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Julia Sepel Loreto

AVALIAÇÃO DOS EFEITOS DO CONSUMO DE UMA DIETA RICA EM SACAROSE EM *Drosophila melanogaster*: ÊNFASE EM ALTERAÇÕES METABÓLICAS E TRANSCRICIONAIS

Santa Maria, RS, Brasil
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Dissertação apresentada ao curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciências Biológicas: Bioquímica Toxicológica**

Orientadora: Profª Drª Nilda Berenice de Vargas Barbosa

Santa Maria, RS

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Aprovada em 09 de novembro de 2021:

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Nilda Berenice de Vargas Barbosa, Drª, UFSM
(Presidente/Orientadora)

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Jeferson Luis Franco, Dr, Unipampa

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Santa Maria, RS
2021

DEDICATÓRIA

A minha família pelo constante carinho, suporte e exemplo.

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After having made a few preparatory experiments, he concluded with a panegyric upon modern chemistry, the terms of which I shall never forget: ‘The ancient teachers of this science,’ said he, ‘promised impossibilities and performed nothing. The modern masters promise very little; they know that metals cannot be transmuted and that the elixir of life is a chimera but these philosophers, whose hands seem only made to dabble in dirt, and their eyes to pore over the microscope or crucible, have indeed performed miracles. They penetrate into the recesses of nature and show how she works in her hiding-places.

(Frankenstein, Mary Shelley)

RESUMO

AVALIAÇÃO DOS EFEITOS DO CONSUMO DE UMA DIETA RICA EM SACAROSE EM *Drosophila melanogaster*: ÊNFASE EM ALTERAÇÕES METABÓLICAS E TRANSCRICIONAIS

AUTORA: Julia Sepel Loreto
ORIENTADORA: Nilda Berenice de Vargas Barbosa

A prevalência do Diabetes mellitus (DM) tem aumentado nas últimas décadas, sendo 90% dos casos de diabetes tipo-2 (DT2), o qual é caracterizado pela resistência à insulina. Além da hiperglicemia, o DT2 está fortemente associado com a obesidade e diversas desordens como doenças cardiovasculares, neuropatias e doenças neurodegenerativas e doenças renais. A patogênese do DT2 envolve a interação de fatores intrínsecos e fatores extrínsecos, como a dieta e a atividade física. A dieta rica em açúcar (HSD) em modelos experimentais tem sido amplamente utilizada para mimetizar fenótipos do DT2. Para analisar o espectro de efeitos gerado pelo consumo de uma HSD, utilizamos a mosca *Drosophila melanogaster* (*D. melanogaster*), um organismo bem consolidado para estudos de distúrbios metabólicos. As drosófilas foram mantidas desde ovo, por toda sua fase de desenvolvimento e até os 7 dias de vida em uma HSD ou dieta controle. O consumo da HSD aumentou os níveis de glicose e triglicerídeos nos indivíduos adultos. A análise de transcriptoma do corpo total revelou que a dieta causou um aumento de biogênese ribossomal e diminuição da expressão de genes relacionados com o metabolismo energético e desenvolvimento, sobretudo o muscular. Além de estar de acordo com a literatura, mostrando que a dieta induz fenótipos de DT2, nosso estudo traz novos achados sobre genes e vias moleculares afetados pela dieta em *D. melanogaster*, os quais podem servir de base para investigações das relações estabelecidas entre a HSD e o DM.

Palavras-chave: Diabetes. transcriptoma. dieta. *D. melanogaster*.

ABSTRACT

EVALUATION OF THE EFFECTS OF CONSUMPTION OF A HIGH SUGAR DIET IN *Drosophila melanogaster*: EMPHASIS ON METABOLIC AND TRANSCRIPTIONAL CHANGES

AUTORA: Julia Sepel Loreto
ORIENTADORA: Nilda Berenice de Vargas Barbosa

The prevalence of diabetes mellitus (DM) has increased in the last decades, with 90% of cases been of type-2 diabetes (T2D), which is characterized by insulin resistance. In addition to hyperglycemia, T2D is strongly associated with obesity and several disorders such as cardiovascular diseases, neuropathies and neurodegenerative diseases, and kidney diseases. The pathogenesis of T2D involves an interaction of intrinsic factors and extrinsic factors, such as diet and physical activity. The high sugar diet (HSD) in experimental models has been commonly used to mimic the phenotype of the human pathology. To analyze the spectrum of effects generated by the consumption of an HSD, we used the *Drosophila melanogaster* fly, a well-established organism for studies of metabolic disorders. The fruit flies were kept from the egg, throughout their developmental stages and their adult lifespan on an HSD or a control diet. The consumption of HSD elevated the levels of blood glucose and triglycerides in adults as young as seven days old. The transcriptome analysis of total body revealed that the diet caused an increase in ribosomal biogenesis and decreased expression of genes related to energy metabolism and development, namely the muscle. Taken together, our results are in agreement with the literature, showing that the diet induced T2D phenotypes, and bring new findings on molecular genes and pathways affected by diet in *Drosophila*, which may serve as a basis for investigating the relationship between HSD and the DM

Keywords: Diabetes. transcriptome. high sugar diet (HSD). *D. melanogaster*.

LISTA DE ABREVIATURAS

AKT: protein kinase B
CREB: cyclic-AMP response element binding protein
CREB: cyclic-AMP response element binding protein
D. melanogaster: *Drosophila melanogaster*
Dilps: *Drosophila* insulin-like peptides
DM: Diabetes Mellitus
DT1: diabetes tipo-1
DT2: diabetes tipo-2
ECVAM: Centro Europeu para Validação de Métodos Alternativos
FOXO: forkheadbox O
HFD: High Fat Diet/dieta rica em gordura
HSD: High Sugar Diet/dieta rica em açúcar
IGF: Fator de crescimento semelhante à insulina
InR: insulin receptor
IPCs: Insulin Producing Cells/ Células produtoras de insulina
SUS: Sistema Único de Saúde
TOR: target of rapamycin

SUMÁRIO

1. INTRODUÇÃO

1.1 DIABETES MELLITUS E DIETA

O Diabetes mellitus (DM) é um distúrbio metabólico caracterizado por um estado de hiperglicemia, causado no diabetes tipo 1 (DT1) por perda total ou parcial da produção de insulina e no diabetes tipo 2 (DT2) pela resistência à insulina. É estimado que 6,4% da população mundial tenha DM, sendo previsto que a patologia será a sétima causa de morte populacional mais comum e com um aumento de milhões de casos em escala global até 2030, tanto em países desenvolvidos quanto em países em desenvolvimento (Shaw et al., 2010; OMS 2019). O DT2 é a forma mais prevalente, constituindo cerca de 90% dos casos de DM no mundo (Koye et al., 2018; Glovaci et al., 2019). Além dos aspectos genéticos, o DT2 tem forte relação com obesidade, doenças cardiovasculares, neuropatias e nefropatias. Estudos epidemiológicos têm indicado que o DT2 também está associado com o desenvolvimento de doenças neurodegenerativas, notoriamente a Doença de Alzheimer (Han et al., 2010; Baglietto-Vargas et al., 2016; Kandimalla et al., 2017). No Brasil, o DT2 representou cerca de 5% da carga de doença nacional em 2008 e entre o período de 2008 á 2010 cerca de 15% dos custos hospitalares do SUS foram destinados ao tratamento da doença (Costa et al., 2017).

O risco de desenvolvimento de DT2 e suas complicações envolve uma combinação de vários fatores, tanto extrínsecos como intrínsecos. Em relação a fatores intrínsecos, são considerados todos os fatores internos que podem regular a produção e liberação de insulina, portanto questões genéticas e até mesmo epigenéticas podem influenciar o risco e progressão da patologia de DM. Até o momento mais de 40 loci foram relacionados ao DT2 em humanos, e esse carácter poligênico da patologia dificulta a compreensão sobre a que nível os fatores genéticos de um indivíduo afetam a etiologia, progressão e tratamento da doença (Ahlqvist et al., 2011).

Em relação aos fatores extrínsecos que podem ser relacionados ao DT2, podemos incluir todos os hábitos de vida que possam vir a alterar os sinais que modulam a produção ou a liberação de insulina ou até mesmo fatores que influenciem a própria via de insulina. Dentre os fatores extrínsecos vê-se uma relação positiva entre a prevalência de DT2 e os seguintes hábitos: alimentação hipercalórica, níveis baixos de movimentação e exercício, alto tempo de tela (tempo gasto geralmente sentado em frente a tela de televisão, computador, tablet ou smartphone), altos níveis de poluição sonora, baixa qualidade ou duração de sono, exposição a fumaça de cigarro por fumo passivo ou ativo e baixa renda (Kolb e Martin., 2017). Embora

exista uma associação entre diversos fatores e o DT2, ainda não é possível estabelecer o nível de influência que cada fator exercerá na etiologia ou progressão da patologia. Por exemplo, ao analisar a incidência de DT2 sob viés de renda na população australiana, encontrou-se que a prevalência da patologia aumentava na camada social com renda baixa, mas parcialmente mediada pelo aumento de prevalência de fumo e baixos níveis de exercício (Williams et al., 2010). No Brasil, uma rede complexa de interações afeta as tendências de prevalência de DM na população; como associações entre baixa renda, índices de desenvolvimento do estado em que o indivíduo reside, gênero e susceptibilidade a obesidade (Diderichsen et al., 2020).

Um dos hábitos de vida intimamente ligado ao desenvolvimento do DM e amplamente estudado é a dieta: o risco de desenvolver DT2 é positivamente associado com o consumo de carne vermelha, carne processada e bebidas adoçadas com açúcar; sendo o consumo desses alimentos associado com um risco três vezes maior de desenvolver a doença (Fardet e Boirie., 2014; Xi e Liu., 2016; Schwingshackl et al., 2017). Alguns alimentos também foram relacionados com a diminuição do risco de desenvolver de DT2 (vegetais, fibras e café, entre outros), no entanto, alguns estudos de meta-análise sinalizam que mais importante que a ingestão de grupos alimentares benéficos é a não ingestão dos grupos alimentares positivamente associados com o aumento de risco de desenvolvimento de DT2 (Schwingshackl et al., 2017). Atualmente é discutido pela comunidade científica e médica se órgãos públicos governamentais não deveriam tomar ações em relação a controle de produtos alimentícios de forma semelhante ao que foi feito com a indústria do tabaco, argumentando que o estado tem o dever de informar e proteger a população de práticas que tragam danos à saúde (Kaldor et al., 2015). Ter uma dieta saudável é tão importante que intervenções no padrão de alimentação e exercício ainda são consideradas as medidas preventivas mais efetivas contra o desenvolvimento do DM, mesmo quando comparados a fármacos (Lovic et al., 2019).

