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Karen C M Moraes, Jacques Montagne

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1 ***Drosophila melanogaster*: a powerful tiny animal model for the study of**
2 **metabolic hepatic diseases**

3
4 **Karen C.M. Moraes^{1*}, Jaques Montagne^{2*},**

5 ¹Laboratório de Sinalização Celular e Expressão Gênica, Instituto de Biociências, Departamento de
6 Biologia Geral e Aplicada, UNESP, Rio Claro, SP, Brazil

7 ²Institute for Integrative Biology of the Cell (I2BC), CNRS, Université Paris-Saclay, CEA, F-91190,
8 Gif-sur-Yvette, France

9
10
11 *** Correspondence:**

12 Karen C.M. Moraes

13 kcm.moraes@unesp.br

14
15 Jaques Montagne

16 jacques.montagne@i2bc.paris-saclay.fr

17
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20
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22

23

24 **Abstract**

25 Animal experimentation is limited by unethical procedures, time-consuming protocols, and high cost.
26 Thus, the development of innovative approaches for disease treatment based on alternative models in
27 a fast, safe, and economic manner is an important, yet challenging goal. In this paradigm, the fruit-
28 fly *Drosophila melanogaster* has become a powerful model for biomedical research, considering its
29 short life cycle and low-cost maintenance. In addition, biological processes are conserved and
30 homologs of ~75% of human disease-related genes are found in the fruit-fly. Therefore, this model
31 has been used in innovative approaches to evaluate and validate the functional activities of candidate
32 molecules identified in *in vitro* large-scale analyses, as putative agents to treat or reverse pathological
33 conditions. In this context, *Drosophila* offers a powerful alternative to investigate the molecular
34 aspects of liver diseases, since no effective therapies are available for those pathologies.
35 Nonalcoholic fatty liver disease is the most common form of chronic hepatic dysfunctions, which
36 may progress to the development of chronic hepatitis and ultimately to cirrhosis, thereby increasing
37 the risk for hepatocellular carcinoma (HCC). This deleterious situation reinforces the use of the
38 *Drosophila* model to accelerate functional research aimed at deciphering the mechanisms that sustain
39 the disease. In this short review, we illustrate the relevance of using the fruit-fly to address aspects of
40 liver pathologies to contribute to the biomedical area.

41

42

43 **Introduction**

44 In modern societies, obesity has become a prevalent problem; according to the World Health
45 Organization (WHO), more than 600 million adults were obese in 2016 (WGO, 2020). Consequently,
46 metabolic dysfunctions have considerably increased, including hepatic diseases, which are one of the
47 top 10 causes of death worldwide (Asrani et al., 2019; Powell et al., 2021; WHO, 2020). These
48 diseases classified as acute or chronic may progress to irreversible damage and malfunction of the
49 liver, while therapeutic strategies are still limited. To address this problem, innovative drugs and
50 natural products have been explored; however, clinical trials have their own limitations, considering
51 patient safety against the adverse side effects of pharmaceutical products, extensive time required for
52 analyses, and ethical issues. Thus, to help investigating new compounds in preclinical stages, the
53 *Drosophila melanogaster* model has become of utmost importance. *Drosophila* genetics have been
54 used for studying many biological processes and is now a powerful alternative model to investigate
55 metabolic changes associated with disease settings. The fruit-fly has a short life cycle, which enables
56 analyses of the effects of drugs in various metabolic routes in a short time (Lee et al., 2020; Men et
57 al., 2016; Tennessen et al., 2014). Furthermore, relevant biological processes and disease-related
58 genes are conserved between human and *Drosophila* (Pandey and Nichols, 2011; Ugur et al., 2016),
59 despite the flies lower genomic complexity compared to that of humans. In this minireview we
60 discuss the advantages offered by the fruit-fly model to investigate hepatic dysfunctions in a
61 translational perspective.

62

63 **The burden of hepatic diseases needs translational science**

64 The liver is a critical organ for the maintenance of body homeostasis; it controls several molecular
65 and metabolic routes and also acts in detoxification processes (Ramos et al., 2021). In metabolic
66 disorders, lipids may accumulate in the hepatocytes contributing to the establishment of fatty liver,
67 also known as hepatic steatosis. This clinical condition, which happens when triacylglycerols (TAGs)
68 represent at least 5% of the liver weight (Ikura, 2014; Powell et al., 2021; Ramos et al., 2021), can
69 remain asymptomatic for several years or progress to more deleterious stages. Nonalcoholic fatty
70 liver disease (NAFLD) is one of the most common forms of liver pathologies affecting ~25% of the
71 world population (Younossi et al., 2018). In addition, drug abuse, environmental contaminants,
72 imbalanced diet, and viral infections may also contribute to the development of NAFLDs (Byrne and
73 Targher, 2015). More dramatically, NAFLD favors the development of chronic hepatitis that

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74 potentially progresses to cirrhosis, increasing the risk for HCC (Friedman et al., 2018; Powell et al.,
75 2021). The worldwide panel of liver pathologies also comprises chronic viral hepatitis, alcoholic
76 liver disease, metabolic and cholestatic liver dysfunctions (Asrani et al., 2019; WGO, 2020). Although
77 NAFLD was identified more than 50 years ago (Parise, 2019; Sanyal, 2019), the therapeutic options
78 to treat or at least to control these diseases rely on long-term procedures and are still limited to
79 weight loss and diet modification, stressing the critical needs of innovative strategies based on fast
80 and reliable preclinical tests.

81 Translational science aims at using the scientific discoveries from preclinical models to set up the
82 bases of clinical trials (Gilliland et al., 2016; van Erk et al., 2021). Knowledge on liver pathologies
83 has now increased, leading to the characterization of the biochemical mechanisms underlying disease
84 progression and to the identification of specific biomarkers (Eslam and George, 2020; Friedman et
85 al., 2018; Samuel and Shulman, 2018). Advances in our understanding of lipid and sugar metabolism
86 along with omics studies and mechanistic investigations, have revealed important regulatory
87 functions of the transcriptions factors sterol-regulatory-element-binding-proteins (SREBPs),
88 carbohydrate-responsive-element-binding-protein (ChREBP), liver-X-receptors (LXRs) and
89 peroxisome-proliferator-activated-receptors (PPARs). In addition, the patatin-like-phospholipase-
90 domain-containing-3 (PNPLA3), a triacylglycerol-lipase, has been shown to contribute to the control
91 of energy expenditure or storage; PNPLA3 expression is regulated by nutritional sources, especially
92 carbohydrates (Bruschi et al., 2017). Furthermore, the mechanistic-target-of-rapamycin (mTOR),
93 which coordinates cell growth at the organismal level, is implicated in metabolic-related disease (Ji et
94 al., 2021; Saxton and Sabatini, 2017). Other studies have demonstrated that epigenetic processes
95 have been linked to liver diseases, but also that the genetic profile of an individual is directly
96 correlated to the severity of the disease, since different alleles encode metabolic enzymes with
97 variable functional activities (Anstee et al., 2019; Ramos et al., 2021). This has opened an unexplored
98 field in the search for innovative therapeutics, including oligonucleotide usage to reduce through
99 RNA-interference (RNAi) the levels of molecules relevant for liver homeostasis. In this context,
100 translational research provides a safe way to test innovative drugs at a large scale using animal
101 models. Rodents, have been long used in preclinical trials, despite the time-consuming analyses,
102 while unpredictable side-effects of the drugs in humans cannot be excluded (Bryda, 2013; Van
103 Norman, 2019). Thus, alternative models provide a golden solution, considering that many societies
104 limit animal experimentation to protect them against cruelty (Doke and Dhawale, 2015; Freires et al.,
105 2017).

106

107 **The fruit-fly model**

108 In this paradigm, the fruit-fly has emerged as a powerful system to study pathological conditions.
109 The *Drosophila* life-cycle comprises four developmental stages: embryonic, larval, pupal and adult.
110 Development from embryo to adult takes about ten days and adult lifespan one to two months
111 (Johnson and Stolzing, 2019), allowing metabolic investigations on hundreds of offspring either at
112 juvenile (larva and pupa) or adult stages. Given its powerful genetics, the *Drosophila* model has
113 proved an efficient strategy for the study of several types of human pathologies, including metabolic
114 (Baenas and Wagner, 2019; Musselman and Kuhnlein, 2018; Perrimon et al., 2016) neurological
115 (Coll-Tane et al., 2019; Link and Bellen, 2020; Mariano et al., 2020; Salazar et al., 2020), cardiac
116 (Birse et al., 2010; Diop et al., 2015; Guida et al., 2019; Piazza and Wessells, 2011), digestive
117 (Musselman and Kuhnlein, 2018; Nayak and Mishra, 2019) and nephrocytic (Millet-Boureima et al.,
118 2018; Rani and Gautam, 2018) comorbidities. In addition, tumor models may be induced in larvae or
119 adult flies by genome manipulation (Dong et al., 2007; Gonzalez, 2013; Hirabayashi et al., 2013;
120 Igaki et al., 2006; Pagliarini and Xu, 2003; Samji et al., 2021). The fruit-fly enables functional large-
121 scale analysis to validate relevant molecules and biomarkers in a faster way than with rodent-based
122 studies (Fernandez-Hernandez et al., 2016; Papanikolopoulou et al., 2019; Bangi, 2020; Bossen et
123 al., 2019; Richardson and Portela, 2018).

