Origin and Development of the Adipose Tissue, a Key Organ in Physiology and Disease

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Adipose tissue is a dynamic organ, well known for its function in energy storage and mobilization according to nutrient availability and body needs, in charge of keeping the energetic balance of the organism. During the last decades, adipose tissue has emerged as the largest endocrine organ in the human body, being able to secrete hormones as well as inflammatory molecules and having an important impact in multiple processes such as adipogenesis, metabolism and chronic inflammation. However, the cellular progenitors, development, homeostasis and metabolism of the different types of adipose tissue are not fully known. During the last decade, Drosophila melanogaster has demonstrated to be an excellent model to tackle some of the open questions in the field of metabolism and development of endocrine/metabolic organs. Discoveries ranged from new hormones regulating obesity to subcellular mechanisms that regulate lipogenesis and lipolysis. Here, we review the available evidences on the development, types and functions of adipose tissue in Drosophila and identify some gaps for future research. This may help to understand the cellular and molecular mechanism underlying the pathophysiology of this fascinating key tissue, contributing to establish this organ as a therapeutic target.

Keywords: WAT, BAT, beige adipocytes, adipose tissue, adipose stem cells, drosophila, fat body development, adipithelial cells

INTRODUCTION

In this manuscript we review the past and recent literature on the origin, development, types and function of mammalian adipose tissue and put it in relation to physiological and disease conditions such as obesity, diabetes, lipodystrophies or cancer-associated cachexia. We identify the gaps that need to be addressed regarding the origin and development of this tissue and propose Drosophila as a suitable model organism to explore those open questions. We review the existing evidences on the origin, development and function of the adipose tissue (AT) of this organism, making a clear distinction between the embryonic and larval stages and the adulthood, when the developmental programmes are finished. As we considered there are two different scenarios where play different actors and also where same actors could play different roles. We also review the available studies in Drosophila on the above-mentioned metabolic diseases.

MAMMALIAN ADIPOSE TISSUE AND ASSOCIATED DISEASES

The lifestyle of developed countries, where population have an easy access to high caloric food and decreased physical exercise, has played important roles into the rise of obesity and Type 2 Diabetes
Mellitus (T2DM) to the category of pandemics (Doria et al., 2008; Guilherme et al., 2008; Saltiel, 2012). Global prevalence of overweight and obesity combined has risen by 27.5% for adults and 47.1% for children between 1980 and 2013 (Ng et al., 2014). Furthermore, excess body weight is one of the major risk factors contributing to the global incidence of disease worldwide. According to the International Diabetes Federation (IDF), more than 371 million people across the globe have diabetes and this number is predicted to rise to over 550 million by 2030. Adipose tissue (AT) plays also important roles in other diseases. For instance, lipid storages at AT are susceptible to be wasted by tumour-secreted molecules, a fact known as cancer-associated cachexia (Rydén and Arner, 2007; Tsoli et al., 2016). This process is featured by increased systemic inflammation, general metabolic dysfunction, and elevated resting energy expenditure (Fearon et al., 2011). Cachexia affects 50–80% of cancer patients and accounts for up to 20% of cancer deaths. It is estimated that death normally ensues when weight loss exceeds 30–40% (Arthur et al., 2014). In this scenario, the understanding of adipocytes’ development will improve our knowledge on metabolic diseases such as obesity and T2DM, as well as on anomalous metabolic states that lead to chronic inflammation. (Stephens, 2012; Wu et al., 2013; Hotamisligil, 2017). Understanding in deep the biology of the AT will allow the development of potential therapies targeting thermogenesis as a means of increasing energy expenditure.

The Adipose Tissue as a Regulator of Energy Homeostasis in Mammals

Energy is fundamental for life. Therefore, storage and homeostasis of energy are key processes for any organism. In the mammalian body, including that of humans, the energy that is neither consumed nor converted into glycogen is stored in form of neutral lipids in the adipocytes of the AT, more specifically in the lipid droplets (LDs). The LDs are key organelles controlling fat storage and mobilization (Olofsson et al., 2008; Beller et al., 2010). They consist of a core of neutral lipids (triglyceride and cholesterol esters) surrounded by a monolayer of phospholipid and cholesterol in which several proteins are embedded (Thiam and Beller, 2017).

Importantly, the AT is a plastic organ able to adapt to different physiological circumstances to ensure energy distribution among the different needs: metabolism, thermogenesis and lactation (Cinti, 2018). AT can grow by either increasing the number of adipocytes, which depends on adipocyte stem cells (ASC) or increasing the LDs size. In fat oxidizing tissues, LDs expansion is supported by specific mitochondria, known as peridroplet mitochondria. Those remain bound to the LD even after the homogenization the tissue and show specific features such as enhanced bioenergetic capacity, reduced β-octidation capacity, supported LD expansion by providing ATP for triacylglycerides (TAG) synthesis, and maintenance of a distinct protein composition due to low fusion-fission dynamics (Benador et al., 2018).

In mammals there are different types of ATs: white AT (WAT), designed for energy storage, and brown AT (BAT), intended to dissipate energy and generate heat (Berry et al., 2013; Gupta, 2014). WAT is classified in subcutaneous or visceral WAT, depending on its anatomical location (Cleal et al., 2017). The two types of WATs have distinct developmental timing, microscopic appearance, molecular signature and certainly biological function. Subcutaneous WAT may protect against certain aspects of metabolic dysfunction (Snijder et al., 2003a; Snijder et al., 2003b). Visceral WAT is associated with metabolic complications and appears to increase the risk of T2DM, hyperlipidemia and cardiovascular disease (Grauer et al., 1984). Increasing number of evidences converge on to the idea that within mammalian bodies there are different AT depots, which present a vast heterogeneity among them, and contain the ASC that support their homeostasis (Cleal et al., 2017).

Until recently, the functions of the BAT were associated to the neonatal period (Novak et al., 1971) and the scientific community thought that this type of fat was not present during the adulthood. A bit more a decade ago, BAT was identified in adult humans and it was found to be reduced, on mass and activity, in obese and diabetic patients (Cypess et al., 2009; Cypess et al., 2013; Lidell et al., 2013; Alcalá et al., 2019). This finding pointed out to the idea of enhancement of BAT preadipocyte differentiation and proliferation as a therapeutic strategy to fight obesity (Alcalá et al., 2019). Later evidences converged on to the idea that adult human BAT shares molecular characteristics with murine “beige” cells rather than classical brown cells (Wu et al., 2012; Cannon et al., 2020).

“Beige” or “brite” adipocytes, also known as Beige Adipose Tissue (BeAT), are energy-burning adipocytes, with “brown-like’ features, such as increased mitochondrial content, multilocular storing of LDs, and the ability to burn off lipids as heat (Ikedo et al., 2018). BeAT is found in different spots of the adult body within the WAT (Cypess et al., 2009; Cypess et al., 2013; Lidell et al., 2013; Wu et al., 2012; Whittle et al., 2011). Although beige adipocytes share some markers with brown adipocytes, they also show specific markers different from those of both brown and white adipocytes (Table 1) (Sharp et al., 2012; Waldén et al., 2012; Ussar et al., 2014).

In spite of their distinct functions, WAT and “Beige AT” (BeAT) share the ability for reciprocal, reversible transdifferentiation to tackle special physiologic needs. Thus, chronic need for thermogenesis induces browning and chronic

| TABLE 1 | Factors expressed in differentiated adipocytes. |
|---|---|
| Marker | Adipocyte type |
| LEP, ASC1 | White |
| TEMEM26, HOXC9, TBX1 | Beige |
| UCP1, PRDM16, P2RX5 | Beige and brown |
| ZIC1, Lhx8 | Brown |
| ADIPQ | White, beige and brown |

**Abbreviations**: ADIPQ, adiponectin; ASC1, asc-type amino acid transporter 1; HOXC9, homeobox C9; LEP, leptine; Lhx8, LIM homebox 8; P2RX5, purinergic Receptor P2X; ligand-gated ion channel 5; PRDM16, PR domain containing 16; TEMEM26, transmembrane protein 26; TBX1, t-box 1; UCP1, uncoupling protein 1; ZIC1, zinc finger protein of the cerebellum 1.
positive energy balance induces whitening (Cohen and Spiegelman, 2016; Cinti, 2018). Different signals, after birth and adult state, will determine BeAT differentiation. While Platelet activating factor and Interleukin 6 (IL-6) are determining factors in BeAT development after birth, β-adrenergic stimulation and IL-4 are active during adulthood (Chung et al., 2017; Finlin et al., 2017; Babaei et al., 2018; Yu et al., 2019; Hoang et al., 2021). Interestingly, a switch from BeAT to WAT underlies cancer-associated cachexia, triggered by the parathyroid-hormone-related protein (Kir et al., 2014; Petruzzelli and Wagner, 2016).

