Emerging models for studying adipose tissue metabolism

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Abstract

Understanding adipose metabolism is essential for addressing obesity and related health concerns. However, the ethical and scientific pressure to reduce or eliminate animal testing, aligning with the 3Rs, has triggered the implementation of diverse *in vitro* and *in vivo* models for analysing anomalies in adipose metabolism. In this review, we will address this issue from various perspectives. Traditional adipocyte cell cultures, whether animal or human-derived, offer a fundamental starting point. These systems have their merits but may not fully replicate *in vivo* complexity. Established cell lines are valuable for high-throughput screening but may lack the authenticity of primary-derived adipocytes, which closely mimic native tissue. To enhance model sophistication, spheroids have been introduced. These three-dimensional cultures better mimicking the *in vivo* microenvironment, enabling the study of intricate cell-cell interactions, gene expression, and metabolic pathways. Organ-on-a-Chip (OoC) platforms take this further by integrating multiple cell types into microfluidic devices, simulating tissue-level functions. In particular, adipose-OoC provides dynamic environments with applications spanning drug testing to personalized medicine and nutrition. Beyond *in vitro* models, genetically amenable organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and Zebrafish larvae have become powerful tools for investigating fundamental molecular mechanisms that govern adipose tissue functions. Their genetic tractability allows for efficient manipulation and high-throughput studies. In conclusion, a diverse array of research models is crucial for deciphering adipose metabolism. By leveraging traditional adipocyte cell cultures, primary-derived cells, spheroids, and OoCs, as well as organism models, we bridge the gap between animal testing and a more ethical, scientifically robust, and human-relevant approach, advancing our understanding of adipose tissue function and its impact on health.

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; 3Rs principles, Replacement, Reduction, and Refinement; 5-HT, 5-hydroxytryptamine; AKH, adipokinetic hormone; AKT, protein kinase B; AOoC, adipose Organ-on-a-Chip; ASCs, adipose-derived stem cells; ATGL, adipose triglyceride lipase; C/EBPs, CCAAT/enhancer-binding proteins; CPL-1, cathepsin L-like protease; CRISPR-Cas9, Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9; DAF-2, DAuer Formation abnormal-2; dILPs, Drosophila insulin-like peptides; faf2, fatty acid synthase-associated factor 2; FAs, fatty acids; FDA, Food and Drug Administration; FFA, free fatty acids; foigr, foie gras; FOXO, Forkhead box O; GH, growth hormone; HFD, high-fat diet; HFD, high-fat diets; hMADS, human multipotent adipose derived stem; HSD, high sugar diet; HSL, hormone-sensitive lipase; IBMX, 1-methyl-3-isobutylxanthine; IIS, insulin/IGF-1 signalling; INP, insulin-like peptides; IR, insulin resistance; kat1, ketothiolase 1; LC-PUFA, long-chain-PUFA; Luc2, Luciferase 2; MEFs, mouse embryonic fibroblasts; MSCs, mesenchymal stem cells; MTAs, material transfer agreements; MUFA, monounsaturated fatty acids; NAFLD, non-alcoholic fatty liver disease; NFD, normal fat diet; NGM, nematode growth media; NHR, nuclear hormone receptor; NIH, National Institutes of Health; Nlaz, Neural Lazarillo; Nrf1/NFE2L1, Nuclear factor erythroid 2-related factor 1 / Nuclear factor, erythroid derived 2, like 1; OA, oleic acid; OoC, Organ-on-a-Chip; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; pkc1, protein kinase C1; PLs, phospholipids; pnpla3, patatin-like phospholipase domain containing 3; PPARs, peroxisome proliferator-activated receptors; PPAR-γ, peroxisome proliferator-activated receptor gamma; Pref-1, preadipocyte factor-1; PUFA, polyunsaturated fatty acids; RNAi, RNA interference; ROS, reactive oxygen species; SGBS, Simpson-Golabi-Behmel syndrome; SREBP, sterol regulatory element binding protein; SVF, stromal vascular fraction; T3, triiodothyronine; Tg, transgenic; TGs, triglycerides; tm6sf2, transmembrane 6 superfamily member 2; trappc11, transport protein particle 11; Upd2, Unpaired 2; WAT, White adipose tissue; WHO, World Health Organization.

1. Introduction

The World Health Organization (WHO) defines obesity as a global health emergency resulting from an imbalance in energy intake and storage [1,2]. Projections indicate that by 2030, 51% of the humanity may be obese, and consequently affecting both health and healthcare systems on a worldwide scale [1,2]. Obesity is characterized by the excessive expansion of adipose tissue, resulting in the excessive storage of lipidic nutrients within adipose tissues [3]. Adipose tissue is of particular importance due to its ability to secrete various substances that regulate metabolic pathways, including hormones, cytokines, and adipokines [4]. Current sedentary lifestyles and prolonged overnutrition results in the deposition of excessive free fatty acids (FFA) in adipose tissue, and when adipocytes reach their maximum capacity for fat storage, and insulin's effectiveness diminishes due to insulin resistance, lipolysis is triggered within adipocytes [5]. This process results in the release of lipids in the form of FFA into the circulatory system, with subsequent deposition in peripheral tissues, potentially contributing to lipotoxicity and compromising their proper function [6]. This scenario leads to the generation of excess reactive oxygen species (ROS) and pro-inflammatory signals, which play a crucial role in the development of obesity associated comorbidities including metabolic syndrome, insulin resistance (IR), cardiovascular diseases, hypertension, and dyslipidemia, among others [6]. Therefore, it is crucial to obtain a deeper understanding of adipose tissue metabolism and the factors involved in its dysregulation. Given the complexity of obtaining human adipose tissue samples, as it requires invasive surgery, various animal models are available, including mouse, rat, and hamster models, which have thus far satisfactorily met this requirement [7,8]. However, the inability of animal models to fully predict therapeutic responses in humans constitutes a significant challenge [9]. Furthermore, the increasing awareness among the general population regarding the reduction in the use of animals in research, coupled with initiatives from both the United States Food and Drug Administration (FDA) [10] and the European Union legislation (European Directive 2010/63/EU) [11] strongly promotes the development of innovative strategies for the study of adipose tissue metabolism in an animal-free experimental context. The ethical and scientific obligation to reduce or eliminate

animal testing is underscored by the 3Rs principles—Replacement, Reduction, and Refinement. This imperative has spurred the development of alternative models, ensuring more humane and efficient methods for scientific research and testing, aligning with evolving ethical standards.

In this review, we will examine various alternative models for the study of adipose tissue metabolism, from traditional two-dimensional (2D) *in vitro* cultures using established adipocytes cell lines, to co-cultures, and more advanced three dimensional (3D) approaches such as spheroids and Organ-on-a-Chip (OoC) systems, and the use of lower non-mammalian organisms like the nematode (*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*), or Zebrafish larvae (*Dario rerio*).

2. Adipose tissue metabolism

White adipose tissue (WAT) serves a central role in maintaining the body's energy and glucose balance [12], both at the organ and systemic levels with two main functions. First, adipose tissue stores energy in the form of triglycerides (TGs) and regulates their distribution and mobilization throughout the body [13]. Prolonged of excessive TGs amount storage leads to an increase in lipid droplet size, resulting in adipose tissue expansion and obesity [14]. Conversely, during periods of scarcity or increased energy expenditure, adipocytes hydrolyze TGs into glycerol and fatty acids through lipolysis [15]. These released products travel through the bloodstream, influencing lipid distribution and contributing to whole-body energy balance by infiltrating other organs like muscle, liver, and more [16] (Fig. 1).

