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Drosophila as a model to study the genetic mechanisms of obesity-associated heart dysfunction

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Abstract

Obesity and cardiovascular disease are among the world's leading causes of death, especially in Western countries where consumption of high caloric food is commonly accompanied by low physical activity. This lifestyle often leads to energy imbalance, obesity, diabetes and their associated metabolic disorders, including cardiovascular diseases. It has become increasingly recognized that obesity and cardiovascular disease are metabolically linked, and a better understanding of this relationship requires that we uncover the fundamental genetic mechanisms controlling obesity-related heart dysfunction, a goal that has been difficult to achieve in higher organisms with intricate metabolic complexity. However, the high degree of evolutionary conservation of genes and signalling pathways allows researchers to use lower animal models such as *Drosophila*, which is the simplest genetic model with a heart, to uncover the mechanistic basis of obesity-related heart disease and its likely relevance to humans. Here, we discuss recent advances made by using the power of the *Drosophila* as a powerful model to investigate the genetic pathways by which a high fat diet may lead to heart dysfunction.

Keywords: triglycerides • obesity • heart dysfunction • genetic control

Introduction

Vertebrate animal models (rodents, frogs and fish) have contributed greatly to our understanding of human biology and physiology [1–4]. However, these models are genetically and metabolically complex, which limits their ability to provide insight into fundamental processes. Invertebrate model organisms, such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* are genetically simpler, have less redundancy, and less complex metabolism; they have thus been instrumental in dissecting fundamental pathways and mechanisms [5–9]. Among the invertebrate models, *Drosophila* is the only one with a heart, it has a short lifespan, and there are established genetic tools for analysing heart function [10, 11]. Importantly, most gene families and signalling pathways are highly conserved between flies and humans [12], making *Drosophila* the organism of choice among the invertebrate models to perform heart function-related studies. The *Drosophila* heart develops in a homologous fashion to vertebrate hearts, including that of humans [13], and functions in an analogous manner to pump the blood (haemolymph) through the body cavity in an open circulatory system [14]. Therefore, despite the fact that the mature *Drosophila* and vertebrate hearts are quite different morphologically, the initial developmental processes and the basic functional properties and molecular constituents are remarkably conserved [11, 13, 15]. This is exemplified by the *tinman* gene, first discovered in the fly [16], which turned out to be the first gene found critical for heart determination in all species [17–19]. Since the discovery of *tinman*, *Drosophila* has been established as a model of many forms of congenital heart disease, cardiomyopathies and of cardiac ageing.

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Here, we discuss the use of *Drosophila* as a model for studying obesity and heart dysfunction as a consequence of excess dietary fat intake.

High fat diet feeding of *Drosophila* causes excess fat accumulation

The critical organs for glucose and lipid metabolism in vertebrates and mammals also have counterparts in insects, including *Drosophila* [20, 21]. For example, the functions of the liver and adipose tissue in mammals are assumed by the fat body and specialized oenocyte cells in the fly. The fly also has insulin producing cells [22, 23] and cells producing the glucagon-like hormone AKH [24], which are analogous to mammalian pancreatic beta and alpha cells respectively. Collectively, these tissues integrate genetic, environmental and dietary signals to control energy balance and metabolic homeostasis in the fly [25–27]. This suggests that the central mechanisms controlling fat and glucose metabolism are preserved in *Drosophila* and likely participate in the development of obesity and obesity-related heart dysfunction.

The worldwide epidemic of diabetes and its associated metabolic disorders are at least partly caused by consumption of high caloric fat- and sugar-enriched foods. Recently, we established an obesity model, in which *Drosophila* are fed a high fat diet (HFD; 30% coconut oil-enriched food) for several days [28]. Like humans, flies on HFD display symptoms of obesity that include increased levels of triglyceride (TG), the main form of cellular lipid storage, as well as increased levels of glucose and insulin, a hallmark of insulin resistance. The HFD-induced rise in TG levels is accompanied by increased signalling through the target of rapamycin (TOR) pathway, decreased expression of adipose triglyceride lipase (ATGL, encoded by the *Bmm* gene in flies) and a corresponding increase in fatty acid synthase (FAS) expression (Fig. 1; [28]). Although the mechanism by which high fat-supplemented food induces symptoms of obesity and the metabolic syndrome remains to be determined, it is interesting to note recent findings that a high sugar diet also induces excess TG accumulation and associated heart defects in flies ([29]; J. Na, R.B., K. Ocorr and R. Cagan., unpubl. data).