124 Most of the genes and metabolic routes involved in human hepatic diseases are conserved in
125 *Drosophila* (Table 1). The conservation between fly and mammalian genes is of utmost interest for
126 translational studies aiming for a deeper understanding of cellular dysfunctions for which,
127 investigation is technically restricted in mammalian models and impossible in humans.

128 In contrast to mammals, most invertebrates do not contain an organ equivalent to the liver, although
129 hepatic functions are conserved. In insects, the fat body (FB) has long been considered as the liver
130 counterpart (Li et al., 2019), although recent studies suggest that the oenocytes also accomplish
131 hepatic-related functions (Gutierrez et al., 2007; Storelli et al., 2019). In *Drosophila*, the FB is an
132 organ that spreads throughout the entire organism and oenocytes are groups of cells located
133 underneath the abdominal external cuticle. In humans, dietary nutrients are transferred to the liver
134 through a portal system and lipids are transported through the lymph stream as chylomicrons.
135 *Drosophila* has an open circulatory system so that nutrients crossing the intestinal epithelium enter
136 the haemolymph, and thus, the body-wide distribution of the FB favors nutrient uptake (Figure 1A).

137 Further, given its eating sources (mostly rotting fruits), the fruit-fly needs a powerful detoxification
138 system that involves the gut, the FB and the oenocytes (Huang et al., 2019; Iredale, 2010; Yang et al.,
139 2007). Over the past two decades, several studies on metabolic hepatic dysfunctions have taken
140 advantage of the fly model (Ghosh et al., 2020; Hader et al., 2003; Hofbauer et al., 2020; Liao et al.,
141 2020; Villanueva et al., 2019) to describe the functional activities of molecules relevant for human
142 pathologies (Hmeljak and Justice, 2019; Heier and Kuhnlein, 2018; Perrimon et al., 2016; Ugur et al.,
143 2016).

144

145 **Homeostatic dysfunctions in NAFLD**

146 Type 2 diabetes mellitus (T2DM) is another metabolic-related disease progressing exponentially. The
147 T2DM hallmark is the development of insulin resistance, due to impaired response to the hormone or
148 its reduced production by pancreatic β -cells (Boland et al., 2017; Li et al., 2021), thereby resulting in
149 high glucose levels, increased general oxidative stress, vascular problems, and serious secondary
150 perturbations in the physiology and the metabolism of the body (Chatterjee et al., 2017; Galicia-
151 Garcia et al., 2020). In diabetic patients, lipid metabolism is modified, and the liver may accumulate
152 fatty acids (FAs), which facilitates the establishment of NAFLD (Kuchay et al., 2020; Sanyal, 2019).

153 In NAFLD, the metabolism of both lipid and glucose in hepatic cells is disrupted (Sanyal, 2019; Shen
154 et al., 2017). The accumulation of lipids in the hepatocytes results from changes in lipid uptake, *de*
155 *novo* synthesis of FAs, β -oxidation and export of very-low-density-lipoproteins (VLDL) (Ipsen et
156 al., 2018; Sanyal, 2019), which together affect the serum levels of TAG and cholesterol and may
157 provoke instability of body homeostasis. Conversely, these changes in lipid metabolism, which are in
158 part connected to insulin resistance in the hepatic tissue, reinforce the persistence of liver
159 dysfunction, indicating that cellular metabolism is integrated at the organismal level (Chao et al.,
160 2019; Sanyal, 2019). Under a normal and healthy homeostatic environment, hepatic cells use
161 pyruvate to produce energy through the citric-acid cycle in mitochondria. During this process the
162 exceeding energy is exported from the mitochondria as citrate to generate palmitic acid that can be
163 used as a precursor of membrane lipids or esterified to TAGs and stored in cytosolic lipid droplets
164 (LDs) (Currie et al., 2013; Ramos et al., 2021). These hepatic lipid stores can be remobilized through
165 the β -oxidation pathway in response to energy demands, or exported from the liver in water-soluble
166 VLDL particles comprising apolipoproteins, cholesterol and phospholipids (Ipsen et al., 2018).
167 However, under conditions of metabolic dysfunction, excess LDs may accumulate in the liver and

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168 trigger the NAFLD, which favors lipotoxicity and subsequently the development of inflammatory
169 processes and an increase in the levels of reactive oxygen species. Together, these perturbations
170 provoke mitochondrial dysfunction and disease progression, leading to insulin resistance, T2DM and
171 metabolic syndrome (Rosato et al., 2019).

172 Binding of insulin to its cognate receptor activates the mTOR signaling network (Meng et al., 2021;
173 Yoon, 2017), which acts through a feedback loop to dampen the insulin response by inactivating
174 insulin-receptor-substrate-1 (IRS1) and repressing the expression of the insulin receptor (InR) and
175 insulin-like-growth-factor-1 (IGF1). Therefore, in the context of metabolic dysfunction, the mTOR
176 signaling network is poorly activated and the subsequent absence of the negative feedback loop
177 sustains an auto-amplification of the insulin resistance process. In this pathological condition, a
178 number of regulatory proteins are synthesized by the hepatocytes to counteract homeostasis
179 disruption, including Notch-receptor-2, insulin-like-growth-factor-binding-proteins (IGFBPs), the
180 potassium-inwardly-rectifying-channel-subfamily-J-member-11, chemokines and kinases (Tamarai et
181 al., 2019).

182 The PPAR nuclear receptors that can act as lipid sensors to regulate metabolic homeostasis are
183 potential targets for the development of drug therapy to NAFLD (Liss et al., 2017). The human
184 genome encodes three PPAR members (α , β/δ and γ), which are differentially expressed in tissues,
185 although each of them may impinge on liver metabolism. PPAR α is largely expressed in the liver
186 where it controls several aspects of FA homeostasis; it is frequently found at low level in patients
187 with steatosis. PPAR β/δ is mainly expressed in muscle cells, but at lower levels in adipocytes and
188 hepatocytes. PPAR γ , which is mainly expressed in adipose tissue, is upregulated in the NAFLD
189 pathological condition to dampen dysfunction of lipid and glucose metabolism. However, these
190 unbalanced production of PPARs may contribute to insulin resistance and metabolic syndrome (Liss
191 et al., 2017; Corrales et al., 2018), supporting the use of PPAR agonists to treat NAFLD {Seo, 2008
192 #1332; Yoo, 2021 #1331}. The utilization Glucagon-likepeptide-1 (GLP-1) receptor agonists is
193 another therapeutic strategy currently explored in clinical trials { Dai, 2020 #1334; Lv, 2020 #1333}.
194 GLP-1 is secreted by enteroendocrine cells after meal ingestion to potentiate insulin secretion and
195 suppress glucagon production. The secreted bioactive forms act systemically, since the GLP-1
196 receptor (GLP-1R) is expressed in a number of tissues {Dai, 2020 #1334}. The utilization of GLP-1R
197 agonists exhibiting a longer half-life compare to that of the genuine hormone has been approved for
198 T2DM treatment {Andersen, 2018 #1335}. These agonists have positive effects on steatogenesis in
199 T2DM patient {Teshome, 2020 #1336}, but controversial results about the benefit yet restrain their

200 usage in NAFLD treatment {Lv, 2020 #1333}. Finally, a number of molecules secreted by the
201 adipose tissue may affect inflammatory processes, insulin resistance and NAFLD progression.
202 Adiponectin is one such factor that is down-regulated in response to hepatic stress and constitutes a
203 promising target to treat steatosis, since studies demonstrated that increased levels of this adipokine
204 ameliorate NAFLD (Scherer et al., 1995; Shabalala et al., 2020). Given the diversity of signals that
205 may induce metabolic deregulations in hepatic cells, genetic investigations using alternative *in vivo*
206 models are needed to decipher the organ interconnections that elicit the deleterious condition of
207 NAFLD.