Second, in addition to its role as a fuel reservoir, adipose tissue functions as an endocrine organ [17], producing various bioactive molecules, such as adipokines (including leptin [18], adiponectin [19], visfatin [20], apelin [21], vaspin [22], hepcidin [23], chemerin [24], and omentin [25]), which communicate with other organs (muscle, liver, pancreas, brain, …) through endocrine signaling [12]. Thus, adipose tissue plays a pivotal role in obesity-related conditions and dysfunction in the biosynthesis, assembly, secretion, and signaling of adipokines is associated with obesity and related disorders [26], such as insulin resistance, cardiovascular disease, diabetes, depression, and cancer.

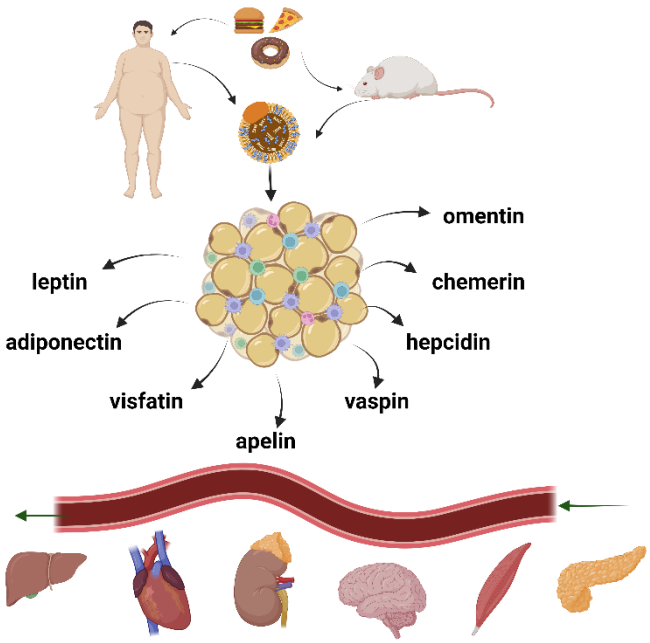

Fig. 1. Schematic representation of the importance of adipose tissue in the regulation of metabolism. The Western lifestyle, characterized by sedentary behaviour coupled with excessive calorie intake, especially from fast and high-fat food, leads to an excess of energy that accumulates in the form of triacylglycerides in adipose tissue. Besides serving as an energy reservoir, adipose tissue secretes numerous factors and hormones with autocrine, paracrine, and endocrine effects, influencing distant tissues such as the liver, heart, kidneys, brain, muscular system, pancreas, among others. In obesity, the storage limit of triacylglycerides can be exceeded, leading to dysregulation of adipose tissue with systemic effects. Additionally, various animal models (rat, mouse, hamster, etc.) have been used in recent decades to better understand the involvement of adipose tissue in obesity and its associated comorbidities.

3. *In vitro* models of adipocytes to study adipose metabolism

3.1. Animal derived cell models

The cellular prototype that characterizes adipose tissue is the mature adipocyte [27]. However, adipose tissue comprises a diverse array of cells in addition of mature adipocytes, including preadipocytes, macrophages, fibroblasts, pericytes, blood cells, endothelial cells, smooth muscle cells, mesenchymal stem cells (MSCs), and adipose precursor stem cells [28]. These cells collectively form what is known as the stromal vascular fraction (SVF). Adipocytes are originated from MSCs present in the SVF, and this differentiation process is known as adipogenesis, which progresses from lipoblasts to preadipocytes and finally to mature adipocytes [29] (Fig. 2). This differentiation process is intricate and involves multiple steps, including the activation of specific adipogenic

transcription factors, including preadipocyte factor-1 (Pref-1) [30], peroxisome proliferator-activated receptor gamma (PPAR-γ) [31], fork-head box O (FOXO) [32], CCAAT/enhancer-binding proteins (C/EBPs) [33], and sterol regulatory element binding protein (SREBP)[34] that drive gene expression and adipocyte development [28]. This multi-step process, although not yet fully understood, was initially described several decades ago using 3T3-L1 cells obtained from disaggregated Swiss mouse embryos and which display a fibroblast-like morphology [35], observing that high insulin concentrations could stimulate adipogenesis.

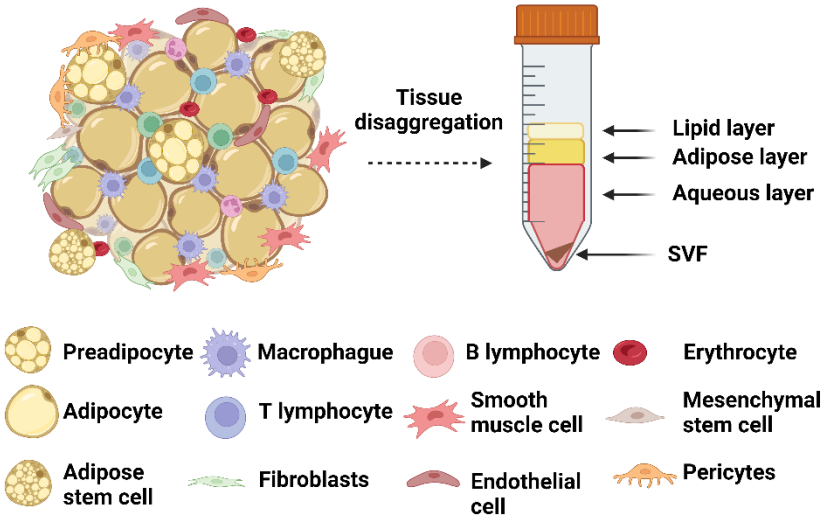

Fig. 2. Adipose tissue is a mixture of different cell types. While the characteristic cell type of adipose tissue is the mature adipocyte (characterized by a single lipid droplet that occupies almost the entire cytoplasm, with the nucleus displaced and very few mitochondria), there is a great cellular diversity that allows adipose tissue to exhibit plasticity in response to various stimuli and situations. This includes cells from the immune system (macrophages, lymphocytes, etc.), muscle cells, endothelial cells, red blood cells, pericytes or fibroblasts, as well as preadipocytes, adipocyte stem cells, or mesenchymal stem cells. This variety of different cell types, in addition to mature adipocytes, can be easily obtained after disaggregation of adipose tissue followed by gentle centrifugation. This process yields a precipitate containing the stromal vascular fraction (SVF), while mature adipocytes are separated into an upper phase (adipose phase). Above, a small lipid phase appears as a result of the release of lipids after the lysis of some mature adipocytes. All this cellular diversity positions adipose tissue as a source for obtaining stem cells, to regulate immune response, and control systemic metabolism.

Over the following years, investigations into the underlying mechanisms of adipogenesis in mouse-derived adipocyte cell lines led to the identification of highly pro-adipogenic compounds, including 1-methyl-3-isobutylxanthine (IBMX)

[36], PPAR-γ agonists (*e.g.*, rosiglitazone) [37], and dexamethasone [38]. In the case of human-derived adipose cell lines, additional pro-adipogenic elements such as triiodothyronine (T_3), transferrin, cortisol, or fetuin were also found to be necessary [39] (Fig. 3).