Ainda assim, existe uma tendência mundial ao alto consumo de alimentos ultraprocessados, alimentos que são formados a partir da extração de outros alimentícios ou de maneira sintética; e geralmente com adições de açúcares e gorduras. Alimentos ultraprocessados atualmente fazem parte da dieta de crianças, adolescentes e adultos em diversos países de culturas distintas e o aumento de consumo desses alimentos nas últimas décadas é comumente associado com aumento de doenças metabólicas como obesidade, um fator de risco para DM (Juul e Hemmingsson, 2015; Mandoura et al., 2017; Bohara et al., 2021). No Brasil de forma semelhante a tendencia mundial, cerca de 30% da energia consumida é

proveniente de alimentos ultraprocessados, sem distinção entre idade e condição socio-econômica (Louzada et al., 2015). Esses dados podem ser preocupantes, já que alimentos ultraprocessados não são ideais por possuírem baixo teor de fibra, alto nível de sódio, carboidratos simples, altas taxas de gorduras saturadas, eventualmente gorduras trans, além de medidas altas de açúcar adicionado (Monteiro et al., 2018). Uma medida importante para análise de produtos ultraprocessados é justamente o de açúcar adicionado, a quantidade de açúcar(es) adicionado(s) na produção do alimento, que estão além daqueles já presentes na sua matéria-prima. Um trabalho de 2014 feito nos Estados Unidos demonstrou que os açúcares adicionados representam cerca de 14% da energia total da dieta diária americana, sendo que um terço desse valor é proveniente somente de bebidas adoçadas com açúcar (refrigerantes, energéticos, etc.) (Drewnowski e Rehm, 2014).

Inúmeros estudos têm relacionado a dieta, especificamente alimentos ultraprocessados, com patologias como DT2, obesidade, doenças cardiovasculares, câncer, síndrome do intestino irritável, depressão, entre outras (Elizabeth et al., 2020). Entretanto, o conhecimento de como diferentes dietas podem trazer malefícios ou benefícios a um organismo ainda está em construção e debate. Ainda não foi elucidado se o que causa malefícios e riscos a patologias como o DT2 é o nível de processamento do alimento, os valores nutricionais da dieta, a quantidade calórica ou até mesmo grupos de alimentos em si (como açúcares adicionados, carne vermelha e carne processada, por exemplo).

1.2 MODELOS DE DM EXPERIMENTAL: *DROSOPHILA MELANOGASTER* COMO ORGANISMO ALTERNATIVO

As pesquisas translacionais vêm auxiliando com precisão a compreensão da relação do DT2 com a dieta. A utilização de um background genético conhecido em uma situação controlada facilita a identificação de fenótipos, bem como alterações em vias metabólicas e comportamentais. Neste contexto, dietas ricas em açúcar (HSD, do inglês High Sugar Diet) são facilmente reproduzidas e amplamente usadas na pesquisa com diferentes modelos experimentais para induzir fenótipos semelhantes ao DT2 e compreender as implicações da dieta e da doença sobre o funcionamento metabólico de diversos organismos (Musselman L. P., 2011; King et al., 2012).

Um dos animais utilizados com sucesso nesse modelo de HSD é a mosca da fruta, *Drosophila melanogaster* (*D. melanogaster*), que de acordo com o Centro Europeu para a

Validação de Métodos Alternativos (ECVAM), é consolidada como modelo animal alternativo para a pesquisa de genes alvos de várias doenças (Benford e Hanley, 2000). Além de compartilhar cerca de 60% dos genes, a *D. melanogaster* e os humanos conservam vias metabólicas e de neurotransmissão em comum, bem como, mecanismos de regulação de ritmos circadianos e processos de aprendizagem e memória (Benton, 2008).

Particularmente para o estudo de doenças metabólicas como o DM, cabe salientar que nas moscas a via insulina/IGF é conservada e modula muitos processos associados ao metabolismo, a reprodução e a longevidade (Pasco e Leopold, 2012). O genoma da mosca contém genes homólogos de componentes da via de sinalização da insulina, incluindo genes dilps (peptídeos insulin-like), receptor de insulina (InR), substrato para o receptor de insulina (Chico), e várias proteínas e fatores de transcrição regulados pelo hormônio, como (proteína quinase AKT), Fator de Transcrição da família forkheadbox (Foxo), transdutor coativador do fator de transcrição CREB (TOR), e outros (Figura 1) (Oldham e Hafen, 2003; Morris et al., 2012; Pasco e Leopold, 2012).

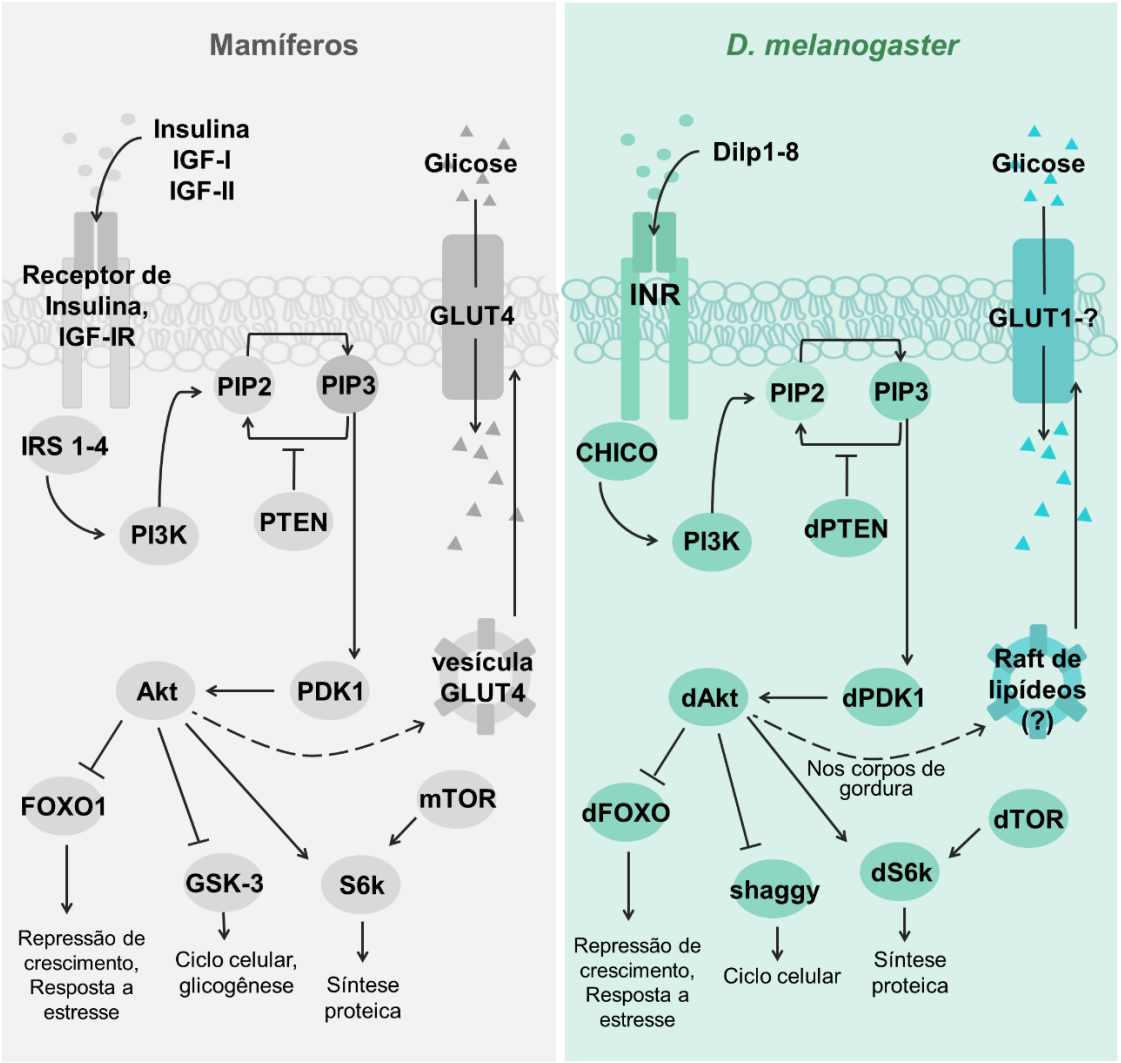

Figura 1- Ilustração comparativa entre via de insulina em humanos e a via de insulina em *D. melanogaster*. Pontos de interrogação (?) sinalizam hipóteses em trabalho na literatura. Baseado e adaptado de revisões de Garofalo (2002) e de Alfa e Kim (2016).

A homologia entre a via de insulina humana e a via de insulina em *D. melanogaster* é alta, talvez com a maior distinção nos dilps, já que há 8 peptídeos diferentes com funções levemente distintas nas drosófilas, embora semelhantes a insulina humana. O considerado mais próximo a insulina humana é o dilp5, que inclusive se liga e ativa o InR humano. O dilp6 é considerado o de função mais distinta da insulina e mais próximo ao fator de crescimento (Nassel et al., 2015). Existe também uma distinção tempo-espacial na produção de dilps: em sua maioria são expressos nos IPCs (dilp2, 3 e 5) (do inglês “insulin producing cells” ou células que produzem insulina), mas também podem ser produzidos nos músculos e intestinos (dilp3), corpos de gordura (dilp6), ovários e túbulos de malpighian (dilp5) ou em estágios larvais (dilp1, 7 e 8) (Nassel et al., 2015). Dilp1, 2, 3 e 5 são co-expressos durante o período larval, e embora cada um tenha seu próprio padrão de expressão, a proposta atual analisa que possa existir uma

redundância de funções, i. e regulação de processos parecidos em tempos distintos do ciclo de vida (Gronke et al., 2010). Embora a via de insulina entre a *D. melanogaster* e mamíferos seja semelhante, certamente uma limitante é a diversidade de função e expressão dos dilps, fato que precisa ser levado em conta ao comparar funcionamentos de vias e alterações de expressão.