The Adipose Tissue in Signalling and Inflammation in Mammals

AT is a large endocrine organ, insulin sensitive, that secretes around 600 different adipokines, the hormones that act on distant organs (Table 2) (Lehr et al., 2012a; Cinti, 2018) as well as a vast diversity of other signalling molecules, such as metabolites, lipids, non-coding RNAs or extracellular vesicles (Funcke and Scherer, 2019). Leptin and adiponectin are well known hormones secreted by adipocytes (Cinti, 2018). Leptin inhibits appetite, stimulate thermogenesis, enhance fatty acid oxidation, decrease glucose, and reduce body weight and fat. Adiponectin mediates the insulin-sensitizing effect (Yadav et al., 2013). Omentin, secreted by non-adipocyte cells in the AT, acts as insulin-sensitizing factor and it has been reported to have anti-inflammatory, anti-atherogenic and anti-cardiovascular disease properties (Tan et al., 2010). Dipeptidyl peptidase IV, secreted by visceral white adipocytes from obese individuals, seems to be associated to insulin-resistance development (Lamers et al., 2011; Lehr et al., 2012b).

AT is not only composed by adipocytes but also comprises as well other cell types such as preadipocytes, fibroblasts, stromal cells, T-cells, granulocytes, macrophages and monocytes (Hotamisligil, 2017). During the last years, several adipokines and cytokines secreted by AT and also other molecules of signalling pathways linking AT metabolism and immune system, have been identified. For example resistin, a hormone secreted by macrophages M1, pro-inflammatory phenotype, that infiltrate obese adipocytes, produces a notable effect on systemic metabolism by acting as a crosstalk between obesity-
inflammation and metabolic diseases (Schwartz and Lazar, 2011). Likewise, the dysfunction of AT is associated with the secretion of multiple molecules that mediate the inflammatory response. In fact, stressed adipocytes from obese ATs activate the inflammasome system, which could induce a chronic low-grade inflammation. Inflammation appears in other tissues besides AT, including brain, liver, airways and pancreatic islets. This inflammatory state is known as “metaflammation” because it contributes to several immunometabolic diseases, including T2DM, cardiovascular disease, asthma, neurodegenerative disease, cancer and lipodystrophies (Hotamisligil, 2017; Cinti, 2018).

The Origin and Development of the Mammalian Adipose Tissue

A great number of studies, most of them carried out in vitro, shed light on the factors involved in differentiation of white, brown and beige adipocytes, such as peroxisome proliferator-activated receptor gamma (PPARγ), CCAAT/enhancer binding proteins (C/EBP), krupper-like factors (KLFs), peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α) or nuclear factor I A (NFIA) (reviewed at (Hiraike et al., 2017; Cinti, 2018; Hiraike et al., 2020). However, the initial commitment of mesenchymal progenitors to the adipocyte lineage remains less explored (Billon et al., 2007).

A comprehensive understanding of the origin of white/brown/beige adipocytes differentiation is of upmost interest given the potential to induce browning AT in obese patients (Cypess and Kahn, 2010). It is accepted that animals with more BAT are more resistant not only to obesity but also to T2DM (Kopecký et al., 1996; Collins et al., 1997; Guerra et al., 1998; Almind et al., 2007). Conversely, animals without functional BAT are prone to obesity and T2DM (Lowell et al., 1993; Bachman et al., 2002; Feldmann et al., 2009). Attempts to block lipid storage or inhibiting WAT development failed, as several studies have shown that this strategy drives fat accumulation in organs not specialized for fat storage. This condition is known as “ectopic lipid” and has been associated with insulin resistance and the development of T2DM (Gastaldelli, 2011).

BAT, WAT and BeAT can develop from both neural crest and mesoderm, specifically BAT develops from paraxial mesoderm and WAT and Beige AT does from lateral plate mesoderm (Billon et al., 2007). Periaortic arch adipose tissue (PAAT), which is composed of both BAT and WAT, is derived from multiple cell lines, being neural crest cells the main contributors (Fu et al., 2019). Furthermore, brown adipocytes could arise from Myogenic Factor 5 (MYF5)-expressing myogenic precursor cells by the action of PRDM16 (PRD1-BFI-RIZ1 homologous domain containing 16). PRDM16 controls a bidirectional cell fate switch between skeletal myoblasts and BeAT cells and it is also responsible for beige cells found within WAT depots (Seale et al., 2008; Enerbäck, 2009; Lshibashi and Seale, 2010; Petrovic et al., 2010; Seale et al., 2011). PRDM16 gene expression is downregulated by miR-149-3p during fasting conditions allowing the switch from subcutaneous to visceral AT (Ding et al., 2016).

The specific origin of the beige adipocytes remains to be clarified. Some evidences support the idea that they arise from unique precursor cells (Wu et al., 2012), but others suggest that they may arise from white adipocytes in a process referred to as transdifferentiation (Cinti, 2012; Cinti, 2018).

Moreover, developed spots of both BAT and WAT present a remarkable adipocytes’ heterogeneity. Low- and high-thermogenic brown adipocytes with distinct features and functions coexist in BAT. Low-thermogenic brown adipocytes, unlike the high-thermogenic ones, show low Ucp1 and Adipoq expression, larger lipid droplets, lower mitochondrial content and are functionally specialized in fatty acid uptake (Song et al., 2020). Similarly, functional heterogeneity is found when comparing subcutaneous and omental preadipocytes, which show distinct capacities for replication, adipogenesis and apoptosis (Tchkonia et al., 2005).

To study in deep all aspects of adipocyte biology is required to know the molecular properties of adipose precursor cells and the development of adipocytes. The identification and characterization of the ASC population is fundamental to understand AT development, formation and maintenance.

Equally important is to study how the ASC contribute to the homeostasis and maintenance of the AT under both normal energy intake and excess nutrient load. In the same way, it would be critical to know the growth factors and developmental signalling pathways altering ASC behaviour and adipocyte formation. Furthermore, although some factors determining fat cells fate has been described, most of these studies have been carried out in vitro and their role in vivo has not been explored (Berry et al., 2013; Gupta, 2014; Jung et al., 2019).

Open Questions on the Origin and Function of the Adipose Tissue

One of the most important open questions is the origin of the AT: which factor(s) are required for the specification of the AT primordium, how proliferation of ASC is regulated and how ASC differentiate into adipocytes. The identification and characterization of the ASC population is fundamental to understand AT development, formation and maintenance.

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Last but not least, the growing number of functions in which the mammalian AT is involved require further studies, being the use of model systems an important tool to be exploited (Hotamisligil, 2017).

Drosophila as a Model to Study Adipose Tissue

The ability to store nutrients, mainly in form of TAG, is conserved from yeast to human (Kadereit et al., 2008) (Figure 1). Even though a better knowledge on AT from an evolutionary perspective could improve our understanding on the development, function and dysfunction of this organ, this theme...
remain as a neglected enigma (Ottaviani et al., 2011). TAG synthesis and lipolysis related genes are already present in unicellular organisms such as Saccharomyces cerevisiae or Candida parapsilosis (Zweytick et al., 2000; Neugnot et al., 2002; Daum et al., 2007). Caenorhabditis elegans also stores TAG in form of LDs in the intestinal epithelial cells (Watts, 2009).