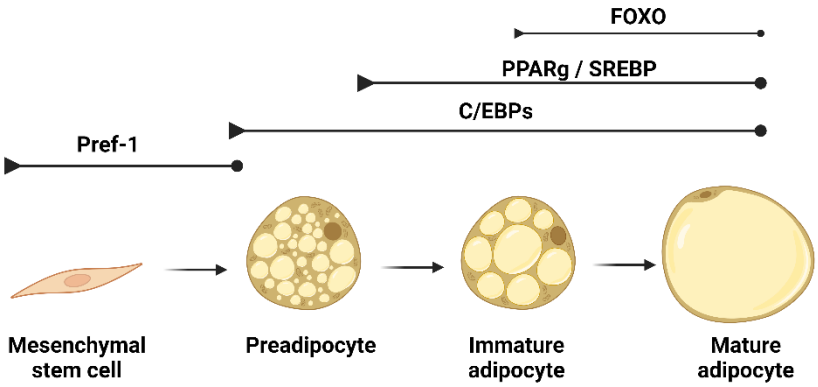

Fig. 3. Adipogenesis process. The differentiation of preadipocytes from mesenchymal stem cells involves a sequential activation of transcription factors, including Pref1, C/EBPs, PPAR-γ, SREBP, and FOXO. This orchestrated process leads to the maturation of adipocytes, regulating adipogenesis and lipid metabolism in a controlled manner. This process that occurs in adipose tissue has been replicated in cellular models for preclinical studies by adding various triggers such as insulin, including 1-methyl-3-isobutylxanthine (IBMX), PPAR-γ agonists, dexamethasone, triiodothyronine (T3), transferrin, cortisol, or fetuin. Pref-1, preadipocyte factor-1; PPAR-γ, peroxisome proliferator-activated receptor gamma; C/EBPs, CCAAT/enhancer-binding proteins; SREBP, sterol regulatory element binding protein; FOXO, fork-head box O.

Taken together, all these years of research on adipocyte cellular models have allowed the establishment of various animal-origin cell lines (mainly rodents, such as 3T3-L1 [35], 3T3-F442A [40], C3H/10T1/2 [41], OP9 [42], OP9-K [43], Ob17 [44]), as well as those derived from mouse embryonic fibroblasts (MEFs) [45] or SVF [46]. Murine preadipocytes have traditionally served as a helpful tool for exploring various facets of adipocyte biology and adipogenesis [28]. Primary animal-derived cell cultures (*e.g.*, SVF) are fibroblast-shaped cells that, under the appropriate conditions, can differentiate into mature adipocytes. Primary cell cultures offer distinct advantages, as they can be derived from different anatomical locations or depots, from animals of varying ages, allowing for the investigation of depot- or age-specific adipogenic or secretory mechanisms. In contrast, preadipocyte cell lines (*e.g.*, 3T3-L1, 3T3-F442A, …) lack the capacity to address these specific aspects [47]. It is worth noting that these animal-derived

primary adipose models present some limitations: *1*) they cannot proliferate in culture; *2*) more difficult to transfect with DNA; *3*) present large TGs stores that disrupt biochemical and microscopy analyses; *4*) they may present some genetic variations among source animals; and *5*) the labour-intensive isolation procedure increase complexity. Furthermore, variances can be observed among preadipocytes from mouse, rat, and other animals [48]. In contrast, animal derived preadipocytes (*e.g.*, 3T3-L1) resolve some of these limitations. These animal derived well-established cell lines offer several advantages, including ease of culture, cost-effectiveness, higher passage tolerance, and homogeneity in the cell population [49]. Consequently, it provides a consistent response to treatments and changes in experimental conditions. These cells lines have been extensively used for assessing the effects of drug compounds and food ingredients on adipogenesis, investigating the molecular mechanisms of adipogenesis, and exploring potential applications for obesity treatment [50]. Additionally, these well-established cell lines are worthwhile for co-culture and 3D-cell studies, as well as high-throughput compound screening [51]. However, these cell lines have some limitations, such as a time-consuming initial subculture process, a minimum two-week requirement for full-adipogenic differentiation [52], a loss of adipogenic potential after extensive passaging [42], and confluent cells are challenging to transfect [42].

3.2. Human derived cell models

While traditional preferences leaned towards animal cell models for adipogenesis studies, human cell models derived from the adipose tissue of patients with specific medical conditions are now gaining prominence in *in vitro* adipose metabolism research [53]. The reason behind this shift is the superior reliability of human cells in reflecting human conditions, particularly obesity and its metabolic complications. These cell models generally start with the isolation of human multipotent adipose derived stem (hMADS) cells obtained from the SVF of subcutaneous adipose tissue [54] to establish different cell lines such as Chub-S7 [55] PAZ6 [56], LiSa-2 [57], Simpson-Golabi-Behmel Syndrome (SGBS) [58]. Human primary preadipocytes offer an exceptional model for investigating adipocyte biology and the alterations related to obesity. They closely resemble the adipose tissue environment, capturing depot-specific characteristics,

including variations in the adipogenic potential of visceral and subcutaneous preadipocytes [28]. These differences may arise from the specific depot of origin. Their ability to reflect the characteristics of their donors makes them valuable for studies examining variations among individuals, such as those related to obesity, weight loss, or age [59,60]. Furthermore, human preadipocytes exhibit the advantage of not requiring extensive *in vitro* proliferation before differentiation. Another noteworthy aspect is their successful differentiation in serum-free conditions, allowing researchers to explore the impact of particular compounds on adipogenesis without interference from highly variable serum components [61]. Despite these merits, human preadipocytes have certain limitations. They are typically available in limited quantities and possess a restricted capacity for renewal [62]. In addition, accessibility to human cell lines remains limited, mainly due to their scarcity in the commercial market and the need for specific material transfer agreements (MTAs) to acquire them. Furthermore, the use of traditional cell cultures in adipose metabolism research has limitations due to the oversimplified environment they provide [28]. Adipose tissue is a complex, 3D-structure, and 2D cultures fail to replicate its physiological conditions accurately, conditions that can be partially addressed in the following models.

3.3. Co-cultures, spheroids, and OoC

Co-culturing adipocytes with other cell types and 3D culture systems represent esteemed tools for unravelling the intricate metabolic interactions between adipose tissue and different tissues (Fig. 4). These research approaches provide a more realistic understanding of the factors and pathways that could be targeted by novel pharmaceutical and food bioactive interventions aimed at addressing obesity and its associated health issues [61]. Many co-culture investigations involving adipocytes and other cell types have primarily concentrated on exploring the connections between obesity, insulin resistance, and inflammation [63]. One way to assess these relationships is by co-culturing macrophages with adipocytes, as the direct cell-to-cell contact can trigger an inflammatory response in the adipocytes [51,64]. The feasibility of co-cultivating mature adipocytes with various cell types, including myocytes [65], endothelial cells [66], neurons [67], colorectal cancer cells [68], among others, has been investigated. These studies observed direct interactions influencing the production of several secretory

factors, cell growth, and cellular development. Recent progress in various technologies, such as biomaterials, stem cell innovations, 3D bioprinting, and microfluidic systems, has significantly enhanced the modelling of adipose tissue, both under normal physiological circumstances and in pathological states [69]. The use of 3D-cell culture models has become increasingly important in studying adipocyte behaviour and function across diverse approaches [70,71] advancing our understanding of the management of obesity. 3D-cell culture involves engineering functional tissues with living cells and biomaterials. Despite its technical challenges, it offers numerous advantages over 2D-cell culture [63], including maintaining cellular morphology, enhancing differentiation, improving cell viability and responsiveness to stimuli, and replicating native-like drug metabolism, gene expression, protein synthesis, and cell proliferation [53,72]. Some 3D models of adipose tissue have been developed using matrix- and scaffold-based constructs, scaffold-free spheroids, microfluidic devices, and OoC systems [73]. These models provide *in vitro* relevance for studying adipogenesis, adipocyte metabolism, obesity, and obesity-related diseases [74]. For example, scaffold-free spheroid cultures closely mimic *in vivo* adipogenesis [75]. Bioprinted 3D adipose tissue models enable research into the crosstalk between adipocytes and cancer cells, impacting tumour growth, invasion, metastasis, and cancer-related pathologies [76]. OoC technology provides a high-throughput platform for pharmacological testing or bioactive compounds studies, and the inquiry of post-translational modifications in a physiologically relevant context [77,78]

Researchers have made significant strides in employing 3D-cell culture techniques to study adipose tissue and its related phenomena. These methods, although technically more challenging, offer numerous advantages compared to traditional 2D-cell cultures [79,80]. They preserve cellular morphology, support well-characterized differentiation, enhance cell viability and reactivity to stimuli, and replicate native-like processes such as drug metabolism, gene expression, protein synthesis, and cell proliferation [81]. In the quest to create more physiologically relevant environments for cell cultures, various strategies have been explored. For instance, a study by Brännmark and colleagues investigated the impact of employing matrices made of aligned and randomly oriented polycaprolactone fibres for populations of adipose-derived stem cells (ASCs)

undergoing adipocyte differentiation [82]. They found that ASCs differentiated on aligned polycaprolactone fibre matrices exhibited an increased level of maturity compared to traditionally cultured cells [82].