Considerando as semelhanças com a regulação do metabolismo humano, diversos modelos de estudos já foram desenvolvidos em *D. melanogaster* para investigar a patogênese do DM, como por exemplo o uso de mutantes, principalmente interrompendo a transcrição de dilps ou do próprio InR. Modelos que interrompem a transcrição de dilps geralmente são considerados como modelos de DT1, já que geram uma deficiência de dilps ao alterar sua produção, semelhante a deficiência de insulina em humanos; enquanto modelos que alteram o InR geralmente são considerados como modelos de DT2, já que mantém os níveis de dilps normais ou até elevados ao mesmo tempo que diminuem a atividade da via de insulina (Alfa e Kim, 2016). Ainda assim, mesmo com esses recursos transgênicos, o modelo de DT2 gerado por dieta é o considerado mais próximo a DT2 em humanos, já que gera um fenótipo obesogênico e uma resistência na via de insulina, fenômeno observado por diversos grupos (Tabela 1) (Skorupa et al., 2008; Musselman et al., 2011; Morris et al., 2012; Alfa e Kim, 2016).

Particularmente com HSD, nosso grupo já registrou fenótipos classicamente associados ao DM2 em *D. melanogaster*: o consumo de HSD contendo 30% de sacarose (HSD-30%) induziu um atraso de desenvolvimento, em concordância com trabalhos da literatura (Musselman et al., 2011; Alfa e Kim, 2016); e um aumento nos níveis de glicose, triglicerídeos e de expressão do dilp5 (Ecker et al., 2017).

Tabela 1- Exemplos de fenótipos desenvolvidos em modelos de DM usando *D. melanogaster*

Modelo	Insuficiência	Fenótipos principais	Referências
Deficiência de insulina (modelos de DT1)	Completa remoção dos IPCs	○ atraso de desenvolvimento, ○ tamanho corporal reduzido, ○ hiperglicemia, ○ sensibilidade à insulina preservada	Rulifson et al., 2002; Wessells et al, 2004; Haselton et al, 2010;
	Inativação parcial dos IPCs		Broughton et al., 2005; Haselton et al., 2010
	Disrupção genética dos dilps2, 3 e 5		Groenke et al., 2010;
	Disrupção genética dos dilps2, 3 e 5		Zhang et al., 2009; Groenke et al., 2010;
Resistência à insulina (modelos de DT2)	Mutantes heterozigotas para o Receptor de Insulina	○ Secreção de dilps elevada, ○ níveis normais de glicemia,	Tatar et al., 2001; Park et al., 2014
	Expressão reduzida do Receptor de Insulina nos corpos de gordura		Park et al., 2014
Resistência à insulina induzido por dieta (modelos de DT2)	HSD – dietas com alta concentração de açúcar	○ Obesidade, ○ níveis elevados de dilps inicialmente seguido por diminuição, ○ hiperglicemia, ○ resistência à insulina,	Skorupa et al., 2008; Musselman et al., 2011; Morris et al., 2012;
	HFD – dietas com alta concentração de gordura	○ Obesidade, ○ níveis elevados de dilps inicialmente, ○ hiperglicemia, ○ resistência à insulina, ○ toxicidade cardíaca	Birse et al, 2010

Adaptado de Alfa e Kim (2016).

1.3 ANÁLISE TRANSCRIPTÔMICA NO ESTUDO DO DM

Abordagens ômicas como a genômica e a transcriptômica têm contribuído de forma notória para a identificação das bases moleculares associadas à ocorrência e progressão de diferentes patologias, incluindo o DM (Lawlor et al., 2017; De Jesus e Kulkarni, 2019). A técnica de transcriptoma utiliza-se da sequência de todos os componentes transcritos em uma determinada amostra, ou seja, detecta o nível de transcrição de todos os genes de uma única vez (Jenkinson et al., 2016), representando no contexto de DT2 x HSD uma ferramenta poderosa para estudar as vias metabólicas que estão sendo expressas dentro das situações limitadas pelo estudo. Em Drosófilas, já foram publicados trabalhos que possuem o intuito de compreender os efeitos de diferentes dietas no funcionamento de órgãos específicos, como antenas (Jung et al., 2018), corpos de gordura ou adipócitos (Musselman et al., 2018), cabeças das moscas (Hemphill et al., 2018); ou efeitos da dieta sob a fase larval (Williams et al., 2015) ou senescência (Doruszuk et al., 2012; Whitaker et al., 2014; May e Zwaan, 2017; Zhang et al.,

2018; Li et al., 2019; Teltumbade et al., 2020); ou até mesmo comparações entre dietas (Reed et al., 2014; Nazario-Yepiz et al., 2017; Osborne e Dearden, 2017; Camus et al., 2019; Mateus et al., 2019); ou abordando outros aspectos em relação a dieta e mudanças transcricionais em drosófilas (Branco e Lemos, 2014; Musselman et al., 2018; Azuma et al., 2019). No entanto, até o momento não encontramos uma análise de transcriptoma sendo utilizada para identificar vias moduladas por uma dieta HSD em *D. melanogaster*.

2. HIPÓTESE E JUSTIFICATIVA

Considerando a necessidade de estudos que apontem, de forma geral, vias de sinalização e genes preferencialmente impactados pelo consumo de uma dieta rica em açúcar, este estudo foi delineado para obter, através da técnica de transcriptoma e recursos de análise ontológica, um panorama geral de como esta dieta poderia estar afetando a expressão de genes relacionados a diferentes processos biológicos em *D. melanogaster*. Nossa hipótese é de que a dieta induza mudanças de expressão em vias e genes específicos relacionados com o DT2 de humanos, e que o modelo proporcione uma visão mais precisa para o delineamento de estudos translacionais, bem como conhecimento dos impactos da deita sobre aspectos fisiológicos da *D. melanogaster*.

3. OBJETIVOS

3.1 OBJETIVO GERAL

Usando *D.melanogaster* como organismo modelo, identificar genes e processos biológicos afetados pelo consumo de uma dieta rica em sacarose.

3.2 OBJETIVOS ESPECÍFICOS

- Averiguar se o consumo da HSD induz fenótipos associados com hiperglicemia, através dos níveis de marcadores bioquímicos (glicose e triglicerídeos);
- Identificar as alterações gerais de expressão gênica induzidas pela HSD;
- Investigar a relação translacional entre as alterações de expressão gênica induzidas pela HSD e as condições da patologia de DT2;

4. ARTIGO CIENTÍFICO

Os resultados obtidos do presente estudo estão apresentados sob a forma de um Artigo Científico. Neste constam as seções: Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas.

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Human type 2 diabetes mellitus-associated transcriptional disturbances in a high-sugar diet long-term exposed *Drosophila melanogaster*

Julia Sepel Loreto [a], Sabrina Antunes Ferreira [a], Daniel MP Ardisson-Araújo [a, b, *], Nilda Vargas Barbosa [a, *]

[a] Programa de Pós-graduação em Bioquímica Toxicológica, Universidade Federal de Santa Maria, Avenida Roraima, 1000, 97105–900 Santa Maria, RS, Brazil
[b] Laboratório de Virologia de Insetos, Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, Avenida Roraima, 1000, 97105-900 Santa Maria, RS, Brazil

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ABSTRACT

Type 2 Diabetes mellitus (T2DM) is a multifactorial and polygenic disorder with the molecular bases still idiopathic. Experimental analyses and tests are quite limited upon human samples due to the access, variability of patient's conditions, and the size and complexity of the genome. Therefore, high-sugar diet exposure is commonly used for modeling T2DM in non-human animals, which includes invertebrate organisms like the fruit fly *Drosophila melanogaster*. Interestingly, high-sugar diet (HSD) induces delayed time for pupation and reduced viability in fruit fly larvae hatched from a 30% sucrose-containing medium (HSD-30%). Here we carried out an mRNA-deep sequencing study to identify differentially transcribed genes in adult fruit fly hatched and reared from an HSD-30%. Seven days after hatching, flies reared on control and HSD-30% were used to glucose and triglyceride level measurements and RNA extraction for sequencing. Remarkably, glucose levels were about 2-fold higher than the control group in fruit flies exposed to HSD-30%, whereas triglycerides levels increased 1.7-fold. After RNA-sequencing, we found that 13.5% of the genes were differentially transcribed in the dyslipidemic and hyperglycaemic insects. HSD-30% up-regulated genes involved in ribosomal biogenesis (*e.g. dTOR*, *ERK* and *dS6K*) and down-regulated genes involved in energetic process (*e.g. Pfk*, *Gapdh1*, and *Pyk* from pyruvate metabolism; *kdn*, *Idh* and *Mdh2* from the citric acid cycle; *ATPsynC* and *ATPsynß* from ATP synthesis) and insect development. We found a remarkable down-regulation for *Actin* (*Act88F*) that likely impairs muscle development. Moreover, HSD-30% up-regulated both the *insulin-like peptides 7* and *8* and down-regulated the *insulin receptor substrate p53*, *isoform A* and *insulin-like peptide 6* genes, whose functional products are insulin signaling markers. All these features pointed together to a tightly correlation of the T2DM-like phenotype modeled by the *D. melanogaster* and an intricate array of phenomena, which includes energetic processes, muscle development, and ribosomal synthesis as that observed for the human pathology.

1. Introduction

Diabetes mellitus (DM) encompasses a group of metabolic disorders that share the common phenotype of hyperglycemia, insulin resistance, and hyperinsulinemia. The most common subtype is the type 2 DM (T2DM), which comprises about 90% of all cases worldwide and represents a major public health issue due to growing prevalence and severity of associated comorbidities (WHO, 2019). The precise pathophysiological processes underlying T2DM etiology remains idiopathic as the disorder harbors a multifactorial repertoire (Prasad and Groop, 2019). However, unhealthy diet and lifestyle have been recognized as key risk factors for T2DM development and progression along with genetic and epigenetic background (Kota et al., 2012; Ling and Rönn, 2019). The close relationship between genetic background and adverse environmental issues suggests that unhealthy habits interact with genes to cause the disorder (Jenkinson et al., 2016).