Excess fat accumulation is associated with heart dysfunction in *Drosophila*

After two centuries of scientific observation and debate (reviewed in [30]), it has become clear that obesity and diabetes in humans promote the development of secondary diseases, including heart dysfunction, high blood pressure, atherosclerosis and fatty liver disease, collectively known as the metabolic syndrome. How obesity causes symptoms of metabolic syndrome, in particular cardiomyopathies, is still largely unknown. The recently developed fly models of obesity-related heart dysfunction have begun to reveal some of the genetic mechanisms involved.

Recent advances in the measurement of heart function in adult *Drosophila* have made it possible to determine whether obesity induces heart dysfunction in *Drosophila,* as it does in humans. Indeed, the Semi Automated Optical Heart Analysis (SOHA) method developed by Ocorr and colleagues [11, 31] has revealed some answers. HFD feeding reduces cardiac output (fractional shortening), causes partial conduction block and induces regions of non-contractility (reminiscent of an infarct-like state) in the majority of these 'fatty' hearts [28], strongly suggesting that HFD also significantly impair heart function in flies. These HFD-induced changes in the regular heartbeat pattern are illustrated in the M-mode traces from SOHA: under HFD feeding conditions the heart rate is faster and more erratic than observed under normal feeding conditions (Fig. 2). These findings demonstrate that, as for humans, flies exposed to prolonged HFD feeding (days in flies) exhibit severe cardiac dysfunction, which suggests that the underlying mechanisms by which dietary intake affects metabolic homeostasis and heart function may indeed be conserved between *Drosophila* and humans.

The next step to understanding the relationship between dietary fat intake and cardiovascular disease is to investigate the molecular mechanisms that link altered fat metabolism to dysregulated heart function. In this regard, Birse *et al.* [28] have shown that the insulin-TOR pathways are key factors in mediating fat accumulation in the whole organism and in the heart itself, and the subsequent development of heart dysfunction (Fig. 1). For example, genetic manipulations that reduce insulin-TOR pathway activity prevent the HFD-induced accumulation of fat, and importantly, these interventions also protect against heart dysfunction. Moreover, when insulin-TOR signalling was diminished only in the fly's heart, excess fat still accumulated in the rest of the body, but the HFD-induced cardiac abnormalities were abolished. These findings suggest that cardiac-specific interventions of major metabolic regulators (such as the insulin-TOR pathways) may represent new therapeutic avenues for targeting obesity-related heart dysfunction.

Two other studies, also using a genetic approach to modulate fat accumulation, have uncovered new factors in the relationship between fat metabolism and heart dysfunction in *Drosophila* [32,33]. In one study a new feedback inhibitory loop for TOR signalling was found to involve the transcriptional regulation of the fly homolog of the Sestrins (*dSesn*) through TOR-dependent activation of the FoxO transcription factor. dSesn seems to act as a rheostat to stimulate AMPK activity, which in turn inhibits TOR function. Thus, in the absence of *dSesn* TOR was hyperactivated, leading to excess fat accumulation and ensuing heart defects; these effects were reversed (rescued) by addition of the TOR inhibitor rapamycin or the AMPK activator AICAR (Fig. 1; [32]).

In the second study, ablation of the gene *easily shocked* (*eas*), which encodes a key enzyme in the synthesis of the membrane phospholipid phosphatidylethanolamine (PE), also caused excess fat accumulation and cardiac defects [33], a phenotype reminiscent of *dSesn* mutants. *eas* mutant flies have low PE levels, and exhibit increased processing of the transcription factor SREBP to its active mature (nuclear) form m-SREBP (Fig. 1; [34]). m-SREBP activates transcription of lipogenic genes which results in elevated TG levels and impaired heart function [33]. Remarkably, hearts from *eas* mutants display a similar phenotype to those of HFD-fed flies including tachycardia [28]. In addition, overexpression of m-SREBP in the heart