The ability to, not only store fat, but also to secrete endocrine factors is already present in molluscs. Haliothis fulgens or Helix aspersa store TAG in the midintestinal gland known as hepatopancreas. This also secretes a glucose lowering level hormone, Phe-Met-Arg-Phe-amide (FMRFa), belonging to the evolutionarily conserved RF-amide neuropeptide family (Rösser and Kiss-Tóth, 2014). Neuropeptide FF (NPFF) an anorexigenic peptide, also member of this family, is critical to keep a basal NPY gene expression at arcuate nucleus and promote diet-induced thermogenesis, coupling energy homeostasis with energy partitioning to AT and bone tissue (Zhang et al., 2018). Moreover, NPFF is able to promote macrophage M2, anti-inflammatory phenotype, activation and increase the proliferation of murine and human adipose tissue macrophages (Waqas et al., 2017). Latter in evolution, the AT, in addition to serve as a fat storage and endocrine tissue, is also involved in immunity, as is the case of insects. The lipid storage tissue in insects is known as fat body (FB), which is an endocrine-secreting organ involved in nutrient sensing, development, metabolism, immunity and reproduction (Doane, 1960; Dean et al., 1985; Charroux and Royet, 2010).

WAT is present in most of vertebrate taxa, including fish, amphibian, reptiles and mammals (Gesta et al., 2007; Todorčević et al., 2009; Imrie and Sadler, 2010). Lampreys, at the base of vertebrates’ evolution, present fat cells with similar morphological characteristic to white and brown adipocytes (Müller, 1968), which is in conflict with the generally accepted idea about BAT is not present in cold-blooded vertebrates.

BAT is larger in small mammals, such as mouse, than in human. A recent debate points out to mice BAT, rather than the human adult BAT, as a classic defined BAT and, therefore, considers mouse the best model to study the development of this tissue (Cannon et al., 2020).

However, considering the complexity of the AT in mammals, a simpler model than mouse is required to study the development and determination of this tissue. A model organism that allows performing genetics analysis in vivo would be suitable to address those open questions. In this regard, Drosophila represents a good model for the study of AT based on its genetics accessibility, the lower complexity of the AT and the functional conservation of this tissue along evolution.

Drosophila melanogaster has been used for more than 100 years to study conserved biological processes and decipher the molecular and genetic basis of multicellular organisms, as well as a vast number of human diseases (Yamaguchi and Yoshida, 2018). Several studies have established Drosophila as a model to study obesity and metabolic diseases [reviewed at (Musselman et al., 2011; Pasco and Léopold, 2012; Na et al., 2013)]. Important, molecules and signalling pathways involved in the regulation of metabolism and physiology of the AT in mammals are conserved in Drosophila. For instance, Drosophila insulin/insulin like growth factor signalling (IIS) acts as a conserved satiety pathway promoting glucose uptake by peripheral tissues (Saltiel and Kahn, 2001) and sustaining sugar and lipid anabolic processes (Kim and Rulifson, 2004; Buch et al., 2008). Glucagon-like peptide adipokine hormone (Akh) signalling, conversely, is activated in response to reduced nutrient availability and promotes mobilization of energy reserves (Kim and Rulifson, 2004; Lee and Park, 2004; Bharucha et al., 2008).

Short neuropeptide F (sNPF) is a functional homolog of mammalian orexigenic Neuropeptide Y (Nässel and Wegener, 2011), and its overexpression in sNPF-producing neurons causes hyperphagia and body fat accumulation in flies (Baumbach et al., 2014). Conversely, downregulation of this gene in sNPF-positive neurons reduces food intake (Lee et al., 2004). More recently, Drosophila has been demonstrated to be a good model to study T2DM. Flies fed on high sugar diet (HSD) develop diabetes showing increased levels of glucose in their hemolymph (blood-like system), insulin resistance and heart dysfunction (Palanker Musselman et al., 2011; Pasco and Léopold, 2012; Na et al., 2013). Drosophila has also been used as a model to identify new regulators of mammalian glucose metabolism (Ugrankar et al., 2015) and has made important contributions

FIGURE 1 | Evolution of the adipose tissue (AT). Presence of fat cells, fat organs and proper AT is indicated in different phyla and species throughout evolution.
to understand the main components of signalling pathways involved in tumour development, including the cancer associated cachexia (Figueroa-Clarevega and Bilder, 2015; Enomoto et al., 2018; Saavedra and Perrimon, 2019).

Studies to fully understand the regulation of lipid metabolism in Drosophila are ongoing. It is well known that lipids are taken by adipocytes from the hemolymph, and are esterified and stored as TAGs and cholesterol esters. Moreover, the Drosophila FB, functionally equivalent to mammalian AT and liver, carries out glycolysis and lipogenesis using carbohydrates (Figure 2) [reviewed at (Arrese and Soulages, 2010)]. Also, cellular lipid uptake as well as lipid transport and lipoprotein metabolism has been well studied in Drosophila (Parra-Peralbo and Culi, 2011; Palm et al., 2012; Rodríguez-Vázquez et al., 2015; Yin et al., 2021). Furthermore, fly mutants in Lipin, a phosphatidate phosphatase required for normal insulin pathway signalling that plays a central role in FB function and energy metabolism, Seipin, a transmembrane protein with roles in ER calcium homeostasis and lipid storage, or Sik3 (Salt-inducible kinase 3), a kinase involved in lipid catabolism by regulating bmm gene expression show reduced lipid content and lipodystrophy (Li et al., 2019). All together make this model organism suitable to study different types of lipodystrophies.

As Edward O. Wilson said in “Letters to a young Scientist” for, each biological question there is a suitable system for discovering the answer (Wilson, 2013). In that case, we, as Azeez and collaborators, think that Drosophila melanogaster is a suitable system to identify the primordium of AT and the population of ASCs in adults, as well as to characterize the adult AT in order to understand the adipocyte biology (Azeez et al., 2014). As a proof of principle, Pospisilik et al (Pospisilik et al., 2010) found Hedgehog as a determinant of Brown versus White adipose cell fate, using Drosophila as a model system. In addition, a well-established cell lineage tracing system, G-TRACE, has been a key tool for exploring origin, development and differentiation of tissues in Drosophila (Evans et al., 2009), only very recently available in mammalian model systems (Berry et al., 2013; Jung et al., 2019).

ORIGIN AND DEVELOPMENT OF EMBRYONIC FAT BODY PRECURSORS

The Drosophila FB arises from the embryonic mesoderm (Hartenstein and Jan 1992). At stage 11, the progenitor fat cells arise from nine bilateral clusters of cells in the inner mesodermal layer that span the parasegments 4 through 12 and the mesoderm separates in the splanchnopleure and somatopleure. The somatopleure will give rise to the FB, somatic musculature and other cell types (Campos-Ortega and Hartenstein, 1985). FB cells’ lineage can be traced by analyzing the expression patterns of the genes Adh, Collagen type IV alpha 1 (Col4a1), the steroid hormone receptor seven up (svp) and serpent (srp), as well as the enhancer-trap line 29D that exhibits an expression pattern restricted to developing embryonic fat cells (Hoshizaki et al., 1994) (Tables 3, 4). This enhancer-trap line allowed tracing the fat-cell lineage to nine bilateral cluster of cells within the emerging mesoderm, representing the progenitor fat cells. The svp-positive cells at stage 12 identified early precursor fat cells, and the expression of Adh and Col4a1 was used to identify the terminal fat cell differentiation at stage 15. By late stage 15/16 embryo, mature fat cells coalesce into a single cell thick FB layer throughout the abdomen and form three domains: the lateral FB, the dorsal fat cell projection, and the ventral collar (Miller et al., 2002). Finally, the expression of the GATA-like transcription factor Srp is a marker for the early stages of fat cell development (Sam et al., 1996). Other enhancer traps-lines that drive expression in fat cells at larval and adult stages are 3-76a, X8-157a, and l(3)2E2. Interestingly, l(3)2E2 regulates svp gene expression (Hoshizaki et al., 1995). Therefore, svp and the gene(s) near to the enhancer trap 29D were suggested to be key factors for determination and differentiation of embryonic FB (Tables 3, 4) (Hoshizaki et al., 1994).