Beyond individual's behavior, T2DM is also a polygenic disorder (Prasad and Groop, 2019) and the still-growing 'omics'-wide approaches like genomics and transcriptomics have contributed successfully to identify the molecular bases associated with illness occurrence and progression (Lawlor et al., 2017; De Jesus and Kulkarni, 2019). For instance, RNA-seq analysis of neutrophils and peripheral blood from

* Corresponding authors at: Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Bioquímica Toxicológica, 97105-900 Santa Maria, RS, Brazil.
E-mail addresses: daniel.araujo@ufsm.br (D.M. Ardisson-Araújo), nvbarbosa@yahoo.com.br (N.V. Barbosa).

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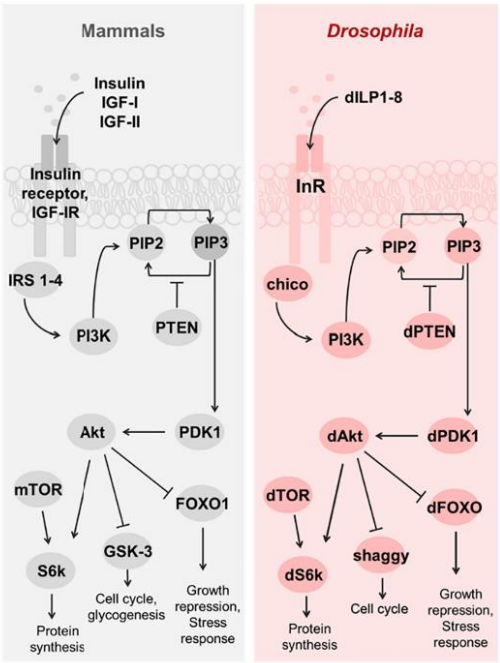


Scheme 1. Overview of insulin signaling markers conserved among *D. melanogaster* and mammals. Drosophila Insulin-like peptides (dILPs) interact with an Insulin Receptor (InR) homolog similarly to the mammalian pathway, where the main difference is the specific role each of the dILPs fulfill. The InR activates the insulin receptor substrate homolog Chico that signalize itself by means of the phosphatidylinositol kinase homolog (PI3K) to the phosphoinositide-dependent kinase 1 homolog (dPDK1). Phosphatase and tensin homolog (dPTEN) role are also conserved among both fruit flies and mammals and may inhibit PI3K effects. dPDK1 signalizes to the Akt kinase homolog (dAkt) that among other properties has three important roles: (*i*) inhibits the forkhead box, sub-group O homolog (dFOXO) and (ii) inhibits the glycogen synthase kinase 3 homolog (Shaggy or GSK-3), and (*iii*) activates the Ribosomal protein S6 kinase homolog (dS6k). dS6k activity can also be regulated by the Target of rapamycin homolog (dTOR), which regulates growth in a nutrient dependent manner.

T2DM individual compared with healthy ones revealed transcriptional modulation of specific set of genes like inflammatory- and lipid-related genes (Kleinstein et al., 2019). Also, RNA-seq analysis of skeletal muscle of T2DM individuals revealed molecular modulation of epigenetic-associated gene players, myogenesis dysregulation, down-regulation of muscle function-associated genes, and up-regulation of inflammation and extracellular matrix components (Väremo et al., 2017). Besides, meta-analysis approaches have provided genomic/transcriptomic links between T2DM and the pathogenesis of other diseases. In T2DM and Alzheimer's disease patients, a complex set of genes related with insulin and interleukin-mediated signaling pathways was found (Mirza et al., 2014). However, experimental analysis and test are quite limited upon human samples due to the access, the variability of patient's conditions, and the size and complexity of the genome.

A recurrent experimental protocol to model and investigate human T2DM-associated phenotypes relies on a simple basis of high-sugar content diet (HSD) intake by an organism model (King, 2012; Moreira, 2013; Ecker et al., 2017). For instance, the fruit fly *Drosophila melanogaster* has been shown to be a suitable model organism to modeling T2DM. For instance, the insulin signaling pathways in *D. melanogaster* is conserved when compared to mammals (Garofalo, 2002) and includes the insulin-like peptides (dILPs) and receptor (InR), the insulin receptor substrates (IRS) as well as similar downstream components involved in cell growth, cell cycle, and protein synthesis control (as referred in Scheme 1). HSD exposure induces hyperglycemia, increase in storage/circulating lipids, and insulin resistance in the fruit fly, which are recognized hallmarks of T2DM (Ecker et al., 2017; Álvarez-Rendón et al., 2018). HSD also induced developmental deficits and defective responses to insulin in both larvae and adult flies (Skorupa et al., 2008; Musselman et al., 2011; Pasco and Léopold, 2012; Pendse et al., 2013). Moreover, a 30% sucrose-containing HSD (HSD-30%) affected insect pupation, hatched-larvae viability, body weight, hyperglycemia and relative transcript levels of genes associated with the insulin pathway (Ecker et al., 2017).

Although diet itself is considered to be one of the top-10 experimental factors that changes the transcriptional levels of housekeeping genes in insects (Lü et al., 2018), there is no study showing a landscape of differentially transcribed genes in *D. melanogaster* when exposed to HSD. Most transcriptomic studies with fruit fly and HSD focus on the effect of diet on specific tissues, including fat bodies, head, and antennae (Hemphill et al., 2018; Jung et al., 2018; Musselman et al., 2018) or the effect of some compounds on the transcription of epigenetic and senescence markers (Doroszuk et al., 2012; Branco and Lemos, 2014; May and Zwaan, 2017; Azuma et al., 2019; Teltumbade et al., 2020). Here we used an RNA-Seq approach on adult flies hatched from and reared on an HSD-30% to identify genes differentially transcribed and characterize the molecular networks associated with modeling T2DM. We found that a long-term HSD-30% exposure caused increase of glucose and triglycerides levels in fruit flies. Moreover, we found an altered transcription in genes associated with ribosomal synthesis, energetic pathways, and muscle development processes.

2. Materials and methods

2.1. Fly stock and husbandry

Flies of the Oregon-R strain were kept in 2.5 × 6.5 cm bottles underneath-containing 30 g standard corn medium at a constant temperature of 24 ± 1 °C, with relative humidity of 60% and light/dark cycle of 12 h. The standard food was based on corn medium contained 44% coarse and 35% medium corn flour, 11% wheat germ, 8% sucrose, 0.5% milk powder, 0.5% NaCl, 0.5% soybean flour, 0.5% rye flour, 0.8% of methyl p-hydroxybenzoate antifungal (Nipagin®) and lyophilized yeast. The components were dissolved in a proportion of 1:3 dry

Table 1
Nutritional components and caloric content from control and HSD-30% diet.

Diets	Cornflour g/L	Wheat germ g/L	Powdered milk g/L	Salt g/L	Soybean flour g/L	Rye flour g/L	Sucrose g/L	Total		C:P ratio	P:C ratio
								g/L	Kcal		
Control	220,5	27,6	1,1	1,32	1,1	1,32	22,05	275	916,9	8,52	23,89
HSD-30%	220,5	27,6	1,1	1,32	1,1	1,32	404,05	657,5	2406,6	0,117	0,042

C:P carbohydrate-to-protein ratio.
P:C protein-to-carbohydrate ratio.

medium/water and simmered for 3–5 min. After cooling, the mixture was dispensed into vials (3 mL) for the two groups. Flies from control group were raised from eggs until adult phase on standard corn medium (2.5% sucrose), while flies from HSD group in the corm medium plus 30% sucrose (HSD-30%). After hatching on control and HSD-30% media, the flies from viable larvae were placed in flasks with the respective diets again until reach seven days old for the biochemical analyses and RNA extraction. The sucrose concentrations on diets were based in a previous study performed by Ecker et al. (2017). Fresh food was prepared every 2 days. Diet composition and its carbohydrate/protein ratio are showed in Table 1.

2.2. Measurement of insect whole body glucose and triglycerides contents

Glucose and triglycerides were measured in 7 days old adult flies hatched from both the control diet and the HSD-30% according to the protocol described in Ecker et al. (2017). Briefly, twenty flies were cold-anesthetized in ice, and then the whole-body pool homogenized in glucose measuring buffer (5 mM Tris [pH 6.6] 2.7 mM KCl, 137 mM NaCl) or triglyceride measuring buffer (0.02 M TFK [pH 7.4] + 0.5% Tween 20). The homogenates were centrifuged at 10.500 $\times g$ for 3 min and the supernatant (10 μL) incubated with the specific buffer at 37 °C for 25 min. Triglycerides and glucose levels were determined using Labtest assays according to supplier instructions (Triglyceride Liquiform Kit and Glucose Liquiform Kit). The levels of glucose and TG from body samples were normalized by protein using the method of Lowry et al. (1951).

2.3. RNA-seq

For RNA-seq analysis, three replicates per group (control and HSD-30%) were used with 7 days-old flies each (30 individuals per replicate with the proportion of 1:1 male/female). The RNA quality was accessed in a Nanodrop® spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and quantified in a Qubit 4TM fluorimeter (Thermo Fisher Scientific) with the QubitTM RNA HS Assay kit (Thermo Fisher Scientific). The RNA integrity value was inferred with the Bionalizer 2100 (Agilent, Santa Clara, California, USA) using the RNA 6000 nano kit (Agilent). The messenger RNA was isolated using the Dynabeads ™ mRNA Purification Kit (Thermo Fisher Scientific). The sequencing was carried out on the Ion GeneStudio S5 equipment (Thermo Fisher Scientific) using the Ion Total RNA-Seq Kit v2 kit (Thermo Fisher Scientific) for making the libraries that were identified with barcodes and loaded onto an Ion 540 ™ chip (Thermo Fisher Scientific). 5–6 Gb of sequences were obtained for each sample.