208

209 **Homeostasis and metabolism in *Drosophila***

210 In *Drosophila*, the FB synthesizes and stores glycogen and TAG-containing LDs (Figure 1A)
211 (Garrido et al., 2015; Gronke et al., 2003; Yamada et al., 2018). In the gut, dietary lipids are
212 hydrolyzed to glycerol, free FAs and monoacylglycerols and taken up into the enterocytes through a
213 molecular mechanism yet poorly characterized (Holtof et al., 2019; Miguel-Aliaga et al., 2018;
214 Toprak et al., 2020). In the enterocytes, these molecules are converted to diacylglycerols that are
215 loaded in the haemolymph on apolipoprotein particles, functioning as lipid vehicles to the FB (Palm
216 et al., 2012). However, FB cells deficient for fatty-acid-synthase fail to accumulate LDs, indicating
217 that *de novo* FA synthesis is essential for lipid storage in these cells (Garrido et al., 2015).
218 Conversely, fasting induces mobilization and consumption of lipid stores from the FB. The perilipins
219 Lsd1 and Lsd2 envelop LDs and protect them from lipolysis, whereas the lipase, Brummer, catalyzes
220 TAG hydrolysis (Bi et al., 2012; Gronke et al., 2005; Gronke et al., 2007). Concurrent to fasting-
221 induced lipid hydrolysis, oenocytes accumulate LDs (Figure 1B), suggesting that remobilized lipid
222 stores efflux from the FB and are taken up and oxidized in the oenocytes (Gutierrez et al., 2007). This
223 process is similar to the remobilization of lipid stores from adipocytes to hepatocytes induced by
224 fasting in mammals (Iredale, 2010; Yamada et al., 2020). Congruently, enzymes responsible for
225 ketone body biogenesis are highly expressed in oenocytes (Huang et al., 2019), although it has not
226 been formally demonstrated whether other tissues (FB, muscles) may also perform β -oxidation to
227 supply energy demand (Parvy et al., 2012). This energy mobilization process is regulated by target-
228 of-rapamycin (TOR), which is present in two distinct complexes, TORC1 and TORC2; the former
229 directly responds to nutrients, whereas the latter is a component of the insulin signaling pathway. The
230 intermediates of this signaling network are conserved in the fruit-fly (Table 1), although the TORC1

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231 and insulin/TORC2 signaling branches can work independently in most *Drosophila* tissues, including
232 the FB (Devilliers et al., 2021).

233 The FB also acts as a nutrient sensor to coordinate overall body growth and homeostasis through the
234 production of insulin-like-peptides (Dilps) by a cluster of neurosecretory cells (NSCs) (Colombani et
235 al., 2003). Moreover, similar to mammals, sugar metabolism in flies is modulated by an
236 insulin/glucagon-like axis (Figure 1A-B) (Oh et al., 2019). The *Drosophila* genome encodes eight
237 Dilps (Brogiolo et al., 2001), Dilp2 and Dilp5, which are produced in the NSCs, are the major
238 regulators of sugar homeostasis (Rulifson et al., 2002). Genetic ablation of the NSCs results in an
239 overall reduced body size and in an increase in the levels of glucose and trehalose (disaccharide of
240 glucose) that is the main circulating sugar in insects. This phenotype resembles the metabolic defects
241 provoked by insulin deficiency in Type-I-diabetic patients. Conversely, the *Drosophila* adipokinetic
242 hormone (AKH) produced by the neuroendocrine corpora cardiaca (CC) controls circulating sugar
243 levels in a glucagon-like manner (Kim and Rulifson, 2004). Both NSCs and CC secrete their
244 products into the haemolymph, close to the *Drosophila* heart equivalent, thereby favoring hormone
245 distribution throughout the entire body. Secretion of AKH in *Drosophila* by the CC depends on an
246 ATP-K⁺-dependent channel that directly responds to circulating sugar levels (Kim and Rulifson,
247 2004). A pair of glucose-sensing neurons plays a pivotal role in coordinating NSC and CC functions
248 by activating Dilp secretion, while inhibiting AKH secretion (Figure 1A-B)(Oh et al., 2019).

249 Furthermore, secretion of Dilps by the NSCs strongly relies on FB messengers that relay nutritional
250 cues (Colombani et al., 2003; Delanoue et al., 2016). The JAK-STAT ligand Unpaired2, is one such
251 messenger, which can be functionally replaced using molecular genetic tools by human leptin,
252 showing that as in mammals, fat cells produce an hormone in response to nutrient load to control
253 feeding physiology (Rajan and Perrimon, 2012). Therefore, although the mammalian liver equivalent
254 is not a discrete organ in *Drosophila*, hepatic functions and dysfunctions are closely conserved: the
255 FB appears to be in charge of hepatic functions related to feeding, whereas the oenocytes accomplish
256 functions related to fasting. Further, both in mammals and flies, integration of the TOR signaling
257 network at the organismal level is central in controlling lipid and sugar metabolism in response to the
258 nutritional status (Sanguesa et al., 2019; Schmitt et al., 2015).

259 Most of the mammalian transcriptional regulators involved in NAFLD are conserved in *Drosophila*
260 (Table 1). Mondo, the ChREBP/MondoA homologue, regulates several metabolic routes in response
261 to dietary sugar {Mattila, 2015 #869}. The *Drosophila* SREBP regulates the expression of genes
262 required for FA synthesis (Kunte et al., 2006). However, consistent with insect sterol auxotrophy

263 (Clark and Block, 1959), SREBP activity is not regulated by sterols, but by
264 phosphatidylethanolamine (Dobrosotskaya et al., 2002). The best homologues of LXRs and PPARs
265 are the Ecdysone receptor (EcR) and the nuclear receptor Eip75B, respectively (Parvy et al, 2014).
266 Ecdysone is a steroid hormone that controls developmental transitions, whereas Eip75B is an
267 intermediate of the ecdysone signaling, whose activity depends on nitric-oxide-synthase.
268 Surprisingly, manipulating this signaling pathway in the ecdysone-producing gland, results in a
269 dramatic changes in LD accumulation in FB cells, indicating that this signaling pathway also impinge
270 on lipid homeostasis (Caceres et al., 2012). In summary, the metabolic routes and most of the
271 regulatory genes that play a critical role in NAFLD are conserved in *Drosophila*. The activity of
272 some regulatory gene products varies as compare to that of their mammalian counterparts, but
273 remains connected to basal metabolism. Importantly, the phenotypes induced by loss-of-function of
274 these genes can be used as reference criteria to monitor the efficiency and the adverse effects of drug
275 compounds in preclinical trials.

276

277 **Perspectives**

278 Alternative models have largely contributed to our understanding of biological processes. Recent
279 studies have shed light on potential targets for drug therapy, which need physiological validation
280 prior to clinical trials. Thanks to a plethora of genetics tools to direct in a tissue-specific manner
281 either over-expression or RNAi-inactivation of a gene of interest (Ugur et al., 2016) {Senturk, 2018
282 #1062}, the fruit-fly model emerges as a powerful alternative for large-scale analyses. Collection of
283 transgenic lines targeting a vast majority of the *Drosophila* genes, with a particular focus on the
284 orthologues of disease-related-human genes, are available from Stock centers (Heigwer et al., 2018;
285 Dietzl et al., 2007). This approach validated the functions of relevant proteins, such as CDGSH-iron-
286 sulfur-domain-containing-protein-2, whose haplo-insufficiency causes NAFLD and promotes HCC
287 development (Shen et al., 2017). This approach also identified genes that contribute to fat deposition
288 (Hoffmann et al., 2013; Pospisilik et al., 2010) and whose dysregulation in humans can lead to
289 obesity, diabetes, and NAFLD (Graham and Pick, 2017). Liver diseases are a burden in the modern
290 society, especially NAFLD and HCC; *in vivo* investigations using *Drosophila* in translational
291 approaches will be useful to validate enzymes and other molecules crucial for body homeostasis,
292 increasing the chance to develop innovative therapeutic strategies.

293

294 **Conflict of Interest**

295 The authors declare that the research was conducted in the absence of any commercial or financial
296 relationships that could be construed as a potential conflict of interest.