The development of the FB requires the GATA-like transcription factor Srp, necessary and sufficient for the progression through the early stages and development of fat cells (Saltiel and Kahn, 2001; Buch et al., 2008; Musselman and Kühnlein, 2018). In fact, FB and gonads derive from
| Factor/pathway                              | Abbreviation | Human homolog                                      | Stage | Function                                                                 | References                                                                 |
|--------------------------------------------|--------------|---------------------------------------------------|-------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Adipokinetic hormone (Akh)/Akh receptor signalling | Akh/AkhR     | Functional homolog to glucagon                     | L, A  | Carbohydrate and lipid mobilization                                      | Fu et al., (2019), Seale et al., (2008), Enerbäck (2009), Daum et al. (2007), TM. (1978), Butterworth et al. (1988), Britton and Edgar. (1990), Guerman et al. (2017), Britton et al. (2002), Hoshizaki. (1994) |
| Alcohol dehydrogenase                      | Adh          | 15-hydroxyprostaglandin dehydrogenase              | E     | Fat metabolism                                                           | Todorović et al. (2009)                                                   |
| Bigmax                                     | Bigmax       | Max-lik protein X                                  | L     | Sugar sensing and lipogenesis                                             | Arrese and Soulages. (2010)                                               |
| Brummer lipase                             | Bmm          | Adipose triglyceride lipase, ATGL                  | L, A  | Lipolysis independent of Akh                                             | Yamada et al. (2018)                                                     |
| Cabut                                      | Cbt          | Kruppel-like factors 10 and 11                     | L     | Transcriptional repression upon sugar sensing                            | Arrese and Soulages. (2010)                                               |
| cAMP-responsive element binding protein B  | dCREB2       | CREB/CREM                                          | A     | Akh target. TAG storage modulation                                       | (Seale et al., 2008; Ren et al., 2015)                                    |
| CCHamide-2a                                | CCHA2        | Neuropeptide                                       | L     | Ilp2 and 5 expression and secretion                                       | Ugrankar et al., (2019), Ugrankar et al. (2011)                           |
| Dawdle<sup>a</sup>                         | Daw          | Activin                                            | L     | DILPs secretion, inhibition of carbohydrate and lipase at intestine      | Bi et al. (2012), Beller et al. (2006)                                     |
| Dorsal                                     | Di           | RELA proto-oncogene                                | L     | Toll target, induced by fungi and Gram-positive bacteria                 | Grönke et al. (2010)                                                     |
| Dorsal-related immunity factor             | Dif          | RELA proto-oncogene                                | L     | Toll target, induced by fungi and Gram-positive bacteria                 | Grönke et al. (2010)                                                     |
| DP Transcription Factor                    | DP           | Transcription Factor Dp-1, TDP1                    | L     | Endoreplication                                                          | Baumbach et al. (2014)                                                    |
| Drosophila insulin/insulin like growth factor (IGF) signalling | ISS          | Insulin like signalling                            | L     | Coordination of nutritional status, endoreplicating tissue metabolism and growth. Determination of final body size. Inhibition of immune response | Feldmann et al., (2009), Gastaldelli, (2011), Baumbach et al. (2014), Fu et al., (2019), Lee et al., (2004), TM. (1978), Palanker et al., (2009), Reis et al., (2010), Bartok et al. (2015), Palu and Thummel (2016) |
| E2F Transcription Factor1, 2               | E2f1, E2f2   | E2F Transcription Factor 1-6                       | L     | Endoreplication                                                          | Baumbach et al. (2014)                                                    |
| Ec dysone signalling                       | Ec           | E2F Transcription Factor 1-6                       | L     | Antagonist to ISS, systemic growth inhibition                             | Wathler and Farese, (2012)                                               |
| Elger<sup>a</sup>                          | Egr          | Tumor necrosis factor alpha, TNFalpha              | L     | Activation of JNK-dependent inhibition of lipids production              | Pancretic Hormones (1990)                                                |
| Endoplasmic reticulum degradation enhancing α-mannosidase-like protein 1 | Edem1        | ER degradation enhancing alpha-mannosidase like protein 2 | L     | Systemic insulin signaling maintenance                                    | Isabel et al. (2005)                                                     |
| Extracellularly regulated kinase 7         | Erk7         | Mitogen-activated protein kinase 15                | L     | Growth, lipid storage and adaptation to nutrient shortage Inhibition of Daw expression. Increased lifespan FB development and metabolic homeostasis Induction of lip secretion | Rechmann and Rehorn, (1998); Werthebach et al. (2019); Moore et al. (1998) |
| Forkhead box, sub-group O                 | Foxo         | FOXO3                                              | L, A  | FB development and metabolic homeostasis                                  | Hasygar et al. (2021)                                                    |
| Glass bottom boat                         | Gbb          | Bone morphogenetic protein 7                       | L     | Induction of lip secretion                                                | Hasygar et al. (2021)                                                    |
| Growth blocking peptide 1<sup>a</sup>      | GBP1         | Epidermal growth factors, EGFR                     | L     | Induction of lip secretion                                                | Hasygar et al. (2021)                                                    |
| Growth blocking peptide 2<sup>a</sup>      | GBP2         | Epidermal growth factors, EGFR                     | L     | Induction of lip secretion                                                | Hasygar et al. (2021)                                                    |
| Hepatocyte nuclear factor 4               | Hnf4         | Hepatocyte nuclear factor 4 alpha                  | L     | Carbohydrate metabolism                                                  | Palm et al. (2012), Rodriguez-Vázquez et al., (2015)                      |
| Histone deacetylase 4                     | HDAC4        | hDAC                                               | A     | Akh target under short fasting condition. Lipolysis Binds DILPs extracellularly and inhibits ISS, tumour-mediated FB wasting Immunity, inhibition of growth, reduction of ISS/TOR signalling and TAG storage | Delaunoie et al. (2016), Koyama and Mirth. (2016) Kaderet et al., (2008), Schaffer et al., (1990) Grönke et al., (2010), Post et al., (2018) |
| Imaginal morphogenesis protein-like 2<sup>a</sup> | Impl2       | Insulin-Like Growth Factor Binding Protein 7, IGFBP7 | L, A  | Immunity, inhibition of growth, reduction of ISS/TOR signalling and TAG storage | Fu et al. (2019), Butterworth et al. (1988)                                |
| Immune deficiency signalling              | Imd          | NF                                                 | L     | Regulate glycoen synthesis                                               | Fu et al. (2019)                                                         |
| Insulin like peptide 2                    | Ilp2         | Insulin                                            | L     | Synthesis and release of trehalose into hemolymph                        | Fu et al. (2019)                                                         |
| Insulin like peptide 3                    | Ilp3         | Insulin                                            | L     | Regulate glycoen synthesis                                               | Fu et al. (2019)                                                         |
| Insulin like peptide 5                    | Ilp6         | Insulin                                            | L     | Regulate glycoen synthesis                                               | Fu et al. (2019)                                                         |