2.4. Data processing and differential transcription analysis

The IonTorrent raw sequences were initially trimmed to remove both adapter sequences and low-quality sequencing regions and reads less than 15nt in length using the CLC Workbench Software. The mRNA RefSeq was retrieved from NCBI with a total of 30,704 sequences, including transcription variants. Mapping was carried out in the same software (https://digitalinsights.qiagen.com) with default parameters. The default RNA-Seq parameters of 0.9 for 'minimum length fraction', of 0.8 for 'minimum similarity fraction', and 'maximum number of hits for a read' of 10 were used in the software CLC Workbench. The gene transcription quantification was obtained for each RNA-seq by the RPKM (Reads Per Kilobase Million) method. To confirm the relatedness among libraries, principal component analysis (PCA) and Heat Map were carried out for gene transcription values using the same software. RPKM values were used to establish a transcriptional fold-change (FC), which were used to perform statistical analysis. Therefore, genes were separated into three main categories according their transcription: untranscribed genes (UTG), equally transcribed genes (ETG) and differentially transcribed genes (DTG). A gene was considered as 'untranscribed' when all libraries had an RPKM value equal zero. When a gene was transcribed in the control replicates and repressed to zero in the HSD-30% replicates, that genes was considered repressed and when a gene was not transcribed in the control replicates and presented transcripts in the HSD-30% replicates, that genes was considered activated. A gene was only considered differentially transcribed when SD < 25% of the FCs average. All results were expressed as the FC mean ± standard deviation of the mean (SD) and data derived from the transcriptome were analyzed by ANOVA-like and Student's *t*-test when appropriated. The results were considered significant when $p \leq 0.05$.

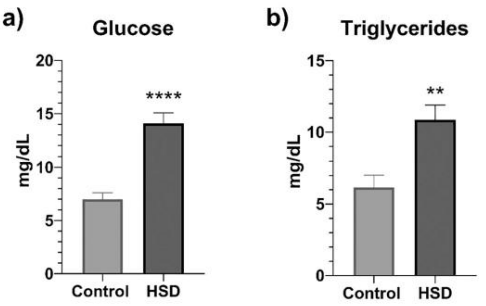


Fig. 1. Glucose and triglycerides levels of flies hatched from and fed on HSD. **(a)** Glucose levels and **(b)** Triglycerides levels. The levels of glucose and triglycerides were measured in whole body samples of flies 7 days after hatching from control or HSD-30% diet. Data are presented as mean ± S.E.M by unpaired *t*-test ($n = 4$). $^{*}p < 0.05$ and $^{***}p < 0.0001$ indicates statistical difference from control.

2.5. Gene ontology analysis

The Metascape (https://metascape.org/) resource was used to generate the Enrichment Ontology clusters for all DTG (Zhou et al., 2019). FC values were separated for a specific (FC > 2.5) and a generalist analysis (FC > 1). Figures provided from the Metascape resource were used with few modifications. The original pictures were included as Supplementary material.

3. Results

3.1. Glucose and triglycerides

We raised individuals of *D. melanogaster* from eggs until adult phase from an HSD-30% to model T2DM-like phenotypes. Glucose levels found in HSD-30% flies were about 2-fold higher than the control flies (Fig. 1A; control: 7 mg/dL; HSD: 14 mg/dL; $p = 0.0001$). Moreover, HSD-30% flies had an increase of 1.7-fold in the triglycerides content when compared to control flies (Fig. 1B; control: 6 mg/dL; HSD: 10 mg/dL; $p = 0.0048$). We checked the levels of glucose and triglycerides in flies 7 days after hatching.

3.2. RNA-seq features

We carried out an RNA-seq analysis to determine the transcriptional profile of whole-body adult fruit flies after long-term HSD-30% exposure. For RNA-seq analysis, three replicates per group (control and HSD-30%) were used with 7 days-old flies each (30 individuals per replicate with the proportion of 1:1 male/female). We extracted the total RNA from body and prepared for mRNA deep-sequencing using the IonTorrent methodology. We obtained 83,608,952 raw reads and we remained with 80,446,936 after trimming (Table S1) that were used for

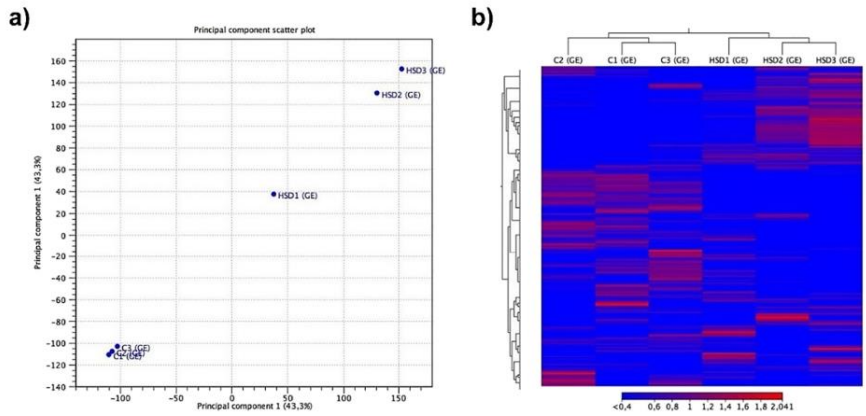


Fig. 2. Analysis of concordance among the control (C1, C2 and C3) and HSD libraries (HSD1, HSD2 and HSD3). **(a)** Principal component analysis plot based on RPKM values for control and HSD libraries reveals similar components that clustered in closely related areas. **(b)** Heat Map based on the RPKM value of all *D. melanogaster* transcripts shows two main clusters that separate control and HSD-30% libraries.

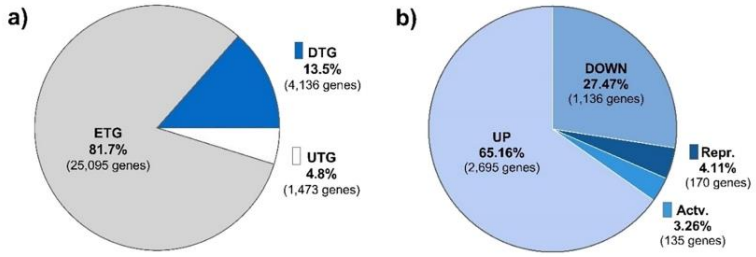


Fig. 3. Percentages of genes with different and equal transcription level. **(a)** Percentage of equally transcribed genes (ETG), differentially transcribed genes (DTG), and untrascribed genes (UTG) for all leveled genes in *D. melanogaster*. **(b)** Percentage considering only the DTGs. Genes were combined into Up-regulated (UP), Down-regulated (DOWN), activated (Actv.) and repressed (Repr.) genes.

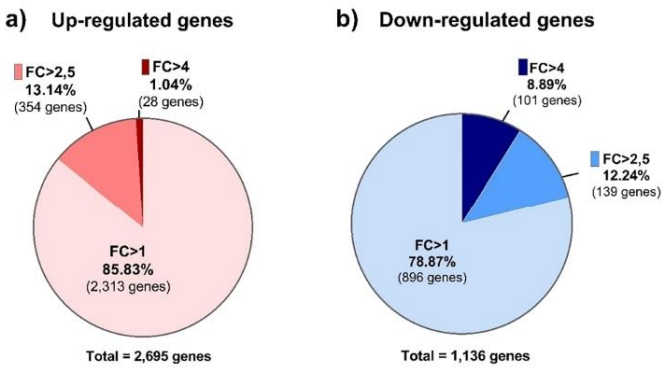


Fig. 4. Percentage of the fold-change (FC) in Up and Down-regulated genes. Total number of **(a)** Up- and **(b)** Down-regulated genes are presented with its specific FC. We divided the genes into three categories based on the FC values: FC > 1, FC > 2,5 and FC > 4. A total of 2695 genes were up-regulated and 1136 down-regulated. Most of genes up- and down-regulated presented an FC < 2.5. For up-regulated genes only about 1.0% presented FC > 4. For down-regulated genes about 9% presented an FC > 4.

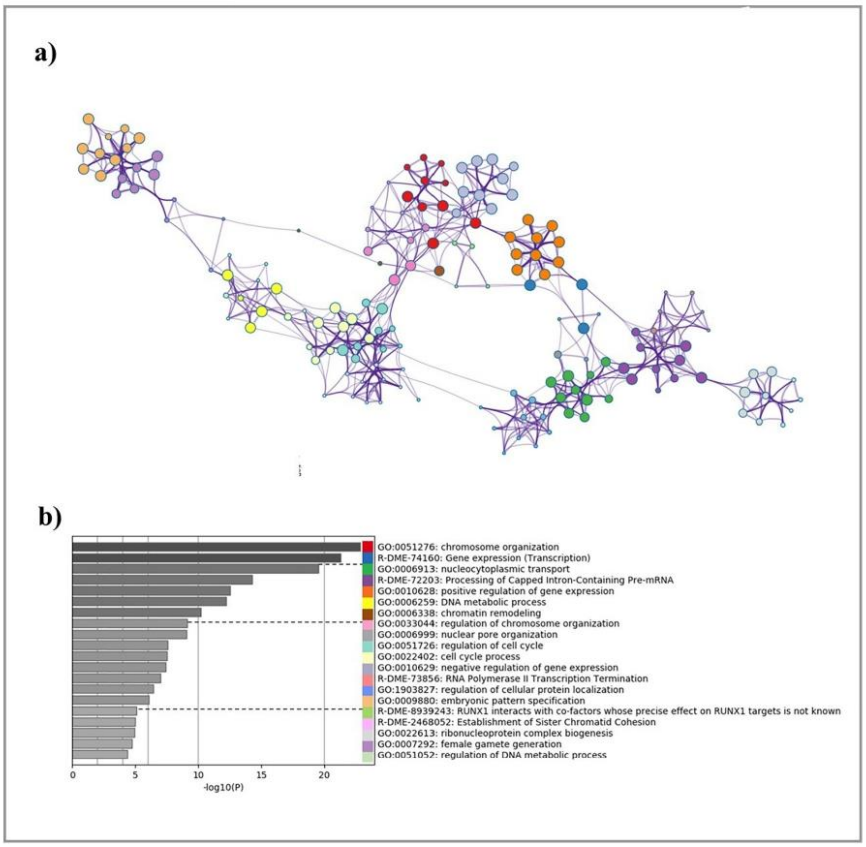


Fig. 5. Functional enrichment ontology analysis of up-regulated genes (FC > 2.5).
(a) Representative network of the clusters from the enriched terms: 'chromosome organization', 'gene expression (transcription)', 'nucleocytoplasmic transport', 'Processing of capped intron-containing Pre-mRNA', 'positive regulation of gene expression', 'DNA metabolic process' and 'chromatin remodeling', among others. The enrichment ontology graph represents each term as a circle node, where the circle size is proportional to the number of DTGs associated to the term. The clusters are represented by different colors and circles of the same color are associated with the same cluster. The edges connect terms that have a similarity score of >0.3 that determines the density of the edge line. **(b)** Bar graph of enriched terms hierarchically colored according to *p*-values. Each term presents the same color code in both layouts. Log10(P) indicates *P*-value in log base 10. Metascape (https://metascape.org/).

further analyses.