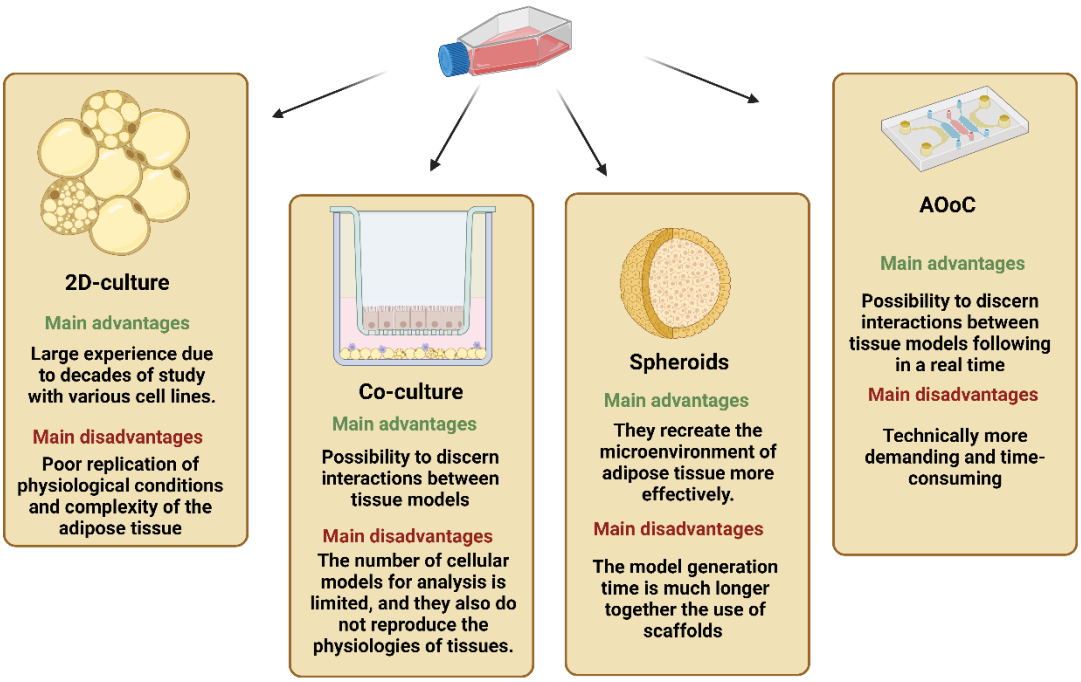

Fig. 4. *In vitro* cell cultures adipose models. The metabolism of adipose tissue has been studied for decades using different *in vitro* cell culture models. Initially, classic two-dimensional (2D) models characterized key factors in regulating major adipose metabolic pathways (*e.g.*, lipogenesis, lipolysis, insulin signaling, …). Subsequently, the introduction of 2D co-cultures, with either adherent or suspended cells, elucidated the relationship between different tissues and mature adipocytes. More recently, the introduction of scaffold-based culture techniques has allowed the generation of adipose spheroid models that more efficiently recreate the three-dimensional microenvironment of adipose tissue. Finally, Adipose-on-a-Chip (AOoC) models have combined these previous techniques on a chip, enabling real-time monitoring of mature adipocyte responses to various stimuli. When integrated into a multi-Organ-on-a-Chip, it allows the assessment of how these responses influence other types of tissues.

Spheroid cultures have emerged as an appreciated technique for differentiating progenitor cells and cultivating differentiated cells within a 3D framework [83]. An adipose spheroid is a cell cluster composed of adipocytes, representing a more physiological model for studying adipose tissue *in vitro* [75]. Adipose spheroids aim to recreate the complex microenvironment where cell-cell and cell-matrix interactions play crucial roles in shaping cellular characteristics like molecular profiles, cell morphology, migration, metabolism, and proliferation [84]. In

contrast, traditional 2D monolayer cultures struggle to capture these interactions accurately [69], prompting the development of 3D cell culture methods. The adoption of 3D methods for primary human cells is a more recent development [85]. These cultures, including spheroid cultures, hollow-fibre bioreactors, and microfluidic OoC systems, are designed to support the maintenance of primary human cells. Adipose spheroid cultures have become a prominent choice for mature human cell culture [86]. In this method, single-cell suspensions are seeded into specialized plates or drops, sometimes incorporating extracellular scaffolds. Over time, these cells aggregate and organize into well-defined spheroidal structures. Compared to traditional 2D cultures, these 3D spheroid scaffolds, containing components from the adipose extracellular matrix, including cellular bioprinting of cell-hydrogel droplets [87], show a greater lipid accumulation (as occurs with 3T3-L1 [88] and 3T3-F442A [89]) and higher expression of specific genes associated with adipocytes [75]. They also exhibit more significant responses to certain stimuli related to lipolysis.

In addition to these methods, various 3D reconstructions of adipose tissue have been attempted using different technologies. Encapsulating hADSCs in hydrogels [90] or fibrin-based constructs [91] has shown promise in maintaining lipid accumulation and the expression of genes associated with adipose tissue. Moreover, hADSC spheroids have been successfully co-cultured with human umbilical vein endothelial cells [92], leading to the formation of vascular structures, which could be beneficial for *in vivo* transplantation and facilitating the development of more complex adipose tissue models. These 3D-spheroid culture systems offer several advantages over traditional 2D cultures. They promote increased differentiation of cells, as evidenced by the expression of adipocyte genes and the formation of unilocular lipid droplets, which are characteristics not typically observed in 2D cultures [71,93]. Consequently, these 3D cultures more closely resemble the function and appearance of adipocytes in their natural environment. They also show promise in efficiently differentiating visceral adipose progenitor cells, a task that proves challenging in 2D culture [94].

The next natural step is to obtain the *in vitro* adipose tissue model in platform, integrating mature adipocytes into a microfluidic device, an adipose OoC (AOoC) [95]. This AOoC system includes chambers connected to a vascular-like

microchannel, safeguarded from shear forces [96]. Customized isolation and injection methods are used to create 3D microtissues with fresh adipocytes in individual chambers, allowing the generation of multiple independent replicate cultures [96]. This approach enables successful monitoring of cell viability, fatty acid metabolism, and drug responsiveness through media analysis and transparent chamber visualization [96]. AOoC has been further improved by seeding several cell cultures into various chip chambers, resulting in the creation of a multicompartment system. Combining multiple systems on a single chip enables the simulation of intricate processes, like the bioaccumulation of hydrophobic chemicals in the compartments of fat cells [95].

The development of beige or brown adipose tissue on a chip, or BAT-on-a-chip, has made use of these similar technologies. Spheroids made from murine brown adipose-derived SVF cells, but not 2D cultures, exhibited robust adipogenic induction of beige/brown biomarkers like uncoupling protein 1 and cell death activator [93]. The creation of a reliable beige/brown adipose 3D model for drug discovery assays is of great importance to biotech and pharmaceutical companies, given the interest in beige/BAT as a target for drug discovery in the context of obesity prevention and treatment [95].

