishment and synthesis of c11-18:1. It should be noted that the L18-LC group also presented a higher flow of Scd1, estimated by the palmitoleic acid/PA ratio, as well as a higher gene expression. In agreement with our results, other authors have described that the activity of Scd1 is in parallel associated with an increase in the amount of its mRNA in the liver [34]. There is evidence that associates an increase in *Scd1* activity with increased lipogenesis, hepatic steatosis, fat accumulation and metabolic disorders. However, these consequences are associated with increased OA and not with c11-18:1 [35]. Some studies inversely correlate the content of c11-18:1 in blood cells with myocardial risk and suppression of lipogenesis [36,37]. In contrast, although total hepatic oleate strongly correlates with TAG accumulation in the liver, as well as with higher adipose tissue and OA content in circulating TAG, an increase in PA has not shown these correlations [35]. It should be noted that in L12 the animals that consumed cherry, regardless of the origin, showed a lower amount of OA in liver than their respective VH. In this sense, some authors affirm that OA induces autophagy in hepatocytes [38], while others propose that due to the high lipotoxicity that it produces in cells, it is rapidly esterified to TAG [39]. Therefore, our results suggest that cherry consumption at L12 would lead to lower toxicity and predisposition to autophagy compared

to VH consumption. However, further studies, such as the analysis of TAG composition, or the activity of autophagic enzymes, are required to clarify this suggestion.

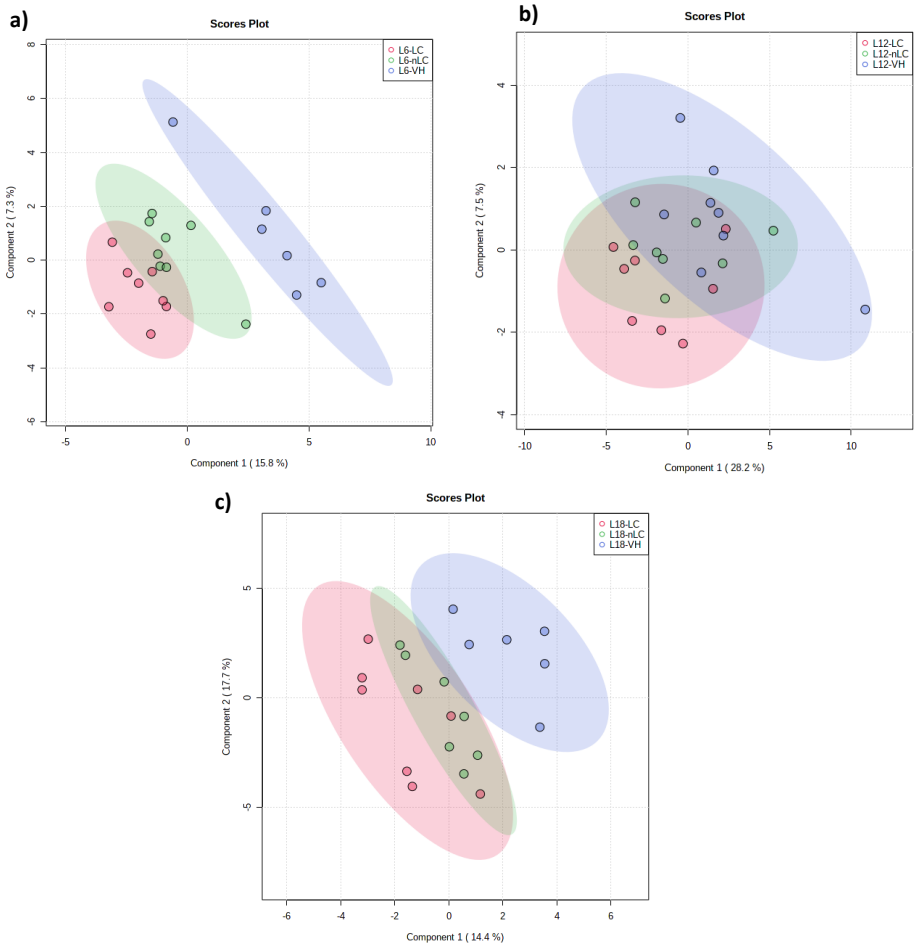

Fig.7. PLS-DA of the forty-nine metabolites (hydrophilic and lipophilic) determined in serum rats Fischer 344 fed with vehicle (VH), local cherry (LC), or non-Local Cherry (nLC), and exposed to different photoperiod with 6 (L6) (**a**), 12 (L12) (**b**) or 18 (L18) (**c**) hours of light.

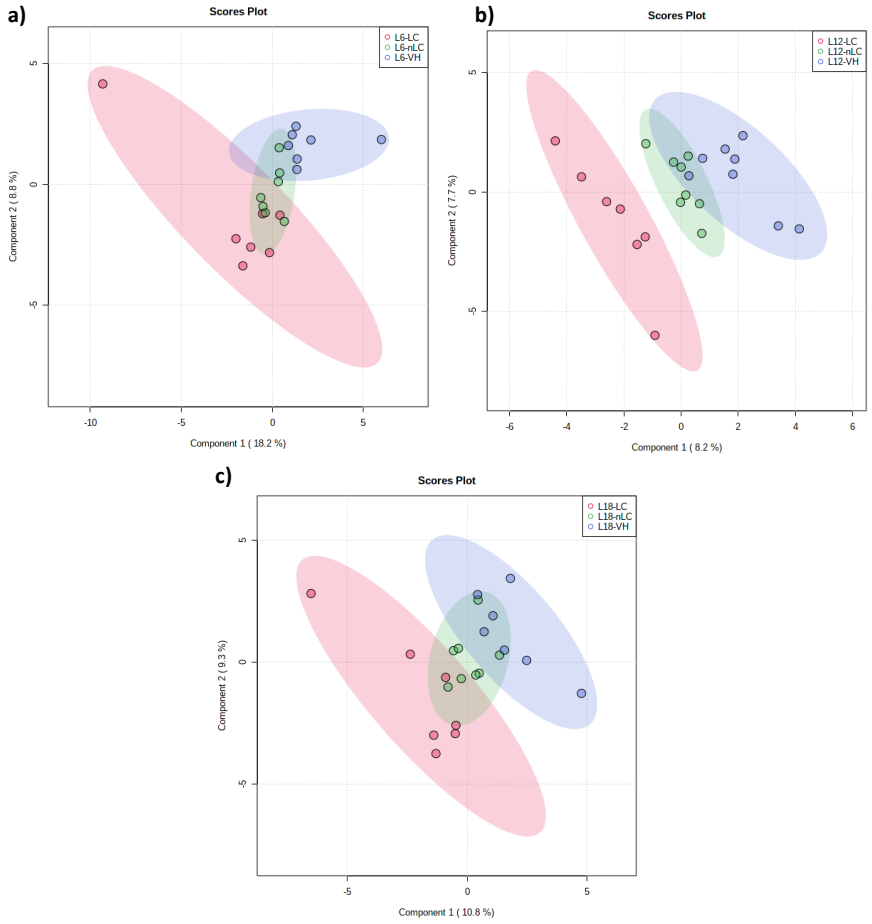

Fig.8. PLS-DA of the fifty-four metabolites (hydrophilic and lipophilic) determined in liver rats Fischer 344 fed with vehicle (VH), local cherry (LC), or non-Local Cherry (nLC), and exposed to different photoperiod with 6 (L6) (**a**), 12 (L12) (**b**) or 18 (L18) (**c**) hours of light.

At the muscular level, the L18-LC group, despite not differing in the amount of SFA, tended to have a higher expression of the FA transporter FAT/CD36 and a higher content of FA 15:0 with respect to its VH. On the contrary, they present less SA. Recently, some authors have positively associated the muscle FA 15:0 level with increased glucose uptake, improved insulin sensitization in muscle cells, and a lower incidence of diabetes [40].

However, a higher SA content is associated with serious metabolic pathologies [41–43] Nevertheless, such an effect was not observed in the group supplemented with nLC at L18, pointing to an influence of the different phenolic content in both fruits. Interestingly, animals exposed to L6 or L12 showed a slightly greater difference in SA compared to their respective VH, despite the absence of a photoperiod effect. These results provide evidence on the importance of the moment of fruit consumption in its season.

On the other hand, MUFAs and PUFAs do not have the same lipotoxic and dysfunctional effect as long-chain SFAs, but instead promote good metabolic health by mitigating the adverse effects of SFA [44,45]. In muscle, for example, unsaturated FAs antagonize the excessive production of free oxygen radicals (ROS) produced by SFA [46,47], promote the synthesis of neutral TAG [48], allow greater oxidation of SFA [49] and prevent the loss of muscle mass [50]. In this sense, the animals from the L18-LC group presented a higher content of palmitoleic acid and c11-18:1 in the muscle in relation to their respective VH. It has been observed that the incubation of muscular cells with palmitoleic acid antagonizes the morphological, biochemical and functional mitochondrial changes produced by SFA [45]. Therefore, these results suggest that the origin of the cherry and its polyphenolic profile could have a differential effect on the metabolism and transport of FA from the liver to the muscle.

Contrarily, those animals that consumed cherries at L12 presented less amount of palmitoleic acid than their respective VH in muscle, while the content of c11-18:1 did not change. Notably, there is an effect of the photoperiod on the proportion of palmitoleic acid, since the VH groups significantly differed in their content depending on the photoperiod, being higher at L12. It should be noted that the proportions of palmitoleic acid in the animals treated with cherries in photoperiods, L12 and L18, are similar. In this sense, other authors observed that polyphenols cause a decrease in MUFAs, mainly palmitoleic acid, *in vitro* in liver cells [11]. Therefore, since the animals that consumed VH at L12 and

L18 differ in the content of palmitoleic acid, while those treated with cherry are similar to each other between the different photoperiods, it could be inferred that the consumption of cherry would modulate the content of MUFAS, mainly palmitoleic acid, regardless of the photoperiod. However, animals exposed to L6 do not show changes between treatments, suggesting that exposure to 12 hours of light and darkness has a more important effect on the level of palmitic acid than the rest of the photoperiods. However, more research is suggested to provide evidence to understand the mechanisms involved.

On the other hand, the L18-nLC animals showed a higher hepatic proportion of cis PUFAs, a higher content of n-6 PUFAs than their respective VH. Specifically, they present more LA and c11,c14-20:2 n-6. However, they also have a higher proportion of ALA and 22:5 n-3 and a lower expression of *Fads2*, which is key for PUFAs synthesis. It should be noted that the pathway analysis reflected an alteration in LA metabolism among these experimental groups. In agreement with our results, other authors observed that the treatment of hepatocytes with delphinidin-3-*O*-glucoside reduced the expression of *Fads2* and the level of EPA [51,52]. The difference in the content of this type of anthocyanidins between the different types of cherries could determine this differential effect. Furthermore, considering the evaluated hepatic hydro and lipophilic metabolites, the L18-nLC group also had higher total cholesterol, free cholesterol, total phospholipids, phosphatidylethanolamine, phosphatidylcholine, sphingomyelin, and asparagine than their respective VH. Sphingolipids, glycerolipids and phospholipids are part of bioactive membrane constituents, which by forming lipid rafts regulate their fluidity and serve as first and/or second messengers of different pathways [53]. The condensation of serine with PA is the first step in the formation of ceramides. Depending on the stimulation and cell homeostasis, ceramides are involved in different metabolic pathways, where phosphatidylcholine and sphingomyelin are involved [26] Considering that all the animals received the same nutritional

composition of FA, the differential effect on the content of PUFAs as well as on the amount of hepatic hydro and lipophilic metabolites would be given by the different types of cherry and the season consumed. In this sense, nLC has a higher amount of flavonols, specifically quercetin, than LC. Flavonols have been documented to modulate the expression of desaturases, lipogenic enzymes, and transcription factors in vitro in hepatocytes and adipocytes. [10,54]. However, there is limited evidence on (poly)phenol intake, circadian rhythm, and hepatic FA profile. Being concerned about the different metabolic pathways in which these lipid substances are involved in (**Fig. 1**), future determinations on the composition of phospholipids, glycerolipids, TAG, VLDL and hepatic lipoproteins are needed to elucidate the molecular and biochemical mechanisms involved.

At the muscular level, a clear effect of seasonal treatment is observed, since both groups that ingested cherry have lower amounts of total PUFAS than their respective VH. Although there are no significant differences in the different FA, the L18-LC animals tended to have lower muscle DHA than the VH. Furthermore, increased gene expression of the adiponectin receptor, AdipoR2, is observed in these animals. In agreement with our results, it has been observed that anthocyanins, which are present in greater quantities in LC, have preventive effects on metabolic disorders, due to their influence on pathways that are mainly involved in adiponectin and AMPK activation [55]. It should be noted that adiponectin, through its receptor, activates the metabolic pathways of AMPK and PPAR, which produces a greater uptake of blood glucose due to a greater translocation of the GLUT4 transporter towards the membrane surface and a greater β-oxidation of FA [56,57]. Consequently, it modulates insulin sensitivity and has an important influence on lipid and glucose homeostasis. L18-nLC animals tend to have a lower proportion of EPA and DHA and a higher gene expression of Cs, which are key in the oxidative metabolism of muscle FA than their respective VH. Considering that n-3 PUFAs are predominantly

oxidized above other FA [58], it can be inferred that cherry consumption, regardless of origin, increases the oxidation of n-3 PUFAs. In this sense, other authors have documented that the consumption of polyphenols, proanthocyanidins, improves the muscular oxidative capacity of obese rats, through the phosphorylation of AMPK [59]. More studies are needed to clarify the mechanisms involved. On the contrary, the animals exposed to L12 that ingested cherries, present a higher level of total cis PUFAs, mainly in PUFAS n-6 than their respective VH. However, there is a clear effect of the photoperiod, since the VH of L12 and L18 were also affected. It should be noted that the animals treated in L12 present similar values as the rest of the groups supplemented in L18, showing once again the influence of fruit consumption in a season that does not correspond to its usual harvest and consumption.

CONCLUSIONS

In the present study we have determined the consequences of the consumption of cherries from different origins and the effect of their intake in different photoperiods, long (L18), standard (L12) and short (L6), on the FA profile and expression of key enzymes and transporters in lipid metabolism in liver and muscle, as well as on hydro and lipophilic serum and hepatic metabolites of Fischer 344 rats. We have observed that the intake of cherry in season, regardless of origin, decreases the content of hepatic SFA, mainly PA, possibly due to a greater unsaturation by action of the Scd1 enzyme. At the same time, a lower content of total muscle PUFAs is observed, possibly due to the action of polyphenols that improve oxidation. In addition, the L18-nLC group showed changes in the metabolism of LA with respect to its VH. On the other hand, the animals that consumed cherry at L12, showed a higher proportion of liver and muscle SFA, mainly SA, and a lower enzymatic flow of *Scd1*. However, no significant changes were observed in animals exposed to L6.

Because FA are involved in multiple cellular functions, more studies are needed to clarify the mechanisms involved.

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UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

SUPPLEMENTARY MATERIAL

Table 1. Nucleotide sequences of primers used for real time quantitative PCR.

Gene	*Forward Primer (5' to 3')*	*Reverse Primer (5' to 3')*	*Tissue*
Acc2	CTTTTCTAGGTCCCCGAGTGA	CTTCCGCTCCAGGGTAGAGTT	*M*
AdipoR2	CCACACAACACAAGAATCCG	CCCTTCTTCTTGGGAGAATGG	*M*
CS	CCGTGCTCATGGACTTGGGCCTT	CCCCTGGCCCAACGTAGATGCTC	*M*
Elovl2	TTTGGCTGTCTCATCTTCCA	GGGAAACCATTCTTCACTTC	*L*
Fads1	TACAGGCAACCTGCAACGTTC	GGTGCCACCTTGTGGTAGTTGT	*L*
Fads2	GGAACCATCGACATTTCCAG	TCTTTATGTCGGGGTCCTTG	*L*
FAT/CD36	GTCCTGGCTGTGTTTGGA	GCTCAAAGATGGCTCCATTG	*M*
Scd1	GAGGAACATCATTCTCATGGTCC	CGTACACGTCATTCTGGAACG	*L*

The table shows the nucleotide sequences of primers used for PCR amplification in liver (L) and gastrocnemius muscle (M) tissues. *Acc2*, Acetyl-CoA carboxylase 2; *AdipoR2*, Adiponectin receptor 2; *CS*, Citrate Synthase; *Elovl2*, Elongation of very-long-chain fatty acid enzyme 2; *Fads1*, Δ-5 desaturase; *Fads2*, Δ-6 desaturase; *FAT/CD36*, fatty acid translocase, homologue of CD36; *Scd1*, Desaturase Δ-9.

Table 2. Liver fatty acid profile of Fischer 344 rats fed a standard diet

Fatty Acid	L6			L12			L18			*2xA*
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
Σ SFA	43,46 ± 0,28 a*	43,10 ± 0,55 a	43,32 ± 0,55 a	41,87 ± 0,96 a*	42,59 ± 0,74 a	43,38 ± 0,36 a*	46,27 ± 0,34 b	43,48 ± 0,72 a	41,89 ± 0,30 a	P, T*, PxT
14:0	0,86 ± 0,10 ab	0,74 ± 0,04 a*b	0,87 ± 0,02 ab	1,00 ± 0,02 a	0,74 ± 0,07 b	0,76 ± 0,04 b	0,93 ± 0,03 ab	0,92 ± 0,09 a	0,88 ± 0,07 ab	T
15:0	0,15 ± 0,02	0,15 ± 0,01	0,17 ± 0,01	0,17 ± 0,01	0,17 ± 0,01	0,16 ± 0,01	0,16 ± 0,01	0,18 ± 0,01	0,19 ± 0,01	
PA	29,40 ± 0,85 ab*	27,89 ± 0,72 a	29,08 ± 0,58 ab	29,00 ± 0,38 a	27,58 ± 0,89 a	28,48 ± 0,27 a	31,42 ± 0,53 b	29,52 ± 0,92 ab*	28,43 ± 0,27 a	P*, T
17:0 ISO	0,28 ± 0,01	0,27 ± 0,04	0,29 ± 0,02	0,26 ± 0,01	0,27 ± 0,03	0,26 ± 0,01	0,37 ± 0,02	0,31 ± 0,02	0,27 ± 0,01	
17:0	0,20 ± 0,04	0,24 ± 0,01	0,23 ± 0,01	0,23 ± 0,02	0,25 ± 0,02	0,25 ± 0,01	0,22 ± 0,01	0,22 ± 0,03	0,25 ± 0,01	
SA	12,85±0,64a b*c	14,07 ± 0,53a	12,95 ± 0,18ab	11,57 ± 0,61b	13,84 ± 0,20a	13,69 ± 0,47a	13,52 ± 0,23c	12,60 ± 0,81a*bc	12,11±0,55bc*	PxT
Σ MUFAS	19,65 ± 2,02 ab **	18,47 ± 1,16 a **	19,91 ± 0,42 ab	22,82 ± 1,41 b	19,10 ± 0,98 a	19,18 ± 0,69 a	19,51 ± 0,46 a	21,33 ± 1,42 ab	20,45 ± 0,77 a	PxT*
c9-14:1	0,07 ± 0,02	0,05 ± 0,01	0,07 ± 0,00	0,08 ± 0,02	0,05 ± 0,01	0,05 ± 0,01	0,07 ± 0,00	0,06 ± 0,02	0,05 ± 0,01	
Palmitoleic acid	5,92 ± 0,70 b	4,44 ± 0,64 a	5,15 ± 0,07 ab	5,34 ± 0,69 ab	4,17 ± 0,40 a	4,29 ± 0,33 a	5,46 ± 0,35 ab	5,97 ± 0,45 b	4,70 ± 0,36 ab*	T*
c9-17:1	0,19 ± 0,03	0,21 ± 0,01	0,22 ± 0,01	0,24 ± 0,03	0,24 ± 0,01	0,22 ± 0,01	0,24 ± 0,01	0,23 ± 0,00	0,23 ± 0,01	
c6-18:1	0,09 ± 0,01	0,11 ± 0,02	0,09 ± 0,01	0,09 ± 0,01	0,09 ± 0,01	0,10 ± 0,01	0,15 ± 0,02	0,14 ± 0,02	0,10 ± 0,01	
OA	9,46 ± 0,89 a	8,40 ± 0,36 a	8,81 ± 0,53 a	11,71 ± 1,03 b	9,04 ± 0,60 a	9,29 ± 0,29 a	8,75 ± 0,27 a	9,39 ± 1,05 a	9,59 ± 0,90 a	
c11-18:1	4,89 ± 0,05 ab	5,00 ± 0,22ab	5,28 ± 0,23 ab	5,11 ± 0,10 ab	5,25 ± 0,11 ab	4,98 ± 0,19 b	4,67 ± 0,16 b	5,29 ± 0,21 a	5,47 ± 0,17 a	T*, PxT*
c12-18:1	0,17 ± 0,03	0,19 ± 0,01	0,20 ± 0,02	0,17 ± 0,01	0,17 ± 0,01	0,17 ± 0,02	0,16 ± 0,02	0,19 ± 0,02	0,20 ± 0,02	

(continuation)

c11-20:1	0,09 ± 0,01 ab	0,08 ± 0,00 a	0,09 ± 0,00 a*b	0,10 ± 0,01 b	0,10 ± 0,01 b	0,09 ± 0,01 ab	0,08 ± 0,01 a	0,08 ± 0,00 a	0,10 ± 0,01 b	P*, PxT
Σ PUFAS-cis	33,84 ± 1,75 ab	36,91 ± 1,31 a	35,05 ± 0,96 ab	32,08 ± 0,51 b	36,87 ± 1,70 a	36,04 ± 0,90 ab*	32,39 ± 0,89 b	33,52 ± 1,66 ab	36,28 ± 0,68 a	T
Σ PUFAS n-6	27,53 ± 1,49 ab	30,08 ± 1,20 a	28,47 ± 0,85 ab	26,76 ± 0,48 ab	30,06 ± 1,53 a	29,44 ± 0,88 a	25,99 ± 0,79 b	26,99 ± 1,28 a*b	29,72 ± 0,61 a	T
LA	10,68 ± 0,38 ab*	11,21 ± 0,95 ab	10,83 ± 0,28 ab	12,32 ± 0,78 b	11,52 ± 0,53 ab	11,10 ± 0,67 ab	9,61 ± 0,43 a	10,27 ± 0,49 a	12,30 ± 0,78 b	PxT*
GLA	0,09 ± 0,01 a	0,10 ± 0,01 ab	0,11 ± 0,00 ab	0,09 ± 0,01 a	0,10 ± 0,01 ab*	0,12 ± 0,00 b	0,11 ± 0,01 ab	0,11 ± 0,00 ab	0,10 ± 0,01 ab	
c11,c14-20:2	0,17 ± 0,01 a	0,20 ± 0,01 a	0,20 ± 0,01 a*b	0,19 ± 0,01 a*b	0,20 ± 0,02 b	0,19 ± 0,02 ab	0,16 ± 0,01 a	0,18 ± 0,01 a	0,21 ± 0,01 b	T
DGLA	0,73 ± 0,06	0,71 ± 0,13	0,69 ± 0,06	0,63 ± 0,03	0,71 ± 0,05	0,68 ± 0,05	0,72 ± 0,00	0,78 ± 0,12	0,62 ± 0,09	
AA	15,39 ± 1,34 ab	17,33 ± 0,47 ab	16,28 ± 0,60 ab	12,88 ± 0,54 b	16,99 ± 1,01 a	16,79 ± 0,19 a	15,22 ± 0,23 ab	15,16 ± 1,36 ab	15,89 ± 0,82 ab	T*
c13,c16-22:2	0,10 ± 0,03	0,10 ± 0,03	0,10 ± 0,03	0,09 ± 0,01	0,09 ± 0,02	0,11 ± 0,03	0,08 ± 0,06	0,14 ± 0,03	0,15 ± 0,02	
c7,c10,c13,c16-22:4	0,37 ± 0,04	0,42 ± 0,03	0,44 ± 0,04	0,41 ± 0,00	0,45 ± 0,04	0,46 ± 0,05	0,41 ± 0,01	0,39 ± 0,02	0,45 ± 0,03	
Σ PUFAS n-3	6,31 ± 0,28 a	6,84 ± 0,16 a	6,58 ± 0,27 a	5,42 ± 0,17 b	6,81 ± 0,18 a	6,60 ± 0,07 a	6,40 ± 0,16 a	6,53 ± 0,40 a	6.55 ± 0.28 a	T, PxT*
ALA	0,14 ± 0,02 a	0,16 ± 0,04 a	0,17 ± 0,01 a	0,25 ± 0,05 b	0,19 ± 0,02 ab	0,16 ± 0,03 a	0,14 ± 0,02 a	0,15 ± 0,03 ab*	0,23 ± 0,03 b	PxT*
EPA	0,37 ± 0,02 ac	0,38 ± 0,06 a	0,32 ± 0,01 ab	0,28 ± 0,02 bc*	0,32 ± 0,02 ab	0,30 ± 0,02 b	0,36 ± 0,01 c	0,37 ± 0,02 ac	0,28 ± 0,02 b	P*, T
c 22:3	0,09 ± 0,00	0,10 ± 0,01	0,12 ± 0,01	0,08 ± 0,00	0,12 ± 0,01	0,11 ± 0,01	0,12 ± 0,01	0,11 ± 0,01	0,09 ± 0,01	