(Continued on following page)
| Factor/pathway                        | Abbreviation | Human homolog | Stage | Function                                                                 | References                                                                 |
|--------------------------------------|--------------|---------------|-------|--------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Insulin like peptide 6a              | Ilp6         | Insulin       | L     | Toll pathway target, repression of DILP2, lifespan extension             | Zimmermann et al. (2004), Heine et al. (2018), DiAngelo and Brinbaum (2009) |
| Insulin like peptide 7               | Ilp7         | Insulin       | L     | Regulation of TAG synthesis                                              | Palu and Thummel (2016)                                                    |
| Kruppel                             | Kr           | BCL6 transcription repressor | L     | Fat determination/differentiation (?)                                   | Todorčević et al. (2009), Palanker Musselman et al. (2011)                |
| Lipid storage droplet-1              | Lsd-1        | Perilipin 2   | L     | Lipolysis                                                                | Evans et al. (2009), Campos-Ortega and Hartenstein (1985)                 |
| Lipid storage droplet-2              | Lsd-2        | Perilipin 2   | L     | Involved in TAG storage                                                  | Hartenstein and Jan 1992, Campos-Ortega and Hartenstein, (1985)            |
| Lipin                               | Lpin         | Lipin 3       | L     | FB development and TAG storage                                          | Hoshizaki et al. (1995)                                                   |
| Liver kinase B1                      | Lkb1         | Liver kinase B1 | A     | Akh/AkhR signalling target under short fasting condition. Lipolysis     | Delanoue et al. (2016), Koyama and Mirth (2016)                           |
| mir-8 stem loop                      | miR-8        | microRNA 200a | L     | Ec signaling target, growth regulation                                  | Schaffer et al. (1990), Hughson et al. (2021), Young and Zeichner (2013) |
| Mondo                               | Mondo        | MLX interacting protein | L     | Sugar sensing and lipogenesis                                            | Enomoto et al. (2018), Saavedra and Perrimon (2019), Arrese and Soufages (2010) |
| Myc                                 | Myc          | MYC proto-oncogene | L     | Ec signaling target, control of glucose and lipid metabolism, Ilp2 secretion | Schaffer et al. (1990), Arrese et al., (2006), Gáliková et al. (2015)          |
| NAD + dependent deacetylase Sirt1    | Sirt1        | Sirtuin 1     | L     | Inhibition of TAG storage                                                | Yin et al. (2021)                                                         |
| NAD + dependent deacetylase Sirtuin 2 | Sirt2      | Sirtuin 2     | L, A | Glucose homeostasis and peripheral insulin sensitivity. Increased lifespan | Rodriguez-Vázquez et al. (2015)                                            |
| No child left behind                | Nclb         | PWP1 homolog  | L     | ERK7 target, growth-promoting downstream effector of mTOR                | Reichmann and Rehorn (1998)                                               |
| PDGF- and VEGF-related factor 1      | Pvf1         | Platelet derived growth factor | A     | Repression of lipid synthesis by activating TOR signaling at oenocytes at the end of AT development. Tumour-mediated FB wasting | Luong et al., (2008), Sousa-Nunes et al., (2011)                          |
| protein 53                           | ps3          | protein 53    | L     | Sensing nutrient stress and metabolic homeostasis, AMPK target           | Grönke et al. (2003)                                                      |
| Relish                              | Rel          | Nuclear factor kappa B subunit 1 | L     | Imd target, induced by Gram-negative bacteria                             | Grönke et al. (2010)                                                      |
| Salt-inducible kinase 3              | Sik3         | SIK family kinase 3 | A     | Akh/AkhR signalling target under short fasting conditions. Insulin target feeding conditions. Lipolysis | Delanoue et al., 2016, Koyama and Mirth, 2016                              |
| Serpent                             | Srp          | GATA binding protein 1 | E     | Fat determination/differentiation                                       | Todorčević et al. (2009), Saltiel and Kahn, (2001), Buch et al., (2008), Musselman and Kühnlein, (2018) |
| Seven up                            | Svp          | Nuclear receptor subfamily 2 group F member 2 | E, L, A | Fat determination. Immunity and xenobiotic response                      | Cermelli et al. (2006)                                                    |
| Slimfast                             | Sif          | Solute carrier family 7 member 1 | L     | Amino acid sensing                                                       | Cermelli et al. (2009)                                                    |
| Snazarus                             | Snz          | Sorting nexin 25 | L     | Activation of TAG storage a t peripheral LD                              | Sam et al. (1996)                                                        |
| Stearoyl-CoA desaturase              | Desat1       | Stearoyl-CoA desaturase 5 | L     | Fatty acids and lipid biosynthesis                                       | Arrese et al. (2006)                                                      |
| Store-operated calcium entry         | SOCE         | Store-operated calcium entry | A     | Akh/AkhR signalling target. TAG storage modulation                       | Petrovic et al. (2010)                                                    |
| Stunted*                             | Sun          | ATP synthase F1 subunit epsilon | L     | TOR signaling target, lip secretion                                    | Ballard et al. (2010)                                                     |
| Sturkopf                             | Sturkopf     | Lipid droplet associated hydrolase | L     | Endocrine physiology regulation (ISS and JH pathway)                    | Miller et al. (2002)                                                      |
| Sugarbabe                            | Sug          | GIL-similar transcription factor | L     | ERK7 target, lipogenic TF                                                | Enomoto et al., (2018), Reichmann and Rehorn (1998)                      |
| Target Of Rapamycine signalling      | TOR          | mTOR signalling | L     | Cellular nutrient sensing                                                | Cermelli et al., (2006), Krahmer et al. (2013), Grönke et al. (2003), Ballard et al. (2010), Lee et al. (2004) |
| Telomere fusion                      | Tefu         | ATM serine/threonine kinase | L     | (Continued on following page)                                           | (Continued on following page)                                             |
TABLE 3 | (Continued) Factors and signalling pathways playing a role in fat body development or function.

| Factor/pathway | Abbreviation | Human homolog | Stage | Function | References |
|----------------|--------------|---------------|-------|----------|------------|
| Triglyceride Lipase | TGL | Lipase A, lysosomal acid type | L | E2F/DP target, inhibition of DNA damage response | Britton et al. (2002) |
| Toll signalling | Toll | Toll-like receptor family signaling | L | Lsd-1 target, Lypolysis, immunity, inhibition of growth, reduction of ISS signalling and TAG storage | Rajan and Perrimon, (2012), Ingaramo et al., (2020) |
| Type IV collagen | Col4a1 | Collagen type IV alpha 1 chain | E | Fat metabolism | Todorčević et al. (2009) |
| Uncouple protein 4C | Ucp4C | Uncouple protein 1 | A | Dissipation of energy in the mitochondria | Rajan and Perrimon, (2012), Ingaramo et al., (2020) |
| Unpaired 2a | Upd2 | JAKSTAT ligand, functional homolog to leptin | L | p53 target, DILPs secretion | Teixeira et al., (2003), Grönke et al. (2003) |

*FB-secreted factors. Abbreviation: NF, not found; E, embryo; L, larvae; A, adult.

TABLE 4 | Enhancer trap lines.

| Enhancer trap | Cells | Cytological location |
|---------------|-------|---------------------|
| 29D | EFC | 58DE |
| l(3)2E2 | EFC, LFC, AFC | 87B (seven up) |
| 3-76a | EFC, LFC, AFC, ADEC | 5CD |
| X8-157a | EFC, LFC, AFC, ADEC | 19D |
| RD721 | LFC, AFC | 58C |
| RD1937 | LFC, AFC | 3CD |
| l(2)0734 | LFC, AFC | Chr 2 |
| l(2)9685 | LFC, AFC | 60F (kruppel) |
| l(2)3552 | LFC, AFC | Chr 2 |
| l(2)10435 | LFC, AFC | Chr 2 |
| l(3)4504 | LFC, AFC | Chr 3 |
| l(3)7842 | LFC, AFC | Chr 3 |
| S3358 | LFC, AFC | 26D |
| rP445 | LFC, AFC | 24A |
| AS3 | LFC, AFC | 25BC |
| RD1272 | LFC, AFC | 64B |
| RD61 | AFC | 54BC |

Abbreviations: EFC, embryonic fat cells; L, larvae fat cells; A, adult fat cells; ADEC, adipose epithelial cells. In parenthesis, genes probably regulated by those enhancers.

mesoderm and abdA allows gonadal mesoderm to develop by repressing Srp function in this region (Moore et al., 1998).