In summary, these 3D adipose tissue models enhance the understanding of adipocyte metabolism, obesity, and related diseases, making them valuable tools for drug testing and research on adipose tissue development and pathologies. However, it is important to note that 3D culture methods are technically more demanding and time-consuming than 2D cultures due to the risk of aggregate damage and the need for costly plates or scaffolds. Recently, genetic model organisms that are not vertebrates, such as nematodes, Zebrafish, and fruit flies, have become significant models for investigating a broad range of human diseases that at least partially addressing some of the weaknesses of *in vitro* cultures.

4. *Caenorhabditis elegans* (*C. elegans*) to study adipose metabolism

C. elegans is a eukaryotic, multi-organ, transparent nematode which feeds on bacteria. It is a small animal with a short lifecycle of 2.5-3 days and a total longevity of around 2–3 weeks at 20ºC. Its genome is completely sequenced,

399 shares 65% of homology with the human genome, and energy-homeostasis
400 related genes are very well conserved [97]. Nematodes are a suitable and
401 versatile *in vivo* model to study the fat metabolism and to identify obesity-
402 promoting or lipid-lowering compounds thanks to its many advantages: they can
403 be manipulated genetically with numerous tools, such as Clustered Regularly
404 Interspaced Short Palindromic Repeats and CRISPR-associated protein 9
405 (CRISPR-Cas9) gene editing and RNA interference (RNAi). It is easy to quantify
406 *C. elegans* lipids by using lipid-specific dyes and *in vivo* fluorescence markers
407 thanks to its transparent condition, which also allows to develop mutant
408 nematodes with reporter genes for a better assessment of gene expression; and
409 last, many mutant strains are available at low cost from the Caenorhabditis
410 Genetic Center (National Institutes of Health (NIH)), and *C. elegans* use for
411 experiments does not require the approval of the Institutional Animal Care and
412 Use Committee [98] (Fig. 5).

413 However, there is also disadvantages with *C. elegans* that the researcher should
414 keep in mind: first, the total lipid species in nematodes remains a mystery to date,
415 although sterols, phospholipids (PLs), and TGs are particularly abundant in
416 nematodes; second, the *C. elegans* lipidome is unique to several lipid classes
417 such as sphingolipids, which brings an inconvenience for the study of the lipid
418 metabolism [99]; and third, despite the nematode genome contains some genes
419 involved in cholesterol synthesis, they seem to not be able to synthesize
420 cholesterol and the nematode growth media (NGM) must be supplemented [100].

421 About nematode's lipid metabolism, they do not have adipose tissue because
422 they store their fat mainly in lipid droplets surrounding the intestine. The lipid
423 droplets are constituted by TGs, sterol esters and delimited by a PLs monolayer
424 [101]. *C. elegans* obtain fatty acids (FAs) from the bacteria in the diet but they
425 have endogenous FAs *de novo* synthesis (lipogenesis). They produce saturated
426 palmitic acid (C16:0) which can be integrated into TGs or PLs, or it can be
427 modified by FAs elongases and desaturases for the synthesis of unsaturated FAs
428 (monounsaturated fatty acids- (MUFA) or polyunsaturated fatty acids (PUFA))
429 [102]. When the utilization of stored triacylglycerols and releasing of free FAs is
430 needed (lipolysis), the nematode uses lipases capable of hydrolysing acyl esters

as the adipose triglyceride lipase (ATGL) and the hormone-sensitive lipase (HSL) [103].

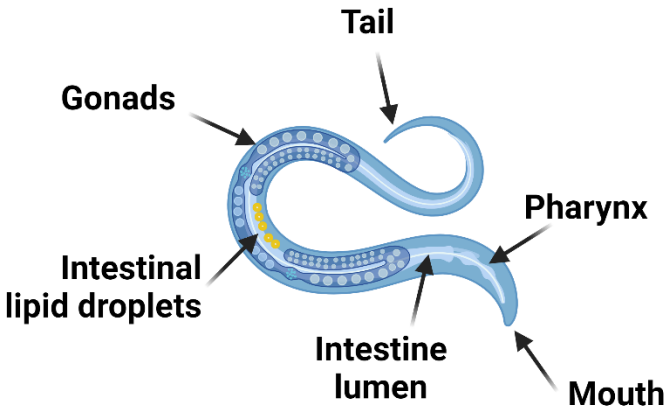

Fig. 5. Anatomical diagram of an adult *Caenorhabditis elegans*. A nematode is depicted, indicating the most important anatomical parts (mouth, tail, pharynx, intestinal lumen, gonads) as well as lipid droplets at the intestinal level.

4.1. *C. elegans* metabolic pathways in fat metabolism

Despite the similarities in fat metabolism between nematodes and mammals, there is some differences that must be underlined. These differences could be both an advantage or a disadvantage depending on the metabolic pathway or substance you are studying.

The insulin/IGF-1 signalling (IIS) pathway is a highly conserved endocrine regulator that controls the accumulation of fat to maintain homeostasis both in *C. elegans* and mammals. Nematodes do not synthesize insulin, but they have 40 insulin-like peptides (INP) that only activate one cell surface transmembrane receptor, DAuer Formation abnormal-2 (DAF-2) [104]. DAF-2 has intrinsic tyrosine kinase activity, whose autophosphorylation leads to the conversion of the membrane phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3) [105], which recruits the downstream effector proteins 3-phosphoinositide-dependent protein kinase-1 (PDK-1), protein kinase B (AKT) kinase 1 (AKT-1) and AKT-2 [106]. These activated serine/threonine kinases can phosphorylate and negatively regulate DAF-16/FoxO which is a member of the FoxO family of Forkhead transcription factors, which are key regulators of growth, metabolism, stress responses, cell cycle control, and longevity in many organisms. DAF-2 deficient *C. elegans* strains are not able to detect INPs and

they have increased fat storage and shifted fat metabolism because part of their nutrient sensing capacity is lacking [102]. Through DAF-16/FoxO many compounds have been identified to regulate the body fat levels. Xiong *et al*. found that Fuzhuan Tea relied on the regulation of the expression of *fat7* by DAF-16/FoxO to reduce the adiposity in *C. elegans* on glucose-rich diets [107]. Moreover, the extract of *Grifola frondosa* also depended on the significant overexpression of DAF-16/FoxO for its fat-reducing activity [108], and a treatment with the flavonoid chrysin improved the lifespan, reduced the fat storage, and promoted DAF-16 expression significantly [109].

Both *C. elegans* and mammals have sensors of intracellular FA levels capable to regulate the lipid metabolism, which are some members of the nuclear hormone receptor (NHR) family of transcriptional regulators. The mammalian genome encodes a total of 48 NHRs, but only the peroxisome proliferator-activated receptors (PPARs) can interact with FAs and other lipids [110]. Peroxisome proliferator-activated receptor alpha (PPARα) stands out as central regulator of energy homeostasis in metabolic tissues, whose activation markedly promotes the uptake, utilization, and breakdown of FAs, leading to the decreased serum levels of TGs and FAs [111]. *C. elegans* contains 284 NHRs but only 15 of which are conserved in mammals, and just NHR-49 is functionally close to PPARα. NHR-49 serves as a key regulator of fat usage, modulating two different metabolic pathways that control the degradation of stored fat and maintain the homeostasis of the saturation of FA [110]. For example, Farias-Pereira et al. demonstrated that isorhamnetin (a flavonol found in dill, kale, and onions) upregulated fat loss through activation of enoyl-CoA hydratase (involved in FA β-oxidation) and ATGL-1 via NHR-49-dependent pathway at transcriptional levels [112]. Similarly, Savova et al. proved that nematodes treated with betulinic acid significantly decreased the lipid accumulation through downregulating key transcription factors, as NHR-49, among other transcriptional effects [113].