(continuation)

c7,c10,c13,c16,c19-22:5	0,91 ± 0,06	0,99 ± 0,04	1,00 ± 0,11	0,92 ± 0,07	0,98 ± 0,05	0,95 ± 0,08	0,80 ± 0,07	0,97 ± 0,13	1,01 ± 0,07	
DHA	4,75 ± 0,23 a	5,16 ± 0,10 a	4,94 ± 0,23 a	3,92 ± 0,10 b	5,13 ± 0,19 a	4,99 ± 0,06 a	5,00 ± 0,20 a	4,87 ± 0,34 a	4,91 ± 0,23 a	T, PxT
Σ NI	1,07 ± 0,15	0,99 ± 0,04	0,90 ± 0,11	0,73 ± 0,08	0,95 ± 0,06	0,93 ± 0,10	0,99 ± 0,04	0,89 ± 0,05	0,78 ± 0,09	

Proportion of fatty acids in liver expressed as % FAME of animals exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). Σ NI: sum of unidentified fatty acids; ΣSFA: saturated fatty acids ΣMUFAs: monounsaturated fatty acids; ΣPUFAs: polyunsaturated fatty acids; AA: arachidonic acid: c4,c8,c11,c14-20:4 n-6; ALA: α-Linolenic acid: c9,c12,c15-18:3 n-3; DGLA: Dihomo-γ-linolenic acid: C8,c11,c14-20:3 n-6; DHA: Docosahexaenoic acid: C4,c7,c10,c12,c16,c19-22:6 n-3; SA: Stearic acid: 18:0; EPA: Eicosapentaenoic acid: c5,c8,c11,c14,c17- 20:5 n-3; GLA: gamma-linolenic acid: c6, cis-9,c12-18:3 n-6; LA: Linoleic acid: c9,c12-18:2 n-6; OA: Oleic Acid: c9-18:1; PA; Palmitic acid: 16:0. Values expressed as mean ± SEM (n=4). P, photoperiod; *T,* treatment; *PxT*, photoperiod and treatment effect. (two-way ANOVA (***2xA***), $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 1.0$ (Post-hoc DMS, one way ANOVA). ** Indicate trend wich pars. # Indicate significant statistical differences $p < 0.05$ and ## indicates trend $0.05 < p < 1.0$ (Student´s t-test).

Table 3. Muscle fatty acid profile of Fischer 344 rats fed a standard diet

Fatty Acid	L6			L12			L18			2xA
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
Σ SFA	39.39 ± 0.44 b	41.30 ± 0.56 a	40.32 ± 0.37 ab*	40.05 ± 0.16 b	40.51 ± 0.29 ab **	41.31 ± 0.92 a **	39.22 ± 0.24 ab	40.83 ± 0.07 ab	39.89 ± 0.45 b	T, PxT*
4:0	0.32 ± 0.07	0.47 ± 0.11	0.48 ± 0.05	0.28 ± 0.06	0.33 ± 0.13	0.53 ± 0.07	0.26 ± 0.03	0.35 ± 0.11	0.33 ± 0.03	
12:0	0.11 ± 0.06	0.08 ± 0.00	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.11 ± 0.02	0.07 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	
14:0	0.83 ± 0.06 a	0.78 ± 0.04 a	0.70 ± 0.02 a	1.09 ± 0.19 b	0.90 ± 0.08 ab	0.80 ± 0.02 a	0.81 ± 0.00 a	0.96 ± 0.09 ab	0.91 ± 0.06 ab	
15:0	0.13 ± 0.01	0.16 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	0.14 ± 0.01*	0.17 ± 0.01*	0.16 ± 0.01	
PA	28.08 ± 0.74 a	29.64 ± 0.60 a*b	29.38 ± 0.59 ab	29.91 ± 0.61 c	29.22 ± 0.42 ac **	30.63 ± 0.53 ac **	28.47±0.46 abc*	29.36 ± 0.53 ab	28.57 ± 0.45 b	P
17:0	0.18 ± 0.02 ab*	0.20 ± 0.01 ab	0.21 ± 0.02 ab	0.18 ± 0.01 a	0.19 ± 0.01 ab	0.19 ± 0.02 ab	0.22 ± 0.00 b	0.21 ± 0.01 ab	0.22 ± 0.01 ab	P
SA	9.30 ± 0.50 a	9.35 ± 0.41 a	8.91 ± 0.55 ab	8.04 ± 0.84 b	9.15 ± 0.21 a	8.42 ± 0.91 a	9.83 ± 0.17 a	8.70 ± 0.64 b	9.17 ± 0.39 ab*	PxT
20:0	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.03	
22:0	0.03 ± 0.01 a	0.03 ± 0.01 a	0.05 ± 0.02 ab	0.01 ± 0.00 a**	0.02 ± 0.00 a	0.05 ± 0.02 ab **	0.02 ± 0.00 a	0.03 ± 0.02 ab*	0.06 ± 0.03 b	T
24:0	0.03 ± 0.00	0.04 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.05 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.04 ± 0.02	

(continuation)

Σ MUFAS	18.15 ± 1.18 ab	16.23 ± 0.54 a	14.62 ± 0.43 a	20.01 ± 4.56 b	18.68 ± 0.67 ab	18.29 ± 1.66 b	16.65 ± 0.77 a	19.40 ± 0.94 ab	19.67 ± 1.53 ab	P
c9-14:1	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.00	0.08 ± 0.03	0.06 ± 0.01	0.40 ± 0.33	0.05 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	
c7-16:1	0.26 ± 0.02	0.27 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.27 ± 0.02	0.31 ± 0.03	0.27 ± 0.00	0.30 ± 0.01	0.28 ± 0.01	
Palmitoleic Acid	3.15 ± 0.26 a	2.79 ± 0.15 a	3.05 ± 0.50 a	4.76 ± 1.02 b	3.68 ± 0.36 ab#	2.96 ± 0.27 a #	3.08 ± 0.18 a	3.91±0.37 ab##	3.62 ± 0.40 ab	
c9-17:1	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.12 ± 0.04	0.15 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	
c6-18:1	0.08 ± 0.01	0.10 ± 0.02	0.10 ± 0.01	0.09 ± 0.01	0.13 ± 0.02	0.13 ± 0.03	0.11 ± 0.02	0.12 ± 0.01	0.11 ± 0.03	
OA	10.10 ± 1.04 ab*	8.53 ± 0.39 ab	7.22 ± 0.28 b	13.39 ± 1.46 c	9.79 ± 0.38 ab	10.98 ± 1.93 ac	8.49 ± 0.53 ab	9.96 ± 0.60 a	10.68 ± 1.25 ab	
c11-18:1	4.25 ± 0.08 ab	4.23 ± 0.09 a	4.20 ±0.06 a	4.44 ± 0.11 ab	4.42 ± 0.05 ab	4.33 ± 0.15 ab	4.34 ± 0.07 ab	4.65 ± 0.06 c	4.56 ± 0.18 bc	P
c12-18:1	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.07 ± 0.00	0.08 ± 0.00	0.03 ± 0.02	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	
c11-20:1	0.08 ± 0.01	0.08 ± 0.00	0.08 ± 0.00	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.02	
Σ PUFAS-cis	40.13 ± 1.74 a	38.81 ± 3.26 ab	43.45 ± 0.21 a	34.67 ± 1.64 c	39.63 ± 0.83 b	39.60 ± 0.40 b	42.19 ± 0.52 a	38.73 ± 0.88 b	39.24 ± 1.26 b	P,PxT
ΣPUFAS n-6	27.66 ± 0.64 a	25.79 ± 2.62 a	27.65 ± 1.43 a	25.15 ± 0.95 b	27.16 ± 0.39 a	27.36 ± 1.21 a	28.46 ± 0.17 a	27.18 ± 0.47 a	27.20 ± 0.85 a	P, PxT*
LA	16.66 ± 0.19 a	15.53 ± 0.32 a*b	15.48 ± 0.53 a*b	16.21 ± 0.39 a	16.23 ± 0.24 a	14.54 ± 0.51 b	16.86 ± 0.47 a	16.48 ± 0.66 a	16.14 ± 0.49 a	P*. T

(continuation)

GLA	0.03 ± 0.01 b	0.04 ± 0.01 ab	0.05 ± 0.01 a	0.04 ± 0.00 ab*	0.04 ± 0.00 ab	0.03 ± 0.00 a*b	0.04 ± 0.01 ab	0.04 ± 0.01 ab	0.04 ± 0.01 ab	
DGLA	0.33 ± 0.03	0.37 ± 0.02	0.39 ± 0.00	0.30 ± 0.04	0.35 ± 0.01	0.33 ± 0.03	0.35 ± 0.02	0.34 ± 0.03	0.33 ± 0.02	
c11,c14- 20:2	0.11 ± 0.01 b	0.13 ± 0.01 ab	0.14 ± 0.02 a	0.12 ± 0.01 ab	0.12 ± 0.01 ab	0.12 ± 0.00 ab	0.13 ± 0.00 ab	0.13 ± 0.00 a	0.13 ± 0.01 a	T*
AA	9.53 ± 0.67 a	11.36 ± 0.02 a	10.54 ± 1.37 ab*	7.46 ± 0.80 b	9.35 ± 0.38 ab*	10.50 ± 0.15 a	10.03 ± 0.32 a	9.22 ± 0.83 ab	9.63 ± 0.57 ab	
c7,c10,c13,c16-22:4	0.53 ± 0.02	0.60 ± 0.03	0.57 ± 0.06	0.47 ± 0.05	0.52 ± 0.02	0.54 ± 0.06	0.51 ± 0.01	0.53 ± 0.03	0.55 ± 0.00	
c4,c7,c10,c13,c16-22:5	0.48 ± 0.04*	0.60 ± 0.04	0.62 ± 0.08 *	0.47 ± 0.07	0.54 ± 0.02	0.48 ± 0.08	0.54 ± 0.03	0.47 ± 0.03	0.51 ± 0.04	
ΣPUFAS n-3	12.46 ± 1.10 a	13.02 ± 0.82 a	14.38 ± 0.32 a	11.14 ± 1.70 b	12.47 ± 0.45 a	10.82 ± 1.53 b	13.73 ± 0.46 a	11.55 ± 0.49 ab	12.04 ± 0.52 ab	P, PxT
ALA	0.29 ± 0.04	0.21 ± 0.03	0.21 ± 0.01	0.33 ± 0.05	0.26 ± 0.01	0.27 ± 0.05	0.27 ± 0.04	0.32 ± 0.06	0.31 ± 0.05	
EPA	0.12 ± 0.01 ab	0.13 ± 0.01 ab	0.14 ± 0.00 a	0.11 ± 0.02 ab	0.13 ± 0.01 ab	0.10 ± 0.01 b	0.13 ± 0.01 ab	0.12 ± 0.01 ab	0.11 ± 0.00a*b ##	
c7,c10,c13,c16,c19-22:5	2.35 ± 0.20	2.81 ± 0.14	2.70 ± 0.41	2.21 ± 0.37	2.42 ± 0.12	2.26 ± 0.33	2.49 ± 0.08	2.39 ± 0.25	2.32 ± 0.14	
DHA	9.78 ± 0.84 ab	9.87 ± 0.76 ab	10.95 ± 0.11 a	8.50 ± 1.38 b	9.65 ± 0.36 ab	8.19 ± 1.22 b	10.85 ± 0.44 a	8.80 ± 0.28 ab	9.30 ± 0.44 ab	
ΣNI	1.13 ± 0.21	1.52 ± 0.25	1. 34 ± 0.12	1.01 ± 0.17	1.35 ± 0.11	1.67 ± 0.35	1.08 ± 0.10	1.25 ± 0.24	1.12 ± 0.03	

Proportion of fatty acids in gastrocnemius muscle expressed as % FAME of animals exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). Σ NI: sum of unidentified fatty acids; ΣSFA: saturated fatty acids ΣMUFAs: monounsaturated fatty acids; ΣPUFAs: polyunsaturated fatty acids; AA: arachidonic acid: c4,c8,c11,c14-20:4 n-6; ALA: α-Linolenic acid: c9,c12,c15-18:3 n-3; DGLA: Dihomo-γ-linolenic acid: C8,c11,c14-20:3 n-6; DHA: Docosahexaenoic acid: C4,c7,c10,c12,c16,c19-22:6 n-3; SA: Stearic acid: 18:0; EPA: Eicosapentaenoic acid: c5,c8,c11,c14,c17- 20:5 n-3; GLA: gamma-linolenic acid: c6, cis-9,c12-18:3 n-6; LA: Linoleic acid: c9,c12-18:2 n-6; OA: Oleic Acid: c9-18:1; PA; Palmitic acid: 16:0. Values expressed as mean ± SEM (n=4). P, photoperiod; *T,* treatment; *PxT*, photoperiod and treatment effect. (two-way ANOVA, $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05< p < 1.0$ (Post-hoc DMS, one way ANOVA). ** Indicate trend wich pars. # indicate significant statistical differences $p < 0.05$ and ## indicates trend $0.05< p < 1.0$ (Student´s t-test) with respective VH.

TABLE 3. Pathway analysis of Liver FA profile of LC vs VH in L18.

Pathway Name	Match Status	p	-log(p)	FDR	Impact
FA elongation	1/39	0.133	0.874	0.467	0.0
FA degradation	1/39	0.133	0.874	0.467	0.0
FA biosynthesis	2/47	0.304	0.516	0.481	0.014
ALA metabolism	1/13	0.330	0.481	0.481	0.333
LA metabolism	1/5	0.343	0.463	0.481	1.0
Biosynthesis of unsaturated FA	10/36	0.516	0.287	0.602	0.0
AA metabolism	1/36	0.865	0.062	0.865	0.332

Liver twenty-seven fatty acids (FA) from animals exposed 18 hours of light (L18) and treated with LC or VH were exposed to pathway analysis using Metaboanalyst. Main metabolic pathways significantly affected, the total amount of metabolites involved in the pathway, the amount of metabolites that have information, the p value, false discovery rate (FDR), and impact, are shown. ALA: alpha-Linoleic acid; LA: Linolenic acid; AA: Arachidonic acid. Kyoto encyclopedia of Genes and Genomes (KEGG) was database utilized.

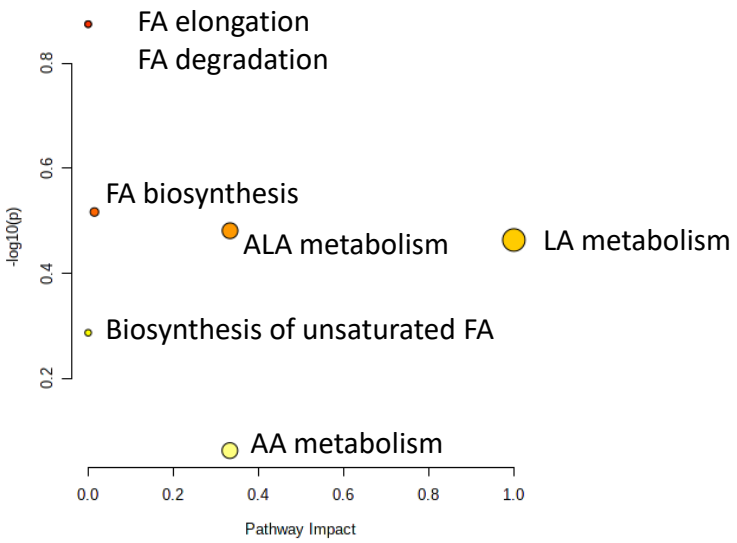

Fig.1 Pathway analysis based on data from liver twenty-seven FA of L18-LC vs L18-VH groups. Overview of pathway analysis from Kyoto encyclopedia of Genes and Genomes (KEGG) database. **AA**, arachidonic acid: c4,c8,c11,c14-20:4 n-6; AL, Linolenic acid: c9,c12-18:2; **ALA:** α-linoleic acid: c9,c12,c15-18:3 n-3.

Table 4. Concentration of representative aqueous serum metabolites analyzed by nuclear magnetic resonance in male Fisher 344.

umol/mM	L6			L12			L18			2xA
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
Leucine	0.11±0.01 abc	0.12±0a	0.13±0a	0.12±0c	0.12±0.01a c	0.11±0.01b c	0.10±0b	0.10±0b	0.1±0b	*P*
Isoleucine	0.08±0a	0.08±0a	0.08±0ac	0.08±0ac	0.08±0c	0.07±0bc	0.07±0b	0.07±0a*b	0.07±0b	*P*
Valine	0.15±0.01 a	0.15±0.01a	0.16±0a	0.15±0.01a b*	0.16±0.01a	0.14±0.01b	0.14±0.01 b	0.13±0b	0.13±0.01b	*P, PxT**
3-Hydroxybutyrate	0.10±0.01	0.01±0.01	0.11±0.01	0.11±0.01	0.11±0.01	0.10±0.01	0.11±0.01	0.11±0.01	0.11±0.01	
Lactate	5.10±0.75 b	3.83±0.3a	4.45±0.21a b	3.01±0.22a	4.18±0.38a b	4.15±0.47a b	5.29±0.35 b	4.81±0.22a *b	5.55±0.7ab	*P, PxT**
Alanine	0.43±0.02 ab	0.41±0.02a	0.46±0.01b	0.42±0.01b	0.43±0.01a *b	0.42±0.03a b	0.41±0.02 b	0.40±0.01a b	0.43±0b	
Acetate	0.11±0.01	0.13±0.01	0.13±0.01	0.13±0.01	0.13±0.01	0.11±0.01	0.11±0.01	0.11±0.01	0.12±0.01	
Methionine	0.04±0	0.05±0	0.05±0	0.04±0	0.05±0	0.05±0	0.05±0	0.04±0	0.04±0	
Glutamate	0.12±0.01	0.11±0.01	0.13±0.01	0.11±0	0.12±0.01	0.12±0.01	0.11±0.01	0.12±0	0.12±0.01	
Pyruvate	0.046±0ab c	0.041±0a	0.05±0b	0.044±0ab	0.049±0b	0.047±0ab	0.053±0c	0.051±0bc	0.052±0bc	P
Glutamine	0.44±0.01 ab	0.42±0.01a b	0.45±0.01a	0.44±0.01a b*	0.44±0.01a b*	0.40±0.02b	0.49±0.02 a*	0.45±0.01a b	0.43±0.01a *	
Citrate	0.09±0*	0.01±0	0.10±0	0.11±0	0.11±0.01	0.11±0.01	0.11±0.01 *	0.10±0	0.11±0.01	P*.T*

(*continuation)*

Asparagine	0.04±0.01 ab	0.05±0.01a	0.05±0a	0.04±0.01a b	0.05±0ab*	0.05±0.01a b	0.04±0ab	0.04±0b	0.05±0ab*	
Dimethylglycin e	0.005±0	0.005±0	0.006±0	0.005±0	0.005±0	0.006±0	0.005±0	0.005±0	0.005±0	
Lysine	0.21±0.01 a	0.22±0.01a	0.25±0.01b	0.23±0.01b	0.23±0.01a b	0.20±0.01c	0.21±0.01 ac	0.19±0c	0.19±0.01c	P, PxT
Creatine	0.21±0.02 a	0.21±0.01a c	0.26±0.01b	0.25±0.01a b	0.23±0.02a	0.24±0.02a b	0.24±0.02 ab	0.18±0.01c	0.19±0.01c	P, PxT
Ornithine	0.10±0.02 b	0.05±0a	0.06±0.01a	0.05±0ac	0.06±0a	0.11±0.02b	0.04±0c	0.08±0.01a	0.06±0.01ac	PxT
Choline	0.02±0a	0.02±0ab	0.02±0ab	0.01±0b	0.02±0ab	0.02±0a	0.01±0b	0.02±0ab	0.02±0ab	
Carnitine	0.05±0ab	0.05±0a	0.05±0ab	0.04±0a	0.05±0ab	0.05±0ab*	0.05±0b	0.04±0a	0.05±0b	P
Betaine	0.11±0.01 ab	0.11±0a	0.12±0.01a	0.10±0a	0.10±0a	0.10±0.01a	0.12±0.01 b	0.13±0.01b	0.11±0.01ab *	P
Taurine	0.41±0.01 a*b	0.36±0.01a	0.43±0.01b	0.38±0.01a b	0.40±0.02a b	0.39±0.03a b	0.42±0.02 b	0.42±0.02a *b	0.42±0.01b	
Glycine	0.187±0.0 1ab	0.177±0.01 a	0.203±0b	0.19±0.01a b	0.189±0.01 ab	0.196±0.01 ab	0.181±0.0 1a	0.183±0.01 a	0.184±0.01a b*	
Glycerol	0.18±0.03 ab	0.14±0.01a *b	0.16±0.01 ab	0.17±0.01a b	0.17±0.01a	0.16±0.01a b	0.16±0.01 ab	0.14±0.01b	0.15±0.02ab	
Theronine	0.11±0.01 a	0.12±0.01a b*	0.13±0b	0.11±0a	0.11±0ab*	0.11±0.01a	0.09±0.01 c	0.11±0ac*	0.11±0a	P,T
Serine	0.18±0a	0.185±0a	0.20±0b	0.19±0a	0.18±0a	0.19±0.01a b	0.19±0.01 ab	0.17±0a	0.18±0.01a	T

(*continuation*)

Glucose	2.44±0.11 ab	2.47±0.06a	2.48±0.05a	2.74±0.11c	2.47±0.06a	2.27±0.08b	2.43±0.07 a	2.45±0.04a b*	2.43±0.07ab	T*,PxT
Succinate	0.03±0ab	0.02±0b	0.02±0a*b	0.02±0b	0.03±0ab	0.03±0ab	0.02±0b	0.03±0ab	0.03±0a	
Tyrosine	0.05±0ab	0.05±0ac*	0.06±0b	0.06±0bc	0.06±0c	0.05±0ab	0.05±0ab*	0.05±0a	0.05±0a	P, PxT*
Histidine	0.04±0ab	0.04±0ab	0.04±0a	0.04±0ab	0.04±0ab	0.04±0ab*	0.04±0ab	0.04±0b	0.04±0b	P*
Tryptophan	0.05±0.01 *	0.04±0	0.04±0	0.04±0	0.04±0	0.035±0.01	0.04±0*	0.04±0.01	0.04±0.01	
Phenylalanine	0.03±0b	0.04±0ab	0.04±0a	0.03±0b	0.04±0ab	0.04±0ab	0.03±0bc	0.03±0c	0.03±0bc	P,T*
Formate	0.04±0a	0.037±0ab	0.04±0ab	0.03±0ab	0.04±0ab	0.03±0ab	0.03±0b	0.03±0ab	0.04±0ab	
Dimethylamine	0.003±0	0.003±0	0.003±0	0.003±0	0.003±0	0.003±0	0.003±0	0.003±0	0.003±0	
Allantoin	0.02±0ab	0.02±0ab	0.02±0a	0.02±0ab	0.02±0b	0.02±0ab	0.01±0a	0.02±0a*b	0.02±0b	

Standard male Fischer 344 rats exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). Data are expressed as the mean ± SEM (n=8). All the serum metabolites were obtained by performing a nuclear magnetic resonance (NMR) analysis. P, photoperiod; T, treatment; PxT, photoperiod and treatment effect. (two-way ANOVA, $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA).