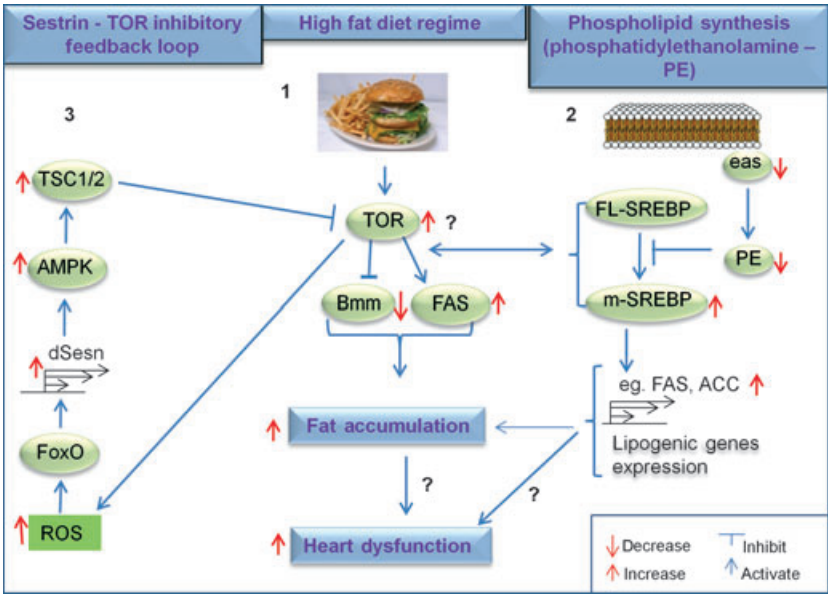

Fig. 1 Impaired lipid metabolism induced by HFD or genetic manipulation induces heart dysfunction. Different mechanisms that initiate fat accumulation also lead to heart dysfunction in *Drosophila*. In 1 (centre), a HFD regime activates the TOR pathway, lowers ATGL/Bmm lipase and increases FAS levels. This leads to fat accumulation and heart dysfunction [28]. In 2 (right), dysregulation of phospholipid synthesis in *easily shocked* (*eas*) mutant flies (decreased production of phosphatidylethanolamine, PE) augments SREBP processing, which leads to accumulation of active (nuclear) SREBP (m-SREBP) and increased expression of lipogenic genes (*e.g.* FAS, ACC). This process also results in fat accumulation and heart dysfunction [33]. Both direct and indirect genetic mechanisms are implicated in fat accumulation and heart dysfunction, demonstrating the need to identify new factors (?) that might reveal how fat accumulation causes heart dysfunction. In 3 (left), a new feedback inhibitory loop involving Sestrin (dSesn in flies) is involved in modulating TOR activity. Increased TOR signaling augments ROS production, which activates the transcription factor FoxO *via* Jun-N-terminal kinase. In turn, FoxO activates *dSesn,* which inhibits TOR activity by activating the AMPK/TSC axis. In *dSesn* mutant flies, this negative feedback loop is diminished, leading to hyperactivation of the TOR pathway and consequently to fat accumulation and heart dysfunction [32].

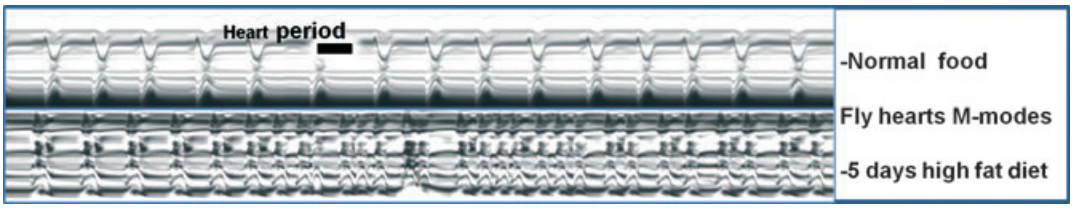

Fig. 2 Heart M-modes from wildtype *Drosophila* fed a normal or high fat diet. The figure shows M-mode traces from high-speed movies of *Drosophila* hearts using the SOHA program [11, 31]. The upper panel shows a cardiac M-mode from a young (2-week-old) fly fed a normal food diet, which shows a regular heart rhythm. The lower panel shows an M-mode from a fly fed a high-fat diet; these hearts beat faster and with an irregular, erratic rhythm. Heartbeat duration (heart period) is represented by the black bars.

mimics the cardiac dysfunction seen in *eas* mutants, whereas cardiac-specific knockdown of SREBP or its lipogenic targets rescues the *eas* cardiac phenotype [33]. Therefore, perturbation of PE synthesis interferes with fat metabolism through SREBP processing, which in turn contributes to heart dysfunction.

Collectively, these studies have shown that both dietary and genetic causes of impaired or altered lipid metabolism can increase fat concentrations and lead to detrimental effects on heart function (Figs 1 and 2). Our understanding of these relationships will be facilitated by studying possible links between the insulin-TOR and SREBP/lipid metabolism pathways, and discovering new mechanisms that link fat accumulation and cardiac dysfunction. Indeed, a recent study in mammalian cells suggested that mTOR complex 1 regulates the SREBP pathway through the nuclear localization of the phosphatidic acid phosphatase Lipin 1 and subsequent nuclear shape [35]. Collectively, these studies will accelerate the identification of new therapeutic targets to protect the heart against the detrimental effects of obesity.