Other important transcription factors of lipid metabolism are the sterol regulatory element binding proteins (SREBPs) which are vital for the uptake, biosynthesis, and oxidative catabolism of lipids. In mammals, SREBP-1 preferentially activates the genes of the FA metabolism, while SREBP-2 preferentially activates the genes of the cholesterol metabolism [114]. Nematodes just possess a single

SREBP (SREBP-1c) ortholog, SBP-1, which is expressed in all metabolic tissues, especially in the endodermal fat-storing cells and intestine. Close to the function of SREBP in mammals, SBP-1 is also required for lipid accumulation in *C. elegans,* and it is a potential target for fat reducing interventions. In *sbp1* knockdown worms, the fat storage and body size were significantly reduced, similar to the characteristics of starvation [115].

In contrast with mammals, nematodes do not need essential FAs in diet since they are able to synthesize a wide range of PUFAs. They express the full range of desaturase activities found in plants (Δ12 and ω3 desaturase) and animals (Δ5 and Δ6 desaturase) as well as ω6 and ω3 PUFA elongase activities found in animals [116]. The ability of nematodes to synthesize a kind of ω-3 and 6 desaturases that are not present in most animals is a great advantage to study the relevance of PUFA for the maintenance of some vital functions. The use of knock-out nematodes for *fat3* which encodes ∆6-desaturase, an enzyme essential for long-chain-PUFA (LC-PUFA) biosynthesis, demonstrated that LC-PUFA are essential for normal neurotransmission in nematodes [117], and Guha *et al*. showed that desaturase capacity of *C. elegans* is a versatile tool to uncover new metabolic pathways and the potential effect of unknown PUFA [118].

Despite nematodes do not have a neuro-endocrine signal as complex as mammals for the control of feeding behaviour, the neurotransmitter serotonin (also known as 5-hydroxytryptamine, 5-HT) seems to have a great relevance in the feeding rate and the body fat content of *C. elegans*. Wild-type nematodes treated with 5-HT showed lower body fat content and an increase of the feeding rate in comparison with the control [119]. The neural serotonin-gated chloride channel termed MOD-1 was identified as a key receptor for control of fat loss, with no effect on food intake. In contrast, three G protein-coupled 5-HT receptors, SER-1, SER-5, and SER-7, were found to modulate food intake and pharyngeal activity, independently of any effects on body fat [120]. However, it must be highlighted that these effects of 5-HT in the feeding behaviour and body fat are independent of its other physiological effects, including those on locomotion, and reproduction rate.

As example of 5-HT relevance, Lin *et al*. described that the inactivation of proteases reduces fat accumulation in *C. elegans* through promoting serotonin signalling. Knockdown nematodes for *cpl1* (cathepsin L-like protease, CPL-1) promoted serotonin synthesis and induced body fat loss in *C. elegans* via central serotonin signalling. Then, circulating levels of the proteases cathepsin correlate significantly with body weight increase in obese individuals probably through inactivation of 5-HT fat metabolism effects [121].

4.2. *C. elegans* adiposity models

Many *C. elegans* mutant strains can be used as fat reduction or accumulation model depending on the metabolic pathways you are interested. With the use of targeted gene mutations and gene silencing expression with RNAi, it has been identified approximately 300 gene inactivation that cause fat reduction and approximately 100 gene inactivation that cause fat accumulation (without significant effects on growth and viability) [102]. In addition, these genes are functionally conserved in mammals stating that the findings in this model would be easily extrapolatable to human research. For example, the deletion of *tub1*, which in mammals codifies for the protein *tubby*, broadly expressed in the central nervous system including the hypothalamus, causes fat accumulation in *C. elegans*. This happens because the gene products of *tub1*, in nematodes, are believed to manage lipid accumulation in cooperation with those of ketothiolase 1 (*kat1*, codifies for Acetyl-CoA acetyltransferase homolog), which govern FA β-oxidation [122].

Haerkens *et al*. performed an interesting study which demonstrates the potential of *C. elegans* as high-throughput model organism to study fat metabolism. Using semi-automated techniques with the mutant strain HBC02 (with an NHR-49/PPARα mutation) as nematode “overweight” model and employing Nile Red as probe (a fluorescent dye for lipid droplets), they made a screening of hundreds of substances and drugs with anti-obesity claims approved by the FDA. As a result, they identified just 4 substances that showed significant reduction of fat levels: Midodrine, Vinpocetine, Fenoprofen and Lamivudine [123].

Furthermore, high-fat diets (HFD) or high-carbohydrate diets can be used with nematodes to reproduce obesity conditions, as is made with rodents and other

552 model organisms. Wang *et al*. reported that nematodes convert excess nutrients
553 into fat after high-sucrose treatment (ranging from 5 to 550 mmol/L) and high-
554 stearic acid treatment (ranging from 5 to 600 µg/mL) [124]. In addition, high-
555 carbohydrate diets have many physiological consequences besides increased fat
556 storage since glucose supplementation in the NGM generally increase ROS,
557 worsens pathologies of nematodes mutant strains, and reduce lifespan of
558 nematodes mainly by altering the IIS pathway [125]. For HFD, stearic acid or
559 excess of cholesterol are generally added in the NGM to mimic an obese model
560 [122,124].

561 MUFA and PUFA supplementation is not used for high-fat diets in nematodes
562 because most of the literature reports beneficial effects after its supplementation
563 in *C. elegans* [126]. Among MUFA, oleic acid (OA) is the most abundant FA in *C.*
564 *elegans* [127] and recently, Castillo-Quan *et al*. described antisteatotic effect after
565 OA supplementation in *C. elegans* through activating the transcription factor
566 SKN-1A (which is a homolog of the mammalian Nrf1/NFE2L1; Nuclear factor
567 erythroid 2-related factor 1 / Nuclear factor, erythroid derived 2, like 1) in the
568 endoplasmic reticulum by a lipid homeostasis response, an unexpected
569 mechanism of signal transduction which may mediate some benefits of the OA-
570 rich Mediterranean diet [128]. Also, PUFA supplementation proved anti-aging
571 effects in nematodes [129,130] and even fat reducing properties [131]. However,
572 the exact effect of unsaturated FAs is still controversial since there is also studies
573 reporting harmful effects after nematodes supplementation with MUFA and PUFA
574 [132] and, long-lived strains of *C. elegans* show a reduction of LC-PUFA
575 abundance [133].

576 **5. *Drosophila melanogaster* (*D. melanogaster*) models to study adipose**
577 **metabolism**

578 The extensive genetic toolkit, short generation times, and the capacity to conduct
579 large-scale genetic screens have made *Drosophila* a valuable tool for studying
580 various human diseases [134,135]. *D. melanogaster*, the fruit fly, emerged as a
581 model for studying metabolism and metabolic diseases. *Drosophila* became an
582 obesity research model in the 1960s following W. W. Doane's discovery of the
583 first obese fly mutant in the *adipose* locus [136]. This model is particularly useful

584 due to its structural and functional similarities to humans in terms of organs,
585 tissues, and biological systems and disease related genes [137] (Fig. 6).
586 Researchers have harnessed the unique advantages of *Drosophila* to delve into
587 the intricate world of obesity and its associated complications. By subjecting
588 these tiny insects to excessive calorie intake similar to the conditions that lead to
589 human obesity with high sugar diet (HSD) [138] and HFD [139]) it has been
590 possible to replicate various aspects of obesity-related disorders [140,141]. When
591 exposed to these diets, *Drosophila* exhibits conditions that closely resemble
592 human hyperglycaemia, insulin resistance [142], and cardiomyopathy [143]
593 making this model a powerful tool to go insight into the effects of various dietary
594 components on metabolic health, particularly the impact of excessive sugar and
595 fat consumption [144]. In addition, genetic interventions can also trigger obesity
596 in flies, separating fat accumulation from alterations in blood sugar levels and
597 carbohydrate processing [145]. One of the key advantages of using *Drosophila*
598 in obesity research is the high degree of genetic conservation between fruit flies
599 and humans, because around of 75% of all known human disease-related genes
600 are conserved in flies [137], which expands its utility in disease research. This
601 genetic similarity allows researchers to explore the underlying genetic factors and
602 molecular mechanisms contributing to metabolic disorders associated with
603 obesity [146]. A major strength of Drosophila is the vast collection of genetic tools
604 that can be used for designing genetic screens to identify genes necessary for
605 controlling obesity. For example, in a genome wide RNAi screening, circa 500
606 obesity genes were identified. From these, some altered obesity and lean
607 phenotypes, and included the *Drosophila* orthologs of glucagon, insulin and TOR
608 signalling pathways [147]. Remarkably, 62% of these obesity genes have human
609 orthologs. Moreover, novel functions were identified such as hedgehog signalling,
610 typically involved in development, implication in white/brown adipocyte
611 determination.