Table 5. Concentration of representative serum lipid metabolites analysed by nuclear magnetic resonance in male Fisher 344.

umol/mM	L6			L12			L18		
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC
Cholesterol	0.34±0.13*	0.23±0.06	0.14±0.04	0.17±0.06*	0.32±0.09	0.25±0.07	0.18±0.05	0.18±0.07	0.22±0.06
Free Cholesterol	0.32±0.07	0.31±0.05	0.22±0.03*	0.25±0.04	0.32±0.05	0.33±0.05	0.25±0.04	0.23±0.04	0.34±0.07 *
Esterified Cholesterol	0.25±0.06	0.25±0.04	0.17±0.02*	0.18±0.03	0.25±0.05	0.27±0.05*	0.19±0.02	0.18±0.04	0.28±0.06 *
TAG	0.49±0.19	0.29±0.06	0.29±0.07	0.27±0.07	0.51±0.15*	0.38±0.11	0.27±0.05	0.27±0.08*	0.4±0.11
DAG	0.1±0.01ab	0.08±0.01a	0.08±0.01a	0.09±0ab	0.1±0.01b	0.08±0.01a	0.09±0.01ab	0.07±0a	0.08±0.01 a
Total Phospholipids	0.54±0.13*	0.4±0.05	0.34±0.05*	0.35±0.05	0.49±0.08	0.41±0.07	0.38±0.06	0.38±0.08	0.4±0.05
Sphingomyelin	0.09±0.03 ab*	0.07±0.02 ab	0.05±0.01 ab	0.04±0.01b	0.09±0.02a	0.06±0.01a b	0.06±0.01 ab	0.07±0.01 ab	0.07±0.02 ab
Total Fatty Acids	6.14±1.27	5.84±0.76	4.63±0.53	4.79±0.57	6.24±0.97	6.29±0.95	4.71±0.49	4.67±0.7	6.38±1.02
Omega-3	0.58±0.12	0.55±0.08	0.42±0.05	0.44±0.06	0.59±0.1	0.6±0.09	0.45±0.06	0.42±0.07	0.61±0.1 1
ARA+EPA	0.9±0.21	0.71±0.1	0.67±0.08	0.66±0.09	0.94±0.18*	0.81±0.12	0.68±0.11	0.59±0.11*	0.84±0.1 6
Oleic Acid	0.96±0.39	0.56±0.16	0.48±0.16	0.49±0.19	0.99±0.32	0.73±0.26	0.48±0.15	0.48±0.2	0.57±0.1 6
Linoleic Acid	0.52±0.22	0.27±0.07	0.24±0.07	0.24±0.08	0.49±0.15	0.36±0.12	0.26±0.07	0.24±0.09	0.3±0.07

DHA	0.14±0.04 ab	0.1±0.02 ab	0.09±0.01a b	0.09±0.01ab *	0.14±0.03b	0.1±0.02 ab	0.1±0.01 ab	0.08±0.01a	0.09±0.0 2 ab
PUFAs	1.43±0.57*	0.82±0.21	0.63±0.17*	0.68±0.22*	1.35±0.4	1.02±0.32	0.76±0.2	0.68±0.25	0.83±0.1 9
MUFAs	2.33±0.97	1.2±0.36	0.75±0.24	1.05±0.43	2.29±0.74	1.65±0.59	1.28±0.34	1.09±0.47	1.29±0.3 7

Standard male Fischer 344 rats exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). DAG: Diacylglycerides; TAG: Triacylglycerides. Data are expressed as the mean ± SEM (n=8). All the liver lipophilic metabolites were obtained by performing a nuclear magnetic resonance (NMR) analysis. Different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA).

Table 6. Concentration of representative aqueous liver metabolites analyzed by nuclear magnetic resonance in male Fisher 344.

umol/gr	**L6**			**L12**			**L18**			**2xA**
	VH	**LC**	**nLC**	**VH**	**LC**	**nLC**	**VH**	**LC**	**nLC**	
Succinate	1.15±0.13 ab	1.1±0.12a	0.96±0.1a	1.12±0.06 ab	1.04±0.12 a	1.34±0.07b	1.19±0.1ab	1.28±0.09 a*b	1.35±0.11b	*P*
Glutamate	1.4±0.15	1.34±0.1	1.43±0.08	1.41±0.15	1.2±0.06	1.37±0.1	1.32±0.12	1.32±0.14	1.45±0.08	
Acetate	0.48±0.05 ab	0.56±0.07 ab*	0.56±0.03 ab*	0.58±0.04 a	0.58±0.05 a	0.43±0.06 b	0.48±0.05ab	0.57±0.04 ab*	0.56±0.06ab*	
Alanine	3.75±0.23a	3.6±0.18ab	3.55±0.13 ab	3.59±0.11 ab	3.29±0.18 b	3.56±0.13 ab	3.47±0.14ab	3.81±0.14a	3.71±0.07ab	
Lactate	6.64±0.43 ab	7.21±0.4 ab	6.89±0.58a b	7.06±0.23 b	6.95±0.54 ab	7.17±0.39 ab	6.89±0.22a	6.96±0.45 ab	7.1±0.42 ab	

3-Hydroxybutyrate	0.3±0.03	0.28±0.02	0.26±0.01	0.32±0.02	0.29±0.03	0.28±0.01	0.26±0.02	0.28±0.01	0.27±0.02	
Valine	0.34±0.02 ab	0.33±0.03 ab	0.32±0.03 ab	0.37±0.03 b	0.24±0.04 a	0.29±0.02a	0.29±0.02a	0.32±0.02 ab	0.3±0.02a	
Leucine	0.77±0.05 ab	0.65±0.09 ab	0.72±0.08a b	0.81±0.06 b	0.61±0.13 a	0.64±0.05a b*	0.66±0.06ab *	0.73±0.07 ab	0.69±0.05ab	
Formate	0.02±0.004 ab	0±0.02ab	0.03±0.002 a	0±0.02ab	0.03±0.00 3 ab	0±0.026b	0.03±0.003a	002±0.001a b	0.02±0.002 ab*	
Choline	0.06±0.004 ab	0±0.048ab	0.04±0.002 ac	0±0.054 ab	0.05±0.00 3c	0±0.046b	0.05±0.003 ac	0±0.037ac	0.05±0.003 ab*	*PxT*
Glycogen	8.12±2.05	5.45±1.13	7.81±2.24*	6.35±1.67	5.27±1.87	5.29±1.65*	6.31±1.27	6.7±1.28	5.76±1.26	
Lysine	0.26±0.02 ab	0.23±0.03a b	0.27±0.02 a*	0.28±0.02 b	0.21±0.04 a	0.24±0.03a	0.24±0.02ab	0.27±0.02b	0.26±0.02ab	
Sarcosine	0.05±0.01 ab	0.06±0.01a	0.06±0a	0.07±0.01 c	0.04±0.01 a	0.05±0ab	0.07±0.01c	0.06±0ab	0.05±0.01ab	T,PxT
Glycine	0.94±0.06a	0.99±0.06 ab	1.02±0.04 ab	1.11±0.07 b	0.98±0.07 ab	0.97±0.06 ab*	1.02±0.06 ab	1.07±0.06 ab	1.03±0.05 ab	
Mannose	0.52±0.09a	0.32±0.06a b	0.54±0.1a	0.43±0.07 ab	0.33±0.08 b	0.35±0.06b	0.56±0.17ab	0.38±0.06 ab	0.33±0.06b	P
Uridine	0.93±0.11	0.74±0.1	0.85±0.09	0.85±0.08	0.64±0.1	0.75±0.1	0.87±0.08	0.85±0.05	0.86±0.05	
Udps	0.59±0.11a	0.65±0.11a b	0.67±0.14 ab	0.87±0.1b	0.65±0.13 ab	0.81±0.08 ab	0.74±0.07ab	0.81±0.12 ab	0.77±0.12 ab	
Carnitine	0.73±0.07b	1±0.07a	0.72±0.08b	1.04±0.08 a	0.8±0.04b	0.83±0.09 ab	0.68±0.07b	0.78±0.06b	0.71±0.05b	P, PxT

Niacinamide	0.21±0.04	0.25±0.03	0.25±0.04	0.23±0.04	0.2±0.03	0.2±0.03	0.25±0.04	0.2±0.05	0.22±0.04
Inosine	0.72±0.1	0.82±0.05	0.78±0.07	0.8±0.08	0.7±0.05	0.73±0.09	0.85±0.08	0.71±0.12	0.73±0.05
Phenylalanine	0.13±0.02ab	0.14±0.02a	0.12±0.02ab	0.12±0.01ab	0.1±0.01b	0.11±0.01ab	0.12±0.01ab	0.11±0.02ab	0.11±0.01ab
Tyrosine	0.13±0.01ab	0.13±0.02a	0.12±0.02ab	0.13±0.02b	0.1±0.01b	0.1±0.01ab	0.12±0.02ab	0.11±0.02ab	0.12±0.01ab
Fumarate	0.07±0.01	0.06±0	0.08±0.01	0.07±0.01	0.08±0.01	0.07±0.01	0.08±0.01	0.06±0.01	0.08±0.01
Glucose-6-Phosphate	18.56±1.8	18.87±1.62	18.81±2.08	19.62±1.08	17.15±1.58	17.57±0.79	19.45±1.9	18.21±2.71	17.8±1.29
Ascorbate	0.84±0.09	0.93±0.07	0.91±0.11	0.99±0.06	0.9±0.07	1.03±0.03	0.89±0.08	0.9±0.15	1.02±0.1
Betaine	1.63±0.19	1.74±0.1	1.87±0.15	1.63±0.12	1.33±0.07	1.86±0.11	2.08±0.16	2.06±0.3	2.07±0.17
Dimethylamine	0.016±0a*b	0.018±0ab*	0.018±0ab	0.018±0a	0.016±0b	0.017±0ab	0.017±0a*b	0.016±0ab*	0.016±0ab
Glutamine	2.89±0.32ab	2.91±0.19ab*	3.05±0.13ab	3.21±0.26ab	2.82±0.29ª	3.07±0.13ab	3.11±0.15ab	3.02±0.42b	3.45±0.2ab
Histamine	0.04±0.01	0.05±0.01	0.04±0.01	0.06±0.01	0.05±0.01	0.06±0.01	0.05±0.01	0.05±0.01	0.05±0.01
Ump	0.08±0.01	0.09±0.01	0.09±0.02	0.12±0.02	0.1±0.01	0.12±0.01	0.1±0.01	0.1±0.02	0.11±0.02
Atp/Adp/Amp	0.29±0.04	0.32±0.05	0.34±0.06	0.38±0.04	0.32±0.03	0.37±0.03	0.34±0.04	0.32±0.07	0.38±0.06
Nad+	0.11±0.02	0.12±0.02	0.13±0.03	0.14±0.03	0.12±0.02	0.17±0.01	0.14±0.02	0.15±0.04	0.19±0.03
Trimethylamine	0.004±0.001	0.005±0.003	0.005±0	0.001±0.005	0.005±0*	0.001±0.005	0.005±0.001	0±0.005*	0.005±0
Asparagine	0.04±0.01a*b	0.04±0.01ab	0.03±0.01a	0.05±0.01a*b	0.04±0b	0.04±0.01b	0.03±0a	0.04±0.01b	0.05±0.01b
Ornithine	0.15±0.02b	0.13±0.01ab	0.15±0.01b	0.16±0b	0.12±0.01a	0.15±0.01a*b	0.14±0.01ab*	0.13±0.02ab	0.13±0.01a

Glutathione	0.71±0.11	0.74±0.11	0.68±0.15	0.79±0.18	0.67±0.02	0.6±0.06	0.68±0.06	0.67±0.09	0.58±0.07	
Histidine	0.3±0.03 ab*	0.34±0a*b	0.34±0.01 ab	0.34±0.02 b	0.3±0.02a	0.3±0.02 ab*	0.31±0.01ab	0.3±0.04 a*b	0.31±0.01ab	PxT *
Uracil	0.02±0.01a	0.02±0.01a	0.03±0.01a	0.02±0a	0.03±0.01 a	0.03±0.01a	0.02±0a	0.06±0.03b	0.03±0.01a	T
Isoleucine	0.18±0.02 ab	0.19±0.02 ab	0.17±0.01 ab	0.2±0.01b	0.17±0.02 ab	0.16±0.01a	0.16±0.01a	0.16±0.02 ab	0.16±0.01ab	
Methionine	0.14±0.02a b	0.15±0.01a b*	0.16±0.01a	0.15±0.01 ab*	0.12±0.01 b	0.13±0.01a b	0.13±0.01ab	0.12±0.02a b	0.12±0.01b	T*
Aspartate	0.01±0b	0.02±0.01a b	0.03±0.01a	0.01±0.01 b	0.01±0b	0.01±0b	0.01±0b	0.01±0b	0±0b	P*, PxT
Creatine	0.13±0.02a	0.12±0.01a	0.14±0.01a	0.14±0.01 a	0.14±0.01 a	0.13±0.01a	0.1±0.01b	0.1±0.01ab	0.12±0.01ab	P
Tryptophan	0.03±0	0.03±0	0.02±0	0.03±0.01	0.03±0	0.02±0	0.03±0	0.02±0	0.03±0	
Xanthine	0.19±0.04	0.16±0.04	0.19±0.05*	0.15±0.04	0.1±0.02	0.1±0.01*	0.15±0.03	0.13±0.04	0.16±0.04	

Standard male Fischer 344 rats exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). Data are expressed as the mean ± SEM (n=8). All the liver hydrophilic metabolites were obtained by performing a nuclear magnetic resonance (NMR) analysis. P, photoperiod; T, treatment; PxT, photoperiod and treatment effect. (two-way ANOVA, $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA).

Table 7. Concentration of representative lipid liver metabolites analyzed by nuclear magnetic resonance in male Fisher 344 rats.

umol/gr	L6			L12			L18			2xA
	VH	LC	nLC	VH	LC	nLC	VH	LC	nLC	
Total Cholesterol	4.26±0.05ab	4.3±0.08a*b	4.02±0.08a	4.1±0.09a	4.29±0.16ab	4.18±0.16a*b	4.13±0.11ab*	4.26±0.1ab*	4.44±0.08b	PxT
Free Cholesterol	3.44±0.08a	3.48±0.08ab	3.28±0.06a	3.17±0.07b	3.51±0.15ab*	3.33±0.09ab	3.31±0.11ab	3.42±0.12ac	3.62±0.06c	P*, PxT*
Esterified Cholesterol	0.55±0.04	0.54±0.04	0.47±0.02	0.56±0.05	0.49±0.06	0.51±0.06	0.53±0.04	0.49±0.02	0.53±0.04	
TAG	4±0.76	4.18±0.63	4.24±0.55	3.87±0.4	4.54±0.74	3.62±0.33	4.67±0.59	4.31±0.39	3.51±0.43	
DAG	5.4±0.2abc*	5.79±0.14a	5.21±0.1b	5.41±0.27abc*	5.64±0.22a	5.04±0.21b	4.92±0.2c	4.96±0.18bc	5.22±0.23abc	P*, PxT*
Total Phospholipids	29.82±0.61a	28.5±0.71a	26.84±0.66ab	29.19±0.97ab	28.39±0.24ab	27.83±1.11ab	27.12±1.17b	28.25±0.55ac	29.44±0.78a	PxT
Phophoethanolamine	8.05±0.21b	6.97±0.3a	7.04±0.3a	7.68±0.39b	7.13±0.18ab	6.91±0.43ab	6.75±0.42a	7.15±0.14ab	7.62±0.39ab	PxT
Phosphatidylcholine	18.43±0.13ac	18.65±0.37a	17.11±0.33b	17.96±0.39abc	17.78±0.45a	17.97±0.61ab	17.8±0.46c	18.66±0.43ac	19.07±0.21ª	PxT
Sphingomyelin	1.05±0.02a	0.98±0.03ab	0.99±0.03ab	0.93±0.04b	1±0.02ab	0.99±0.04ab	0.94±0.04b	1±0.03ab	1.07±0.01ª	PxT
Total Fatty Acids	61±2.56ab	59.47±1.88ab	57.43±1.25b	58.01±1.68a*b	63.5±2.86a	54.73±1.39b	60.12±2.13ab	60.77±2.1a	58.87±2.12ab	PxT

Standard male Fischer 344 rats exposed to different photoperiods (short, L6; standard, 12; long, L18, with 6, 12 and 18 hours of light, respectively) and supplemented with treatment: Local cherry (LC), non-Local cherry (nLC) or vehicle (VH). DAG: Diacylglycerides; TAG:

Triacylglycerides. Data are expressed as the mean ± SEM (n=8). All the liver lipophilic metabolites were obtained by performing a nuclear magnetic resonance (NMR) analysis. P, photoperiod; T, treatment; PxT, photoperiod and treatment effect. (two-way ANOVA, $p < 0.05$); different letters indicate significant statistical differences $p < 0.05$; * indicates trend $0.05 < p < 0.1$ (Post-hoc DMS, one way ANOVA).

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Capítulo IV

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MANUSCRITO 4

In-season consumption of locally produced tomatoes decreases cardiovascular risk indices

Ruiz de Azua, Ma.Josefina1; Cruz-Carrión, Álvaro1; Muguerza, Begoña1; Aragonès, Gerard1; Bravo, Francisca Isabel1; Arola-Arnal, Anna1; Romero, Maria-Paz2; Macià, Alba2; Suarez, Manuel1 *.

1 Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Nutrigenomics Research Group, Tarragona, Spain.

2 Antioxidants Research Group, Food Technology Department, Agrotecnio AGROTECNIO-CERCA Center, University of Lleida, Av/ Alcalde Rovira Roure 191, Lleida, 25198, Spain

*Corresponding author: manuel.suarez@urv.cat

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ABSTRACT

Tomatoes are widely consumed worldwide at any time of the year. However, depending on the variety, they have a characteristic season. In the present study, we evaluate the consequences on serum metabolic markers of consumption of *Ekstasis* tomatoes from different geographical origin and in different seasons in Fischer 344 rats. Furthermore, the hepatic gene expression of key enzymes in lipid metabolism was also evaluated. Animals were classified in 3 photoperiods (L6, L12, and L18) and in 3 treatments (vehicle: VH, Local tomato: LT and non-Local tomato: nLT). Markers of carbohydrate metabolism were affected by the photoperiod, while the gene expression of lipogenic enzymes was affected by both the photoperiod and the treatment. A beneficial effect on cardiovascular risk markers was observed in animals that consumed locally produced tomatoes in season.Keywords: cardiovascular risk, lycopene, phenolic compounds, season, tomato, xenohormesis.

Abbreviations: ACAT: cholesterol acyl transferase; Acc1: Acetyl-CoA carboxylase 1; AI: atherogenic index; AMPK: AMP-activated protein kinase; At.Coef: atherogenic coefficient; cd36: fatty acid translocase, homologue of CD36; ChREBP: Carbohydrate-responsive element-binding protein; Cpt1α: carnitine palmitoyltransferase 1 alpha; CR1: cardiovascular risk 1; CR2: cardiovascular risk 2; DPP-IV: dipepityl-peptidase IV; DW: dry weight; F344: Fischer 344; FA: fatty acid; Fas1, Fatty acid synthase; Fatp5: fatty acid transport protein 5; HDL-c: high-density cholesterol; HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; HOMA: homeostatic model assessment index; IDL-c: Intermediate-density lipoprotein; L12: standard photoperiod; L18: long photoperiod; L6: short photoperiod; LDL-c; low-density cholesterol; LT: Local Tomato; LXR: liver X receptor; MS: mass spectrometry; MTBE: methyl tertbutyl ether; NEFAs: non-esterified fatty acids; nLT: non-Local Tomato; PPAR: Peroxisome Proliferator Activated Receptor; STD: standard diet; TAG: triacylglycerides; TC: total cholesterol; uHPLC: ultra-high performance liquid chromatography; USFs: upstream stimulatory factors; VH: Vehicle.

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INTRODUCTION

The synthesis of phytochemicals by plants is a consequence of exposure to environmental and other external stressors intended to provide a protective and adaptive response. It is known that the content of these compounds, including polyphenols, depends on the external conditions to which plants are exposed in their development and harvest. In this framework, the xenohormesis theory proposes that the heterotrophs that consume plants could detect these molecules, which would provide environmental adaptive signals, regulating pathways that provide resistance to stress. Therefore, the consumption of local and seasonal plant products would be associated with beneficial metabolic adjustments for the forecast of adverse conditions [1].

The Mediterranean diet is a characteristic food pattern that is based on a high consumption of fruits, fresh vegetables, legumes, cereals and whole grains. The dietary patterns of the Mediterranean diet are associated with a protective effect against chronic non-communicable diseases, such as diabetes mellitus, obesity, cardiovascular diseases, allergies, various types of cancer, and neurodegenerative diseases such as Alzheimer's and Parkinson's. Possibly the beneficial effects of its adherence are associated with the large amount of bioactive compounds present, their actions in the cell cycle, apoptosis and angiogenesis and their protective properties against oxidative stress, inflammation and metastasis [2–8].

Tomato is one of the vegetables most consumed within this diet, since it is present not only in fresh form, but it is also found in a wide variety of derivatives such as canned fried tomato, soups, stews and mainly sauces [9]. In 2020, approximately 22 million tons of tomatoes were produced throughout Europe, of which 4 million tons were of Spanish origin [10]. From a nutritional point of view, tomatoes are low in calories, fat and protein, and provide vitamin C, A, folates, and minerals such as sodium, potassium, and magnesium, among

others. In addition, it is a source of significant amounts of phytochemicals, among which carotenoids (lycopene, phytoene, and α and β-carotene) and polyphenols (flavonoids, flavones, flavonones and anthocyanidins) are the main ones [11]. Actually, its content in bioactives is attributed to exert lipid-lowering [12], anti-oxidative [13], anti-cancer [14], anti-inflammatory [11] and protective properties against cardiovascular diseases [15–17]. The amount and type of bioactive compounds in tomatoes is influenced either by genetic and agricultural, environmental conditions during growth and development that generate differences when comparing different species, as well as when comparing the same type of fruit but of different geographical origin [18]. In this sense, it has been observed that the lycopene content varied from 1 to 11 mg / 100 gr, while the amount of phenolic compounds varied between 9 to 27 mg/100g and 25 to 50 mg/100g in tomatoes from India or Spain, respectively [19, 20]. Likewise, a 1- to 4-fold variation in ascorbic acid levels has been documented between tomatoes of the same species [20].