297

298 **Author Contributions**

299 The authors (K.C.M.M. & J.M) contributed equally to the content of the work.

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309

310 **References**

311 Andersen, A., Lund, A., Knop, F.K., and Vilsboll, T. (2018). Glucagon-like peptide 1 in health and
312 disease. *Nat Rev Endocrinol* *14*, 390-403. doi: 10.1038/s41574-018-0016-2

313 Anstee, Q.M., Reeves, H.L., Kotsiliti, E., Govaere, O., and Heikenwalder, M. (2019). From NASH to
314 HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* *16*, 411-428. doi:
315 10.1038/s41575-019-0145-7

316 Asrani, S.K., Devarbhavi, H., Eaton, J., and Kamath, P.S. (2019). Burden of liver diseases in the
317 world. *J Hepatol* *70*, 151-171. doi: 10.1016/j.jhep.2018.09.014

318 Baenas, N., and Wagner, A.E. (2019). *Drosophila melanogaster* as an alternative model organism in
319 nutrigenomics. *Genes Nutr* *14*, 14. doi: 10.1186/s12263-019-0641-y

Drosophila melanogaster in translational studies of liver diseases

- 320 Bangi, E. (2020). Strategies for Functional Interrogation of Big Cancer Data Using *Drosophila*
321 Cancer Models. *Int J Mol Sci* 21. doi: 10.3390/ijms21113754
- 322 Bi, J., Xiang, Y., Chen, H., Liu, Z., Gronke, S., Kuhnlein, R.P., and Huang, X. (2012). Opposite and
323 redundant roles of the two *Drosophila* perilipins in lipid mobilization. *J Cell Sci* 125, 3568-3577. doi:
324 10.1242/jcs.101329
- 325 Birse, R.T., Choi, J., Reardon, K., Rodriguez, J., Graham, S., Diop, S., Ocorr, K., Bodmer, R., and
326 Oldham, S. (2010). High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR
327 pathway in *Drosophila*. *Cell Metab* 12, 533-544. doi: 10.1016/j.cmet.2010.09.014
- 328 Bohni, R., Riesgo-Escovar, J., Oldham, S., Brogiolo, W., Stocker, H., Andrus, B.F., Beckingham,
329 K., and Hafen, E. (1999). Autonomous control of cell and organ size by CHICO, a *Drosophila*
330 homolog of vertebrate IRS1-4. *Cell* 97, 865-875. doi: 10.1016/s0092-8674(00)80799-0
- 331 Boland, B.B., Rhodes, C.J., and Grimsby, J.S. (2017). The dynamic plasticity of insulin production in
332 beta-cells. *Mol Metab* 6, 958-973. doi: 10.1016/j.molmet.2017.04.010
- 333 Bossen, J., Uliczka, K., Steen, L., Pfeifferkorn, R., Mai, M.M., Burkhardt, L., Spohn, M., Bruchhaus,
334 I., Fink, C., Heine, H., *et al.* (2019). An EGFR-Induced *Drosophila* Lung Tumor Model Identifies
335 Alternative Combination Treatments. *Mol Cancer Ther* 18, 1659-1668. doi: 10.1158/1535-
336 7163.MCT-19-0168
- 337 Brogiolo, W., Stocker, H., Ikeya, T., Rintelen, F., Fernandez, R., and Hafen, E. (2001). An
338 evolutionarily conserved function of the *Drosophila* insulin receptor and insulin-like peptides in
339 growth control. *Curr Biol* 11, 213-221. doi: 10.1016/s0960-9822(01)00068-9
- 340 Bruschi, F.V., Tardelli, M., Claudel, T., and Trauner, M. (2017). PNPLA3 expression and its impact
341 on the liver: current perspectives. *Hepat Med* 9, 55-66. doi: 10.2147/HMER.S125718
- 342 Bryda, E.C. (2013). The Mighty Mouse: the impact of rodents on advances in biomedical research.
343 *Mo Med* 110, 207-211.
- 344 Byrne, C.D., and Targher, G. (2015). NAFLD: a multisystem disease. *J Hepatol* 62, S47-64. doi:
345 10.1016/j.jhep.2014.12.012
- 346 Caceres, L., Necakov, A.S., Schwartz, C., Kimber, S., Roberts, I.J., and Krause, H.M. (2011). Nitric
347 oxide coordinates metabolism, growth, and development via the nuclear receptor E75. *Genes Dev* 25,
348 1476-1485. doi: 10.1101/gad.2064111

Drosophila melanogaster in translational studies of liver diseases

- 349 Chao, H.W., Chao, S.W., Lin, H., Ku, H.C., and Cheng, C.F. (2019). Homeostasis of Glucose and
350 Lipid in Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* 20. doi: 10.3390/ijms20020298
- 351 Chatterjee, S., Khunti, K., and Davies, M.J. (2017). Type 2 diabetes. *Lancet* 389, 2239-2251. doi:
352 10.1016/S0140-6736(17)30058-2
- 353 Clark, A.J., and Block, K. (1959). The absence of sterol synthesis in insects. *J Biol Chem* 234, 2578-
354 2582.
- 355 Coll-Tane, M., Krebbers, A., Castells-Nobau, A., Zweier, C., and Schenck, A. (2019). Intellectual
356 disability and autism spectrum disorders 'on the fly': insights from *Drosophila*. *Dis Model Mech* 12.
357 doi: 10.1242/dmm.039180
- 358 Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., and Leopold, P. (2003). A
359 nutrient sensor mechanism controls *Drosophila* growth. *Cell* 114, 739-749. doi: 10.1016/s0092-
360 8674(03)00713-x
- 361 Corrales, P., Vidal-Puig, A., and Medina-Gomez, G. (2018). PPARs and Metabolic Disorders
362 Associated with Challenged Adipose Tissue Plasticity. *Int J Mol Sci* 19. doi: 10.3390/ijms19072124
- 363 Currie, E., Schulze, A., Zechner, R., Walther, T.C., and Farese, R.V., Jr. (2013). Cellular fatty acid
364 metabolism and cancer. *Cell Metab* 18, 153-161. doi: 10.1016/j.cmet.2013.05.017
- 365 Dai, Y., He, H., Li, S., Yang, L., Wang, X., Liu, Z., and An, Z. (2020). Comparison of the Efficacy
366 of Glucagon-Like Peptide-1 Receptor Agonists in Patients With Metabolic Associated Fatty Liver
367 Disease: Updated Systematic Review and Meta-Analysis. *Front Endocrinol (Lausanne)* 11, 622589.
368 doi: 10.3389/fendo.2020.622589
- 369 Delanoue, R., Meschi, E., Agrawal, N., Mauri, A., Tsatskis, Y., McNeill, H., and Leopold, P. (2016).
370 *Drosophila* insulin release is triggered by adipose Stunted ligand to brain Methuselah receptor.
371 *Science* 353, 1553-1556. doi: 10.1126/science.aaf8430
- 372 Devilliers, M., Garrido, D., Poidevin, M., Rubin, T., Le Rouzic, A., and Montagne, J. (2021).
373 Differential Metabolic Sensitivity of Insulin-like-response- and mTORC1-Dependent Overgrowth in
374 *Drosophila* Fat Cells. *Genetics*. doi: 10.1093/genetics/iyaa010
- 375 Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K.,
376 Oppel, S., Scheiblauer, S., *et al.* (2007). A genome-wide transgenic RNAi library for conditional
377 gene inactivation in *Drosophila*. *Nature* 448, 151-156. doi: 10.1038/nature05954

Drosophila melanogaster in translational studies of liver diseases

- 378 Diop, S.B., Bisharat-Kernizan, J., Birse, R.T., Oldham, S., Ocorr, K., and Bodmer, R. (2015). PGC-
379 1/Spargel Counteracts High-Fat-Diet-Induced Obesity and Cardiac Lipotoxicity Downstream of TOR
380 and Brummer ATGL Lipase. *Cell Rep* *10*, 1572-1584. doi: 10.1016/j.celrep.2015.02.022
- 381 Dobrosotskaya, I.Y., Seegmiller, A.C., Brown, M.S., Goldstein, J.L., and Rawson, R.B. (2002).
382 Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila*.
383 *Science* *296*, 879-883. doi: 10.1126/science.1071124
- 384 Doke, S.K., and Dhawale, S.C. (2015). Alternatives to animal testing: A review. *Saudi Pharm J* *23*,
385 223-229. doi: 10.1016/j.jsps.2013.11.002
- 386 Dong, J., Feldmann, G., Huang, J., Wu, S., Zhang, N., Comerford, S.A., Gayyed, M.F., Anders, R.A.,
387 Maitra, A., and Pan, D. (2007). Elucidation of a universal size-control mechanism in *Drosophila* and
388 mammals. *Cell* *130*, 1120-1133. doi: 10.1016/j.cell.2007.07.019
- 389 Eslam, M., and George, J. (2020). Genetic contributions to NAFLD: leveraging shared genetics to
390 uncover systems biology. *Nat Rev Gastroenterol Hepatol* *17*, 40-52. doi: 10.1038/s41575-019-0212-0
- 391 Fernandez-Hernandez, I., Scheenaard, E., Pollarolo, G., and Gonzalez, C. (2016). The translational
392 relevance of *Drosophila* in drug discovery. *EMBO Rep* *17*, 471-472. doi: 10.15252/embr.201642080
- 393 Freires, I.A., Sardi, J.C., de Castro, R.D., and Rosalen, P.L. (2017). Alternative Animal and Non-
394 Animal Models for Drug Discovery and Development: Bonus or Burden? *Pharm Res* *34*, 681-686.
395 doi: 10.1007/s11095-016-2069-z
- 396 Friedman, S.L., Neuschwander-Tetri, B.A., Rinella, M., and Sanyal, A.J. (2018). Mechanisms of
397 NAFLD development and therapeutic strategies. *Nat Med* *24*, 908-922. doi: 10.1038/s41591-018-
398 0104-9
- 399 Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K.B.,
400 Ostolaza, H., and Martin, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. *Int J Mol Sci* *21*.
401 doi: 10.3390/ijms21176275
- 402 Garrido, D., Rubin, T., Poidevin, M., Maroni, B., Le Rouzic, A., Parvy, J.P., and Montagne, J.
403 (2015). Fatty Acid Synthase Cooperates with Glyoxalase 1 to Protect against Sugar Toxicity. *PLoS*
404 *Genet* *11*, e1004995. doi: 10.1371/journal.pgen.1004995
- 405 Ghosh, A.C., Tattikota, S.G., Liu, Y., Comjean, A., Hu, Y., Barrera, V., Ho Sui, S.J., and Perrimon,
406 N. (2020). *Drosophila* PDGF/VEGF signaling from muscles to hepatocyte-like cells protects against
407 obesity. *Elife* *9*. doi: 10.7554/eLife.56969