612 TGs are the main lipid storage form in *Drosophila,* as occurs in humans [148].
613 TGs are a fundamental measure of obesity, and *Drosophila* researchers have
614 developed various methods for quantifying TG levels in these insects. Techniques
615 such as lipid extraction, thin-layer chromatography [149], mass spectrometry
616 [150], and enzymatic assays [151] have enabled the precise measurement of

TGs, aiding in the assessment of obesity in *Drosophila* models. The obesity models developed in *Drosophila* closely replicate the pathophysiology of human obesity, encompassing hyperglycaemia, insulin resistance, cardiac complications, reduced lifespan, and even nephrosis [148]. These parallelisms provide researchers with a unique opportunity to study the progression and interplay of these disorders in a highly tractable system. In addition to its genetic advantages, *Drosophila* exhibits similarities to mammals in terms of energy metabolism [152]. Nutrient absorption through the intestine and the role of skeletal muscles in voluntary energy expenditure are shared features [153]. *Drosophila*'s fat body, a key organ in energy storage and metabolism, serves a dual purpose like the functions of human white adipose tissue and the liver [154]. It stores nutrients such as glycogen and TGs, regulates systemic growth, and influences metabolic state through insulin and TOR signalling pathway. The hormonal regulation of energy metabolism in *Drosophila* involves insulin-like peptides (dILPs) [155] and the glucagon-like adipokinetic hormone (AKH) [156]. While *Drosophila* shares these critical components with mammals, there are also notable differences [146]. In *Drosophila*, nutrients and oxygen are delivered separately through an open circulatory system and a tracheal network, respectively [157]. This distinction adds an extra layer of complexity to the metabolic regulation in *Drosophila*, making it an intriguing model system.

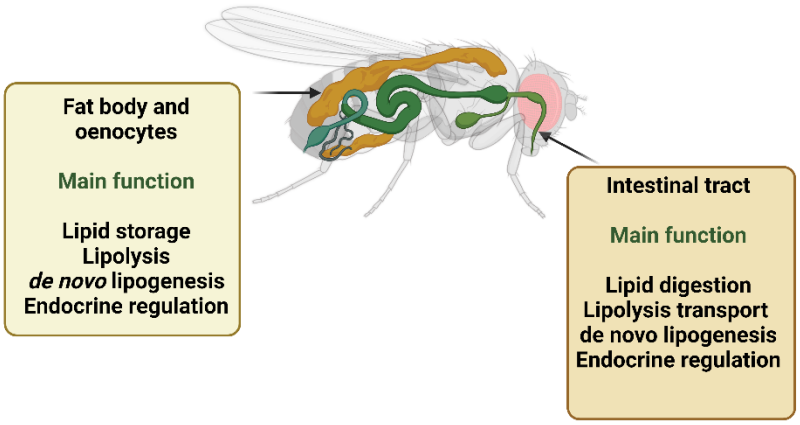

Fig. 6. Anatomical diagram of an adult *Drosophila melanogaster* highlighting the tissues and organs related with the lipid metabolism. The fruit fly centralizes lipid metabolism in the fat body and oenocytes, as well as in the intestinal tract. The main functions of the fat body and oenocytes focus on regulating lipid accumulation and release, similar to human adipose tissue. They also play a crucial role in regulating other tissues and organs through the release of various

endocrine factors. On the other hand, the intestinal tract is more involved in digesting and transporting lipids from the diet, although it is also implicated in de novo lipid generation through de novo lipogenesis. Similar to the fat body, the intestinal tract also performs an endocrine function by releasing different factors and hormones that regulate the metabolism of distant organs (Adapted from [158]).

The fat body in *Drosophila* plays a pivotal role in nutrient storage and energy regulation [159]. It stores glycogen and triglycerides in response to food intake, much like the functions of the mammalian liver and adipose tissue [160]. The breakdown of carbohydrates into glycogen [161] or trehalose [162] and the absorption, transport, and storage of fats in lipid droplets, mainly within the fat body, are crucial metabolic processes. Additionally, the fat body influences nutrient availability, energy storage, and expenditure. For instance, the secretion of cytokines such as Unpaired 2 (Upd2), a member of the Interleukin-6 family of cytokines, in response to feeding influences the systemic metabolic state by impacting dILP secretion [163]. High-sugar diets trigger the release of Neural Lazarillo (NLaz) from the fat body [164], leading to insulin resistance, which mirrors certain aspects of human metabolic disorders. The *Drosophila* fat body, specifically dILP6, promotes lipid uptake in oenocytes during starvation, ensuring coordinated lipid turnover [165]. Oenocytes, located dorsally in the adult abdomen of *Drosophila*, serve as lipid reservoirs, particularly during periods of starvation [165], expressing genes related to various aspects of liver function, including fatty acid metabolism, peroxisomal β-oxidation, ketogenesis, reactive oxygen species metabolism, and xenobiotic metabolism [166,167]. Thus, oenocytes function analogously to mammalian hepatocytes and are essential for larval growth, pupal development, and adult resistance to starvation [168].

To sum up, *Drosophila melanogaster* has become a genetic tool to get insights into adipose metabolism due to its conservation with humans, enabling the study of genetic factors and molecular mechanisms in the context of adipose metabolism. However, its smaller size and differences in some metabolic processes limit direct translation to human physiology.

6. *Danio rerio* (*D. rerio*) models to study adipose metabolism

Zebrafish (*Danio rerio*) is a freshwater fish, traditionally used in ecotoxicology and developmental biology studies. Since its introduction as a modern experimental

model organism, many new tools have been developed for imaging and manipulating metabolic processes in Zebrafish. Given the high genomic and molecular similarities between Zebrafish and other vertebrates including humans, many of the important discoveries in zebrafish are applicable to humans [169].

The key organs that are important for regulation of energy homeostasis and metabolism in mammals are also present in Zebrafish, including digestive organs, adipose tissues, and skeletal muscle (Fig. 7). They have WAT similar as mammals, which can be stored as lipid droplets in visceral, intramuscular, and subcutaneous adipocyte depots. However, Zebrafish is an ectotherm specie, and its metabolic rate is not regulated by environmental temperature, therefore, Zebrafish larvae do not have brown adipocyte tissue [170].

Furthermore, relevant functions in fat metabolism such as appetite regulation, insulin regulation and lipid storage are also well conserved in Zebrafish [171]. Excess of nutrients in Zebrafish causes increased plasma triglyceride levels and hepatic steatosis, and obese Zebrafish also exhibit dysregulation of pathways that control lipid metabolism, including SREBP-1 and PPARs [172].