In the last decade the fundamental role of circadian and circannual rhythms in the regulation of physiological and metabolic processes, having implications in the nutrition and metabolism of living beings, has been claimed. In this sense, chrononutrition studies the interaction of biological rhythms, nutrients and metabolism, focusing on how the biological clock can influence the signaling and modulation of nutrients on the body and in turn, how nutrients and biocompounds present in food can affect our biological clock in the same way [21, 22]. In this regard, the impact of chrononutrition on energy expenditure, thermogenesis, satiety and the levels of serum parameters, linked to the appearance and/or prevention of chronic metabolic diseases such as obesity, overweight, diabetes mellitus, cardiometabolic pathologies, and comorbidities has been evidenced [23–26]. In our previous studies, a differential effect of the consumption of fruits in and out of season was observed. Specifically, the consumption of cherries and tomatoes in-season could prevent oxidative stress

by improving antioxidant status, decreasing serum transaminase activity, and maintaining a constant level of reactive oxygen species and serum malondialdehyde [27, 28]. In addition, the intake of orange out-of-season increased lipogenesis and decreased β-oxidation compared to its consumption in-season [29].

In the present study we aim to evaluate the effects on biochemical parameters and on the gene expression of liver enzymes, of the tomato intake from different areas of Spain and its consumption in different seasons.

MATERIALS AND METHODS

Fruit material

In the present study, *Ekstasis* tomatoes grown and harvested in two different areas of Spain, specifically in, Almería (36°50′17.3″ N 2°27.584′ O; Spain) (nLT: non-Local Tomato) and Tarragona 41°4′29.24″ N 1°3′8.78″ E; Spain) (LT: Local Tomato), were used. Tomatoes were purchased in the autumn, i.e., November 2018, from a local market (Tarragona, Spain) at commercial maturity. Once washed, the edible parts were removed, minced and frozen in liquid nitrogen. They were then ground, mixed and lyophilized using the Telstar LyoQuest lyophilizer (Thermo Fisher Scientific, Barcelona, Spain) at -55 °C. The composition of dietary components in dry weight (DW) was determined. LT presented 0.14g/g DW of proteins; 0.03g/g DW of total lipids; 0.50g/g DW of carbohydrates, of which 0.37 mg/g DW were simple sugars; 0.25g/g DW of dietary fiber and 0.09g/g DW of ash. On the other hand, nLT were characterized by having 0.13g/g DW of proteins; 0.02g/g DW of lipids; 0.52g/g DW of carbohydrates, of which 0.40g/g DW were simple sugars; 0.24g/g DW of dietary fiber and 0.09g/g DW of ash. The profile of phenolic compounds was determined by ultra-high performance liquid chromatography (uHPLC) coupled

with mass spectrometry (MS) and previously described by Cruz-Carrión et al. (2022) [30]. Specifically, we determined total (poly)phenols, flavonoids, caffeic and dihydrocaffeic acid derivatives, free phenolic acids, hydroxybenzoic acid derivatives, hydroxycinnamic derivatives, hydroxycinnamoylquinic acids and phenylpropanoic acid-glycosides Data are shown in Supplementary Table 1. It should be noted that nLT presented a higher concentration of all phenolic compound types, except for total flavonoids.

Determination of lycopene content

Lycopene was extracted from the freeze-dried tomato samples using the method described by Motilva *et al. (2014)* [31]. Samples were weighed (1g) in test tubes and 5 mL of n-hexane/ethanol/acetone extraction solution (50/25/25, v/v/v) was added to solubilize carotenoids. It was vortexed and centrifuged at 9,000 rpm for 15 minutes. To completely extract the lycopene from the matrix, we proceeded with a second vortex for 15 minutes and an ultrasound bath for 30 minutes. The supernatants were decanted with a funnel and 5 mL of water were added. After phase separation, the organic and aqueous phase were collected. With the organic phase the extraction was repeated once more. In the aqueous phase, 5 mL of n-hexane were added and the extraction was repeated. Finally, the organized phases were pooled, the volume was noted and the samples were filtered on a 12:22 micron PVDF filter. The resulting solution (105 mL) was injected into an UPLC-DAD chromatographic system using a YMC carotenoid column (4.6 x 150 mm, 3 µM) (Waters Corp., Milford, MA). A two-phase gradient system of methanol (mobile phase A) and methyl tertbutyl ether (MTBE) (mobile phase B) was used. The gradient started in 55% of mobile phase B, reaching 85% at 6 minutes. The gradient reached initial conditions at 18 minutes and was kept there in isocratic elution for a further 3 minutes. Elution was carried out at a flow rate of 0.5 mL/min and the injection volume was 105 mL Lycopene (All-trans-lycopene,

Extrasynthese) was used as a standard to detect and compare peaks in tomato samples. The standard was dissolved in n-hexane and the calibration curve was calculated. For the detection of lycopene, the chromatograms were recorded at 470 nm. Lycopene was identified based on the retention time of the standard pure compound and comparison with a literature review of the determination of carotene.

Animals

The guidelines for the care and use of animals in the laboratory were followed and all the procedures used were evaluated and approved by the Animal Ethics Committee of the Rovira i Virgili University (Tarragona, Spain) (Project identification code: 9495; file number: FUE-2017-00499873).

Seventy-two 8-week-old male Fischer 344 (F344) rats were used for the present experiment. They were placed in cages and in pairs for 4 days at 22 °C and light/dark cycles for acclimatization. After conditioning, they were randomly distributed in one of the three photoperiods: short photoperiod (n=24, L6, 6 hours of light and 18 hours of darkness); standard photoperiod (n=24, L12, 12 hours of light and 12 hours of darkness); long photoperiod (n=24, L18, 18 hours of light and 6 of darkness). The amount of light hours for each photoperiod was established in order to simulate the days of the different seasons of the year. Thus, L18 would simulate the summer days, L12 the autumn / spring days and L6 the winter days. The animals were fed a standard diet (STD) (A04 SAFE; Panlab, Barcelona, Spain) and water *ad libitum*. After 4 weeks, the animals of each photoperiod were randomly distributed in one of the 3 groups according to the treatment given: LT (n=8), nLT (n=8) or vehicle (VH, n=8). The treatment was carried out for 7 weeks. The supplemented VH was composed of a mixture of sugars that contained the average carbohydrate content of tomatoes used (glucose: fructose 1:1). Regarding the groups supplemented with freeze-dried tomatoes, a dose of 100 mg per body weight diluted in water was daily

supplied. The 3 treatments were administered through a syringe by voluntary licking, guaranteeing the complete taking of the dose. At the end of the experiment, the animals were sacrificed by decapitation, having their last dose of treatment one hour before their death. The liver and serum obtained were stored at -80 °C.

Serum analysis

Blood glucose and lipid levels were quantified by enzymatic colorimetric assays: triacylglycerides (TAG), total cholesterol (TC), high-density cholesterol (HDL-c), low-density cholesterol (LDL-c) (QCA, Amposta, Tarragona, Spain) and non-esterified fatty acids (NEFAs) (WAKO, Neuss, Germany). Circulating insulin levels were also quantified using the rat Insulin ELISA kit (Milipore, Barcelona, Spain).

Cardiovascular risk indices and HOMA index

Using the values of the aforementioned biochemical parameters, the following cardiovascular risk markers were calculated: atherogenic index (AI: [Log (GAD / HDL-c)], cardiovascular risk 1 (CR1: TC / HDL-c), cardiovascular risk 2 (CR2: LDL-c / HDL-c), atherogenic coefficient (At.Coef.: [TC-HDL-c] / HDL-c) Values expressed in mmol/L were used for all determinations.

For the calculation of the HOMA index, the blood glucose and insulin values were used in the following equation: (Blood glucose X Insulinemia) / 22.5.

Hepatic Gene Expression Analysis

For the extraction of total liver RNA, TRIzol Reagent (Thermo Fisher Scientific, Illkirch-Graffenstaden, France) was used and the guidelines indicated by the supplier were followed. The cDNA was synthesized by reverse transcription using High-Capacity cDNA Reverse Transcription (Thermo Fisher Scientific,

Illkirch-Graffenstaden, France). The specific amplification of the cDNA was carried out thanks to the polymerase chain action in real time (RT-qPCR), using iTaq Universal SYBR Green Supermix (Bio-Rad, Barcelona, Spain). Gene expression analysis was performed using primers obtained from Biomers.net (Ulm, Germany). Supplemental Table 2 shows the corresponding sequences. The mRNA concentration of genes related to liver lipid metabolism was studied: Carnitine palmitoyltransferase 1-α (*Cpt1α*), Acetyil-coenzyme A carboxylase (*Acc1*), fatty acid translocase homolog of CD36 (*Cd36*), fatty acid synthase (*Fas1*), sterol regulatory element-binding protein 1 (*Srebp-1c*), hydroxyacyl-CoA dehydrogenase (*Had*), and fatty acid transporter 5 (*Fatp5*). L12-VH group was used as a control group to calculate the relative gene expression, considering that the most characteristic season of the *Ektasis* tomato is autumn and therefore the standard days. Taking into consideration the efficiency of each primers, the method proposed by Pfaffl [32] was used and the *Ppia* gene was used as housekeeping.

Statistical Analysis

The results were expressed as mean ± SEM. SPSS Statistics 22 software (SPSS Inc., Chicago, IL, USA) was used to rule out outliers and for statistical analysis. Normality and homogeneity were evaluated using the Shapiro-Wilk test and Levene's test, respectively. A two-way analysis of variance (two-way ANOVA) was performed to analyze the effect between the two main factors: photoperiod and treatment. A one-way analysis of variance (one-way ANOVA) was used for those cases in which the two-way ANOVA analysis was significant. In this instance, the following groupings between photoperiods and treatments were made: L6-nLT, L6-LT, L6-VH, L12-nLT, L12-LT, L12-VH, L18-nLT, L18-LT, L18-VH and analyzed the differences between means by post-hoc test using DMS. For the comparison analysis between means within the same photoperiod or comparing the same treatment in different photoperiods, a

Student's t-test was also performed. In cases where the requirement of normality or homogeneity was not met, the non-parametric Kruskal-Wallis and Mann-Whitney U tests were performed, as appropriate. The threshold of statistical significance was established at $p < 0.05$; and the trend in $0.05 < p < 0.1$

RESULTS

Lycopene content

As can be seen in Table 1, nLT presented a significantly higher amount of lycopene than LT ($p = 0.050$). Specifically, more than twice the content of this compound was found with respect to LT.

Table 1. Lycopene concentration of freeze-dried tomato samples

	LT	***nLT***
All-trans-lycopene	0.72 ± 0.03 *a*	1.45 ± 0.18 *b*

Concentrations are shown as mg / 100g ± SD. A wavelength of 470 nm from the DAD detector was used.

Biochemical parameters

The results of the biochemical parameters are shown in Table 2. The treatments affected circulating TAG levels in different ways (*T*, p=0.02, two-way ANOVA), mainly in animals exposed to short days, L6. In this sense, those that received LT showed a lower concentration of TAG than those that received nLT or VH ($p = 0.046$; $p = 0.000$, respectively). On the contrary, the L6-nLT animals showed statistically higher amounts with respect to their VH ($p = 0.050$). Furthermore, this group presented a higher concentration of TAG than those who received nLT but in different photoperiods, this difference being significant only with those exposed to L18 ($p = 0.023$; $p = 0.055$; L6-nLT vs L18-nLT; L6-nLT vs L12-nLT, respectively) (Table 2).

On the other hand, the photoperiod tended to affect the concentration of serum NEFAs (*P*, p = 0.085, two-way ANOVA). Specifically, the effect was observed among the animals that were exposed to either L6 or L18. In this sense, the L6-nLT group showed the highest level compared to the L18-nLT group (p = 0.043), whilst no difference was observed between their respective vehicles. Similarly, this difference was also maintained when comparing the animals exposed to L12, although it did not reach statistical significance (p = 0.092) (Table 2).

Although an effect of the photoperiod, treatment or of the interaction of both variables was not observed, there were differences in the concentration of TC between the different experimental groups (Table 2). Specifically, the animals treated with LT in L18 showed the lowest levels of TC in relation to both those that received another treatment in the same photoperiod, and to the animals supplemented with LT in the other photoperiods. However, this difference was only statistically significant with their respective VH and the L12-LT group. (p = 0.029; p = 0.081; p = 0.020, p = 0.052 L18- LT *vs* L18-VH; L18-LT *vs* L18-nLT; L18-LT vs L12-LT; L18-LT vs L6-LT, respectively) (Table 2). It should be noted that tomato consumption, regardless of the origin, in L12 and L6 seems to increase the TC level in relation to their respective VH. On the other hand, in L18, this response was totally contrary even though these differences did not reach statistical significance.

On the other hand, neither HDL-c nor LDL-c levels were significantly affected by the variables, when performing a two-way ANOVA analysis. However, it can be observed in Table 2 that the intake of tomato in L18, regardless of the origin, increases the concentration of HDL-c, this effect being significant with nLT (L18-nLT *vs* L18-VH p = 0.035, L18-LT *vs* L18-VH p = 0.174, Student's t-test). Furthermore, LDL-c levels were also lower in animals that received LT or nLT at L18, although this difference did not reach statistical significance. It is important to highlight that a photoperiod effect has been observed among the

VH of the groups exposed to L18 and L6. Specifically, the L18-VH animals tended to present the lowest levels of HDL-c in comparison with those L6-VH (p = 0.092, Student's t-test).

The two-way ANOVA analysis demonstrated a differential effect on glucose concentration, both in the type of photoperiod and in the type of treatment to which the animals were exposed (*P*, p = 0.003; *T*, p = 0.049). In this sense, the animals exposed to L12 showed higher levels of glucose than those exposed to the other photoperiods, this effect being significant between L12-VH and L18-VH (p = 0.023; p = 0.073; L12-VH *vs* L18-VH; L12-VH *vs* L6-VH, respectively one-way ANOVA). In addition, the L6-LT animals presented the lowest glucose levels compared to the others treated within the same photoperiod, and even with those that consumed this fruits and were exposed to L12 (L6-LT *vs* L6-VH p = 0.024, L6-LT *vs* L6- nLT p = 0.027, L6-LT *vs* L12-LT p = 0.005) (Table 2).

Insulinemia levels were mainly affected by exposure to different photoperiods (*P*, p = 0.045, two-way ANOVA) (Table 2). In general, the groups exposed to L6 had lower concentrations than the exposed to L12. Specifically, the animals treated with VH showed lower insulinemia than those exposed to L12 (p = 0.017 one-way ANOVA). Similarly, the L6-VH group also tended to have a lower insulin concentration than those L18-VH (p = 0.081).

On the other hand, the photoperiod significantly affected the levels of the HOMA index between the experimental groups (*P*, p = 0.008, one-way ANOVA) (Table 2). Specifically, it has been observed that the L12-VH animals presented a statistically higher index compared to those that received the same vehicle, but in the other photoperiods, this difference being statistically significant with those exposed to L6 (p = 0.004; p = 0.063; L12-VH *vs* L6-VH; L12-VH *vs* L18-VH, respectively)). In addition, the L6-VH group also tended to present a lower HOMA index than those exposed to L18 (p = 0.060, Student's test).

Table 2. Plasma parameters and atherogenic indices in Fischer 344 rats exposed to different photoperiods and supplemented with local or non-local tomato lyophilizate or vehicle for 7 weeks.

Serum parametrs	L6			L12			L18			2wA
	VH	nLT	LT	VH	nLT	LT	VH	nLT	LT	
TAG (mmol/L)	1.14 ± 0.11 *c*	1.40 ± 0.07 *a*	0.88 ± 0.09 *b*	1.17 ± 0.09 *c*	1.15 ± 0.10 *a*c*	0.99 ± 0.08 *bc*	0.95 ± 0.08 *bc*	1.10±0.10 *b*c*	0.95 ± 0.08 *bc*	*T*
TC (mmol/L)	1.80 ± 0.16 ab	2.03 ± 0.26 a	2.15 ± 0.25 ab*	1.97 ± 0.20 ab*	2.02 ± 0.23 ab*	2.15 ± 0.25 a	2.11 ± 0.24 a	1.96 ± 0.21 ab*	1.39 ± 0.13 b	
HDL-c	0.75 ± 0.12	0.80 ± 0.21	0.64 ± 0.22	0.50 ± 0.12	0.53 ± 0.01	0.66 ± 0.14	0.38 ± 0.16	0.85 ± 0.11	0.67 ± 0.12	
LDL-c	0.16 ± 0.06	0.14 ± 0.06	0.19 ± 0.08	0.09 ± 0.03	0.15 ± 0.04	0.14 ± 0.03	0.13 ± 0.02	0.08 ± 0.03	0.09 ± 0.02	
NEFAs	0.79 ± 0.10 ab	1.04 ± 0.27 *a*	0.87 ± 0.09 ab	0.78 ± 0.06 *b*	0.76 ± 0.10 *a*b*	0.77 ± 0.11 *b*	0.76 ± 0.08 *ab*	0.70 ± 0.04 *b*	0.62 ± 0.06 *b*	*P**
Glucose	8.87 ± 0.53 *ac**	8.79 ± 0.55 *ac**	7.20 ± 0.27 *b*	10.18 ± 0.55 *c*	9.52 ± 0.64 *ac*	9.22 ± 0.42 *ac*	8.50 ± 0.19 *a*	8.71 ± 0.45 *a*	8.27 ± 0.56 *ab*	*T, P*
Insulin (ng/mL)	5.38 ± 0.68 b	6.27 ± 0.86 ab	6.68 ± 1.24 ab	9.93 ± 1.53 a	7.82 ± 1.10 ab	8.05 ± 1.63 ab	8.80 ± 0.54 ab*	5.78 ± 1.30 ab	8.61 ± 1.60 ab*	*P*
Ratios										
HOMA	0.09 ± 0.01 a	0.11 ± 0.02 a	0.09 ± 0.02 a	0.19 ± 0.04 b	0.14 ± 0.02 ab	0.14 ± 0.03 ab	0.13 ± 0.01 ab*	0.11 ± 0.02 a	0.13 ± 0.03 a	*P*
Atherogenic Index	0.23 ± 0.12	0.21 ± 0.10	0.38 ± 0.14	0.38 ± 0.13	0.29 ± 0.05	0.24 ± 0.13	0.27 ± 0.15	0.14 ± 0.04	0.19 ± 0.09	
CR1	1.97 ± 0.34 b	2.91 ± 0.71 abc*	4.67 ± 1.15 c	3.97 ± 0.51 a	3.34 ±0.53 ab	2.17 ± 0.52 a*b #	4,71 ± 1.59 a	2.09 ± 0.11 b	2.71 ± 0.43 a*b	*PxT*
CR2	0.14 ± 0.07 a*b	0.24 ± 0.12 ab	0.26 ± 0.07 ab	0.10 ± 0.03 b	0.40 ± 0.12 a	0.34 ± 0.13 ab*	0.33 ±0.13 ab	0.13 ± 0.05 ab	0.26 ± 0.09 ab	
At.Coef	0.97 ± 0.34 b	2.24 ± 0.73 abc*	3.67 ± 1.15 c	2.97 ± 0.51 a	2.34 ± 0.53 ab	1.17 ± 0.52 a*b #	3.71 ± 1.59 a	1.09 ±0.11 b	1.71 ±0.43 a*b	*PxT*

Fischer 344 rats fed a standard diet were exposed for 7 weeks to a short, standard or long photoperiod, with 6 (L6), 12 (L12) or 18 (L18) hours of light, respectively, and supplemented with vehicle (VH) or with local tomato, originating in Tarragona (LT) or non-local originating in Almería (nLT). Data are expressed as the mean ± SEM. (n = 8). TAG: triacylglycerides; TC: total cholesterol; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; NEFAs: non-esterified fatty acids; HOMA: Homeostasis model Assessment; CR1 and CR2: cardiovascular risk 1 and 2, respectively; At. Coef: Atherogenic coefficient. Two-way ANOVA analysis (2x2 photoperiod factorial design (L6, L12 or L18) x treatment (VH, nLT or LT), was used to assess the differences between groups. *P*: photoperiod; *T*: treatment effect; *PXT*: effect of interaction. Different letters above the bars indicates significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicates trend ($0.05 < p < 0.1$); # Indicates significative difference with their respective VH ($p < 0.05$ Student´s t-test).

Exposure to different photoperiods and supplementation with tomatoes from different origins jointly exerted a significant effect on CR1 and At.Coef. (*PxT*, p = 0.007, two-way ANOVA) (Table 2). On the one hand, the consumption of LT in season (L12), tended to decrease the CR1 and the At.Coef. in comparison with its VH (p = 0.087, one-way ANOVA; p = 0.031, Student´s t-test). Furthermore, this L12-LT group also showed a decrease in these parameters with respect to those that consumed LT in L6 (p = 0.023). In addition, tomato consumption, regardless of origin, decreased the CR1 and At.Coef. of the animals exposed to L18, this difference being statistically significant between those that received nLT and a trend among those that received LT (CR1: p = 0.029; p = 0.075; At.Coef.: p = 0.023; p = 0.063; respectively). On the other hand, among the animals treated with VH, the exposure to short days (L6) significantly decreased both the CR1 and the At.Coef. in comparison with the exposure to the rest of the photoperiods.

On the other hand, neither AI nor CR2 were significantly affected by exposure to different photoperiods or supplementation with different treatments. However, some differences were observed between the groups. Specifically, the animals that received tomato at L12 or L18, showed a reduction in AI when compared with their respective VH. On the contrary, the LT consumption out of season, in L6, increased this parameter. However, the differences did not reach statistical significance, possibly due to the great variability that exists.

Interestingly, with regard to CR2, the L12-VH group showed a significantly lower risk than those animals treated with any of the two tomatoes under evaluation (L12-VH vs L12-nLT p = 0.037, L12-VH vs L12-LT p = 0.082, Student´s t-test). Similarly, groups exposed to short days and treated with nLT or LT showed higher CR2 than their respective VH. On the contrary, in the L18 animals, tomato consumption, regardless of origin, presented a lower CR2 when compared with their respective VH. However, these differences did not reach statistical significance.

Gene expression of enzymes related to lipid metabolism.

Both the exposure to different photoperiods and the consumption of different treatments had a significant effect on the gene expression of *Acc1* and *Fas1* (*P*, p = 0.001, p = 0.00; *T*, p = 0.028; p = 0.038, respectively). As it can be seen in Figure 1a, the consumption of LT in its consumption season, L12, produced a significant increase in the gene expression of the enzyme *Acc1* with respect to the ingestion of nLT (p = 0.014) while it tended to increase it with respect to those animals that received VH (p = 0.056). Similarly, consumption of LT in L18 also produced this increase in mRNA concentration with respect to its respective VH (p = 0.032) but did not reach statistical significance compared to the group that received nLT (p = 0.052). Furthermore, exposure to different photoperiods had a noticeable effect when observing animals that received LT. In this sense, the animals that ingested LT in L12 or L18 showed a statistically higher gene expression compared to those that received the same treatment, but in L6 (p = 0.034; p = 0.00, respectively).

On the other hand, with regard to the gene expression of *Fas1*, a significant effect of the photoperiod was observed in L18, since L18-VH, L18-nLT as well as L18-LT group presented a higher concentration of hepatic *Fas1* mRNA than the animals that received the same treatments, but in the L6 or L12 photoperiods. In addition, a differential effect of the treatment was observed in those animals that ingested nLT, since in all photoperiods they presented higher gene expression of *Fas1* than the rest of the treatments, this difference being statistically significant only in the animals exposed to long days (p = 0.012, p = 0.055; L18-nLT *vs* L18-VH, L18-nLT *vs* L18-LT, respectively). It should be noted that in all photoperiods the animals that received tomato, regardless of their origin, had a higher *Fas1* gene expression than their respective VH. Except for the L6-LT group, which was the only one that had a lower expression. However, in most cases, they have not reached statistical significance (Figure 1b).