ORIGIN AND DEVELOPMENT OF THE LARVAL FAT BODY

The larval FB is a single cell layer that spreads along the larval body cavity, surrounding the gut and reproductive organs and being exposed to the hemolymph (TM. (1978); Dean et al., 1985). Larval FB contains 2200 cells, a number that remains constant throughout FB development. At larval stages, the FB growth is achieved by increasing cell size through endoreplication cycles, with successive rounds of DNA synthesis without mitosis (Butterworth et al., 1988; Britton and Edgar, 1998). Cell size changes are associated with the accumulation of LDs, glycogen deposits and protein granules. The endocycling progression in the FB cells requires the heteromeric transcription factor complex E2F1/E2F2/DP to repress telomere fusion (tefu) and suppress DNA damage responses (Guarner et al., 2017). In addition, endoreplication in the FB cells is tightly regulated in response to nutrition and depends on IIS (Britton et al., 2002).

Evidences suggest that the development of larval FB might require the expression of various unidentified genes, revealed by the expression of a number of enhancer traps (Table 4) including 3-76a, X8-157a, l(3)2E2. Specifically, the last one regulates the gene expression of svp, suggesting that Svp activity might be involved in that process. kruppel (Kr) expression is not detected in fat cells during embryogenesis, nor during the first- and second-instar stages. However, Kr is expressed in fat cells at the stage previous to metamorphosis and in adults (Table 4). It is possible that Kr serves as a transcriptional regulator in the FB in this last larval instar (Table 3) (Hoshizaki, 1994; Hoshizaki et al., 1994). According to that, it has been found that Kruppel-like factor 11 (KLF11) is a novel browning transcription factor in human adipocytes (Loft et al., 2015).

At the end of the larval development, the FB undergoes a remodelling process with massive autophagy that initiates the pupal transition. The larval FB decreases gradually throughout metamorphosis, and during the first 3 days of adulthood, until no more cells can be observed.

ROLES OF THE LARVAL FAT BODY

The Drosophila larval FB is involved in multiple functions that allow the coordination of the metabolic homeostasis. Larval FB extends as a longitudinal fat sheet at each larval body side. Salivary glands present also an associated-FB whose function is unknown. The most important functions of this tissue include the storage and release of energy, the nutrient sensing function, and the role in the systemic immunity (Figure 3). These functions are regulated by hormones and require the crosstalk of the FB with other tissues. The pathways that adjust the growth rate to the nutritional conditions are the IIS and the target of rapamycin (TOR) pathways, and those involved in the systemic immunity are the Toll and Immune deficiency (Imd) pathways. In the next sections, we review the current knowledge about the role of these signalling pathways and the main factors involved in the different
functions of the larval FB and in its communication with other tissues.

**Store and Release of Energy Reserves**

Similar to the mammalian WAT, the *Drosophila* larval FB stores and releases energy in response to the organism energetic demands. The energy is stored mainly in the form of glycogen and of TAGs, the lipolysis products of those being transported to other tissues to support growth and survival.

**Carbohydrates**

In *Drosophila*, glycogen is the main storage form of carbohydrates and is found in the body wall muscles and in the FB in late larval stages (Baker and Thummel, 2007; Garrido et al., 2015). In addition to glycogen, trehalose is synthesized in the FB and released into the hemolymph. Upon starvation, glycogen is mobilized to maintain the circulating sugar levels (Mattila et al., 2015; Yamada et al., 2018). In mammals, the sensing of sugar at intracellular levels is mediated by the heterodimer formed by the conserved bHLH-Zip transcription factors ChREBP (Carbohydrate Response Element Binding Protein) and MondoA, together with their common partner Mlx (Max-like protein X), which are activated by sugars and promote the conversion of sugars to lipids. They control most of the sugar-responsive genes as well as carbohydrate, amino acid and lipid metabolism (Havula and Hietakangas, 2012; Mattila et al., 2015). In *Drosophila*, the single orthologs of ChREBP/Mondo and Mlx are Mondo and Bigmax, respectively, and this transcriptional network is essential for sugar tolerance also in this organism. Accordingly, the Mondo-Bigmax deficient *Drosophila* larvae presents lethality on any diet containing high levels of sucrose, glucose or fructose (Havula et al., 2013). In addition to the regulation of metabolic genes, Mondo-Bigmax regulate the expression of the TGFβ/Activin ligand Dawdle (Daw), the Gli-similar transcription factor Sugarbabe and the orthologue of mammalian Kruppel-like factors 10 and 11, Cabut (Bartok et al., 2015; Mattila et al., 2015). As detailed in next sections, the intracellular glucose sensing by Mondo-Bigmax is coupled to systemic growth through Daw. Other nutrient sensors involved in sugar tolerance are the nuclear receptor Hnf4 (Hepatocyte nuclear factor 4) and the NAD+-dependent deacetylase Sirtuin 1 and 2 (Sirt1, Sirt2). Hnf4 plays a critical role in carbohydrate metabolism as shown by the *Hnf4* mutant larvae, which display highly elevated circulating glucose and trehalose levels and defects in lipid homeostasis (Palanker et al., 2009; Palu and Thummel, 2016). Sirt2, is required in the FB to maintain glucose homeostasis and peripheral insulin sensitivity by deacetylating and stabilizing Hnf4 through protein interactions (Palu and Thummel, 2016). Moreover, Sirt1 negatively regulates TAG accumulation in the larval FB (Reis et al., 2010).

**Lipids**

TAG is the main lipid form in the FB, which is synthesized from dietary carbohydrates, fatty acids or proteins and is stored in intracellular LDs. Similarly to mammals, LDs of different sizes belong to distinct functional classes, which

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**FIGURE 3** | Schematic representation of the diverse functions of *Drosophila* fat body in larvae. Top, larvae brain represented in soft purple. Abbreviations: Akh Adipokinetic hormone; AkhR, Akh receptor; AMPK, AMP-activated protein kinase; CC, corpora cardiaca; CCHa2, CCHamide-2; Daw, Dawdle; Ec, ecdysone; EcR, ecdysone receptor; Egr, Eiger; GBP1/2, Growth blocking peptide 1/2; ImP2, Imaginal morphogenesis protein late 2; Imd, Immune deficiency; Irl, insulin receptor; miR-8, mir-8 stem loop; PG, prothoracic gland; Sun, Stunted; SvR, Seven up; TOR, target of rapamycin; Upd2, Unpaired 2.
The best characterized LD proteins in the FB during the larval life are Lsd-1 and Lsd-2 (lipid storage droplet-1 and -2), homologous to the mammalian PAT domain protein family (Perilipin, ADRP, and TIP47) (Grönke et al., 2003; Teixeira et al., 2003). Lsd-2 is required for storage of TAG, whereas Lsd-1 stimulates TAG hydrolysis (Bi et al., 2012). The subproteome analysis of LDs of Drosophila FB identified 248 proteins (Beller et al., 2006). Most of them were involved in cellular metabolism but proteins have been identified with diverse biological functions, including intracellular transport, cell organization and cell biogenesis. For instance, the droplet-associated protein Sturkopf has a role in endocrine physiology regulation (Werthebach et al., 2019). The sturkopf mutant adults show a mild decrease in TAG storage levels. However, they fail to adjust their developmental rate to dietary yeast-to-sugar ratio changes, suggesting a function in insulin and juvenile hormone signalling activities. Moreover, distinct spatially LD populations have been described in Drosophila FB: the peripheral LDs, in contact with the plasma membrane, and the larger cytoplasmic medial LDs. The peripheral LD homeostasis is regulated by Snazarus (Snz), which binds to LDs and promotes TAG storage (Ugrankar et al., 2019).