6.1. Zebrafish adiposity models

Obese Zebrafish can be conveniently produced by overfeeding starting from the onset of feeding at 5-day post-fertilization. This is advantageous over rodent models since diet can only be manipulated after weaning, which is at least 3 weeks after birth. Although early fish larvae have no WAT, lipid droplets accumulate in the blood stream and measures such as whole-larval TG level may be used as indicator for quantifying obesity progression [173]. HFD rapidly increases Zebrafish adiposity and chicken egg yolk supplementation is the most used HFD for Zebrafish larvae and juveniles [174,175].

Adult Zebrafish have also been used as an obesity model, since the first diet-induced obese Zebrafish model in 2010 [172]. Meguro *et al*. developed custom high-fat Zebrafish diets containing 20% corn oil or lard, and they demonstrated that these HFD make zebrafish obese [176]. Since then, slightly different protocols of HFDs has been used to study the effects on the fish: Arias-Jayo *et al*. found that a diet enriched with 10% of fat changes the intestinal microbial community composition of Zebrafish, which was correlated with low-grade

inflammation [177]; Carnovali *et al*. prove that an imbalance of fat metabolism generated an osteoporosis-like phenotype in adult Zebrafish after a standard diet with a 20% lard surplus [178]; and, also a standard diet with 20% of lard surplus produced cognitive function impairment and changes in neuronal gene expression in Zebrafish adults [179]. Moreover, Li *et al*. were recently able to establish a non-alcoholic fatty liver disease (NAFLD) model through diet after feeding adult Zebrafish for 30 consecutive days [180].

It is interesting to note that obesity induced by overfeeding normal fat diet differs from that induced by high fat diet. Landgraf *et al*. compared the metabolic phenotype of obesity induced by overfeeding of a normal fat diet (NFD; fish-food, Artemia cysts) to that by HFD (egg yolk powder). Although both increase adiposity, fish with NFD-induced obesity are metabolically healthy. In contrast, fish with HFD-induced obesity are metabolically unhealthy, with glucose intolerance, fatty liver, and preferential increase of visceral fat [181]. This phenomenon correlates with what is observed in humans, which is thought to be due to an increase in visceral fat accumulation instead of subcutaneous fat storage [182].

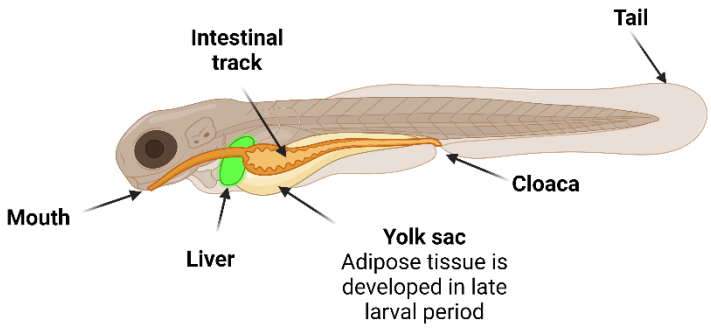

Figure 7. Anatomical diagram of a *Danio rerio* larvae highlighting the tissues and organs related with the lipid metabolism. In Zebrafish larvae, lipid metabolism primarily involves the liver, which serves as a major site for lipid synthesis, storage, and regulation. The intestine plays a crucial role in lipid digestion and absorption from the diet. Adipose tissue contributes to lipid storage, while other tissues, such as muscle and brain, are involved in lipid utilization and energy homeostasis, ensuring overall metabolic balance.

Multiple mutant lines of Zebrafish have been identified to study the genes and pathways contributing to lipid metabolism and adipose tissue regulation. These mutants are often identified because they have fatty liver at larval stages, or

because they have increased adiposity during adulthood [170]. There is some mutant fish mimicking non-alcoholic fatty liver disease (NAFLD) symptoms through different mechanisms. For example, some years ago a mutant named *foie gras* (*foigr*) [183] was identified because it spontaneously developed steatosis due to a mutation in transport protein particle 11 (*trappc11*), a gene which functions in vesicle trafficking and is also implicated in human liver disease [184]. More recently, Shihana *et al*. found that the depletion of patatin-like phospholipase domain containing 3 (*pnpla3*), fatty acid synthase-associated factor 2 (*faf2*) and transmembrane 6 superfamily member 2 (*tm6sf2*) gene expression in larvae through CRISPR-Cas9 knockdown, significantly increased the effects of ethanol (2%) and HFD toxicity by increasing hepatic steatosis and hepatic neutrophil recruitment, proving these genes could be implicated in NAFLD prevention [185]. About mutants with alterations in adipose tissue there are also examples as the mutant Zebrafish known as *vizzini*, which exhibits extremely large subcutaneous lipid droplets although the number of lipid droplets in adipocytes is unchanged. This fat distribution is due to a mutation in growth hormone (GH) 1 gene (*gh1*) leading to a premature stop codon. The phenotype is consistent with GH deficient mice and humans that develop enlarged volume of subcutaneous adipose tissue [186]. In the case of visceral fat, the loss of *plexin d1* function causes a reduction in visceral fat in Zebrafish due to a decrease of lipid droplets size and adipocyte hyperplasia. Consequently, with high fat diet, the mutants preferentially store lipid in subcutaneous adipose tissue and are protected from developing insulin resistance. Despite PLEXIN D1 was identified in humans, the exact mechanism through this gene controls visceral fat was unknown since studies with Zebrafish were developed [187].

Furthermore, the transparent condition of Zebrafish larvae allows the creation of transgenic fishes that can express fluorescent proteins in specific cell types, which are then easily detected and enable monitoring of obesity development and progression [188]. For example, Brun *et al*. studied the energy metabolism disruption using a mutant fish to quantify the expression of the gen protein kinase C1 (*pkc1*); a transgenic (Tg) Zebrafish line where the promoter region of the pck1 gene is driving the expression of the Luciferase 2 (Luc2) reporter gene [189]. However, not all lipid metabolism genes are conserved in sequence and function

in Zebrafish. For example, the leptin protein of Zebrafish is only 19% identical to the human protein. Unlike mammals, leptin, and leptin receptor are not expressed in adipose tissue in Zebrafish. Leptin receptor-deficient Zebrafish primarily have disrupted glucose homeostasis, which is different from phenotypes observed in mammals such as hyperphagia and morbid obesity [190].

To sum up, all these examples of Zebrafish models illustrate the utility of this whole-organism model for mechanistic investigations, drug testing and discovery, as well as food bioactive compounds effects in obesity and lipid metabolism. The power of this model remains largely untapped, and undoubtedly, more mechanistic discoveries of fat metabolism will be made from Zebrafish models.

7. Conclusions

This comprehensive review focus on the diverse array of models available to probe the intricate processes of adipogenesis and adipocyte differentiation *in vitro* and in lower organisms, particularly concerning obesity and adipocyte dysfunction. Exploring and developing diverse adipose tissue models is imperative to bridge the gap between traditional animal studies and human biology. To understand the complexities of obesity and its associated comorbidities, it is essential to have models that closely resemble the human adipose tissue microenvironment. Human-relevant models enable the investigation of diverse situations occurring in obesity, from adipocyte differentiation to metabolic alterations and interactions between adipocytes and surrounding cells. With the rise of 3D cultures, spheroids, OoC platforms, and lower organisms' models, researchers will be able to dissect the cellular, genetic, and molecular basis of obesity. This will be the starting point to recreate the intricate conditions of human adipose tissue, shedding light on the underlying mechanisms of obesity-related health issues and other diseases. These advanced models offer greater precision, contributing to more effective therapeutic strategies and a deeper understanding of this global health challenge.

CRediT authorship contribution statement

Ignasi Mora: Writing – review & editing. Francesc Puiggròs: Writing – review & editing. Florenci Serra: Writing – review & editing. Katherine Gil-Cardoso: Writing

– review & editing. Xavier Escoté: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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