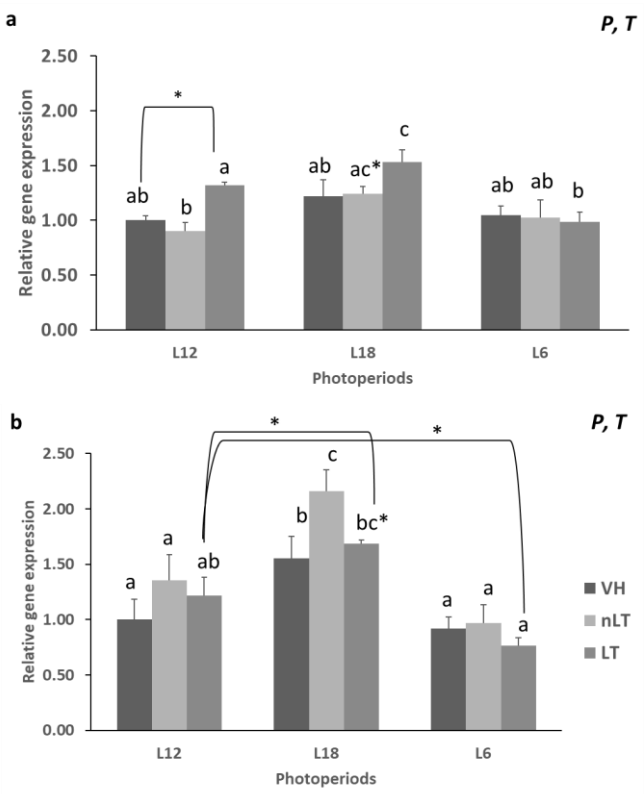

Figure 1 Gene expression of liver lipogenic enzymes. The mRNA levels of Acetyl-coenzyme A carboxylase (*Acc1*) (**a**) and fatty acid synthase (*Fas1*) (**b**) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), non-Local tomatoes (nLT) or Local (LT), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L12-VH group. *P*, photoperiod effect; *T*, treatment effect (two-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicate trend ($0.05 < p < 0.1$).

The interaction between photoperiod and treatment exerted a statistical effect on the levels of gene expression of the enzymes *Cd36* and *Had* (*PxT*: p = 0.030; p = 0.032, two-way ANOVA, respectively). As it can be seen in **Figure 2a**, the *Cd36* mRNA concentration was statistically higher in those animals that consumed nLT in L18, with respect to those that were exposed to the same photoperiod but received the other treatments (p = 0.020, p = 0.018, L18-nLT

vs L18-VH; L18-nLT *vs* L18-LT, respectively). However, in the rest of the groups exposed to L6 or L12 this differential effect was not observed between the treatments. Furthermore, the L18-nLT animals also showed higher *Cd36* gene expression compared to those L6-nLT (p = 0.044). However, on the contrary, the L18-LT group tended to present a lower concentration of mRNA of the enzyme with respect to the L6-LT (p = 0.060).

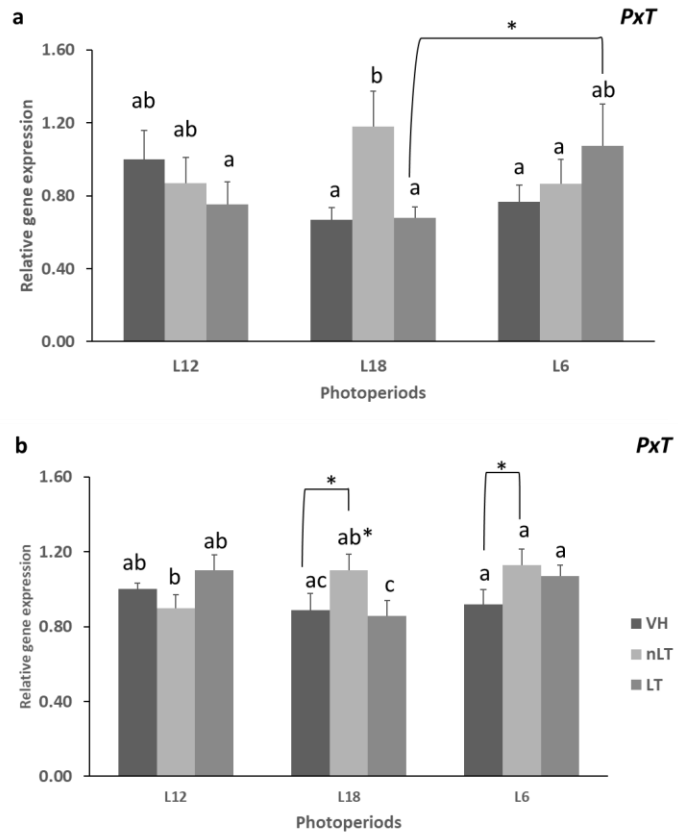

Figure 2. Gene expression of liver lipogenic enzymes. The mRNA levels of translocase homolog of CD36 (*Cd36*) (**a**) and hydroxyacyl-CoA dehydrogenase (*Had*) (**b**) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), non-Local tomatoes (nLT) or Local (LT), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L12-VH group. *P*, photoperiod effect; *T*, treatment effect (two-way ANOVA, p <0.05). Different letters above the bars indicate significant differences (p <0.05) (post-hoc DMS, one-way ANOVA). * Indicate trend (0.05 < p < 0.1).

Similarly, it can be observed in **Figure 2b** that the L18-nLT animals also showed a higher gene expression of the *Had* enzyme compared to those that received LT in the same photoperiod (p = 0.026). Furthermore, both the VH exposed to L18 and those exposed to L6 tended to present a lower concentration of mRNA than the animals treated with nLT in their respective photoperiods (p = 0.053, p = 0.053, respectively). However, those exposed to L12 this relationship was the opposite, although it did not reach statistical significance. In addition, in relation to the groups that received LT, those exposed to L18 were the ones that showed a significantly lower expression with respect to the other photoperiods (p = 0.026; p= 0.050; L12, L6, respectively). On the other hand, in relation to those supplemented with nLT, it was those exposed to L12 that showed a lower concentration of *Had* mRNA compared to L6 or L18.

The gene expression of the *Srebp-1c* protein was significantly affected by exposure to different photoperiods, while the expression of *Cpt1α* tended to be affected by the same variable (*P*, p = 0.005; p = 0.083, two-way ANOVA, respectively). In this sense, as it can be seen in **Figure 3a**, the L6-VH animals were those that presented a lower concentration of mRNA, with respect to the other VH (p = 0.026, p = 0.001; L6-VH *vs* L12-VH, L6-VH *vs* L18-VH, respectively). Similarly, the L6-nLT group tended to present a lower expression of *Srebp-1c* than those exposed to L18 (p = 0.063). On the other hand, among the animals exposed to long days, L18, those that received LT a greater amount of mRNA compared to the rest of the treatments, reaching statistical significance only with the group treated with VH (p = 0.022, p = 0.061; L18-LT *vs* L18-VH, L18-LT *vs* L18-nLT, respectively). No significant differences have been observed between the treatments of the other photoperiods.

In addition, the photoperiod influenced the gene expression of *Cpt1α*, since it has been observed that the animals that received nLT in L6 tended to present a higher concentration of mRNA of the enzyme compared to those that received the same treatment but were exposed to L18 (p = 0.077) (**Figure 3b**).

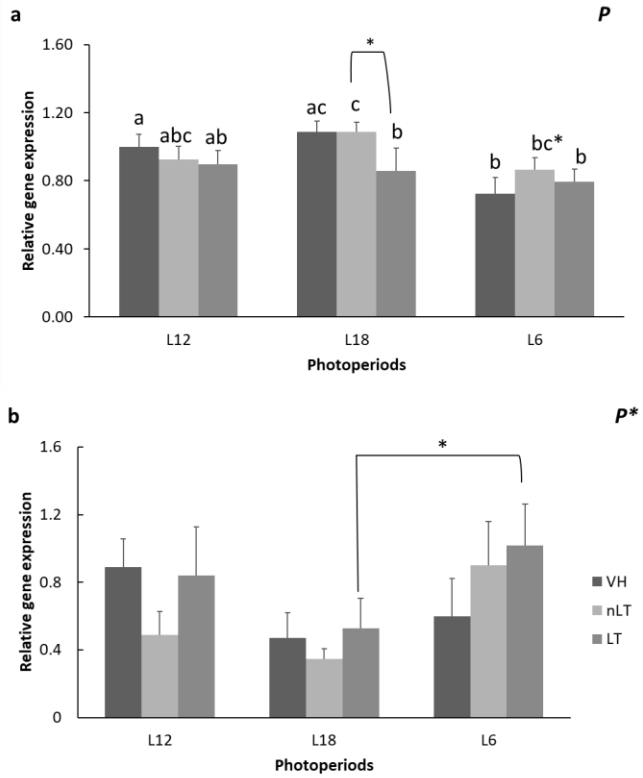

Figure 3. Gene expression of liver lipogenic enzymes. The mRNA levels of sterol regulatory element-binding protein 1 (*Srebp-1c*) (**a**) and Carnitine palmitoyltransferase 1-α (*Cpt1α*) (**b**) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), non-Local tomatoes (nLT) or Local (LT), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L12-VH group. *P*, photoperiod effect (two-way ANOVA, $p < 0.05$). Different letters above the bars indicate significant differences ($p < 0.05$) (post-hoc DMS, one-way ANOVA). * Indicate trend ($0.05 < p < 0.1$).

Although neither the photoperiod nor the treatment significantly affected the *Fatp5* gene expression, it can be seen that there were differences between the treated animals. Specifically, those who received tomato, regardless the origin, in L6, presented a higher concentration of mRNA of the *Fatp5* transporter than their respective VH ($p = 0.014$, $p = 0.010$; L6-VH *vs* L6-nLT, L6-VH *vs* L6-LT, respectively). Similarly, they also tended to show higher gene expression than

the groups treated but exposed to L12. Furthermore, the L18-VH group tended to present a higher amount of mRNA than the L6-VH group, although it did not reach statistical significance (p = 0.094) (**Figure 4**).

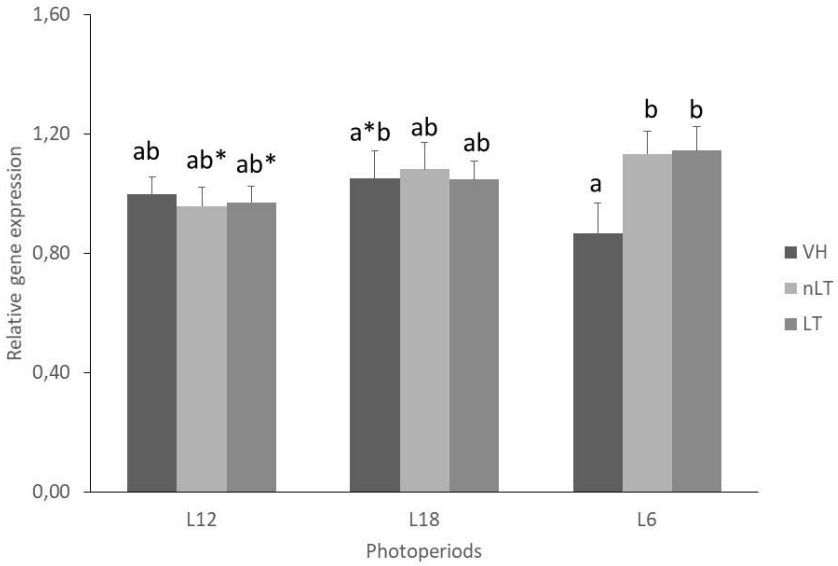

Figure 4. Gene expression of transporter of fatty acids in liver. The mRNA levels of fatty acid transporter 5 (*Fatp5*) of male Fischer 344 rats treated for 7 weeks with vehicle (VH), non-Local tomatoes (nLT) or Local (LT), and exposed to different photoperiods (short: L6, standard: L12 or long: L18). Values expressed as mean ± SEM (n = 8). The values were normalized by the L12-VH group. (two-way ANOVA, p <0.05). Different letters above the bars indicate significant differences (p <0.05) (post-hoc DMS, one-way ANOVA). * Indicate trend (0.05 <p <0.1).

DISCUSSION

In the present study, it has been evaluated how the origin of the tomato and its time of consumption can influence certain metabolic parameters, as well as the gene expression of certain key liver enzymes of lipid metabolism. In addition, we demonstrate how these differences can be given by the

characteristic composition in bioactive compounds that could be determined by the geographical origin of the plant.

First, regarding their content in bioactives, we observed that nLT had a higher content of all-lycopene than LT. Tomato is a climacteric fruit, where ripening is carried out by an increase in oxygen consumption and production of carbon dioxide and ethylene [33]. In fact, the sharp peak in ethylene production is considered one of the most important factors that determine changes in color, texture, aroma and flavor, as well as determining the content of bioactive compounds such as polyphenols and lycopene [34]. Considering that the tomato, once pulled from the plant, continues to breathe, they are generally harvested in early stages of maturation, to avoid reaching the consumer excessively ripe. Consequently, we infer that the higher content of lycopene and total polyphenols in nLT may be due to the fact that they reached the peak of ethylene and concentration of bioactive compounds at times close to their use. On the other hand, because the LTs are nearby, they are harvested in late stages of maturation, where the ethylene peak was recent and therefore begins a stage of decomposition and loss of bioactive compounds. Furthermore, storage conditions, such as light exposure, temperature or proximity to other climacteric fruits that produce and release ethylene, also influence the content of bioactive compounds in tomatoes [35]. In this sense, the amount of polyphenols can vary from 9 to 27 mg/100g and from 25 to 50 mg/100g in tomatoes from India and Spain, respectively, while the amount of lycopene can vary from 0.5 to 15 mg/100 gr according to the variety and its origin [18, 20, 36, 37]. Therefore, the metabolic effects and their health implications may also vary according to the place of origin of the ingested fruit. Despite the differences in the content of bioactive compounds between a tomato of local and non-local origin, it should be noted that according to the *xenohormesis* theory, each fruit will be beneficial for living beings in the same environment in which it was harvested, thanks that the phytochemical composition of each one

would produce adequate adaptive changes for better survival [23, 38]. A decrease in oxidative stress markers and plasma lipids are some of the observed effects of consuming locally produced fruits [27, 28, 39].

The photoperiod affects the lipid and carbohydrate metabolism of all treatments.

In the present study we observed that the photoperiod significantly affects the plasma glucose and insulin concentration, the HOMA index and the hepatic gene expression of *Acc1*, *Fas1* and *Srebp1c*. Furthermore, the photoperiod tends to affect the concentration of plasma NEFAs and the levels of C*pt1α* mRNA. Despite not having a statistically significant effect, differences have also been observed between animals that received the same treatment and that were exposed to different hours of light and darkness per day, as for example in the case of the concentration of TAG or TC. Specifically, it has been observed that those L6-VH animals presented lower amounts of plasma glucose and insulin and consequently a lower HOMA index than those L12-VH. In addition, they also presented a lower gene expression of *Srebp-1c*. Therefore, because the animals exposed to L6 had more hours of activity, it is to be expected that they have less blood glucose than those exposed to L12. It is important to note that glucose and insulin stimulate the transcriptional activation of several lipogenic genes, through complex mechanisms involving phosphorylation, dephosphorylation, and translocation of transcription factors [40]. In this sense, insulin activates the expression and activation of *Srebp-1c* through the PI3-kinase/AKt pathway and consequently stimulates *de novo* lipogenesis [41, 42]. Therefore, the L12-VH group has presented a higher glycemia, which could have produced a greater release of insulin and consequently could have stimulated a greater expression of *Srebp-1c*. However, this higher expression did not result in an induction of lipogenic genes such as *Acc1* and *Fas1*, nor in a higher concentration of plasma TAG. In this sense, it has been observed that

other transcription factors such as USFs, ChREBP and liver X receptor (LXR) are necessary to regulate the transcription of lipogenic genes [40].

On the other hand, the effect of the photoperiod has also been observed among animals that received nLT and were exposed to L6 or L18. Specifically, those exposed to L6 showed significantly higher amounts of serum NEFAs and TAG and tended to higher gene expression of *Cpt1α*. Furthermore, a lower concentration of *Fas1* and *Cd36* mRNA has also been observed, together with a trend towards a lower expression of the *Srebp-1c* protein than the L18-nLT animals. It should be noted that when there is fasting or prolonged periods of exercise, there is hydrolysis of the TAG stored in the different fatty deposits and consequently the plasma NEFAs rise to supply other tissues with energy [41]. Therefore, it is not strange to observe a higher hepatic expression of *Cpt1α* in L6-nLT animals, which is the limiting enzyme of β-oxidation, by catalyzing the passage of plasma fatty acids (FA) to the mitochondria [43]. However, sometimes the final fate of NEFAs is not oxidation but also re-esterification to TAG [44]. However, L6-nLT animals tended to have lower hepatic expression of the *Srebp-1c* protein, which controls the activation of enzymes for the synthesis of TAG and FA, including *Fas1*. Consequently, it is not surprising that L6-nLT animals also have a lower expression of this key enzyme in FA synthesis. In addition, these animals presented a lower expression of *Cd36*, which is a transporter of plasma NEFAs into the hepatocytes [41]. Although it is believed that an increase in plasma NEFAs is directly associated with a greater secretion of TAG [45], there is evidence that states that its increase does not necessarily contribute significantly to an increase in TAG secretion, since FA are not the sole source for their training [46]. Therefore, this increase in TAG is not due to a greater synthesis from NEFAs but to a greater traffic from the liver or other extrahepatic tissues as energy sources. Xie *et al*. (2017) showed that animals exposed to fewer hours of light had greater locomotive activity than those exposed to longer days [47]. In this sense, it has been observed that after

exercise there is a mobilization of lipids from adipose tissue that increases NEFAs/TAG up to two hours later [48]. Therefore, these results are to be expected, because the animals exposed to L6 had more hours of darkness and therefore of movement, expending more energy, while the L18 rats, having more hours of light, had fewer hours of activity, which is reflected in statistical differences of energetic plasma substrates. Some of these responses were also observed among animals exposed to L12-nLT and L6-nLT but did not reach statistical significance. Consequently, more studies on the consumption of energy substrates and the pathways used are necessary.

Among the animals that received LT, an effect of exposure to different photoperiods is also observed, mainly among those that were exposed to short days. On the one hand, the L6-LT group presented a glycaemia and gene expression of *Acc1* significantly lower than those exposed to L12, together with a tendency to a lower expression of *Fas1* and a higher expression of *Fatp5*. Importantly, *Fatp5* localizes to hepatocyte membranes and mediates uptake of long-chain FA from the circulation into the cell [41]. FATP5-ko animals have been shown to reduce FA uptake by up to 50%, indicating the importance of this transporter [49]. Therefore, it can be assumed that the L6-LT animals presented a greater uptake of hepatic FA through *Fatp5*, while they expressed less amount of lipogenic enzyme mRNA. Similarly, the L6-LT animals presented a lower concentration of *Acc1* and *Fas1* mRNA and a higher *Had* level, while they showed a tendency to express more *Cd36* than the L18-LT animals, indicating a lower synthesis and higher FA oxidation. However, these results are not consistent with those reported by other authors since there is evidence that exposure to fewer hours of light produces a greater synthesis of circulating TAG, due to an activation of lipogenic genes, including *Fas1*, because of a low level of NR1D2 [50–52]. In addition, other authors affirm that exposure to L6 produces an alteration in the homeostasis of glucose and NEFAs metabolism, by observing an increase in these parameters and a lower gene expression of

Cd36 and *Had*. However, these studies were carried out with animals fed a standard diet and in the absence of tomato. Consequently, one could think of a particular effect of the treatment, which will be discussed later.

Tomato consumption has differential effects on lipid metabolism depending on its geographical origin.

In the present study, a significant treatment effect has been observed between the biochemical parameters and between the gene expression of key enzymes in lipid metabolism. Specifically, the consequences of tomato consumption on serum markers were evidenced in the L6 photoperiod since the levels of TAG and glucose were affected. On the one hand, the animals that consumed LT had lower levels of TAG and glucose than those that received only VH. In contrast, those who received nLT had higher TAG levels than those who received VH, while blood glucose was not significantly affected. Therefore, a clear effect of the geographical origin of the tomatoes is observed, being the consumption of LT the one that produced a decrease in TAG and glucose in relation to the consumption of VH and nLT. In this sense, there are numerous studies where an improvement in the lipid and glycemic profile is observed when supplementing the animals with tomato. However, most of them use high-fat diet models or animals with an established metabolic pathology such as hepatic steatosis, diabetes mellitus, cardiovascular problems, or obesity to observe these differences [53–58]. Unfortunately, there is little bibliography on the preventive effect of its intake in a healthy animal model and studies that investigate the molecular mechanisms of this effect are required. A hypoglycemic and lipid-lowering effect of the intake of polyphenolic compounds has been observed, because of their ability to inhibit the activity of digestive enzymes and reduce the hydrolysis of carbohydrates and fats [59]. For example, Fan *et al.*, (2013) evidenced an inhibitory effect of dipepityl-peptidase IV(DPP-IV) by polyphenolic compounds of fruits and vegetables [60].

However, in our study, the decrease in TAG and glucose has not been observed in all the groups that consumed tomato, but only in L6-LT. This effect may be due to the higher concentration of flavanols contained in LT compared to nLT observed in our previous publication [30]. In this sense, it has been observed that luteoin acts non-competitively to inhibit the activity of DPP-IV and thus exert a hypoglycemic effect [60].

On the other hand, an effect of the treatment is also observed on the gene expression of enzymes related to lipid metabolism in animals exposed to L18. Specifically, those who received nLT showed a higher concentration of *Fas1* and *Cd36* mRNA, together with a tendency to an increased *Had* expression in relation to their respective VH. Differences were also observed between the groups that received nLT and LT. Notably, *Cd36* is a transmembrane transporter for carotenoids, such as lycopene [61]. Therefore, it is not surprising that the nLT, having presented a greater amount of lycopene than the LT, also had a higher gene expression of its transporter. In this sense, Elvira-Torales *et al*. (2018), observed a higher expression of lipid transporters, including *Cd36* and *Fatp5* in animals that received tomato, assuming a greater entry of lipids to the hepatocyte [62]. In addition, they also observed a LXR gene overexpression, which has an effect at the transcriptional level by stimulating lipid synthesis via SREBP2 and bile acid excretion [53, 62].

The consumption of TL in season, L12, decreases CR1 and At.Coef.

In the present study we observe the metabolic consequences of the intake of tomatoes from different geographical origins together with the effects of its consumption in different photoperiods (*PxT* effect), which simulate different seasonal times. It should be noted that the *Ekstasis* variety used is harvested in the autumn season, which is simulated by the L12 photoperiod. Specifically, those animals that consumed tomato in its characteristic season (L12), did not show any difference in the serum concentration of TC, nor in HDL-c or LDL-c,

regardless the origin. However, they notably presented a lower CR1 and At.Coef. than their respective VH, this difference being significant only with those who ingested LT. In contrast, those exposed to short days, L6, did not present different serum cholesterol levels, but those who consumed LT had higher CR1 and At.Coef. than their respective VH and in relation to their consumption in the other photoperiods. These results would indicate the importance of seasonal consumption and the local origin of the fruit on markers of atherogenic risk. Various authors observed that the determination of CR1 is a more sensitive and specific cardiovascular risk index than the TC concentration, since it is an indicator of the formation of coronary plaques and the thickness of the intima media in the carotid arteries [63, 64]. In addition, several authors observed that At.Coef. is also a good predictor of cardiovascular risk, since it reflects the atherogenic potential of all lipoproteins, since it measures non-HDL-c cholesterol, thus including LDL-C, VLDL-c, and Intermediate density lipoprotein (IDL-c) [65, 66]. Similarly, we previously observed that when tomatoes are consumed in their season, they improve or maintain antioxidant biomarkers, reducing oxidative stress, compared to their consumption in other seasons [28].