Drosophila melanogaster in translational studies of liver diseases

- 408 Gilliland, C.T., Zuk, D., Kocis, P., Johnson, M., Hay, S., Hajduch, M., Bietrix, F., Aversa, G.,
409 Austin, C.P., and Ussi, A.E. (2016). Putting translational science on to a global stage. *Nat Rev Drug*
410 *Discov* *15*, 217-218. doi: 10.1038/nrd.2016.33
- 411 Gonzalez, C. (2013). *Drosophila melanogaster*: a model and a tool to investigate malignancy and
412 identify new therapeutics. *Nat Rev Cancer* *13*, 172-183. doi: 10.1038/nrc3461
- 413 Graham, P., and Pick, L. (2017). *Drosophila* as a Model for Diabetes and Diseases of Insulin
414 Resistance. *Curr Top Dev Biol* *121*, 397-419. doi: 10.1016/bs.ctdb.2016.07.011
- 415 Gronke, S., Beller, M., Fellert, S., Ramakrishnan, H., Jackle, H., and Kuhnlein, R.P. (2003). Control
416 of fat storage by a *Drosophila* PAT domain protein. *Curr Biol* *13*, 603-606. doi: 10.1016/s0960-
417 9822(03)00175-1
- 418 Gronke, S., Mildner, A., Fellert, S., Tennagels, N., Petry, S., Muller, G., Jackle, H., and Kuhnlein,
419 R.P. (2005). Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*. *Cell*
420 *Metab* *1*, 323-330. doi: 10.1016/j.cmet.2005.04.003
- 421 Gronke, S., Muller, G., Hirsch, J., Fellert, S., Andreou, A., Haase, T., Jackle, H., and Kuhnlein, R.P.
422 (2007). Dual lipolytic control of body fat storage and mobilization in *Drosophila*. *PLoS Biol* *5*, e137.
423 doi: 10.1371/journal.pbio.0050137
- 424 Guida, M.C., Birse, R.T., Dall'Agnese, A., Toto, P.C., Diop, S.B., Mai, A., Adams, P.D., Puri, P.L.,
425 and Bodmer, R. (2019). Intergenerational inheritance of high fat diet-induced cardiac lipotoxicity in
426 *Drosophila*. *Nat Commun* *10*, 193. doi: 10.1038/s41467-018-08128-3
- 427 Gutierrez, E., Wiggins, D., Fielding, B., and Gould, A.P. (2007). Specialized hepatocyte-like cells
428 regulate *Drosophila* lipid metabolism. *Nature* *445*, 275-280. doi: 10.1038/s41467-019-08492-8
- 429 Hader, T., Muller, S., Aguilera, M., Eulenberg, K.G., Steuernagel, A., Ciossek, T., Kuhnlein, R.P.,
430 Lemaire, L., Fritsch, R., Dohrmann, C., *et al.* (2003). Control of triglyceride storage by a
431 WD40/TPR-domain protein. *EMBO Rep* *4*, 511-516. doi: 10.1038/sj.embor.embor837
- 432 Havula, E., Teesalu, M., Hyotylainen, T., Seppala, H., Hasygar, K., Auvinen, P., Oresic, M.,
433 Sandmann, T., and Hietakangas, V. (2013). Mondo/ChREBP-Mlx-regulated transcriptional network
434 is essential for dietary sugar tolerance in *Drosophila*. *PLoS Genet* *9*, e1003438. doi:
435 10.1371/journal.pgen.1003438
- 436 Heier, C., and Kuhnlein, R.P. (2018). Triacylglycerol Metabolism in *Drosophila melanogaster*.
437 *Genetics* *210*, 1163-1184. doi: 10.1534/genetics.118.301583

***Drosophila melanogaster* in translational studies of liver diseases**

- 438 Heigwer, F., Port, F., and Boutros, M. (2018). RNA Interference (RNAi) Screening in *Drosophila*.
439 *Genetics* 208, 853-874. doi: 10.1534/genetics.117.300077
- 440 Hirabayashi, S., Baranski, T.J., and Cagan, R.L. (2013). Transformed *Drosophila* cells evade diet-
441 mediated insulin resistance through wingless signaling. *Cell* 154, 664-675. doi:
442 10.1016/j.cell.2013.06.030
- 443 Hmeljak, J., and Justice, M.J. (2019). From gene to treatment: supporting rare disease translational
444 research through model systems. *Dis Model Mech* 12. doi: 10.1242/dmm.039271
- 445 Hofbauer, H.F., Heier, C., Sen Saji, A.K., and Kuhnlein, R.P. (2020). Lipidome remodeling in aging
446 normal and genetically obese *Drosophila* males. *Insect Biochem Mol Biol*, 103498. doi:
447 10.1016/j.ibmb.2020.103498
- 448 Hoffmann, J., Romey, R., Fink, C., and Roeder, T. (2013). *Drosophila* as a model to study metabolic
449 disorders. *Adv Biochem Eng Biotechnol* 135, 41-61. doi: 10.1007/10_2013_196
- 450 Holtof, M., Lenaerts, C., Cullen, D., and Vanden Broeck, J. (2019). Extracellular nutrient digestion
451 and absorption in the insect gut. *Cell Tissue Res* 377, 397-414. doi: 10.1007/s00441-019-03031-9
- 452 Honegger, B., Galic, M., Kohler, K., Wittwer, F., Brogiolo, W., Hafen, E., and Stocker, H. (2008).
453 Imp-L2, a putative homolog of vertebrate IGF-binding protein 7, counteracts insulin signaling in
454 *Drosophila* and is essential for starvation resistance. *J Biol* 7, 10. doi: 10.1186/jbiol72
- 455 Huang, K., Chen, W., Zhu, F., Li, P.W., Kapahi, P., and Bai, H. (2019). RiboTag translomic
456 profiling of *Drosophila* oenocytes under aging and induced oxidative stress. *BMC Genomics* 20, 50.
457 doi: 10.1186/s12864-018-5404-4
- 458 Igaki, T., Pagliarini, R.A., and Xu, T. (2006). Loss of cell polarity drives tumor growth and invasion
459 through JNK activation in *Drosophila*. *Curr Biol* 16, 1139-1146. doi: 10.1016/j.cub.2006.04.042
- 460 Ikura, Y. (2014). Transitions of histopathologic criteria for diagnosis of nonalcoholic fatty liver
461 disease during the last three decades. *World J Hepatol* 6, 894-900. doi: 10.4254/wjh.v6.i12.894
- 462 Ipsen, D.H., Lykkesfeldt, J., and Tveden-Nyborg, P. (2018). Molecular mechanisms of hepatic lipid
463 accumulation in non-alcoholic fatty liver disease. *Cell Mol Life Sci* 75, 3313-3327. doi:
464 10.1007/s00018-018-2860-6
- 465 Iredale, J.P. (2010). Darwin, development, *Drosophila*, and disease. *Hepatology* 51, 1-3. doi:
466 10.1002/hep.23235