3.3. Differentially transcribed genes

We quantified by RNA-seq analysis gene transcriptional modulation elicited in HSD-30% flies compared with the control flies and checked for the convergent robustness of our library. We found a robust concordance among the three libraries of controls (C1, C2 and C3) and also among HSD-30% libraries (HSD1, HSD2 and HSD3) (Fig. 2A), which was inferred from a transcriptional level-based PCA plot analysis. The C1, C2, and C3 libraries clustered in a very restricted area depicting a high convergent data by evaluated principal component. Only the library HSD1 presented a discordance, besides closer to HSD2 than to the controls. We chose for maintaining this library based on the heat map analysis results. By heat map analysis and clustering, we found two main clusters, one formed by the controls and another one formed by HSD-30%, reinforcing the PCA plot results (Fig. 2B).

We quantified gene transcription induced in HSD-30% flies compared to the control flies. We found that 81.73% (25,095 spots) of the transcripts were equally transcribed in both groups, 13.47% were differentially transcribed (DT), and only 4.79% were untranscribed (Fig. 3A). The gene list for each category is included as Supplemental Material (Table S2). From the 4136 DTG, 2695 were up-regulated (65.15%), and 1136 down-regulated (27.46%) (Fig. 3). We also found 170 genes totally repressed (4.11%) and 135 genes activated from zero (3.26%) by the HSD-30% treatment (Fig. 3B, for activated and repressed gene definition see the section Material and Methods). For the up-regulated genes, most of the genes presented a FC < 2.5 (about 85.8%). Only about 1.0% of the genes presented FC > 4 (Fig. 4A). Similarly, for down-regulated genes, most were found to present a FC < 2.5 (about 78.9%) besides about 9% presented down-regulation with a FC > 4 (Fig. 4B). Overall, HSD-30% caused more up- than down-regulation. Nevertheless, the highest FC values were found for the down-regulated genes. Consequently, to interpret the big data generated

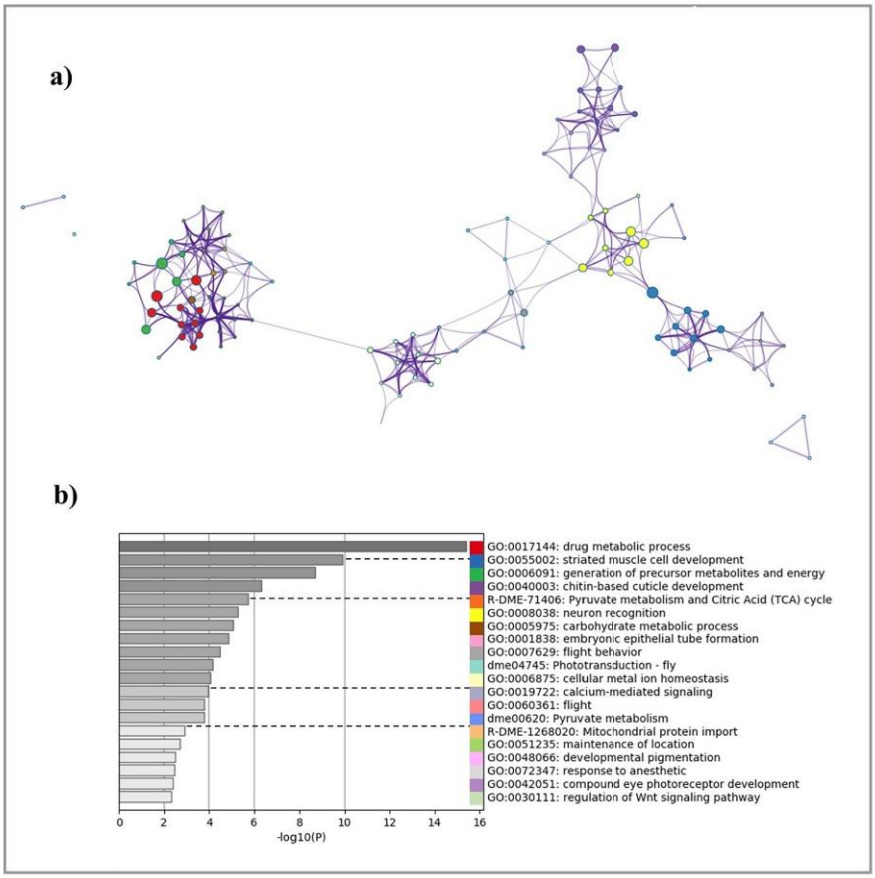


Fig. 6. Functional Enrichment Ontology analysis of Down-regulated genes (FC > 2.5).
(a) Representative network of the clusters from the enriched terms: 'drug metabolic process', 'striated muscle cell development', 'generation of precursor metabolites and energy', 'chitin-based cuticle development' and 'pyruvate metabolism and Citric Acid (TCA) cycle', among others. The enrichment ontology graph represents each term as a circle node, where the circle size is proportional to the number of DTGs associated to the term. The clusters are represented by different colors and circles of the same color are associated with the same cluster. The edges connect terms that have a similarity score of >0.3 that determines the density of the edge line. **(b)** Bar graph of enriched terms hierarchically colored according to *p*-values**.** Each term presents the same color code in both layouts. Log10(P) indicates *P*-value in log base 10. Metascape (https://metascape.org/).

by the RNA-seq analysis and establish a network among DTGs, we submitted individually each list of up- and down-regulated genes to the Metascape analyses.

3.4. Up-regulated genes

Using the list of all up-regulated genes, we found as the top five most enriched terms 'intracellular transport', 'vesicle-mediated transport', 'gene expression (Transcription)', 'regulation of signal transduction', and 'oogenesis' (Supplemental Figs. 9–10). For an enrichment heat map that analyze by a network the Top100 terms, we found that most of the terms were related with 'transcription and translation', 'nuclear metabolism', and 'development of tissues'. Although not in the most enriched areas, some terms related with metabolism and insulin signaling were also found, including 'negative regulation of macromolecule metabolic process', 'TOR signaling', 'regulation of GTPase activity', 'MAPK signaling pathway - fly', 'glycerolipid metabolic process' (Supplementary Fig. 9). Therefore, we investigated the transcription values of some genes commonly related to the TOR and MAPK signaling paths, which have been implicated as cause-effect in experimental T2DM rats (Mariappan et al., 2011). Interestingly, all genes were found to be up-regulated in relation to the control, including two orthologs of *Phosphatidylinositol 3-kinase* (*Pi3K*) (*Pi3K59F* and *Pi3K68D* both with FC = +1.4), *Akt1* (isoform A: FC = +1.9), *Target of Rapamycin* (*dTor;* isoform A: FC = +1.7), *Ribosomal protein S6 kinase 1* (*S6k*, isoform A: FC = +1.2), and *Ribosomal protein S6 kinase 2* (*S6kII*, isoform A: FC = +2.3). Considering MAPK signaling pathway alone, we found an up-regulation of the *downstream of receptor kinase* (*drk*, also known as *Grb2;* isoform C: FC = +1.4), *Ras oncogene at 85D* (*Ras85D*, also known as *Ras*, with FC = +1.7), and *Rolled* (*rl*, also known as *MAPK* or *ERK;* isoform D: FC =

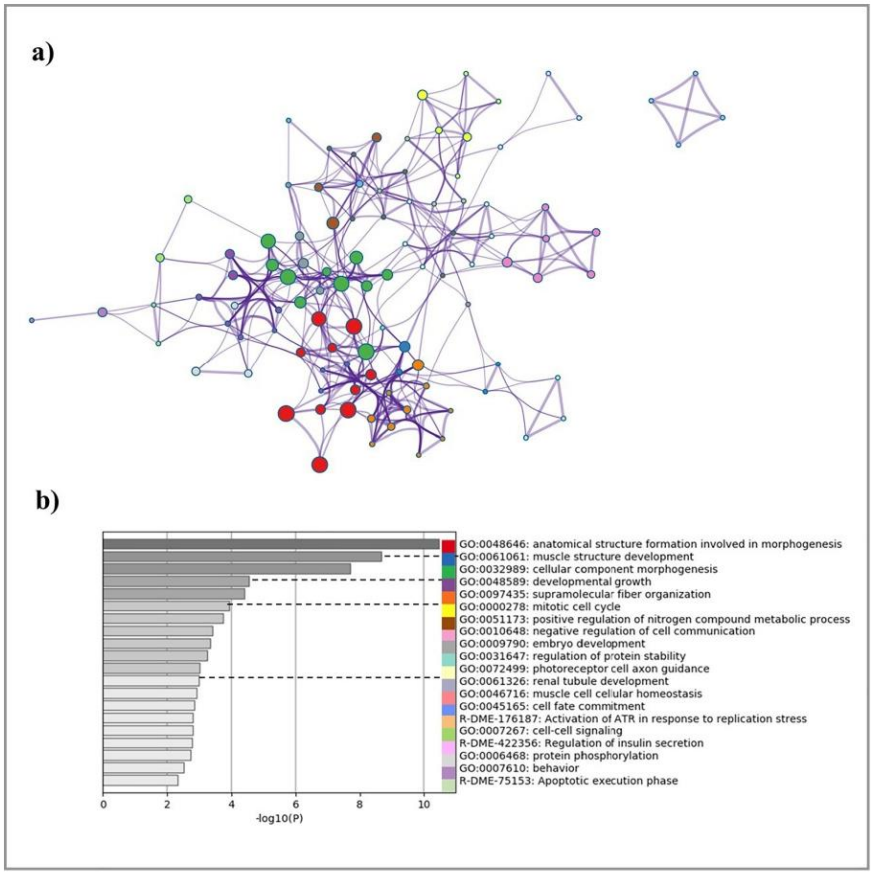


Fig. 7. Functional Enrichment Ontology analysis of activated genes.
(a) Representative network of the clusters from the enriched terms: 'anatomical structure formation involved in morphogenesis', 'muscle structure development', 'cellular component morphogenesis', 'developmental growth' and 'supramolecular fiber organization', among others. The enrichment ontology graph represents each term as a circle node, where the circle size is proportional to the number of DTGs associated to the term. The clusters are represented by different colors and circles of the same color are associated with the same cluster. The edges connect terms that have a similarity score of >0.3 that determines the density of the edge line. **(b)** Bar graph of enriched terms hierarchically colored according to *p*-values. Each term presents the same color code in both layouts. Log10(P) indicates *P*-value in log base 10. Metascape (https://metascape.org/).