Interestingly, the regulation of lipid homeostasis is coupled to FB development and growth. For example, Lipin, which converts phosphatidate to diacylglycerol, is required for normal FB development and TAG storage (Ugrankar et al., 2011). Loss of Lipin in Drosophila leads to severe defects in the development of the FB with changes in cell nucleus, mitochondria, autophagosome formation and size of LDs. Similarly, the Drosophila BMP-5,7 orthologue, glass bottom boat (gbb), is also required for the development of the larval FB and for maintaining proper metabolism. gbb mutants exhibit developmental delay and altered FB morphology with reduced total lipid, glucose and trehalose levels (Ballard et al., 2010). A recent study shows that the FB expression of the atypical MAP kinase, Erk7 (Extracellularly regulated kinase 7), inhibits cell autonomous and systemic growth and lipid storage. Erk7 expression is upregulated by fasting and, therefore, contributes to the adaptation to nutrient shortage. Erk7 regulates the subcellular localization of the chromatin binding protein No child left behind (Nclb), a growth-promoting downstream effector of mTOR, and inhibits the expression of the lipogenic transcription factor gene sugarbabe (Hasygar et al., 2021).

The Insulin/Glucagon Axis
The energy storage in the FB during the larval development is required during low nutrient conditions and for the survival during the non-feeding periods, such as before and during metamorphosis and during the early stages of adulthood. The maintenance of the metabolic homeostasis requires the communication between the nutrient-storing FB and the consuming tissues.

In mammals, the main hormones that regulate the mobilization of fat and glucose are insulin and glucagon (Freychet P. 1990). Insulin is secreted by pancreatic β cells in response to high blood sugar levels, which triggers glycogen synthesis. Under low sugar levels pancreatic α cells release glucagon and triggers the breakdown of glycogen. Glucagon is also a lipolytic hormone that regulates fatty acids, ketone bodies and TAG.

In Drosophila, the insulin/glucagon axis is well conserved and involves the insulin-like peptides (Iilps) and the glucagon-like peptide Akh (Schaffer et al., 1990; Semaniuk et al., 2021). The mobilization of carbohydrate and lipid energy reserves from the FB in response to starvation is regulated by Akh/AkhR, which is produced by the neurosecretory cells of the corpora cardiaca (Kim and Rulifson, 2004; Lee and Park, 2004; Isabel et al., 2005). For carbohydrate mobilization, Akh/AkhR stimulates, through glycogen phosphorylase, the conversion of stored glycogen to hemolymph trehalose, which is important during the nonfeeding periods and during adult flight. The lipid mobilization through the action of Akh/AkhR, led to the phosphorylation of Lsd-1, which activates the Triglyceride Lipase (TGL) (Arrese et al., 2006; Arrese and Soulages, 2010). However, the role of Akh/AkhR is not completely elucidated as some reports suggest that Akh/AkhR is dispensable for lipid homeostasis in third instar larvae (Lee and Park, 2004; Gáliková et al., 2015). A recent report shows that, although in nutrient abundant conditions Akh/AkhR is dispensable during larval development, in low nutrient stress conditions Akh/AkhR signalling alters larval development and the adult metabolism and behaviour (Hughson et al., 2021).

In mammals, the mobilization of fatty acids from TAG storage is coordinated by the hormone-sensitive lipase (HSL) and the Patatin Like phospholipase Domain Containing 2 (PNPLA2, also known as ATGL) (Zimmermann et al., 2004; Young and Zechner, 2013). Interestingly, ATGL-dependent lipolysis of WAT triggers a systemic insulin release, which is essential for the replenishment of BAT energy storage in mice (Heine et al., 2018). In Drosophila, independently of Akh/AkhR signalling, the Brummer (Bmm) lipase, homolog of mammalian ATGL, converts the accumulated TAG to fatty acids (Grönke et al., 2005).

Nutrient Sensor and Systemic Growth
The FB acts as a sensing organ that coordinates the metabolic and physiological responses to the nutrient status of the organism. The FB relays the nutrient information through the secretion of humoral factors to the insulin-producing cells (IPC), which secrete Iilps to control the systemic ISS.

Signalling Pathways in the Fat Body Regulating Body Growth
In Drosophila FB, the IIS and the TOR pathways regulate nutrient uptake, storage and metabolism. In addition, there is a crosstalk between the steroid hormone 20-hydroxyecdysone (ecdysone) and those pathways. Furthermore, the FB is the main sensor of internal oxygen levels that control organismal growth.
The Drosophila genome encodes eight Ilps (Grönke et al., 2010): Ilp2, 3 and 5 are produced by IPCs in the brain and are functionally comparable to insulin; Ilp6, produced in the FB, is related to mammalian Insulin Growth Factors, IGFs (Okamoto et al., 2009; Slaidina et al., 2009); Ilp7 and Ilp8 are relaxin-like peptides (Grönke et al., 2010). Similar to mammalian insulin, Ilps are able to regulate circulating levels of carbohydrates in the hemolymph. Insulin is a positive regulator of fat cell mass, acting through changes in both cell number and lipid storage (DiAngelo and Birnbaum, 2009). Ilp2 and Ilp5 regulate glycogen deposition, Ilp3 is responsible for the synthesis and release of trehalose into hemolymph and Ilp5 and Ilp7 regulate the synthesis of TAG (Kim and Rulifson, 2004; Post et al., 2018; Semaniuk et al., 2021). In addition, IIS/Pi3K (Phosphatidylinositol 3-kinase) signalling coordinates nutritional status with endoreplicating tissues metabolism and growth (Britton et al., 2002). Thus, insulin regulates the critical weight, a checkpoint that occurs early in third instar larvae that determines the final body size (Mirth and Riddiford, 2007).

Mammals and Drosophila use the TOR pathway for cellular nutrient sensing, playing an important role in the balance of energy storage. The TOR kinase activity depends on amino acid availability and mediates protein synthesis, amino acid import, ribosome biogenesis and autophagy (Saxton and Sabatini, 2017). Consequently, Tor mutant larvae showed reduced size and glucose and lipid storage levels, larvae showing a transparent phenotype (Colombani et al., 2003; Luong et al., 2006).

In addition, there is crosstalk between IIS and ecdysone. Ecdysone signalling in the FB antagonizes IIS and promotes autophagy (Rusten et al., 2004; Colombani et al., 2005). Furthermore, ecdysone modulates organisinal growth through a FB relay that attenuates systemic insulin signalling (Colombani et al., 2005; Arquier et al., 2008; Honegger et al., 2008; Jin et al., 2012; Lee et al., 2018).

Humoral Fat Body Derived Signals
In Drosophila and other animals, the organisms require sensing the levels of oxygen to adapt their systemic growth to the environmental conditions. A central regulator for the maintenance of oxygen homeostasis is the hypoxia-inducible factor 1 (HIF-1), a heterodimeric transcription factor composed of the oxygen regulated HIF-1α and the constitutively expressed HIF-1β. In presence of oxygen, HIF-1α is hydroxylated by HIF prolyl hydroxylase (Hph), targeting it for ubiquitin-dependent proteasomal degradation. In hypoxia, HIF-1α is stabilized and induces the expression of target genes that regulate growth and metabolism (Semenza, 2014).

To link the organisinal growth to the nutrient availability, the FB produces signalling molecules that promote or inhibit the insulin secretion from IPCs (Figure 3 and Table 3). Some of these factors and neuropeptides are secreted in response to dietary fats and/or sugars such as Unpaired 2 (Upd2), Daw and CCHamide-2 (CCHA2).

Upd2, a JAK/STAT cytokine (Rajan and Perrimon, 2012), binds to its receptor Dome (Homeless) on GABAergic neurons, releases the inhibition of IPCs and promotes Ilp secretion. Recently, an essential role for adipose p53 in sensing nutrient stress and maintaining metabolic homeostasis has been reported (Ingaramo et al., 2020). Under nutrient deprivation and high-sugar diet, p53 is activated in the FB and represses the expression of Upd2. This AMP-activated protein kinase (AMPK)-dependent p53 activation leads to modulation of Ilp2 levels, systemic insulin/TOR signalling and autophagy induction (Ingaramo et al., 2020). Another response to the consumption of sugar is the release by the FB of the activin-like factor Daw, which promotes the secretion of Ilps through the TGF-β/activin receptor Baboon (Babo) (Ghosh and O’Connor, 2014). In addition, Daw released from the FB signals to the intestine where inhibits the expression of carbohydrases and lipases by enhancing Smad on X (Snox) levels (Chng et al., 2014). The sugar induced gene expression of Daw is mediated by Mondo-Bigmax, whereas Foxo (forkhead box, sub-group O) negatively regulates its expression (Bai et al., 2013; Mattila et al., 2015). A third mechanism by which carbohydrates promote Ilp expression and secretion is through CCHA2, a neuropeptide induced in the FB by proteins and sugars. When released, the CCHA2 peptide promotes the secretion of Ilp2 and Ilp5 via its receptor, CCHA2R, expressed in the IPCs (Ren et al., 2015; Sano et al., 2015).