On the other hand, even though a significant *PxT* effect was not observed on TC levels, in the animals exposed to L18 and supplemented with some type of tomato, they showed a lower concentration of TC than their respective VH. However, in the other photoperiods this difference was not observed, showing an overall effect of both the photoperiod of consumption and the origin of the tomato. In this sense, serum cholesterol levels depend on the balance between the consumption of products that contain it and *de novo* cellular synthesis. The enzymes 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and cholesterol acyl transferase (ACAT), as well as the cellular receptors for LDL-c, play a key role in the regulation of cholesterol metabolism [67, 68]. In agreement with our findings, Navarro-Gonzáles *et al*. (2014), also observed a

hypocholesterolemic effect in animals that consumed tomato juice together with a normal diet, attributing this effect to an inhibition in the activity and of the HMGCR enzyme, thanks to an inhibition post-transcription [69, 70]. Some phenolic compounds, such as flavonoids, have a high affinity with the HMG-CoA enzyme, either by hydrophobic Van de Walls bonds or by the numerous -OH groups, which produce a union of these bioactive compounds, causing inhibition and consequent decrease in cholesterol synthesis [70, 71]. It should be noted that according to our previous publication, LT has a greater amount of total flavanols than nLT, which could explain this decrease. Furthermore, Elvira-Torales *et al*. (2018), observed in rats supplemented with tomato juice, an increase in the mRNA expression of the liver X receptor (LXR) which stimulates the excretion of bile acids by activating CYP7A1 [62]. Therefore, a decrease in the synthesis and a greater excretion would be the possible mechanisms by which the tomato would exert its hypocholesterolemic effect. However, this effect is not observed in the other animals treated in the rest of the photoperiods, so it could be inferred in an overall effect of the treatment and the exposure to the long photoperiod.

CONCLUSIONS

In conclusion, in this study we have obtained evidence that the metabolic effects of tomato consumption depend not only on the season of consumption, but also on the geographical origin of the fruit. Specifically, (**a**) in the tomato consumption season, L12, a beneficial effect of the intake of LT was observed on the cardiovascular risk markers CR1 and At. Coef.; (**b**) off-season, in L6, caused an opposite effect on these parameters; (**c**) however, L6-LT caused a decrease in blood glucose and TAG; (**d**) On the contrary, consumption of nLT in L6 increased them; (**e**) and in L18 caused an increase in the gene expression of *Cd36*, *Had* and *Fas1*. These effects could be due to the higher lycopene content

found in nLT. However, the same effect was not observed in all photoperiods. Consequently, more studies on the interaction of these bioactive compounds with other metabolic enzymes are required to understand the variation that exists between the consumption of phytochemicals, the use of energy substrates and the number of hours of light and darkness per day.

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SUPPLEMENTARY MATERIAL

Table 1. Concentration of (poly)phenolic compounds in local (LT) and non-local (nLT) Ekstasis tomatoes.

Compound	LT	nLT
Flavonoids	399.32	283.37
Caffeic and dihydrocaffeic acid derivatives	185.34	214.49
Free phenolic acids	71.90	120.22
Hydroxybenzoic acid derivatives	102.34	89.42
Hydroxycinnamic acid derivatives	867.26	881.67
Hydroxycinnamoylquinic acids	1149.83	1560.35
Phenylpropanoic acid-glycosides	586.30	596.02
(Poly)phenolic compounds	3362.30	3745.54

The results are expressed as µg/g dw. Table was adapted from Cruz-Carrión et al. (2002).

Table 2. Nucleotide sequences of primers used for real time quantitative PCR.

Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')
Acc1	TGCAGGTATCCCCACTCTTC	TTCTGATTCCCTTCCCTCCT
Cd36	GTCCTGGCTGTGTTTGGA	GCTCAAAGATGGCTCCATTG
Cpt1α	GCTCGCACATTACAAGGACAT	TGGACACCACATAGAGGCAG
Fas1	CTATTGTGGACGGAGGTATC	TGCTGTAGCCCAGAAGAG
Fatp5	CCTGCCAAGCTTCGTGCTAAT	GCTCATGTGATAGGATGGCTGG
Had	ATCGTGAACCGTCTCTTGGT	AGGACTGGGCTGAAATAAGG
Srebp-1c	CCCACCCCCTTACACACC	GCCTGCGGTCTTCATTGT

The table shows the nucleotide sequences of primers used for PCR amplification. *Acc1*, acetyl CoA carboxylase; *Cd36*, fatty acid translocase, homologue of CD36; Cpt1α, carnitine palmitoyltransferase 1 alpha; Fas1, sterol regulatory element-binding protein 1; *Fatp5*, fatty acid transport protein 5; *Had*, hydroxyacyl-CoA dehydrogenase; *Srebp1c*, sterol regulatory element-binding protein 1c.

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María Josefina Ruiz de Azua

6. DISCUSIÓN GENERAL

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DISCUSIÓN

Para la realización de la presente tesis doctoral se ha partido de la hipótesis de **que el consumo de frutas de origen local, específicamente cerezas y tomates, así como su ingesta en temporada, provocan un efecto metabólico favorable extra, especialmente, en el metabolismo lipídico.** En consecuencia, el objetivo principal **fue evaluar si el origen geográfico y/o la época de consumo de estas frutas afectan el metabolismo lipídico y parámetros metabólicos** asociados en un modelo animal saludable.

Se ha evidenciado que los mamíferos poseen un mecanismo molecular que controla y modula el ritmo circadiano de determinadas funciones biológicas y fisiológicas del organismo. En tal sentido, existen factores genéticos y ambientales que determinan cómo los ritmos circadianos y estacionales influyen sobre el metabolismo energético y el comportamiento alimentario. Asimismo, el fotoperiodo y la cantidad de horas de luz diarias son los estímulos de mayor importancia que regulan la sensibilidad estacional de los organismos [1,2]. Por ejemplo, se ha observado que la grasa corporal se incrementa significativamente durante el invierno en latitudes alejadas del ecuador, donde las temperaturas, el clima, las horas del día, el nivel de actividad física y gasto energético, presentan marcadas fluctuaciones durante el año, siendo en invierno mucho menores que en verano [3]. En consecuencia, estas variables pueden impactar negativamente sobre el desarrollo de alteraciones y patologías metabólicas crónicas. Sin embargo, en la actualidad, existen muy pocos estudios al respecto y faltan evidencias que describan los mecanismos moleculares por los cuales se lleva a cabo esta regulación. Por lo tanto, el uso de modelos animales para la profundización en el estudio de los cambios a nivel metabólico que se producen mediante la exposición a diferentes horas de luz diaria ayudaría a la comprensión de los mecanismos subyacentes y a preservar

el estado de salud. Si bien existen diversas especies que muestran respuestas rápidas a los fotoperiodos, como los hámster, la limitada información genética disponible en bases de datos, sumado a la escasez de herramientas moleculares y bioquímicas para su estudio, hace que sea un impedimento su utilización [4,5]. No obstante, en los últimos años la utilización de ratas Fischer 344 ha facilitado el estudio de la exposición a diferentes fotoperiodos, ya que muestran una marcada respuesta fisiológica y reproductiva a las variaciones estacionales de la duración del día [6,7]. Cabe destacar, que la especie va a determinar el fotoperiodo crítico para el cambio entre los fotoperiodos inhibitorios o estimulantes [8].

En tal sentido, el grupo de investigación de Nutrigenómica ha demostrado en trabajos previos que la exposición a un fotoperiodo corto, con 6 horas de luz, altera significativamente el metabolismo glucídico y la captación y oxidación de AG en el hígado, y que produce una menor expresión génica de enzimas lipogénicas, adipogénicas y claves en la lipólisis en el tejido adiposo retroperitoneal, en comparación con animales expuestos a fotoperiodo estándar, con 12 horas de luz [9]. Asimismo, en la presente tesis, considerando el objetivo general, **evaluamos los efectos metabólicos a nivel sérico, hepático y muscular, con énfasis en el metabolismo lipídico, que provoca la exposición a diferentes fotoperiodos.** Si bien también el fotoperiodo corto, L6, generó alteraciones en el metabolismo glucídico, observamos alteraciones más evidentes en el metabolismo lipídico, destacando las diferencias observadas entre la exposición a fotoperiodo largo, L18, en comparación con el estándar, L12. En base a los resultados, sugerimos que L18 estimula la lipogénesis *de novo*, debido a una mayor expresión génica de la enzima *Fas1*. Específicamente, hemos evidenciado que la exposición a L18 resulta en mayores alteraciones sobre la expresión génica de enzimas metabólicas analizadas (*Cpt1α, FAT/cd36, Fas1, Scd1*) y en el flujo enzimático de síntesis de los AG (estimado a partir de las relaciones EPA/ALA, DHA/ALA, AA/AL, AA/GLA)

en comparación con el grupo control L12 (Manuscrito 1). Si bien, en estos animales no se registraron cambios en el peso corporal ni en la ingesta de alimentos [10], otros autores observaron que la exposición a fotoperiodos de más de 12 horas, dan como resultado una respuesta fisiológica y una mayor expresión génica de hormonas tiroideas hipotalámicas y de genes de señalización, que se refleja en un aumento de masa corporal, principalmente a través de la masa magra, en comparación con días más cortos [6]. No obstante, se ha evidenciado que el incremento de peso varía en función del tipo de depósito, con la especie y cepa de los animales. En tal sentido, los hámster de Djungarian incrementan sus tejido adiposo blanco, como respuesta a una insensibilidad a la leptina durante los días largos [11], mientras que las ratas Sprague-Dawley no varían el peso corporal ante las mismas cantidad de horas de luz al día [12].

Como consecuencia de la escasa información sobre la repercusión de la exposición a diferentes horas de luz sobre el perfil de AG de tejidos metabólicos claves, hemos analizado cómo afecta el fotoperiodo a la proporción de AG en el hígado y en el músculo esquelético, así como hemos intentado dilucidar los mecanismos implicados en estos efectos. En tal sentido, los AG son los componentes estructurales principales de las membranas celulares, que no sólo le otorgan el grado de fluidez, sino que también al formar las balsas lipídicas, interactúan con otras moléculas y funcionan como segundos mensajeros implicados en respuestas celulares. En consecuencia, los AG determinarán tanto el progreso de procesos fisiológicos como también jugarán un papel crítico en la prevención o desarrollo de diversas patologías [13,14]. En concreto, la exposición a L18 resulta en una mayor proporción de ácidos grasos saturados (AGS) en hígado, principalmente AP y AE en comparación con L12. Además, encontramos diferencias en el nivel de los AG poliinsaturados (PUFAS) n-3 hepáticos, sugiriendo que la exposición lumínica extrema (ya sean muchas horas, L18, o pocas, L6) podría disminuir la β-oxidación de dichos AG, o podría

provocar una mayor síntesis. Considerando que la β-oxidación depende de la capacidad de captar AG al interior celular y de la capacidad de las enzimas oxidativas, la tendencia a una menor expresión génica de *Cpt1α* y *FAT/cd36* por parte de los animales L18 apoyarían esta hipótesis. Sin embargo, los cambios no son tan evidentes al comparar los animales control con L6 (Manuscrito 1).

A nivel muscular, también se han evidenciado efectos metabólicos diferenciales a la cantidad de horas de luz diaria. Nuevamente los cambios más evidentes han sido entre los animales expuestos a L12 y L18. En concreto, aquellos L18 mostraron menores niveles de AGS principalmente menor proporción de AP, lo cual podría resultar en una mayor sensibilidad a la insulina y menor glucemia, que aquellos expuestos a L12. Sin embargo, también se puede inferir una mayor oxidación muscular ya que los animales L18 presentaron mayor proporción de PUFAs n-3 (DHA y 22:5 n-3) los cuales se asocian con mayor oxidación glucídica y lipídica [15]. No se observaron cambios en la expresión génica de las enzimas claves en estos procesos metabólicos, probablemente debido a la gran variedad de factores que la modulan. En este sentido, más estudios son necesarios para comprender los mecanismos implicados. Es importante destacar el hecho de que, dado que las ratas son animales nocturnos que desarrollan sus principales actividades durante las horas de oscuridad, al estar expuestas a 18 horas de luz presentarían un estado de mayor sedentarismo. En tal sentido, algunos autores observaron una significativa menor actividad locomotora en animales que fueron expuesto a días largos en los puntos ZT10 y ZT14 en comparación con los grupos control o los expuestos a días cortos [16]. Además, al no haberse documentado diferencias en la cantidad de energía y alimento consumido entre los grupos en trabajos anteriores [10], se podría pensar que los animales expuestos a L18 ingieren mayor kcal/hora que el resto, siendo esta diferencia el doble y el triple, en comparación con los L12 y L6 respectivamente. Por lo tanto, a pesar de que globalmente consuman las mismas kcal a lo largo del día, la respuesta

metabólica sería diferente al ser más corta la duración del tiempo de ingesta. Otros autores han observado que pocas pero grandes ingestas en el día producen cambios adaptativos en el metabolismo energético, que se reflejan en una más rápida absorción de glucosa y grasas por el intestino, mayor síntesis de glucógeno y mayor lipogénesis [17]. Sin embargo, es llamativo que las diferencias sean más significativas al comparar el grupo L18 con el L12 que con el L6, sugiriendo un efecto de mayor adaptación de estos últimos (Manuscrito 1). En tal sentido, se ha observado que las ratas Fischer 344 podrían reportar cierto acostumbramiento a la exposición crónica a fotoperiodo corto sobre el peso corporal y parámetros reproductivos [12,18].

Por otro lado, en los últimos años se ha evidenciado que algunos nutrientes y compuestos alimentarios pueden modular los ritmos biológicos. Aunque los mecanismos no estén del todo claros, se sabe que tanto el tipo de dieta, la presencia de determinados biocompuestos, como el momento de ingesta influyen en el ritmo circadiano y circanual de determinados procesos fisiológicos, incluyendo la digestión, el metabolismo y el gasto energético [19–21]. Por lo tanto, en el presente trabajo de investigación, se planteó el objetivo de ***evaluar las consecuencias sobre parámetros metabólicos del consumo de cerezas y tomates (ricos en compuestos bioactivos) de diferentes orígenes geográficos, en diferentes fotoperiodos.***

Al respecto, el consumo de cereza muestra consecuencias moduladoras del fotoperiodo. Particularmente, la cantidad de luz diaria afectó la expresión génica de *Fas1* hepática, siendo significativamente mayor en L18 que en L12 o L6 (Manuscrito 1). Sin embargo, el consumo de nLC en L6, fuera de temporada, produjo una expresión génica similar a la observada en L18, al mismo tiempo que se diferenciaba de su respectivo VH (Manuscrito 2). Asimismo, el contenido de ácido palmitoleico muscular fue menor en aquellos animales expuestos a días largos, L18, en comparación con el grupo control, L12. Sin embargo, cuando los animales expuestos a días estándar consumieron cereza,

independientemente el origen, los valores disminuyeron a valores similares a los animales expuestos a L18, neutralizando el efecto fotoperiodo. Lo mismo ocurre con el contenido de PUFAs totales y PUFAs n-6, ya que los animales que consumieron cereza fuera de temporada, en L12, mostraron un nivel significativamente menor de estos AG, pero que fueron similares a los animales expuestos a L18 (Manuscrito 3). Además, los animales que consumieron cereza en temporada no mostraron cambios significativos en los niveles de AA en comparación con su VH, sin embargo, su consumo fuera de temporada, en L12, los incrementó. No obstante, en estos últimos también incrementó el contenido de PUFAs n-3, principalmente a través del aumento de DHA, en comparación con el VH, alcanzando valores similares al de los animales expuestos a L18 (Manuscrito 3). En tal sentido, Gilbert y col., observaron que el consumo de cereza fuera de temporada afecta la fisiología del tejido adiposo, estimulando la acumulación lipídica debido a una regulación a la baja de la expresión de genes implicados en la absorción y oxidación de grasas por los adipocitos [22]. Además, otros autores observaron una modulación de la leptina hipotalámica asociada al consumo de cereza pero sólo cuando fue consumida fuera de temporada [23].

Similarmente, el consumo de tomate de origen local en temporada L12, disminuye el CR1 y At.Coef, pero en L6, produce un efecto inverso. Por el contrario, su ingesta en L18, tiende a disminuirlo y se asemeja a los valores alcanzados en L12. Por lo tanto, estos resultados recalcan una vez más la importancia del consumo de fruta en temporada (Manuscrito 4). Recientemente hemos demostrado que la ingesta en temporada de tomate también resulta en un efecto beneficioso, ya que se ha observado un menor estado oxidativo, reflejado por una concentración de ROS y MDA inferior en comparación que cuando se consume fuera de temporada [24]. Otros autores también estudiaron los efectos del consumo de fruta en y fuera de temporada. Al respecto, un incremento de la masa grasa fue observado en animales que

consumieron naranja fuera de temporada, sugiriendo que percibían señales de otro fotoperiodo y así adaptaban el fenotipo característico de la temporada de consumo de la fruta [25].

Por otro lado, la teoría de *xenohormesis* expone que la composición fitoquímica característica de las plantas, dada por las condiciones ambientales y externas en la cual se ha desarrollado, va a proporcionar a los seres heterótrofos que la consuman una información adaptativa a aquel contexto [26]. Sin embargo, en la actualidad, gracias a la globalización y a la facilidad de accesibilidad a productos de diversos orígenes y en cualquier época del año, las señales adaptativas de una fruta o vegetal de un sitio, no corresponderían con el contexto en donde se consume el alimento. Por lo tanto, se podría pensar en "señales erróneas" que tendrían algún tipo de consecuencia en el organismo [27]. Cabe destacar, que investigaciones llevadas a cabo en el grupo de investigación evidenciaron una composición fitoquímica diferente entre las cerezas estudiadas [10]. Además, también se han analizado la composición individual de compuestos fenólicos en las cerezas (datos aún no publicados) que demuestran la existencia de diferencias significativas a nivel cualitativo en algunas especies en función del origen geográfico.

En tal sentido, en el presente trabajo evidenciamos efectos diferenciales entre una misma fruta de diversos orígenes, considerando que todos se alimentan con la misma dieta. Específicamente, los animales que consumieron LC en temporada, L18, mostraron una mayor concentración de HDL-c sérico y menor nivel de SFA junto con una mayor expresión génica de *Scd1, Acc1, Fas1* en comparación con su respectivo VH en el hígado. También mostraron una mayor proporción muscular de los AG: 15:0, ácido palmitoleico, y *c11*-18:1, junto con una mayor concentración de mRNA de *FAT/cd36* y *AdipoR2* y menor nivel de DHA en músculo (Manuscrito 2 y 3). Al respecto, la cereza de origen local presenta mayor contenido en antocianinas y flavanoles, polifenoles que fueron asociados con beneficios metabólicos [28,29]. Desafortunadamente, la

limitada información sobre el efecto de estos polifenoles en modelos animales sanos y alimentados con dieta estándar, imposibilita la comparación de resultados. Sin embargo, se hallaron efectos hipolipemiantes, principalmente en la expresión de enzimas lipogénicas hepáticas y mayor excreción de ácidos biliares fecales, en animales que recibían una dieta alta en grasa y que eran suplementados con estos biocompuestos [30–32]. En concordancia con los resultados del presente trabajo, se ha observado que el consumo de un extracto de antocianinas produjo una activación de AMPK a nivel no sólo muscular, sino que también a nivel hepático y en el tejido adiposo [33]. Otros autores, evidenciaron que una suplementación de flavonoles, provoca una mayor cantidad de mitocondrias, mayor respiración mitocondrial, reflejado en una mayor actividad de los complejos de la cadena respiratoria del ciclo de Krebs, una tendencia a una mayor oxidación de palmitoil-carnitina y expresión de *cpt1β* en el músculo gastrocnemio [34,35].

Por otro lado, el efecto causado por nLC en temporada fue distinto que LC, ya que los animales mostraron en el hígado una menor proporción de AGS, AP y EPA, y una tendencia a menor AE. También mostraron un mayor nivel de c11-20:1, PUFAs totales y PUFAs n-6, de AL, 20:2 (n-6), ALA, 22:5 (n-3), mayor contenido en colesterol total, colesterol libre, fosfolípidos totales, fosfatidiletanolamina, esfingomielina, asparagina y menor expresión génica de *Fads2* que su respectivo VH. Además, presentaron menor nivel de DHA y EPA, junto una mayor expresión génica de *Cs* muscular (Manuscrito 3). Al respecto, la nLC cuenta con mayor contenido de flavonoles, y ácidos hydroxybenzoicos y hydroxycinnamicos (datos no publicados). En tal sentido, otros autores observaron que los flavonoles, principalmente la quercitina, induce una ω-oxidación de FA hepáticos y una mayor expresión de PGC1α en el músculo esquelético, el cual promueve la biogénesis mitocondrial y promueve la oxidación [36,37]. Otros mostraron un efecto hipolipemiante del ácido gálico, al disminuir significativamente la concentración de colesterol, TAG y daño

oxidativo [38]. Sin embargo, estos estudios fueron realizados en animales que recibían una dieta rica en grasas y con desordenes metabólicos, por lo que dificulta una comparación directa de los resultados.

En tal sentido, la concentración de polifenoles de las cerezas está determinada por múltiples factores que van desde las condiciones ambientales donde fue cultivada la fruta y su variedad, las condiciones de transporte y almacenamiento, como temperatura, exposición lumínica, humedad hasta el tiempo que trascurre hasta que es consumida [39].

Otro factor a tener en cuenta es la composición nutricional de la dieta estándar. En tal sentido, luego de decidir analizar el perfil lipídico de AG en los tejidos, analizamos la ficha técnica de la casa comercial del alimento brindado a los animales (Anexo). Considerando que las ratas consumen en promedio alrededor de 40 gr de alimento al día, y que 1 kg de alimento proporciona 15000 mg de AL y 1200 mg ALA, en consecuencia los animales no sólo recibieron una dieta deficiente en AG esenciales, sino que también recibieron una dieta con un ratio AL:ALA de aproximadamente 12:1. En tal sentido, la proporción de AG esenciales determina la conversión de PUFAS n-3 o n-6 (EPA y AA, principalmente), y su incorporación a los fosfolípidos de membrana, lo cual también influye en la predisposición a procesos pro- o antinflamatorios. Se ha observado que una ratio mayor a 5 incrementa la incorporación de AA en los tejidos, mientras que una ratio menor a 5 aumenta la incorporación de EPA [46]. En consecuencia, el uso de esta dieta podría ser considerado una debilidad del trabajo y se plantea su no utilización para próximos experimentos, con tal de corroborar los resultados obtenidos. Independientemente de esta situación, dado que todos los animales fueron alimentados con la misma dieta, las diferencias en la proporción de los PUFAs, debido a la exposición a diferentes horas de luz y/o por la incorporación de fitoquímicos que podrían interferir en diversas vías metabólicas, son evidentes.