***Drosophila melanogaster* in translational studies of liver diseases**

- 467 Ji, D., Zhao, Q., Qin, Y., Tong, H., Wang, Q., Yu, M., Mao, C., Lu, T., Qiu, J., and Jiang, C. (2021).
468 Germacrone improves liver fibrosis by regulating the PI3K/AKT/mTOR signalling pathway. *Cell*
469 *Biol Int*. doi: 10.1002/cbin.11607
- 470 Johnson, A.A., and Stolzing, A. (2019). The role of lipid metabolism in aging, lifespan regulation,
471 and age-related disease. *Aging Cell* 18, e13048. doi: 10.1111/acel.13048
- 472 Kim, S.K., and Rulifson, E.J. (2004). Conserved mechanisms of glucose sensing and regulation by
473 *Drosophila corpora cardiaca* cells. *Nature* 431, 316-320. doi: 10.1038/nature02897
- 474 Kuchay, M.S., Choudhary, N.S., Mishra, S.K., and Misra, A. (2020). Nonalcoholic fatty liver disease
475 should be considered for treatment allocation in standard management algorithms for type 2 diabetes.
476 *Diabetes Metab Syndr* 14, 2233-2239. doi: 10.1016/j.dsx.2020.11.015
- 477 Kunte, A.S., Matthews, K.A., and Rawson, R.B. (2006). Fatty acid auxotrophy in *Drosophila* larvae
478 lacking SREBP. *Cell Metab* 3, 439-448. doi: 10.1016/j.cmet.2006.04.011
- 479 Lee, J.Y., Lee, J.H., and Cheon, C.K. (2020). Functional Characterization of Gomisin N in High-Fat-
480 Induced *Drosophila* Obesity Models. *Int J Mol Sci* 21. doi: 10.3390/ijms21197209
- 481 Li, S., Yu, X., and Feng, Q. (2019). Fat Body Biology in the Last Decade. *Annu Rev Entomol* 64,
482 315-333. doi: 10.1146/annurev-ento-011118-112007
- 483 Li, X.D., He, S.S., Wan, T.T., and Li, Y.B. (2021). Liraglutide protects palmitate-induced INS-1 cell
484 injury by enhancing autophagy mediated via FoxO1. *Mol Med Rep* 23. doi:
485 10.3892/mmr.2020.11786
- 486 Liao, S., Amcoff, M., and Nassel, D.R. (2020). Impact of high-fat diet on lifespan, metabolism,
487 fecundity and behavioral senescence in *Drosophila*. *Insect Biochem Mol Biol*, 103495. doi:
488 10.1016/j.ibmb.2020.103495
- 489 Link, N., and Bellen, H.J. (2020). Using *Drosophila* to drive the diagnosis and understand the
490 mechanisms of rare human diseases. *Development* 147. doi: 10.1242/dev.191411
- 491 Liss, K.H., and Finck, B.N. (2017). PPARs and nonalcoholic fatty liver disease. *Biochimie* 136, 65-
492 74. doi: 10.1016/j.biochi.2016.11.009
- 493 Lv, X., Dong, Y., Hu, L., Lu, F., Zhou, C., and Qin, S. (2020). Glucagon-like peptide-1 receptor
494 agonists (GLP-1 RAs) for the management of nonalcoholic fatty liver disease (NAFLD): A
495 systematic review. *Endocrinol Diabetes Metab* 3, e00163. doi: 10.1002/edm2.163

Drosophila melanogaster in translational studies of liver diseases

- 496 Mariano, V., Achsel, T., Bagni, C., and Kanellopoulos, A.K. (2020). Modelling Learning and
497 Memory in *Drosophila* to Understand Intellectual Disabilities. *Neuroscience* 445, 12-30. doi:
498 10.1016/j.neuroscience.2020.07.034
- 499 Mattila, J., Havula, E., Suominen, E., Teesalu, M., Surakka, I., Hynynen, R., Kilpinen, H., Vaananen,
500 J., Hovatta, I., Kakela, R., *et al.* (2015). Mondo-Mlx Mediates Organismal Sugar Sensing through the
501 Gli-Similar Transcription Factor Sugarbabe. *Cell Rep* 13, 350-364.
502 doi: 10.1016/j.celrep.2015.08.081
- 503 Men, T.T., Thanh, D.N., Yamaguchi, M., Suzuki, T., Hattori, G., Arii, M., Huy, N.T., and Kamei, K.
504 (2016). A *Drosophila* Model for Screening Antiobesity Agents. *Biomed Res Int* 2016, 6293163. doi:
505 10.1155/2016/6293163
- 506 Meng, Z., Liu, X., Li, T., Fang, T., Cheng, Y., Han, L., Sun, B., and Chen, L. (2021). The SGLT2
507 inhibitor empagliflozin negatively regulates IL-17/IL-23 axis-mediated inflammatory responses in
508 T2DM with NAFLD via the AMPK/mTOR/autophagy pathway. *Int Immunopharmacol* 94, 107492.
509 doi: 10.1016/j.intimp.2021.107492
- 510 Miguel-Aliaga, I., Jasper, H., and Lemaitre, B. (2018). Anatomy and Physiology of the Digestive
511 Tract of *Drosophila melanogaster*. *Genetics* 210, 357-396. doi: 10.1534/genetics.118.300224
- 512 Millet-Boureima, C., Porras Marroquin, J., and Gamberi, C. (2018). Modeling Renal Disease "On the
513 Fly". *Biomed Res Int* 2018, 5697436. doi: 10.1155/2018/5697436
- 514 Musselman, L.P., and Kuhnlein, R.P. (2018). *Drosophila* as a model to study obesity and metabolic
515 disease. *J Exp Biol* 221. doi: 10.1242/jeb.163881
- 516 Nayak, N., and Mishra, M. (2019). Simple techniques to study multifaceted diabetes in the fly
517 model. *Toxicol Mech Methods* 29, 549-560. doi: 10.1080/15376516.2019.1634171
- 518 Oh, Y., Lai, J.S., Mills, H.J., Erdjument-Bromage, H., Giammarinaro, B., Saadipour, K., Wang, J.G.,
519 Abu, F., Neubert, T.A., and Suh, G.S.B. (2019). A glucose-sensing neuron pair regulates insulin and
520 glucagon in *Drosophila*. *Nature* 574, 559-564. doi: 10.1038/s41586-019-1675-4
- 521 Oldham, S., Montagne, J., Radimerski, T., Thomas, G., and Hafen, E. (2000). Genetic and
522 biochemical characterization of dTOR, the *Drosophila* homolog of the target of rapamycin. *Genes*
523 *Dev* 14, 2689-2694. doi: 10.1101/gad.845700

Drosophila melanogaster in translational studies of liver diseases

- 524 Pagliarini, R.A., and Xu, T. (2003). A genetic screen in *Drosophila* for metastatic behavior. *Science*
525 302, 1227-1231. doi: 10.1126/science.1088474
- 526 Palm, W., Sampaio, J.L., Brankatschk, M., Carvalho, M., Mahmoud, A., Shevchenko, A., and Eaton,
527 S. (2012). Lipoproteins in *Drosophila melanogaster*--assembly, function, and influence on tissue lipid
528 composition. *PLoS Genet* 8, e1002828. doi: 10.1371/journal.pgen.1002828
- 529 Pandey, U.B., and Nichols, C.D. (2011). Human disease models in *Drosophila melanogaster* and the
530 role of the fly in therapeutic drug discovery. *Pharmacol Rev* 63, 411-436. doi:
531 10.1124/pr.110.003293
- 532 Papanikolopoulou, K., Mudher, A., and Skoulakis, E. (2019). An assessment of the translational
533 relevance of *Drosophila* in drug discovery. *Expert Opin Drug Discov* 14, 303-313. doi:
534 10.1080/17460441.2019.1569624
- 535 Parise, E.R. (2019). Nonalcoholic Fatty Liver Disease (Nafld), More Than a Liver Disease. *Arq*
536 *Gastroenterol* 56, 243-245. doi: 10.1590/S0004-2803.201900000-45
- 537 Parvy, J.P., Napal, L., Rubin, T., Poidevin, M., Perrin, L., Wicker-Thomas, C., and Montagne, J.
538 (2012). *Drosophila melanogaster* Acetyl-CoA-carboxylase sustains a fatty acid-dependent remote
539 signal to waterproof the respiratory system. *PLoS Genet* 8, e1002925. doi:
540 10.1371/journal.pgen.1002925
- 541 Parvy, J.P., Wang, P., Garrido, D., Maria, A., Blais, C., Poidevin, M., and Montagne, J. (2014).
542 Forward and feedback regulation of cyclic steroid production in *Drosophila melanogaster*.
543 *Development* 141, 3955-3965. doi: 10.1242/dev.102020
- 544 Perrimon, N., Bonini, N.M., and Dhillon, P. (2016). Fruit flies on the front line: the translational
545 impact of *Drosophila*. *Dis Model Mech* 9, 229-231. doi: 10.1242/dmm.024810
- 546 Piazza, N., and Wessells, R.J. (2011). *Drosophila* models of cardiac disease. *Prog Mol Biol Transl*
547 *Sci* 100, 155-210. doi: 10.1016/B978-0-12-384878-9.00005-4
- 548 Pospisilik, J.A., Schramek, D., Schnidar, H., Cronin, S.J., Nehme, N.T., Zhang, X., Knauf, C., Cani,
549 P.D., Aumayr, K., Todoric, J., *et al.* (2010). *Drosophila* genome-wide obesity screen reveals
550 hedgehog as a determinant of brown versus white adipose cell fate. *Cell* 140, 148-160. doi:
551 10.1016/j.cell.2009.12.027
- 552 Powell, E.E., Wong, V.W., and Rinella, M. (2021). Non-alcoholic fatty liver disease. *Lancet*. doi:
553 10.1016/S0140-6736(20)32511-3