+2.6).

We carried out the same Metascape analysis focusing only on up-regulated genes with FC > 2.5 (Fig. 5) to filter the results for the most affected genes. We found 'chromosome organization', 'gene expression (Transcription)', 'nucleocytoplasmic transport', 'processing of capped intron-containing pre-mRNA' and 'positive regulation of gene expression' as the top 5 most enriched terms, all with -10log10(P). This is in accordance with the fact that HSD-30% induced an abroad upregulation when compared to the number of downregulated genes. By enrichment heat map analysis of the Top100 genes, most terms were also found to be related with process of 'nuclear metabolism' and 'transcription and translation' (Supplementary Fig. 1–2).

3.5. Down-regulated genes

Using the list of all down-regulated genes for a generalist analysis (FC > 1), we found as the top5 most enriched terms 'ATP metabolic process', 'nucleoside triphosphate biosynthetic process', 'inorganic cation transmembrane transport', 'cellular component morphogenesis', and 'myofibril assembly', all with −10 log10(P) (Supplemental Figs. 11–12). The enrichment heat map generated with the Top100 genes revealed more types of biological processes altered by the HSD-30% when compared to the up-regulated genes (Supplementary Fig. 11). The down-regulated genes were divided into two main terms, which included 'energetic process' and 'development' and four minor terms, including 'behavior', 'starch and sucrose metabolism', 'response to abiotic stimulus', and 'neutrophil degranulation'.

When considered only the down-regulated genes with a FC > 2.5

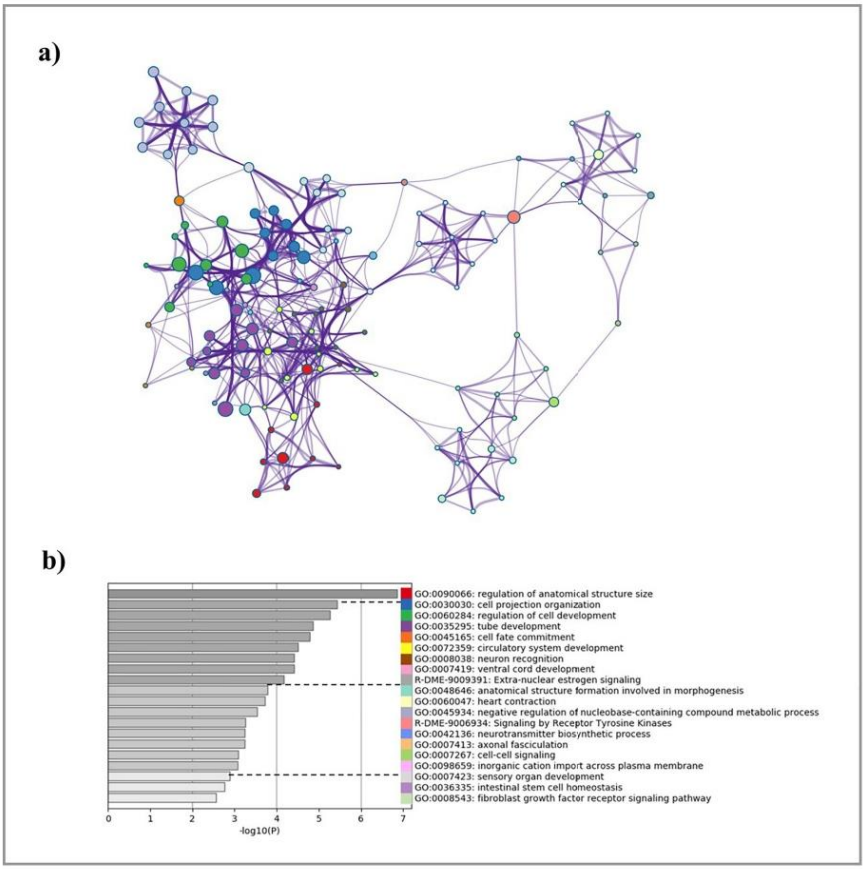


Fig. 8. Functional Enrichment Ontology analysis of repressed genes.
(a) Representative network of the clusters from the enriched terms: 'regulation of anatomical structure size', 'cell projection organization', 'regulation of cell development', 'tube development', 'cell fate commitment', 'circulatory system development' and 'neuron recognition', among others. The enrichment ontology graph represents each term as a circle node, where the circle size is proportional to the number of DTGs associated to the term. The clusters are represented by different colors and circles of the same color are associated with the same cluster. The edges connect terms that have a similarity score of >0.3 that determines the density of the edge line. **(b)** Bar graph of enriched terms hierarchically colored according to p-values. Each term presents the same color code in both layouts. Log10(P) indicates P-value in log base 10. Metascape (https://metascape.org/).

(Fig. 6), we found 'drug metabolic process', 'striated muscle cell development', 'generation of precursor metabolites and energy', 'chitin-based cuticle development' and 'pyruvate metabolism and citric acid (TCA) cycle' as the top5 most enriched terms, all with −5 log10(P). Analyzing the Top100 enrichment heat map of down-regulated genes (Supplementary Fig. 3–4), we found that most terms were related to three major categories, including 'energy metabolism', 'muscle and epidermis development', and 'behavior'.

Other terms associated with the down-regulated genes were linked mainly with 'flight behavior' and 'circadian rhythm', two biological processes linked with the 'muscular system' and 'energy use'. *Actin 88F* (*Act88F*, FC = -43.7) presented the highest FC value among the down-regulated genes in HSD-30% when compared to the control. Other forms were also down-regulated by HSD-30%: *Actin 79B* (*Act79B*, isoform A: FC = -18.2), *Actin 57B* (*Act57B*, FC = -3.9) and *Actin 42A* (*Act42A*, FC = -1.5). We also investigated the transcription values of genes involved in major energetic pathways, including the pyruvate metabolism, the tricarboxylic acid (TCA) cycle, and the electric transport chain (nuclear-coded components). On the pyruvate metabolism there was a down-regulation of the following genes: *Phosphofructokinase* (*Pfk;* isoform D:FC = -4.9; and isoform C: FC = -1.5), *Glyceraldehyde 3 phosphate dehydrogenase 1* (*Gapdh1,* isoform B: FC = -3.3) and *Pyruvate kinase* (*Pyk*, FC = -4.2). For the TCA cycle, we observed a down-regulation of *knockdown* (*kdn*, also known as *citrate synthase* or *CS;* isoform A: FC = -5.5); the two orthologs of *Isocitrate dehydrogenase 3* (*Idh3*), *Idh3a* (isoform C:FC = -3.5; and isoform A:FC = -3.4), and *Idh3b* (isoform B:FC = -2.6); and *Malate dehydrogenase 2* (*Mdh2*, FC = -2.9). We also analyzed the transcription values of the *ATP synthase* (*ATPsyn*) from the electric transport chain. From the 11 subunits of *ATPsyn*, five were down-regulated: *ATPsynG* (FC = -2.3), *ATPsynβ* (FC = -3.3), *ATPsynC*

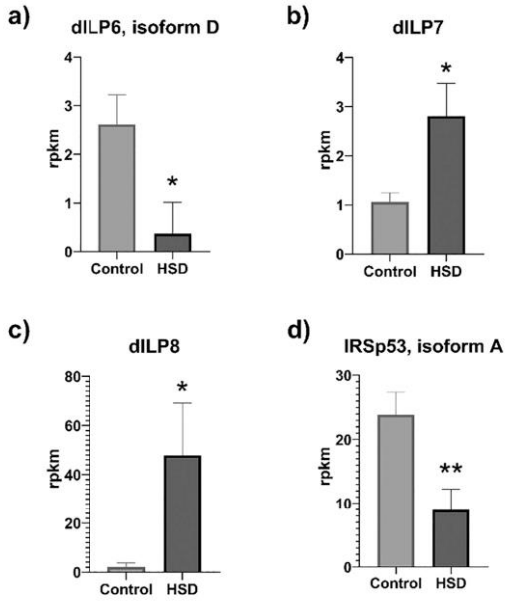


Fig. 9. Effects of HSD-30% on transcription levels of genes related to the insulin signaling pathway. **(a)** *dILP 6*, *isoform D*, **(b)** *dILP 7*, **(c)** *dILP 8* and **(d)** *IRS p53*, *isoform A*. Data are presented as RPKM and expressed as mean ± SD by Student's *t*-test, $^{*}p \leq 0.05$ indicates statistical difference from control.

(isoform A: FC = -4.9 and isoform E: FC = -2.3), *ATPsynE* (FC = -2.4), and *ATPsynγ* (isoform B: FC = -3.5; and isoform C: FC = -3.4).

3.6. Activated and repressed genes

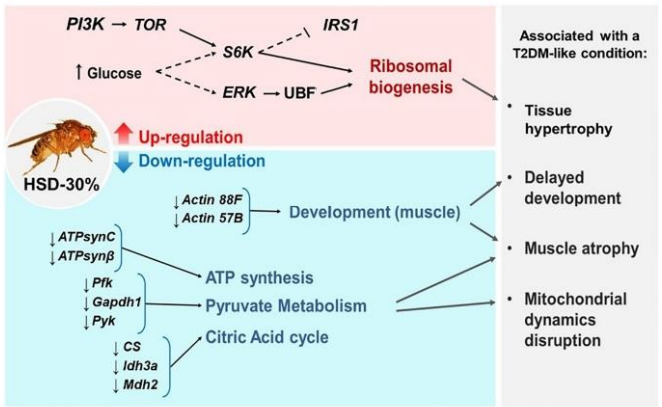
We also analyzed individually by the Metascape analysis the activated and repressed genes. We considered activated when a gene was totally untranscribed in the control and we found transcripts in the HSD-30% replicates and the opposite for the repressed ones. The enrichment heat map of the activated genes (Fig. 7) showed 'anatomical structure formation involved in morphogenesis', 'muscle structure development', 'cellular component morphogenesis', 'developmental growth', and 'supramolecular fiber organization' as the top 5 most statistically enriched terms, all with −4 log10(P). Most of the terms were linked with development, with a few exceptions like 'positive regulation of nitrogen compound metabolic process', 'regulation of protein stability' and 'regulation of insulin secretion' (Figs. S5 and S6). For the enrichment heat map of the repressed genes (Fig. 8), we found 'regulation of anatomical structure size', 'cell projection organization', 'regulation of cell development', 'tube development' and 'cell fate commitment' as the top 5 most enriched terms, all with −5 log10(P). Most of the repressed genes were also associated with development (Figs. S7 and S8).