Las consecuencias metabólicas del consumo de tomates también difirieron en algunos parámetros en relación con el origen (Manuscrito 4). Al respecto, sólo los animales LT disminuyeron los TAG y glucosa sérica en L6 con respecto al VH. Considerando que LT posee mayor cantidad de luteoína [40], podría inferirse el efecto hipolipemiante e hipoglucémico a la inhibición de la actividad de dipepitil-peptidasa y a la estimulación de la expresión de PPARα hepática reportado por varios autores [41,42]. Sin embargo, estas evidencias son en base a modelos con dietas que provocan desordenes metabólicos. La escasez de estudios sobre modelos saludables imposibilita la comparación de resultados. En el resto de los parámetros séricos no hubo diferencias entre los dos tipos de tomates. Además, la mayor expresión génica de *Fas1*, *FAT/cd36*, junto con una tendencia a mayor expresión de *Had* hepática, fue encontrada en los animales que recibieron nLT en L12 con respecto al resto de los tratamientos. En tal sentido nLT presenta mayor concentración de licopeno que LT, el cual ingresa a las células a través del trasportador FAT/cd36, sugiriendo su mayor expresión. Además, se ha observado que animales suplementados con jugo de tomate, estimula la sobreexpresión del receptor X hepático (LXR), el cual estimula la lipogénesis *de novo* a través de la inducción transcripcional de SREBP1c y sus genes diana [43,44].

Cabe destacar que la ingesta de tomate o cereza, ya sea local o no local o dentro o fuera de su temporada característica, no va a implicar un efecto deletéreo sobre la salud, ya que el consumo de frutas siempre es beneficioso, no solo por la cantidad de compuestos bioactivos sino porque son fuente de fibra, vitaminas, minerales y agua [45]. Sin embargo, con estos resultados exponemos la importancia de la priorización de aquellos productos de origen local y por lo tanto de temporada, ya que imparten un beneficio extra al organismo, al aportar una sincronía y armonía con el entorno que nos rodea. En otras palabras, cuando los ritmos externos sincronizan los relojes internos de los organismos, se produce una mejora en patrones de comportamiento,

crecimiento y estado de salud, debido a que estos ritmos proporcionan una representación interna de la duración del día, optimizando la fisiología y el comportamiento según las distintas demandas de los fotoperiodos.

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UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

7. CONCLUSIONES

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

CONCLUSIONES

La presente tesis ha evaluado como el origen geográfico de las frutas y su fotoperíodo de consumo impacta sobre el metabolismo de animales saludables. En base a un análisis cuantitativo de varios parámetros metabólicos sobre tejidos claves relacionados con el metabolismo energético, principalmente el hígado y músculo esquelético, con énfasis en el metabolismo lipídico, se puede concluir que:

1. La exposición a diferentes fotoperíodos afecta parámetros séricos, la expresión génica de enzimas lipogénicas y de enzimas relacionadas con la instauración de ácidos grasos, así como también el perfil de ciertos ácidos grasos hepáticos y musculares.

    1.1. La exposición a fotoperíodo largo induce lipogénesis *de novo* en el hígado, tiende a generar una menor β-oxidación y estimula una mayor acumulación de ácidos grasos saturados y PUFAS n-3 que el fotoperíodo estándar, posiblemente por incremento de la expresión génica de *Fas1,* una tendencia a menor expresión de Cpt1α y FAT/cd36, junto a una menor insaturación por parte de Sdc1 y una relación de DHA/ALA incrementada.

    1.2. La exposición a fotoperíodo estándar genera una menor acumulación de ácidos grasos saturados y PUFAS n-3 in el hígado, debido a una mayor β-oxidación y/o menor síntesis, mientras que genera una acumulación de ácidos grasos saturados en el músculo esquelético.

    1.3. La exposición a días largos afecta el metabolismo del ácido linoleico, posiblemente a través de la modulación de la síntesis de la fosfatidilcolina, comparado con los días con 12 horas de luz.

1.4. La exposición a un fotoperíodo largo afecta la biosíntesis de ácidos grasos insaturados, posiblemente por una menor expresión génica de Scd1, en comparación con un fotoperíodo estándar.

1.5. Los efectos de la exposición a diferentes horas de luz son menos evidentes en el músculo esquelético.

1.6. Efectos diferenciales son observados mayoritariamente entre el fotoperíodo estándar y el largo, sugiriendo que la exposición crónica a un fotoperíodo corto causa un acostumbramiento metabólico.

2. Tanto el origen geográfico de las cerezas dulces *Brooks*, como la exposición a diferentes fotoperíodos afectan parámetros metabólicos en animales saludables.

2.1. El consumo de cerezas dulces en temporada, simulado por el fotoperíodo largo, independientemente el origen, disminuye el contenido de ácidos grasos saturados hepáticos (principalmente el ácido palmítico) y el nivel de PUFAS n-3 en el músculo esquelético, posiblemente debido a una mayor insaturación por parte de la enzima Scd1 hepática, y a una mayor oxidación muscular, respectivamente.

2.2. El origen geográfico de la cereza determina diferentes consecuencias metabólicas, dependiendo de la temporada consumida. Específicamente, en el fotoperíodo largo, el cual es la temporada de consumo, las cerezas de procedencia local incrementan la expresión génica del transportador de ácidos grasos en el músculo FAT/cd36, como así también la proporción del ácido graso 15:0, el ácido palmitoleico y el *c*11-18:1.

2.3. La cereza de origen no local, consumida en temporada, altera el metabolismo del ácido linoleico y e incrementa la concentración de metabolitos lipofílicos hepáticos comparado con su respectivo vehículo.

2.4. El consumo de cereza local incrementa el HDL-c y la expresión génica de las enzimas hepáticas *Acc1* y *Fas1* en el fotoperíodo largo, mientras que disminuye los triacilglicéridos cuando se consumen en fotoperíodo corto, comparando con el consumo de sus respectivos vehículos.

2.5. El consumo de cereza, independientemente el origen, en el fotoperíodo estándar, provoca un nivel de ácido palmitoleico y una proporción de PUFAs totales a nivel muscular, como así también un nivel de PUFAs n-6 hepáticos, similar al observado con el consumo de cereza en temporada (fotoperíodo largo).

3. El origen geográfico del tomate *Ekstasis*, como así también el fotoperíodo consumido, tiene un efecto diferencial sobre marcadores de la salud metabólica.

3.1. El consumo de tomate de origen local, en temporada, simulada por el fotoperíodo estándar, disminuye el riesgo cardiovascular 1 y el coeficiente aterogénico, en comparación con su respectivo vehículo.

3.2. El consumo de tomate, independientemente el origen, en días largos, disminuye la concentración sérica del colesterol total.

3.3. El consumo de tomate de origen local, fuera de temporada, en días cortos disminuye la concentración de triglicéridos, alcanzando valores

similares a cuando se consume en temporada, sin embargo, la ingesta de tomate de origen no local tiene efecto opuestos.

3.4. La ingesta de tomate local baja la glucosa sanguínea y el colesterol total fuera de temporada, en los fotoperíodos cortos y largos, respectivamente.

3.5. El consumo de tomate no local fuera de temporada, en días largos, incrementa la expresión génica del transportador de ácidos grasos FAT/cd36 y de la enzima lipogénica *Fas1*, y tiende a aumentar la expresión de *Had.*

En base a los resultados obtenidos in esta tesis, el consumo de frutas, ricas en compuestos bioactivos, de origen local, lo que potencia su consumo en temporada, supone no solo un consumo sostenible, sino que permite alcanzar una armonía y sincronía con el medio ambiente que nos rodea, consiguiendo así un mayor estado de salud y bienestar.

CONCLUSIONS

The present work has evaluated how the geographical origin of fruits and their photoperiod of intake impact the metabolism of healthy animals. Based on the quantitative analysis of various metabolic parameters of key tissues related with the energy metabolism, namely liver and skeletal muscle, with emphasis on lipid metabolism, it can be concluded that:

1. Exposure to different photoperiods affects serum parameters, the gene expression of lipogenic enzymes and of enzymes related to fatty acid unsaturation, as well as the profile of certain liver and muscle fatty acids.

    1.1. Long photoperiod exposure induces *de novo* lipogenesis in the liver, tends to generate less β-oxidation, and stimulates greater accumulation of saturated fatty acids and n-3 PUFAs than standard photoperiod, possibly due to increased *Fas1* gene expression, to a tendency to lower expression of Cpt1α and FAT/cd36, with a lower unsaturation by Sdc1 and an increased DHA/ALA ratio.

    1.2. Exposure to standard photoperiod generates a lower accumulation of saturated fatty acids and n-3 PUFAs in the liver, due to greater β-oxidation and/or less synthesis, while it generates an accumulation of saturated fatty acids in skeletal muscle.

    1.3. Exposure to long days affects linoleic acid metabolism, possibly through the modulation of phosphatidylcholine synthesis, compared to days with twelve hours of daylight.

    1.4. Exposure to long photoperiods affects the biosynthesis of unsaturated fatty acids, possibly by downregulation of *Scd1* expression, compared to standard photoperiod.

1.5. The effects of exposure to different photoperiods are less evident in skeletal muscle.

1.6. Differential effects are observed to a greater extent between the standard and long photoperiods, suggesting that chronic exposure to a short photoperiod causes metabolic habituation.

2. Both the geographical origin of sweet *Brooks* cherries, as well as exposure to different photoperiods affect metabolic parameters of healthy animals.

2.1. The consumption of sweet cherries in season, simulated by the long photoperiod, regardless of origin, decreases the content of hepatic saturated fatty acids (mainly palmitic acid) and the level of PUFAS n-3 in skeletal muscle, possibly due to a higher unsaturation by the hepatic Scd1 enzyme, and greater muscle oxidation, respectively.

2.2. The geographical origin of the cherry determines different metabolic consequences, depending on the season consumed. Specifically, in a long photoperiod, that is, consumption in-season, locally produced cherries increase the expression of the fatty acid transporter in muscle FAT/cd36, as well as the proportion of fatty acids 15:0, palmitoleic acid and c11-18:1.

2.3. The cherry of non-local origin, consumed in-season alters the metabolism of linoleic acid, and increases the concentration of hepatic lipophilic metabolites compared to its vehicle.

2.4. Local cherry consumption increases HDL-c and hepatic gene expression of *Acc1* and *Fas1* in the long photoperiod, while it decreases triacylglycerides when consumed in a short photoperiod, compared to their respective vehicles.

2.5. Out of season, in a short photoperiod, the intake of cherries, regardless of origin, increases insulin and the HOMA index. In addition, the consumption of those of non-local origin increases triacylglycerides.

2.6. Cherry consumption, regardless of origin, in standard photoperiod, causes a level of palmitoleic acid and a proportion of total PUFAs in muscle, and hepatic n-6 PUFAs like those caused by its consumption in season (long photoperiod).

3. The origin of the tomato *Ekstasis*, as well as the photoperiod of consumption, have a differential effect on markers of metabolic health.

3.1. Intake of locally sourced tomato, in season, simulated by the standard photoperiod, decreases cardiovascular risk 1 and the atherogenic coefficient, compared to respective vehicle.

3.2. Tomato intake, regardless of origin, on long days, decreases total serum cholesterol concentration.

3.3. The consumption of tomato of local origin, out of season, in short days, decreases the concentration of triacylglycerides, reaching values similar to its consumption in season, however, that of non-local origin has opposite effects.

3.4. The local tomato lowers blood glucose and total cholesterol, out of season, in short and long photoperiods, respectively.

3.5. Non-local tomato, out of season, in long days, increases hepatic expression of the fatty acid transporter FAT/cd36 and of the lipogenic enzyme *Fas1*, and tends to increase *Had* expression.

Based on the results obtained in this thesis, the consumption of fruits, rich in bioactive compounds, of local origin, and thus also promoting their intake in season, supposes not only a sustainable consumption but also that allows us to achieve harmony and synchrony with the environment that surrounds us, thus achieving a greater state of health and well-being.

UNIVERSITAT ROVIRA I VIRGILI
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María Josefina Ruiz de Azua

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

8. ANEXO

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METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

Composición nutricional de dieta estándar

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
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A04/A04C/R04 FICHA TÉCNICA ALIMENTO

Composición nutritiva

Aporte calórico (kcal/kg): 2900

COMPUESTOS/ NUTRIENTE	CANTIDAD (%)
Proteínas	16.1
Hidratos de Carbono	60.4
Fibras	3.9
Lípidos	3.1
Minerales, Cenizas	4.6
Humedad	11.9

Composición centesimal

ALIMENTO	CANTIDAD (%)
Proteínas Animales (Pescado)	4.0
Proteínas Vegetales (Soja, Levadura)	8.0
Mezcla Vitamínica Y Mineral	3.9
Cereales, Salvado Y Harinilla	84.1

Composición nutritiva exhaustiva (valor medio)

APORTE DE AMINOÁCIDOS (mg/kg)

Arginina	9000
Cistina	2500
Lisina	7200
Metionina	2800
Triptófano	1900
Glicina	8100

APORTE DE ÁCIDOS GRASOS (mg/kg)

Acido Palmítico	5900
Ácido Palmitoleico	150
Ácido Esteárico	600
Ácido Oleico	4800
Ácido Linoleico	15000
Ácido Linolénico	1200

COMPOSICIÓN MINERAL (mg/kg)

Fosforo	5500
Calcio	7300
Sodio	2500
Potasio	6000
Magnesio	1600
Manganeso	70
Hierro	270
Cobre	16
Zinc	55

COMPOSICIÓN VITAMÍNICA (mg/kg)

VIT. A	7500 UI
VIT. D3	1000 UI
VIT. B1	5.0
VIT. B2	6.5
VIT. B3	16.5
VIT. B6	2.6
VIT. B12	0.010
VIT. E	30 UI
VIT. K3	2.5
VIT. PP	75
Ácido Fólico	0.35
Biotina	0.08
Colina	1600

La información nutricional fue adaptada de la ficha técnica brindada por la casa comercial: https://safe-lab.com/safe_en/products/diets.php

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Listado de publicaciones

UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

Artículos:

- Seasonal consumption of cherries from different origins affects metabolic markers and gene expression of lipogenic enzymes in rat liver: A preliminary Study.

Autores: Ma. Josefina Ruiz de Azua, Álvaro Cruz-Carrión, Begoña Muguerza, Anna Arola-Arnal and Manuel Suarez.

Nutrients 2021, 13(10), 3643; https://doi.org/10.3390/nu13103643

- Tomatoes consumed in-season prevent oxidative stress in Fischer 344 rats: Impact of geographical origin.

Autores: Álvaro Cruz-Carrión, Ma Josefina Ruiz de Azua, Francisca Isabel Bravo, Gerard Aragonès, Begoña Muguerza, Manuel Suárez, Anna Arola-Arnal.

Food & Function, Issue 18, 2021,12, 8340-8350; https://doi.org/10.1039/D1FO00955A

- (Poly)phenolic composition of tomatoes from different growing locations and their absorption in rats: a comparative study.

Autores: Álvaro Cruz-Carrión, Luca Calani, Ma Josefina Ruiz de Azua, Pedro Mena, Daniele Del Rio, Manuel Suárez, Anna Arola-Arnal.

Food Chemistry, Volume 388, 15 September 2022, 132984, https://doi.org/10.1016/j.foodchem.2022.132984

- Oxidative stress in rats is modulated by seasonal consumption of sweet cherries from different geographical origins: Local vs. non-local.

  Autores: Álvaro Cruz-Carrión, Ma. Josefina Ruiz de Azua, Miquel Mulero, Anna Arola-Arnal and Manuel Suárez.

  Nutrients 2020, *12*(9), 2854; https://doi.org/10.3390/nu12092854

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METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

Artículo en preparación

Beneficial effects of bioactive compounds on the prevention or attenuation of liver steatosis induced by diets in experimental animal models

Ruiz de Azua, Ma.Josefina[1]; Rodriguez, Romina M[1];Sain, Juliana[2], Gerstner, Carolina[2]; Fariña, Ana C.[2]; Bernal, Claudio A. [2-3]; Muguerza, Begoña[1]; Suarez, Manuel[1] *;Mulero; Miquel[1.]

[1] Nutrigenomics Research Group, Departament de Bioquímica i Biotecnologia, Universitat Rovira i Virgili, 43007 Tarragona, Spain.

[2] Cátedra de Bromatología y Nutrición, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, Santa Fe, Argentina.

[3] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Santa Fe, Argentina.

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a disease with a complex pathophysiology, which involves several metabolic conditions ranging from obesity, type 2 diabetes mellitus, insulin resistance, hepatic steatosis or dyslipidemia, among others. In recent years, the incidence and prevalence of NAFLD has increased worldwide, currently affecting 25% of the world

population, thus being a significant health and economic burden. It has been shown that certain fatty acids, vitamins and bioactive compounds present in natural foods have beneficial effects for the prevention, attenuation and reversal of the symptoms associated with this disease. This review summarizes the biochemical and molecular mechanisms involved in the etiology and progression of the disease, the animal models used for their study, as well as the evidence on the beneficial effects associated with the consumption of food components.

Posters

- IX Seminario sobre nutrición y estilos de vida saludables, Barcelona, Julio 2019.

Efecto del consumo de frutas y verduras de proximidad sobre parámetros metabólicos en distintos fotoperiodos.

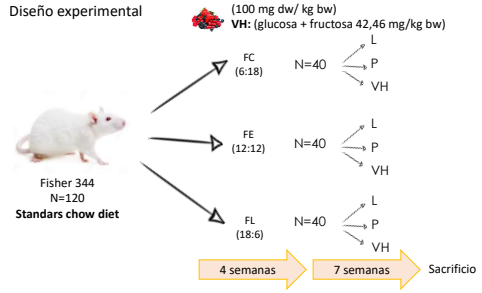
M. Josefina Ruíz de Azua, Manuel Suárez

Universitat Rovira i Virgili, Departamento de Bioquímica y Biotecnología, Grupo de Investigación en Nutrigenómica, Tarragona, España.

Introducción

Las frutas y verduras ricas en compuestos polifenólicos informarían las condiciones externas del momento de cosecha a los heterótrofos que las consumen. La ingesta de frutas procedentes de lejanía (L) y fuera de temporada, produce cambios metabólicos en comparación con el consumo de las de proximidad (P) en su periodo estacional. A su vez, la exposición a diferentes fotoperiodos (corto (FC), estándar (FE) y largo (FL), con 6, 12 y 16 horas de luz respectivamente) produce modificaciones en parámetros metabólicos.

Materiales y métodos



Análisis colorimétrico en muestras de suero de la concentración de:

Triglicéridos (TAG) | Glucosa | Colesterol

Resultados

Efecto del fotoperiodo y del tratamiento sobre los niveles de TAG, glucosa y colesterol en suero

TAG

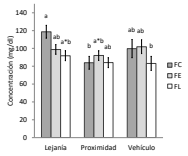


Gráfico 1. Efecto combinando los diferentes tratamientos y los fotoperiodos sobre los niveles de TAG.

Glucosa

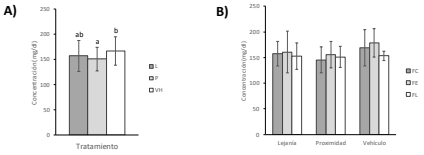


Gráfico 2. Efecto de los tratamientos (A) y el efecto de los tratamientos junto con los distintos fotoperiodos sobre los nieles de glucosa (B).

Colesterol

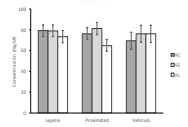


Gráfico 3. Efecto combinando los diferentes tratamientos y los fotoperiodos sobre los niveles de colesterol.

Conclusión

Existe un efecto significativo del tratamiento y del fotoperiodo sobre los niveles de TAG. El consumo de fruta de L en FC, produce un aumento de los TAG, en comparación con el consumo de la misma fruta de P. Sin embargo no se observan cambios en los demás parámetros metabólicos. El consumo de fruta de L produce un aumento de la glucosa sanguínea, independientemente del fotoperiodo expuesto.

- 14th Congress of the International Society of Nutrigenetics/Nutrigenomics, Septiembre 2021.

Sweet cherry consumption affects metabolic parameters and gene expression depending the origin and the photoperiod in which it is ingested.

M.J Ruíz de Azua [a], A. Cruz-Carrión [a]; A. Arola-Arnal [a]; Manuel Suárez [a]

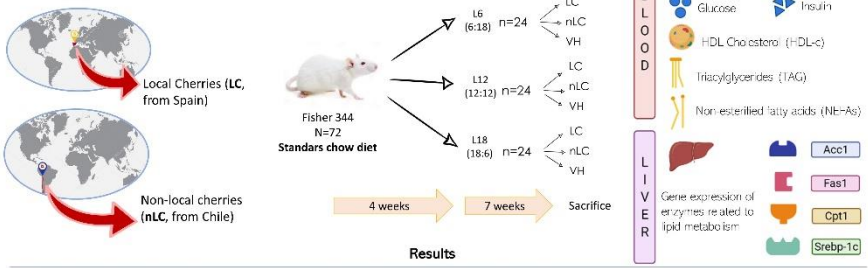
[a]-Universitat Rovira i Virgili, Departamento de Bioquímica y Biotecnología, Grupo de Investigación en Nutrigenómica, Tarragona, España.

Background

The phytochemical composition of fruits depends on the external conditions from where it was cultivated and harvested. Moreover, it is known that biological rhythms have an impact on the bioactivity of these compounds. Therefore, the consumption of fruits from different origins, as well as their intake in different seasons, could have differential effects on health.

Objective

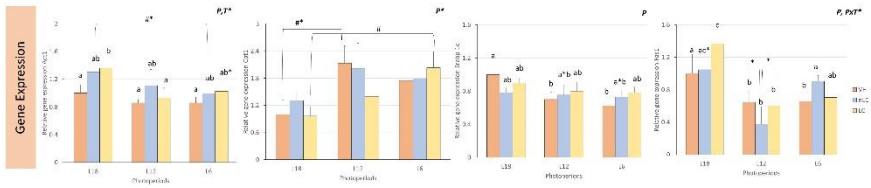
Evaluate the effects of consuming cherries from different geographic origins and on their intake in different seasons



Results

Serum Parameters	L6		L12			L18				
	VH	nLC	LC	VH	nLC	LC	VH	nLC	LC	2wA
TAG (mmol/L)	1.14 ± 0.11 a*b	1.42 ± 0.12 a	1.03 ± 0.14 b	1.16 ± 0.08 a*b	1.11 ± 0.08 b	1.11 ± 0.10 b	0.95 ± 0.08 b	1.00 ± 0.10 b	0.96 ± 0.10 b	P
HDL-c (mmol/L)	0.75 ± 0.11 ab	0.82 ± 0.17 ab	0.72 ± 0.17 ab	0.65 ± 0.18 ab	0.78 ± 0.17 ab	0.45 ± 0.08 ab*	0.38 ± 0.16 a	0.89 ± 0.19 a*b	0.94 ± 0.29 b	-
NEFAs	0.79 ± 0.10 a	0.79 ± 0.05 a	0.69 ± 0.09 ab	0.78 ± 0.06 a	0.79 ± 0.06 a	0.76 ± 0.07 a	0.76 ± 0.08 a	0.55 ± 0.05 b	0.56 ± 0.06 b	P
Glucose	9.38 ± 0.69 ab	8.69 ± 0.43 ab	8.46 ± 0.46 ab*	9.91 ± 0.55 b	8.32 ± 0.92 a	8.06 ± 0.52 a	8.50 ± 0.19 ab*	8.24 ± 0.57 a	8.49 ± 0.19 ab*	T*
Insulin (ng/mL)	5.37 ± 0.63 b	9.86 ± 1.69 a	10.65 ± 1.56 a	8.95 ±1.65 ab*	9.22 ± 1.71 a	9.07 ± 1.45 ab	9.14 ± 1.32 ab*	8.12 ± 1.03 ab	7.07 ± 0.77 ab	PxT*
HOMA	0,14 ± 0.05 b	0,16 ± 0.03 a	0,17 ± 0.03 a	0,17 ± 0.04 a	0,15 ± 0.04 ab	0,13 ± 0.02 ab	0,11 ± 0.02 a*b	0,12 ± 0.02 ab	0,11 ± 0.01 ab	PxT*

Table 1. Serum parameters in Fisher 344 rats. Data are expressed as the mean ± SEM. (n = 8). Two-way ANOVA analysis (2x2 photoperiod factorial design (L6, L12 or L18) x treatment (VH, LC or nLC), was used to assess the differences between groups. P: photoperiod; T: treatment effect; PXT: effect of interaction. Different letters above the bars indicate significant differences (p <0.05) (post-hoc DMS, one-way ANOVA). * Indicates trend (0.05 <p <1).