Drosophila melanogaster in translational studies of liver diseases

- 554 Rajan, A., and Perrimon, N. (2012). *Drosophila* cytokine unpaired 2 regulates physiological
555 homeostasis by remotely controlling insulin secretion. *Cell* *151*, 123-137. doi:
556 10.1016/j.cell.2012.08.019
- 557 Ramos, L.F., Silva, C.M., Pansa, C.C., and Moraes, K.C.M. (2021). Non-alcoholic fatty liver disease:
558 molecular and cellular interplays of the lipid metabolism in a steatotic liver. *Expert Rev*
559 *Gastroenterol Hepatol* *15*, 25-40. doi: 10.1080/17474124.2020.1820321
- 560 Rani, L., and Gautam, N.K. (2018). *Drosophila* Renal Organ as a Model for Identification of Targets
561 and Screening of Potential Therapeutic Agents for Diabetic Nephropathy. *Curr Drug Targets* *19*,
562 1980-1990. doi: 10.2174/1389450119666180808114533
- 563 Richardson, H.E., and Portela, M. (2018). Modelling Cooperative Tumorigenesis in *Drosophila*.
564 *Biomed Res Int* *2018*, 4258387. doi: 10.1155/2018/4258387
- 565 Rosato, V., Masarone, M., Dallio, M., Federico, A., Aglitti, A., and Persico, M. (2019). NAFLD and
566 Extra-Hepatic Comorbidities: Current Evidence on a Multi-Organ Metabolic Syndrome. *Int J*
567 *Environ Res Public Health* *16*. doi: 10.3390/ijerph16183415
- 568 Rulifson, E.J., Kim, S.K., and Nusse, R. (2002). Ablation of insulin-producing neurons in flies:
569 growth and diabetic phenotypes. *Science* *296*, 1118-1120. doi: 10.1126/science.1070058
- 570 Salazar, J.L., Yang, S.A., and Yamamoto, S. (2020). Post-Developmental Roles of Notch Signaling
571 in the Nervous System. *Biomolecules* *10*. doi: 10.3390/biom10070985
- 572 Samji, P., Rajendran, M.K., Warriar, V.P., Ganesh, A., and Devarajan, K. (2021). Regulation of
573 Hippo signaling pathway in cancer: A MicroRNA perspective. *Cell Signal* *78*, 109858. doi:
574 10.1016/j.celsig.2020.109858
- 575 Samuel, V.T., and Shulman, G.I. (2018). Nonalcoholic Fatty Liver Disease as a Nexus of Metabolic
576 and Hepatic Diseases. *Cell Metab* *27*, 22-41. doi: 10.1016/j.cmet.2017.08.002
- 577 Sanguesa, G., Roglans, N., Baena, M., Velazquez, A.M., Laguna, J.C., and Alegret, M. (2019).
578 mTOR is a Key Protein Involved in the Metabolic Effects of Simple Sugars. *Int J Mol Sci* *20*. doi:
579 10.3390/ijms20051117
- 580 Sanyal, A.J. (2019). Past, present and future perspectives in nonalcoholic fatty liver disease. *Nat Rev*
581 *Gastroenterol Hepatol* *16*, 377-386. doi: 10.1038/s41575-019-0144-8

Drosophila melanogaster in translational studies of liver diseases

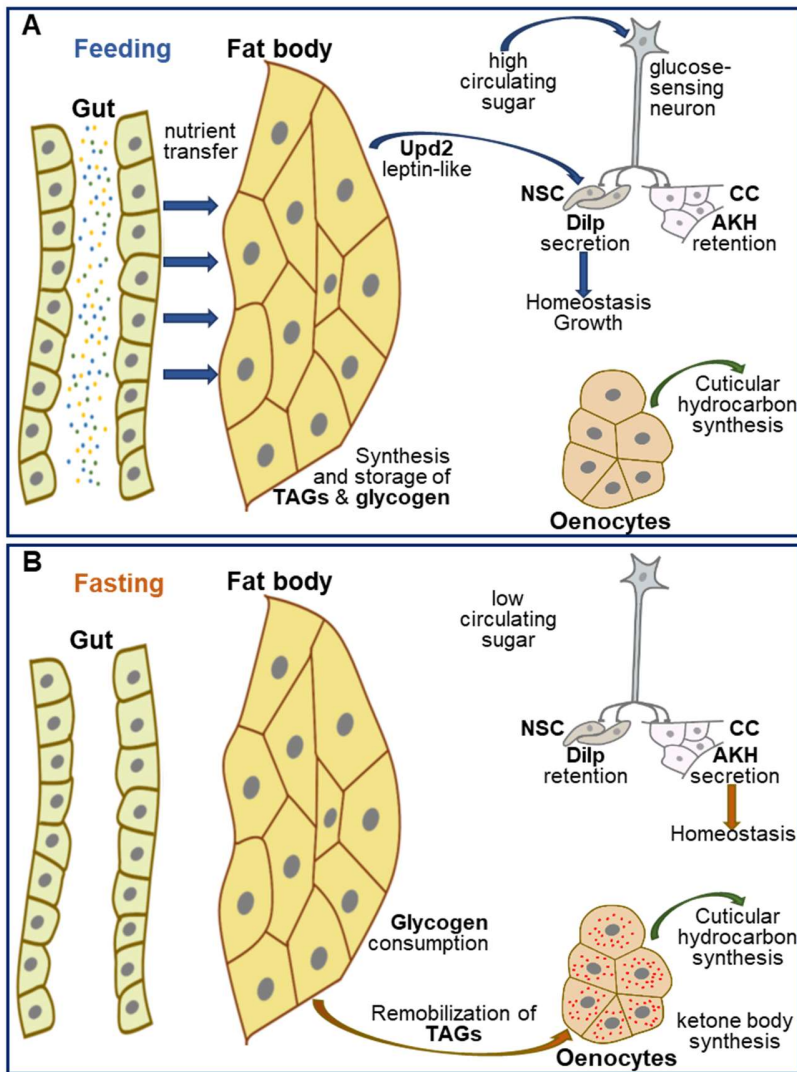
- 582 Saxton, R.A., and Sabatini, D.M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell*
583 *168*, 960-976. doi: 10.1016/j.cell.2017.02.004
- 584 Scherer, P.E., Williams, S., Fogliano, M., Baldini, G., and Lodish, H.F. (1995). A novel serum
585 protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* *270*, 26746-26749. doi:
586 10.1074/jbc.270.45.26746
- 587 Schmitt, S., Ugrankar, R., Greene, S.E., Prajapati, M., and Lehmann, M. (2015). *Drosophila* Lipin
588 interacts with insulin and TOR signaling pathways in the control of growth and lipid metabolism. *J*
589 *Cell Sci* *128*, 4395-4406. doi: 10.1242/jcs.173740
- 590 Senturk, M., and Bellen, H.J. (2018). Genetic strategies to tackle neurological diseases in fruit flies.
591 *Curr Opin Neurobiol* *50*, 24-32. doi: 10.1016/j.conb.2017.10.017
- 592 Seo, Y.S., Kim, J.H., Jo, N.Y., Choi, K.M., Baik, S.H., Park, J.J., Kim, J.S., Byun, K.S., Bak, Y.T.,
593 Lee, C.H., *et al.* (2008). PPAR agonists treatment is effective in a nonalcoholic fatty liver disease
594 animal model by modulating fatty-acid metabolic enzymes. *J Gastroenterol Hepatol* *23*, 102-109. doi:
595 10.1111/j.1440-1746.2006.04819.x
- 596 Shabalala, S.C., Dlodla, P.V., Mabasa, L., Kappo, A.P., Basson, A.K., Pheiffer, C., and Johnson, R.
597 (2020). The effect of adiponectin in the pathogenesis of non-alcoholic fatty liver disease (NAFLD)
598 and the potential role of polyphenols in the modulation of adiponectin signaling. *Biomed*
599 *Pharmacother* *131*, 110785. doi: 10.1016/j.biopha.2020.110785
- 600 Shen, Z.Q., Chen, Y.F., Chen, J.R., Jou, Y.S., Wu, P.C., Kao, C.H., Wang, C.H., Huang, Y.L., Chen,
601 C.F., Huang, T.S., *et al.* (2017). CISD2 Haploinsufficiency Disrupts Calcium Homeostasis, Causes
602 Nonalcoholic Fatty Liver Disease, and Promotes Hepatocellular Carcinoma. *Cell Rep* *21*, 2198-2211.
603 doi: 10.1016/j.celrep.2017.10.099
- 604 Storelli, G., Nam, H.J., Simcox, J., Villanueva, C.J., and Thummel, C.S. (2019). *Drosophila* HNF4
605 Directs a Switch in Lipid Metabolism that Supports the Transition to Adulthood. *Dev Cell* *48*, 200-
606 214 e206. doi: 10.1016/j.devcel.2018.11.030
- 607 Tamarai, K., Bhatti, J.S., and Reddy, P.H. (2019). Molecular and cellular bases of diabetes: Focus on
608 type 2 diabetes mouse model-TallyHo. *Biochim Biophys Acta Mol Basis Dis* *1865*, 2276-2284. doi:
609 10.1016/j.bbadis.2019.05.004
- 610 Tennesen, J.M., Barry, W.E., Cox, J., and Thummel, C.S. (2014). Methods for studying metabolism
611 in *Drosophila*. *Methods* *68*, 105-115. doi: 10.1016/j.ymeth.2014.02.034