3.7. Insulin signaling genes

We also explored the FC values for the genes related to the insulin signaling, which are hallmarker genes modulated in a T2DM context and included the *insulin-like peptides* (*dILPs 1, 2, 3, 4, 5, 6, 7*, and *8*), the *insulin receptor isoforms* (*InR A*, *B*, *C*, and *D*), and the *insulin receptor substrates* (*IRS p53 isoform A*, *Chico isoform A* and *B*) (Fig. 9). Among dILPs, HSD-30% caused an up-regulation in the transcription of *dILP 7* and *8* (+2.64-fold and +22.28-fold respectively). *dILP 6* gene, whose product functions as a negative feedback in the control of dILPs levels between central nervous system (CNS) and fat-bodies, was down-regulated by HSD-30% (−7.07-fold) (Fig. 9). Among insulin receptor substrates, we found that HSD-30% down-regulated the transcription of the *IRS p53*, *isoform A* (+2.66-fold). The transcription of the *InRs* and *Chico* isoforms were not significantly affected by HSD-30% (data not shown).

4. Discussion

Here we have induced a chronic hyperglycemia in *D. melanogaster* individuals to investigate the molecular basis underlying human T2DM-like phenotype. Fruit fly individuals hatched from and raised on HSD-30% were found to present high levels of glucose and triglycerides in the adult phase. Using a transcriptomic approach, we found that HSD-30% affected the transcription of 13.47% from the total genes in *D. melanogaster*, with most been up-regulated, including several genes related to the TOR and MAPK signaling paths. Remarkably, among the down-regulated genes *Actin* was found to be the most affected, a gene related to muscle formation. Importantly, we also found that HSD-30% significantly affected the transcription of hallmarker genes of the insulin signaling pathway, causing an upregulation of *dILP 7* and *8*, and a down-regulation of *dILP 6* and *IRS p53, isoform A*. All these features point



Scheme 2. Schematic image of major findings addressed by the transcriptomic approach and their association with a T2DM phenotypes in humans. Down regulation encompassing genes associated with 'muscle development' like *Actin 88F* and *Actin 57B*; 'ATP synthesis' like *ATPsynC* and *ATPsynB*; 'pyruvate metabolism' like *Pfk*, *Gapdh1*, Pyk and 'citric acid cycle' like CS, *Idh3a*, *Mdh2* are presented in a blue background. Up-regulation encompassing genes associated 'ribosomal biogenesis' *via* TOR and MAPK signaling like *Pi3k*, *TOR*, *ERK* and *S6K* genes are presented in a red background.

together to a tightly correlation of the T2DM-like phenotype reached by the *D. melanogaster* model and the human pathology.

HSD-30% induced down-regulation in genes associated with both energetic and developmental processes (Scheme 2). Changes in energetic pathways have been implicated in the pathogenesis of diabetes and likely related to mitochondrial dysfunctions (Kemppainen et al., 2016; Rovira-Llopis et al., 2017; Silzer and Phillips, 2018). Decreased intermyofibrillar mitochondrial content and oxidative energy metabolism are phenomena that occur together with the skeletal muscle health declining observed in T1DM and T2DM human subjects (D'Souza et al., 2013; Perry et al., 2016). Here we found transcriptional suppression of genes related to the three main energetic pathways, including pyruvate metabolism, TCA cycle, and transporter electron chain.

The down-regulation found toward developmental processes are in sync with phenotypes found in *D. melanogaster* fed on HSD, such as delayed metamorphosis and decreased larval and adult sizes (Kemppainen et al., 2016; Navrotskaya et al., 2016; Ecker et al., 2017). *Actin 88F* was the most down-regulated gene found with −43-fold along with three other isoforms. As addressed, muscle skeletal atrophy is a key complication in T2DM, commonly associated with mitochondrial dynamic disruptions (Fujimaki and Kuwabara, 2017; Gan et al., 2018). Nevertheless, muscle atrophy is not only related to the suppression of metabolic process like genes related to energetic pathways. The loss of muscle mass by T2DM subjects has also been associated with protein metabolism abnormalities, including decreased protein synthesis and activation of proteolytic pathways as ubiquitin–proteasome and autophagy-lysosome (Cohen et al., 2014; Perry et al., 2016). Supporting these findings, a microarray analysis on mRNAs derived from muscles atrophying from different causes, including diabetes in rats, revealed also the contribution of upregulation of ubiquitin-proteasome system and down-regulation of ATP synthesis processes (Lecker et al., 2004; Perry et al., 2016). Therefore, the negative modulation on *Actin* gene found here, seems to be also an important atrophic marker under diabetic conditions. Together, our results on down-regulated genes reveal an intricate relationship between the transcriptional modulation of oxidative energy pathways and muscle formation induced by the T2DM-like phenotype model. Moreover, regarding the insulin pathway, null fruit flies mutants of *dILP 6* have significant reduction in body weight and delay in egg-to-adult development (Grönke et al., 2010), strengthening the findings on phenotype profiles found in flies exposed to HSD and also the down regulation of dILP6 verified herein in HSD30% flies.

Metabolic and developmental processes also appeared in the ontological analysis of the up-regulated genes. Genes from this category were associated with nuclear events. Development of tissues appeared in the top enrichment terms. '*TOR-signaling*' and '*MAPK signaling pathway*' were also among the up-regulated genes (Scheme 2). Interestingly, we found genes coding for ribosomal proteins with the highest RPKM values among the up-regulated genes (Scheme 2). Augmented ribosomal biogenesis seems to be related to tissues growth and proliferation in both physiological and pathological conditions. In *D. melanogaster*, HSD-30% increased the up-regulation of ribosomal proteins induced by bisphenol (BPA) exposure, phenomenon that was associated with the formation of the mitotic spindle to cell division disrupted by BPA (Branco and Lemos, 2014). This is in consonance with our findings in which the terms 'chromosome organization' and 'chromatin remodeling' appeared in the list of up-regulated gene. In diabetes, renal hypertrophy is a recognized manifestation require increased protein synthesis. Data from high-glucose-treated glomerular epithelial cells and renal tissue from type 1 and type 2 diabetic rodents have revealed high-glucose stimulates rDNA transcription activation *via* Ser388 phosphorylation of upstream binding factor (UBF), a nucleolar factor that regulates rDNA transcription in an Erk- and p70S6 kinase-dependent manner (Hannan et al., 2003; Mariappan et al., 2011). mTOR *via* p70S6 kinase also controls several steps of ribosome biogenesis, and under renal hypertrophy Erk and mTORC1 axis seem share common signaling on UBF phosphorylation (Lee et al., 2007; Mariappan et al., 2011). Besides, the activation of TORC1 components has been shown to be involved with insulin resistance induction in mammalian cells (Shah et al., 2004a, 2004b). This cross-talking among ERK/MAPK, TOR, and ribosomal protein S6 kinase on protein synthesis also appeared here as an important branch up-regulated by HSD-30% (Scheme 2).

Overall, the set of activated and repressed genes also encompassed phenomena linked with T2D-phenotypes. Most of the terms found for both categories were associated with development, including muscle development, in a similar manner to observed to down-regulated and up-regulated genes addressed above. In the repressed genes, one of the most enriched terms was 'regulation of anatomical size'. These data reinforce literature reports showing that larvae and adult flies reared on high sucrose diet exhibit a marked reduction in the size (Ecker et al., 2017).

5. Conclusions

Long-term exposure to HSD-30% causes gene transcription modulation in hyperglycemic and dyslipidemic *D. melanogaster* in a similar fashion as that observed for human T2DM. Genes associated with ribosomal synthesis, energetic and muscle development processes were among the most affected hallmarks (Scheme 2). We believe the transcriptomic approach in *D. melanogaster* allowed an overview of the network genomic phenomena induced by the consumption of a diet rich in sucrose and provides important data that could be explored for insect physiology and behavior and also for translational studies, especially due to the size of the genome and the evolutionary conservation of physiological mechanisms between the two organisms. Although future studies are necessary to elucidate the molecular mechanisms underlying T2DM phenotypes HSD-induced, our findings support the relevance of *D. melanogaster* hyperglycemic model as a suitable strategy for further research in DM field.

Declaration of competing interest

The authors declare no conflict of interest.

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5. CONCLUSÕES

O consumo da dieta rica em açúcar induziu fenótipos semelhantes ao DT2 em *D. melanogaster*, alterando diversos processos metabólicos e moleculares do organismo. A dieta promoveu mudanças profundas a nível de transcrição, marcadamente diminuição de transcrição de genes relacionados a funcionalidade mitocondrial, formação muscular e desenvolvimento.
A diversidade de efeitos causados nas moscas pelo consumo da dieta mostra respostas que fatores ambientais podem exercer sobre a saúde de um organismo, reiterando a importância de estudos sobre a alimentação e hábito de vida. Embora sempre precisemos saber as limitações de pesquisas translacionais, neste trabalho demonstramos diversas semelhanças e vantagens do modelo para a área.

6. PERSPECTIVAS

No presente trabalho identificamos alterações de genes relacionados à dinâmica mitocondrial, formação de músculo e biogênese ribossomal. Como distúrbios mitocondriais estão amplamente relacionados com a patogênese do DT2, é de interesse relacionar as alterações transcricionais observadas aqui com a funcionalidade mitocondrial. Além disso, vimos diversos genes relacionados com desenvolvimento alterados, mas não foi possível inferir sobre esse assunto, já que as moscas usadas para o trabalho estavam em fase adulta. Neste sentido, uma avaliação dos efeitos da dieta sob a fase larval, mudanças de expressão gênica e possível conexão entre distúrbios mitocondriais e atrasos de desenvolvimento são também perspectivas de trabalho.

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