Conclusions

Cherry consumption was associated with metabolic benefits. Specifically, the concentration of serum TAG and hepatic lipogenic enzymes were affected both by the geographical origin of the fruit and by the photoperiod of consumption.

PECT NUTRISALT; 2017PMT PIPT 64; 2018PMT PIPT 20,and Serra Húnter fellow.

- I Encuentro intersectorial sobre innovación y calidad en la alimentación, EIICA, online, 30 de septiembre y 11 de octubre, 2021.

EIICA2021 I ENCUENTRO INTERSECTORIAL SOBRE INNOVACIÓN Y CALIDAD EN LA ALIMENTACIÓN

EL CONSUMO DE TOMATES EN TEMPORADA DISMINUYE EL RIESGO CARDIOVASCULAR

Ruiz de Azua, M.J [1]; **Cruz-Carrión, A** [1]; , **Arola, A.** [1]; **Suarez Recio Manuel** [1].
[1] **Universitat Rovira i Virgili, Tarragona, España.**

OBJETIVOS

Determinar los efectos metabólicos del consumo de tomate de diferente origen geográfico (distinta marca fitioquímica) en diferentes momentos del año.

MATERIALES Y MÉTODOS

72 ratas Fisher 344 machos fueron divididas en tres fotoperiodos: **L6, L12, L18**, con 6, 12 y 18 horas de luz respectivamente, para simular las diferentes estaciones. Luego de 4 semanas de adaptación, fueron divididas en 3 grupos según el tratamiento recibido: **nLT** (no-Local Tomate Ekstasis, de Almería, del sur de España), **LT** (Local tomate Ekstasis, de Tarragona, nordeste de España) y **VH** (vehículo) (n=8). Fueron alimentadas con dieta estándar y agua ad libitum. Luego de 7 semanas de tratamiento fueron sacrificadas. Se determinaron parámetros bioquímicos séricos (triacilglicéridos (TAG) (nmol/L), ácidos grasos no esterificados (NEFAs) (nmol/L), glucosa (nmol/L), colesterol total (TC), colesterol HDL y LDL, insulina), índice de riesgo cardiovascular 1 (CR1) (TC/HDL), coeficiente aterogénico (AT. Coef.) (TC-HDL/HDL) y expresión de enzimas hepáticas claves en el metabolismo lipídico.

RESULTADOS Y CONCLUSIONES

El consumo de **LT** en temporada (representado por **L12** dado que la variedad Ekstasis es típica de otoño) disminuyó el riesgo cardiovascular I y el coeficiente aterogénico. La ingesta de **nLT** fuera de temporada (**L6**) incrementó los TAG y NEFAs.
En los animales del grupo **L18-LT** se observó una expresión génica de enzimas lipogénicas mayor, mientras que el **L18-nLT** mostró una mayor expresión de enzimas oxidativas que su respectivo VH.
Los resultados obtenidos indican la importancia de ingerir frutas y verduras de proximidad y en temporada.

Tabla 1. Parámetros séricos y medidas de riesgo aterogénico.

Datos expresados como promedio. F: fotoperiodo T: tratamiento effect; FXT: interacción de efectos. Diferentes letras indican diferencias estadísticamente significativas (p <0.05) (post-hoc DMS, one-way ANOVA) * indica tendencia 0.05 < p <1.0.

	L6			L12			L18			2wA
	VH	nLT	LT	VH	nLT	LT	VH	nLT	LT	
TAG	1.14 *c*	1.40 *a*	0.88 *b*	1.17 *a*c*	1.15 *a*c*	0.99 *bc*	0.95 *bc*	1.10 *b*c*	0.95 *bc*	***T***
NEFAs	0.79 *ab*	1.04 *a*	0.87 *ab*	0.78 *b*	0.76 *a*b*	0.77 *b*	0.76 *ab*	0.70 *b*	0.62 b	***F****
Glucose	8.87 *ac**	8.79 *ac**	7.20 *b*	10.18 *c*	9.52 *ac*	9.22 *ac*	8.50 *a*	8.71 *a*	8.27 ab	***T, F***
Insulin (ng/mL)	5.38 *b*	*6.79 ab*	*6.01 ab*	8.95 *a*	8.61 *ab**	8.05 *ab*	8.80 *ab**	5.38 *b*	6.76 ab	***F***
HOMA	0.09 *a*	0.11 *a*	0.08 *a*	0.21 *b*	0.14 *ab**	0.14 *a*	0.14 *ab**	0.11 *a*	0.13 a	***F***
CR1	2.05 *b*	2.31 *a*b*	3.55 *a*	3.97 *c*	3.34 *ab*c*	2.17 *a*b*	2.80 *abc*	2.09 *b*	2.71 abc*	***FxT***
At.Coef	1.05 *b*	1.31 *a*b*	2.55 *a*	2.63 *a*	2.34 *a*	0.71 *b*	1.80 *ab*	1.09 *a*b*	1.71 ab	***FxT***

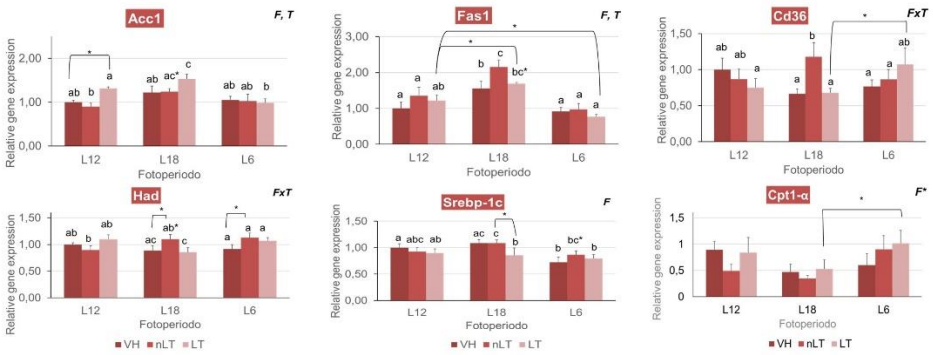


Figura 1. Expresión génica de enzimas hepáticas relacionadas con el metabolismo lipídico. Datos expresados como promedio + SEM. Diferentes letras sobre las barras indican diferencias estadísticamente significativas (p <0.05) (post-hoc DMS, one-way ANOVA) * indica tendencia 0.05 < p <1.0

- I Jornada de Nutracéutica, Tarragona, Marzo 2022.

EL CONSUMO DE TOMATES EN TEMPORADA DISMINUYE EL RIESGO CARDIOVASCULAR

Ruiz de Azua, M.J [1]; Cruz-Carrión, A [1]; , Arola, A. [1]; Suarez Recio Manuel [1].
[1] Universitat Rovira i Virgili, Tarragona, España.
Grup de Recerca NUTRIGENÒMICA URV

INTRODUCCION

Las frutas y verduras presentan una composición característica de fitoquímicos, polifenoles principalmente, que depende de las condiciones ambientales donde fueron desarrollados. Esta marca polifenólica serviría como señal adaptativa del medioambiente para los heterótrofos que los consumen. Además, el momento de la ingesta de vegetales puede tener efectos diferenciales. Por lo tanto, el tomate, ampliamente consumido durante todo el año y con diversos orígenes, podría ocasionar respuestas metabólicas particulares dependientes de la época de consumo.

OBJETIVOS

Determinar los efectos metabólicos del consumo de tomate de diferente origen geográfico (distinta marca fitioquímica) en diferentes momentos del año.

LT: Local tomate (Tarragona)
nLT: no-Local Tomate (Almería)
(100 mg dw/ kg bw)
VH: (glucosa + fructosa 42,46 mg/kg bw)

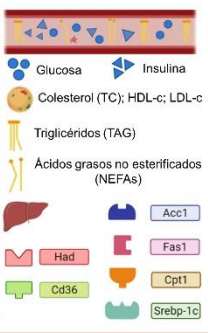
MATERIALES Y MÉTODOS

Fisher 344
N=72
Standars chow diet

L6 (6:18) n=24 → LT, nLT, VH
L12 (12:12) n=24 → LT, nLT, VH
L18 (18:6) n=24 → LT, nLT, VH

4 semanas → 7 semanas → Sacrificio

Riesgo cardiovascular 1 (CR1): TC / HDL-c;
Riesgo cardiovascular 2 (CR2): LDL-c / HDL-c;
Coeficiente aterogénico (At.Coef): [TC-HDL-c]/HDL-c



RESULTADOS

Tabla 1. Parámetros séricos y medidas de riesgo aterogénico.

	L6			L12			L18			2wA
	VH	nLT	LT	VH	nLT	LT	VH	nLT	LT	
TAG	1.14±0.11 c	1.40±0.07 a	0.88±0.09 b	1.17±0.09 c	1.15±0.10 a*c	0.99±0.08 bc	0.95±0.08 bc	1.10±0.10 b*c	0.95±0.08 bc	T
NEFAs	0.79±0.10ab	1.04±0.27 a	0.87±0.09 ab	0.78±0.06 b	0.76±0.10 a*b	0.77±0.11 b	0.76±0.08 ab	0.70±0.04 b	0.62±0.06 b	F*
Glucosa	8.87±0.53 ac*	8.79±0.55 ac*	7.20±0.27 b	10.18±0.55 c	9.52±0.64 ac	9.22±0.42 ac	8.50±0.19 a	8.71±0.45 a	8.27±0.56 ab	T, F
Insulina (ng/mL)	5.38±0.68 b	6.27±0.86 ab	6.68±1.24 ab	9.93±1.53 a	7.82±1.10 ab	8.05±1.63 ab	8.80±0.54 ab*	6.78±1.30 ab	8.61±1.60 ab*	F
HOMA	0.09±0.01 a	0.11±0.02 a	0.09±0.02 a	0.19±0.04 b	0.14±0.02 ab	0.14±0.03 ab	0.13±0.01 ab*	0.11±0.02 a	0.13±0.03 a	F
CR1	1.97±0.34 b	2.91±0.71 abc*	4.67±1.15 c	3.97±0.51 a	3.34±0.53 ab	2.17±0.52 a*b	4,71±1.59 a	2.09±0.11 b	2.71±0.43 a*b	FxT
CR2	0.14±0.07 a*b	0.24 ± 0.12 ab	0.26±0.07 ab	0.10±0.03 b	0.40±0.12 a	0.34±0.13 ab*	0.33±0.13 ab	0.13±0.05 ab	0.26±0.09 ab	
At.Coef	0.97±0.34 b	2.24±0.73 abc*	3.67±1.15 c	2.97±0.51 a	2.34±0.53 ab	1.17±0.52 a*b	3.71±1.59 a	1.09±0.11 b	1.71±0.43 a*b	FxT

Datos expresados como media ± *SEM (n=8)*. F: fotoperíodo T: tratamiento; FxT: interacción de efectos. Diferentes letras indican diferencias estadísticamente significativas (p <0.05) (post-hoc DMS, one-way ANOVA) * indica tendencia 0.05 < p <1.0.

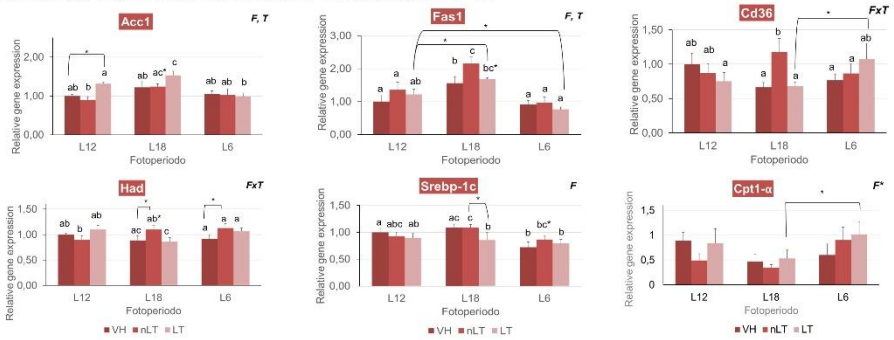
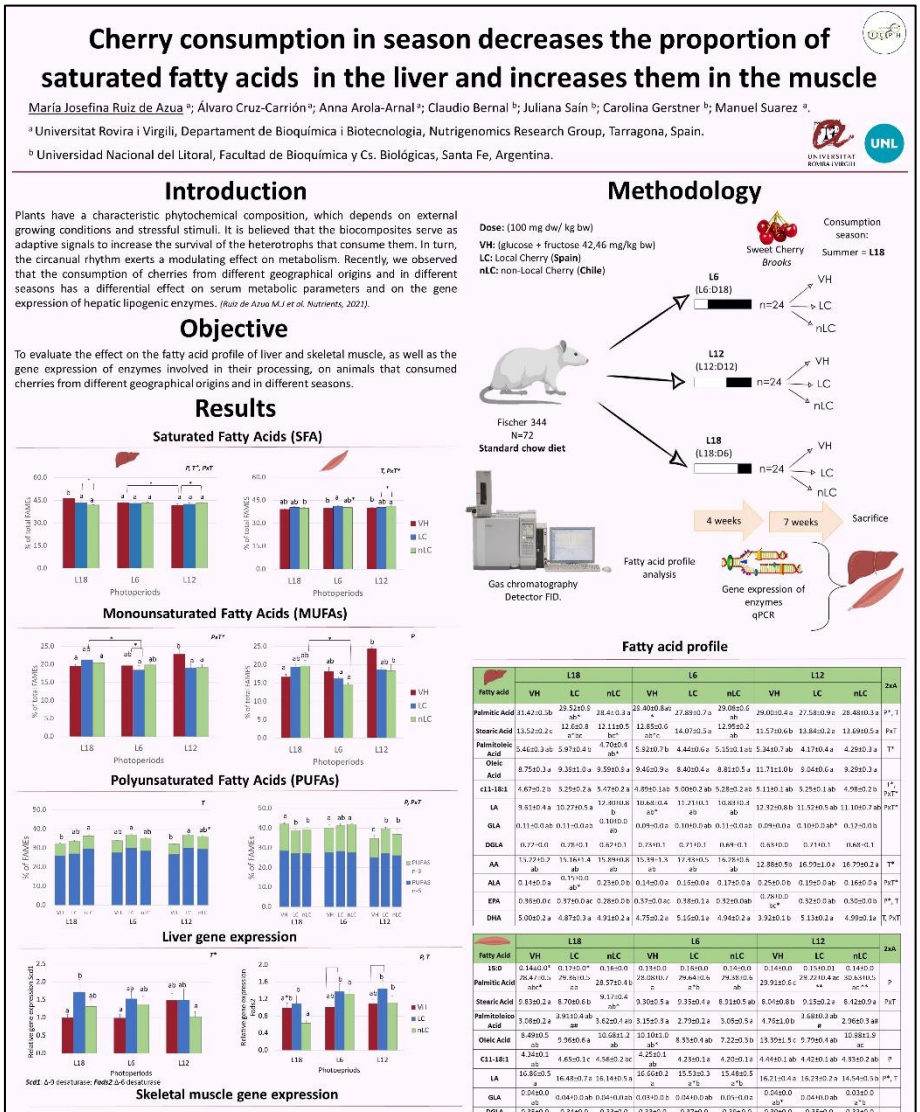


Figura 1. Expresión génica de enzimas hepáticas relacionadas con el metabolismo lipídico. Datos expresados como promedio + SEM. Diferentes letras sobre las barras indican diferencias estadísticamente significativas (p <0.05) (post-hoc DMS, one-way ANOVA) * indica tendencia 0.05 < p <1.0

CONCLUSION

Los resultados obtenidos indican la importancia de consumir frutas y vegetales de proximidad y temporada, específicamente sobre la salud cardiovascular.

- The 10th International Conference on Polyphenols and Health, Londres, Abril 2022.



UNIVERSITAT ROVIRA I VIRGILI
METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

Curriculum Vitae

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MARIA JOSEFINA RUIZ DE AZUA

PHD CANDIDATE IN NUTRITION AND METABOLISM – TARRAGONA, SPAIN

OBJECTIVE

I am interested in studying the metabolic changes produced by the consumption of diferent nutrients with the aim of preventing nutritional pathologies and being able to carry out personalized nutrition.

APTITUDES

Adaptability, responsibility, communicativeness, patience, team and individual work

LENGUAGES

Spanish ●●●●●

Portuguese ●●●●○

English ●●●○○

Catalán ●●○○○

CONTACT

+34 675399651
Maria Josefina Ruiz de Azua
josefinarda@gmail.com
ORCID: 0000-0002-4591-8064

EDUCATION

PHD CANDIDATE IN NUTRITION AND METABOLISM IN NUTRIGENOMICS' RESEARCH GRUP • ON GOING • UNIVERSITY ROVIRA I VIRGILI (URV, TARRAGONA), SPAIN.
Effect of seasonal consumption of local and non-local fruits and vegetables on serum metabolic parameters, liver gene expression and on the lipid profile of muscle and liver tissue.
Supervised by Dr Manuel Suarez Recio.
PhD internship: National University of the Litoral (UNL), Argentina. October 2020- April 2021.

MSC IN NUTRIGENOMICS AND PERSONALIZED NUTRITION • 2017-2018 • UNIVERSITY OF BALEARIC ISLAND, SPAIN.
First- Class Honour: 8.42/10
Final Project: Consequences of dermatan sulfate supplementation on liver tissue in mice fed a high-fat diet. Laboratory of Molecular Biology, Nutrition and Biotechnology (LBNB).
Supervised by Dr. Maria Luisa Bonet Piña.

BSC IN NUTRITION • OCTOBER 2016 • NATIONAL UNIVERSITY OF THE LITORAL (UNL), SANTA FE, ARGENTINA.
First – Class Honour: 7.88/10.
Final project: Nutritional labeling proposal for trans fatty acids in complex foods. Department of Food Science and Nutrition.
Supervised by Dr. Claudio Adrian Bernal.
BSC internship: Human Nutrition. Federal University of Rio Grande do Sul. july 2014 – january 2015.

MARIA JOSEFINA RUIZ DE AZUA

PHD CANDIDATE IN NUTRITION AND METABOLISM – TARRAGONA, SPAIN

MAIN TASKS

- Assistance in rodent studies and evaluation of GTT and draw saphenous blood.
- Development of biochemical and molecular techniques (RNA extraction of liver, PCR, qPCR, quantification of biochemical parameters, liver lipid extraction, NMR, gas chromatography, histological analysis of adipose tissue).
- Development of foods techniques (lipid extraction, lipid quantification, lipidic profile analysis, HPLC)
- Statistical analysis of results (SPSS, MataboAnalyst).
- Teaching assistant.

PUBLICATIONS

- idative stress in rats is modulated by seasonal consumption of sweet cherries from different geographical origins: local vs non-local. Nutrients 2020, 12 (9), 2854; https://doi.org/10.3390/nu12092854
- Tomatoes consumed in-season prevents oxidative stress in Fischer 344 rats: impact of geographical origin. https://pubs.rsc.org/en/content/articlelanding/2021/FO/D1FO00955A
- Seasonal consumption of cherries from different origins affects metabolic markers and gene expression of lipogenic enzymes in rat liver: a preliminary study. https://www.mdpi.com/2072-6643/13/10/3643
- (Poly)phenolic composition of tomatoes from different growing locations and their absorption in rats: a comparative Study. Article accepted by Food Chemistry, not yet published.
- Benefits of the consumption of local fruits and vegetables on metabolic parameters in different photoperiods. XI seminar on nutrition and healthy lifestyles. Barcelona 2019.
- Sweet cherry consumption affects metabolic parameters and gene expression depending on the origin and the photoperiod in which it is ingested (Poster). 14th Congress of the International Society of Nutrigenetics/Nutrigenomics (ISNN). Rumania, September 2021.
- The consumption of tomatoes in season decreases cardiovascular risk (Poster). I Encuentro Intersectorial sobre Innovación y Calidad en la Alimentación (EIICA), Argentina, octubre 2021.
- Efecto benéfico del consumo de tomate en temporada sobre la salud cardiovascular. (Poster). Jornada de Nutracéutica, Tarragona, Spain.

Cherry consumption in season decreases the proportion of saturated fatty acids in the liver and increases them in the muscle (Poster). The 10th International Conference on Polyphenols and Health.

MARIA JOSEFINA RUIZ DE AZUA

PHD CANDIDATE IN NUTRITION AND METABOLISM – TARRAGONA, SPAIN

LEADERSHIP

Volunteer in teaching and support program for university students.

University National of Litoral (2015-2016).

CONTACT

+34 675399651
Maria Josefina Ruiz de Azua
josefinarda@gmail.com
ORCID: 0000-0002-4591-8064

REFEREES

MANUEL SUAREZ RECIO, PHD
Dean of faculty of oenology
Nutrigenomics Research Group
Department of Biochemistry and Biotechnology, Campus Sescelades.
Universitat Rovira i Virgili
43007-Tarragona, SPAIN
P: +34 977 55 8630
E: manuel.suarez@urv.cat

MARIA LUISA BONET PIÑA, PHD
Professor of Biochemistry and Molecular Biology.
Co-director of MSC in nutrigenomics and personalized nutrition.
Laboratory of Molecular Biology, Nutrition and Biotechnology - University of the Balearic Islands (UIB).
P: +34 971 172734.
E: luisabonet@uib.es

CLAUDIO ADRIAN BERNAL, PHD
Independent Researcher CONICET.
Professor of Bromatology and Nutrition.
Faculty of Biochemistry and Cs. Biological. National University of the Litoral. Santa Fe, Argentina.

P: +54 0342-4575211
E: cbernal@fbcb.unl.edu.ar

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METABOLIC CONSEQUENCES OF CONSUMPTION OF FRUITS FROM DIFFERENT ORIGINS AND IN DIFFERENT PHOTOPERIODS: IMPACT ON THE LIPIDIC METABOLISM
María Josefina Ruiz de Azua

Hoy en día, tenemos acceso a alimentos de todas partes del mundo, en cualquier época del año. Específicamente, se puede conseguir frutas y verduras típicas de otros sitios, sin conocer qué consecuencias produce en nuestro organismo. En tal sentido, las plantas poseen una composición fitoquímica característica que depende de las condiciones ambientales dónde se desarrolla. Estos biocompuestos proporcionan señales para los heterótrofos que las consumen, con el fin de que puedan adaptarse favorablemente a las condiciones imprescindibles del entorno. Además, la cantidad de horas de luz diaria impacta en el metabolismo, a través de la modulación de la transcripción génica de determinadas enzimas. En la presente tesis se evaluó el efecto de la exposición crónica a diferentes horas de luz, así como también el efecto del consumo de cerezas y tomates de distinto origen geográfico y en distintos fotoperiodos, simulando diferentes temporadas de consumo, sobre parámetros metabólicos séricos y hepáticos, sobre la expresión de enzimas relacionadas con el metabolismo lípidos y sobre el perfil de ácidos grasos en higado y en músculo. Observamos que tanto el origen, como el fotoperiodo de consumo poseen efectos diferenciales sobre los parámetros evaluados.

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