Drosophila melanogaster in translational studies of liver diseases

- 612 Teshome, G., Ambachew, S., Fasil, A., and Abebe, M. (2020). Efficacy of Glucagon-Like Peptide-1
613 Analogs in Nonalcoholic Fatty Liver Disease: A Systematic Review. *Hepat Med* 12, 139-151. doi:
614 10.2147/HMER.S265631
- 615 Toprak, U., Hegedus, D., Dogan, C., and Guney, G. (2020). A journey into the world of insect lipid
616 metabolism. *Arch Insect Biochem Physiol* 104, e21682. doi: 10.1002/arch.21682
- 617 Ugur, B., Chen, K., and Bellen, H.J. (2016). *Drosophila* tools and assays for the study of human
618 diseases. *Dis Model Mech* 9, 235-244. doi: 10.1242/dmm.023762
- 619 van Erk, M., van Luijk, J., Yang, F., Leeuwenburgh, S.C.G., Sanchez-Fernandez, M.J., Hermans, E.,
620 Felix Lanao, R.P., and van Goor, H. (2021). A Systematic Review and Meta-Analyses on Animal
621 Models Used in Bone Adhesive Research. *J Orthop Res*. doi: 10.1002/jor.25057
- 622 Van Norman, G.A. (2019). Limitations of Animal Studies for Predicting Toxicity in Clinical Trials:
623 Is it Time to Rethink Our Current Approach? *JACC Basic Transl Sci* 4, 845-854. doi:
624 10.1016/j.jacbts.2019.10.008
- 625 Villanueva, J.E., Livelio, C., Trujillo, A.S., Chandran, S., Woodworth, B., Andrade, L., Le, H.D.,
626 Manor, U., Panda, S., and Melkani, G.C. (2019). Time-restricted feeding restores muscle function in
627 *Drosophila* models of obesity and circadian-rhythm disruption. *Nat Commun* 10, 2700. doi:
628 10.1038/s41467-019-10563-9
- 629 WGO (2020). Global burden of liver disease: A true burden on health sciences and economies!! ,
630 W.G. Organisation, ed. [https://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-](https://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-point-of-view-articles-collection/global-burden-of-liver-disease-a-true-burden-on-health-sciences-and-economies)
631 [point-of-view-articles-collection/global-burden-of-liver-disease-a-true-burden-on-health-sciences-](https://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-point-of-view-articles-collection/global-burden-of-liver-disease-a-true-burden-on-health-sciences-and-economies)
632 [and-economies](https://www.worldgastroenterology.org/publications/e-wgn/e-wgn-expert-point-of-view-articles-collection/global-burden-of-liver-disease-a-true-burden-on-health-sciences-and-economies)
- 633 WHO, W.H.O. (2020). The top 10 causes of death. [https://www.who.int/news-room/fact-](https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death)
634 [sheets/detail/the-top-10-causes-of-death](https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death)
- 635 Yamada, T., Habara, O., Kubo, H., and Nishimura, T. (2018). Fat body glycogen serves as a
636 metabolic safeguard for the maintenance of sugar levels in *Drosophila*. *Development* 145. doi:
637 10.1242/dev.158865
- 638 Yamada, T., Hironaka, K.I., Habara, O., Morishita, Y., and Nishimura, T. (2020). A developmental
639 checkpoint directs metabolic remodelling as a strategy against starvation in *Drosophila*. *Nat Metab* 2,
640 1096-1112. doi: 10.1038/s42255-020-00293-4

***Drosophila melanogaster* in translational studies of liver diseases**

- 641 Yang, J., McCart, C., Woods, D.J., Terhzaz, S., Greenwood, K.G., ffrench-Constant, R.H., and Dow,
642 J.A. (2007). A *Drosophila* systems approach to xenobiotic metabolism. *Physiol Genomics* *30*, 223-
643 231. doi: 10.1152/physiolgenomics.00018.2007
- 644 Yoo, J., Jeong, I.K., Ahn, K.J., Chung, H.Y., and Hwang, Y.C. (2021). Fenofibrate, a PPARalpha
645 agonist, reduces hepatic fat accumulation through the upregulation of TFEB-mediated lipophagy.
646 *Metabolism* *120*, 154798. doi: 10.1016/j.metabol.2021.154798
- 647 Yoon, M.S. (2017). The Role of Mammalian Target of Rapamycin (mTOR) in Insulin Signaling.
648 *Nutrients* *9*. doi: 10.3390/nu9111176
- 649 Younossi, Z., Anstee, Q.M., Marietti, M., Hardy, T., Henry, L., Eslam, M., George, J., and
650 Bugianesi, E. (2018). Global burden of NAFLD and NASH: trends, predictions, risk factors and
651 prevention. *Nat Rev Gastroenterol Hepatol* *15*, 11-20. doi: 10.1038/nrgastro.2017.109
- 652



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654 **Figure 1: Hepatic-like homeostasis in *Drosophila*.** (A) In fed *Drosophila*, nutrients are transferred
 655 to the appropriate organs, in particular the FB, which acts as a nutrient sensor to coordinate
 656 organismal growth and homeostasis. The FB synthesizes and stores glycogen and TAGs, and secretes
 657 several proteins, including Upd2, which can be replaced experimentally by mammalian leptin; Upd2
 658 and glucose-sensing neurons contribute to regulating the secretion of Dilps and AKH to control
 659 growth and homeostasis. (B) In fasting *Drosophila*, FB glycogen and TAGs are remobilized, the
 660 latter being transferred to the oenocytes to produce ketone bodies, while glucose sensing neurons
 661 potentiate AKH secretion and Dilp retention in neurosecretory cells to maintain homeostasis.

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Human	<i>Drosophila</i>	Function in <i>Drosophila</i>	Reference
ChREBP MondoA	Mondo	Regulates sugar usage	<i>Havula et al., 2013</i>
SREBPs	SREBP	Regulates genes of FA synthesis	<i>Kunte et al., 2006</i>
LXRs	EcR	Receptor for the steroid hormone ecdysone	<i>Parvy et al., 2014</i>
PPARs	Eip75B	Intermediate in steroid hormone signaling	<i>Parvy et al., 2014</i>
PNPLA3	Brummer	Performs TAGs breakdown	<i>Gronke et al., 2005</i>
Perilipins	Lsd1 Lsd2	Envelop LDs	<i>Bi et al., 2012</i>
Apolipo- proteins	apoLpp apoLTP MTP	Performs lipid transport throughout hemolymph Performs lipid loading on Lpp in enterocytes Performs lipid loading on Lpp in FB cells	<i>Palm et al., 2012</i>
mTOR	TOR	Regulates growth in response to nutrients	<i>Oldham et al., 2000</i>
Insulin IGF1 IGF2	DILPs	Regulate growth and metabolism	<i>Brogiolo et al., 2001</i>
IGFBP	Imp-L2	Modulates DILP activity	<i>Honegger et al., 2008</i>
InR IGF-R	InR	Receptor for the DILPs	<i>Brogiolo et al., 2001</i>
IRS1	chico	Regulates body growth and aging	<i>Bohni et al., 1999</i>
Glucagon	AKH	Regulates dietary sugar response	<i>Kim and Rulifson, 2004</i>
Leptin	Unpaired2	As for leptin, Udp2 is a ligand for the receptor of the JAK/STAT pathway	<i>Rajan and Perrimon, 2012</i>
ACC1 ACC2	ACC	rate-limiting enzyme for fatty acid synthesis	<i>Parvy et al., 2012</i>
FASN	FASN1 FASN2 FASN3	Ubiquitous FA-synthesis Oenocyte-restricted FA-synthesis Oenocyte-restricted FA-synthesis	<i>Garrido et al., 2015</i>

663 **Table 1: *Drosophila* homologues of human gene products involved in metabolic and liver**
664 **disease.**