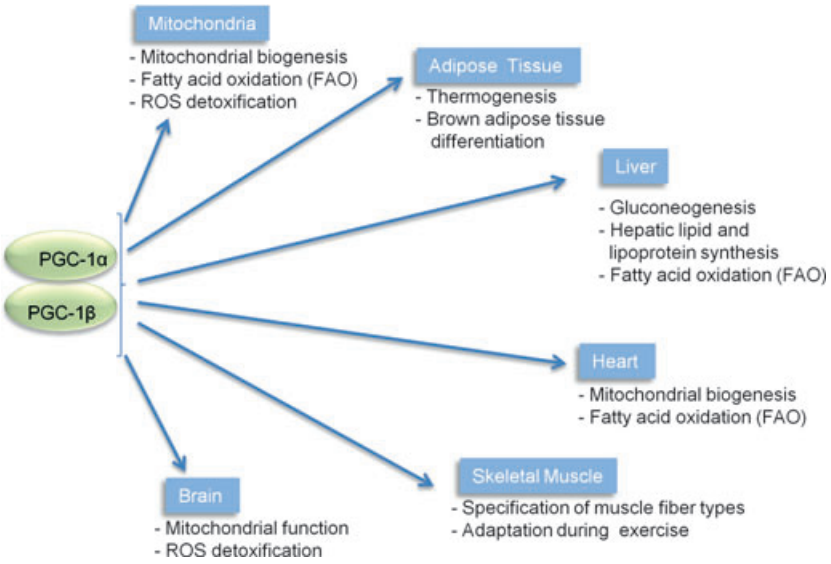

Fig. 3 Summary of PGC-1 metabolic functions in mammals. PGC-1 family members play essential roles in mitochondrial biogenesis, as well as in the control of the electron transport chain and fatty acid oxidation (FOA), both occurring inside the mitochondria.

New players in the fat–heart relationship

To identify new modifiers of obesity-associated heart dysfunction in *Drosophila*, we have conducted a screen of genes that are implicated in various aspects of lipid metabolism, to find new gene functions that modulate the degree of fat accumulation and have potentially detrimental effects on heart function. Several candidates are emerging as interesting players, among them a potential fly homolog of the PPARγ Coactivator-1 (PGC-1) genes. In mammals, PGC-1 genes (PGC-1α, PGC-1β and PRC) are involved in numerous metabolic functions, including mitochondrial biogenesis and function, fatty acid oxidation, muscle fibre determination, gluconeogenesis and thermogenesis (Fig. 3; [36–41]). In mammals, the functions of PGC-1 family members in mitochondrial biogenesis and fatty acid oxidation have been shown to be essential in satisfying the high energy needs in the neonatal and the adult heart [42–44]

In *Drosophila*, only one gene has been identified as a *bona fide* homolog of the PGC-1 genes; *Spargel* [45]. This offers the opportunity to study its role in flies without the complications of potential redundancies and functional complexities found in mammals. Spargel has recently been implicated in nutrient responses during larval development in *Drosophila*, where it appears to be an important player in mitochondrial function, as it is in mammals. [45, 46]

Interestingly, flies with partial loss-of-function of *Spargel* exhibit even more fat accumulation than do wildtype flies in response to a HFD, whereas flies with ubiquitous overexpression of *Spargel* are leaner than wildtype flies and are protected from excessive fat accumulation when fed a HFD (S.B. Diop and R. Bodmer, unpubl. data). Flies with reduced *Spargel* function also exhibit the same heart defects as are induced by a HFD (S.B. Diop and R. Bodmer, unpubl. data). Therefore, increased fat accumulation in flies with decreased *Spargel* function is correlated with an increase in heart defects, whereas the heart is protected from the effects of obesity when Spargel is overexpressed, suggesting that this co-activator plays an important role in HFD-induced obesity and heart dysfunction. It will be interesting to see how Spargel function relates to other modulators of the fat–heart relationship, as we discussed above.

Concluding remarks

The studies discussed in this review have shown that the mechanisms by which a high caloric diet induces heart dysfunction is remarkably conserved from mammals to *Drosophila*. Genetic studies showed the insulin-TOR and SREBP pathways play a central role in this process, and new genes are currently being investigated using the genetic and analytical tools now available in *Drosophila*. This model system is well suited to dissect genetic interactions and identify new players that link impairment of lipid metabolism to a dysfunctional heart. This will ultimately help design new therapeutic strategies to protect the heart against the deleterious effects of high caloric diets or genetic causes of obesity.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

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