TOR-dependent FB humoral signals couple Ilp2 and Ilp5 secretion from the IPCs with amino acid intake and some humoral factors are secreted in response to dietary amino acids such as Stunted (Sun), Eiger (Egr) and the Growth blocking peptides GBP1 and GBP2 (Colombani et al., 2003; Honegger et al., 2008; Gémardin et al., 2009; Rajan and Perrimon, 2012; Ghosh and O’Connor, 2014; Sano et al., 2015; Agrawal et al., 2016; Delanoue et al., 2016; Koyama and Mirth, 2016). Interestingly, amino acid-dependent TOR signalling derived from the FB controls neural stem cell proliferation independent from IPCs-derived Ilps. In the developing central nervous system, embryonic and larval neuroblasts undergo proliferative phases, intercalated with periods of a quiescent state, that is reversible by dietary amino acids (Britton and Edgar, 1998). The TOR-mediated amino acid sensing induces a secreted FB signal that activates the expression of Ilps in glial cells. The local glial Ilps signal on adjacent neuroblasts via the IIS/Pi3K/TOR pathway and control their reactivation (Chell and Brand, 2010; Sousa-Nunes et al., 2011).

Furthermore, ecdysone signalling in the FB modulates insulin dependent systemic growth through the regulation of Myc, microRNA miR-8 and ImpL2 (Ecdysone-inducible gene L2), a member of the immunoglobulin superfamily homolog to the Insulin-Like Growth Factor Binding Protein 7, IGFBP7 (Arquier et al., 2008; Honegger et al., 2008; Hyun et al., 2009; Delanoue et al., 2010; Jin et al., 2012; Lee et al., 2018).

The FB is a sensor tissue for amino acid levels and coordinates growth of peripheral tissues through a humoral mechanism (Colombani et al., 2003). Hence, the downregulation of the Slimfast (Slif) amino acid transporter within the FB is sufficient to induce a general reduction in the rate of larval growth (Colombani et al., 2003). In response to dietary amino acids, the peptide Sun is released from the FB (Delanoue et al., 2016). Sun binds to Methuselah (Mth), a secretin-incretin receptor on IPCs, and stimulates the secretion of Ilps. On the other hand, under
conditions of low amino-acid concentrations, Egr, a Drosophila tumor necrosis factor alpha (TNF-alpha) orthologue is released from the larval FB (Agrawal et al., 2016). This cytokine signals through its receptor Grindelwald (Grnd) on the larval IPCs to activate the JNK-dependent inhibition of Ilps production. The expression of the endoplasmic reticulum (ER) degradation enhancing α-mannosidase-like protein 1 (Edem1) in the FB is also crucial for maintaining systemic insulin signalling, since its down-regulation results in the accumulation of Ilp2 in the IPCs and reduced systemic insulin signalling. The reduction in Edem1 levels is crucial for survival during starvation as lowering edem1 expression levels facilitates the activation Eiger on IPCs and the reduction in IIS. In addition, Edem1 regulates Upd2 to manage the metabolic status (Pathak and Varghese, 2021). Moreover, Growth-blocking peptides 1 and 2 (GBP1 and GBP2) are epidermal growth factors-like cytokines secreted by the FB upon availability of dietary amino acids (Koyama and Mirth, 2016). Recently, it was shown that these adipose tissue factors regulate Ilps secretion by silencing a pair of inhibitory neurons that synapse with IPCs (Meschi et al., 2019).

During late larval life, increased levels of ecdysone affect also systemic growth. Myc expression in the Drosophila FB triggers a cell autonomous mechanism that controls glucose and lipid metabolism to favour the storage of nutrients (Parisi et al., 2013). During the late third instar, ecdysone signalling represses Myc function inhibiting systemic growth. This suggests a humoral factor released downstream of Myc that relays information to control IIS (Delanoue et al., 2010). The ability of FB Myc activity to affect IPC Ilp2 secretion depends on stearoyl-CoA desaturase (Desat1) activity, an enzyme necessary for production of fatty acids and lipid biosynthesis (Parisi et al., 2013). The increased levels of ecdysone suppress the body growth also through the regulation of FB microRNA mir-8 (Hyun et al., 2009; Jin et al., 2012). Multiple peptide hormones regulated by mir-8 may contribute to Drosophila growth (Lee et al., 2015). Among them, the IGF-like factor Ilp6 and the Imaginal morphogenesis protein Late 2 (Impl2) are upregulated in the FB of mir-8 null mutant larvae. Ilp6 expression from larval FB represses secretion of Ilp2 from IPCs and extends lifespan (Bai et al., 2012). Before and during pauripation or in response to starvation, Ilp6 communicates the FB with other organs. For example, it promotes the growth of imaginal discs, which gives rise to adult organs, and the lipid uptake in oenocytes, cell clusters of ectodermal origin that regulate lipid metabolism (Chatterjee et al., 2014). Nutritional restriction also increases the levels of ecdysone, which triggers the production of Impl2 in the FB (Lee et al., 2018). In response to nutrient limitation, the FB nutrient sensor function, which restricts the growth of peripheral tissues, is complemented by the release of nutrients through autophagic degradation of the FB cytoplasm. This provides other tissues with a source of nutrients necessary for survival. Thus, under conditions of low TOR signalling, autophagy promotes normal cell function and survival (Scott et al., 2004).

To adapt the systemic growth to the environmental conditions, the FB integrates the oxygen and amino acids levels through the Hph/HIF-1α and Hph/TOR pathways. In hypoxia, the FB release HIF-1α-dependent humoral factors that inhibit Ilps expression and secretion from the IPCs, thereby restricting the systemic growth. Moreover, independently of HIF-1α, Hph is required for nutrient-dependent TOR activation (Texada et al., 2019). To allow adults viability in hypoxia, the larval FB inhibits TORC1 signalling and reorganizes the lipid storage (Lee et al., 2019). A recent study showed that FOXO is a hypoxia inducible factor that mediates tolerance to low oxygen by inducing immune-like responses in the FB (Barretto et al., 2020).

**Systemic Immunity**

The Drosophila FB coordinates not only the nutrient storage and the animal growth but also the humoral immune response. In Drosophila, the infection by microbes induces the secretion of antimicrobial peptides (AMP) by the FB, which are controlled by the Toll and Imd pathways (De Gregorio et al., 2002). The Toll-NF-κB signalling, which triggers the nuclear translocation of Dif (Dorsal-related immunity factor) and Dorsal, is induced by fungi and Gram-positive bacteria, whereas infection by Gram-negative bacteria leads to the processing and transport or Relish via the Imd pathway. To support the immune activation, the FB increases its volume, expands the ER and alters its metabolism, shifting from lipid metabolism to membrane phospholipid synthesis (Martinez et al., 2020). These changes, induced by Toll signalling to sustain AMP synthesis and secretion, may become detrimental if maintained over long periods due to insufficient nutrient storage. Thus, the expression of a constitutively active Toll receptor in the larval FB inhibits the whole organismal growth, disrupts the insulin signalling in the FB and reduces the TAG storage (DiAngelo and Birnbaum, 2009; Roth et al., 2018; Suzawa et al., 2019). Similarly, persistent activation of the Imd pathway in the larval FB diminished IIS/TOR activity, which resulted in decreased TAG levels and impaired whole animal growth (Davoodi et al., 2019). Moreover, increasing insulin signalling in the FB leads to decreased immune gene expression and, vice versa, decreasing insulin signalling leads to increased immune gene expression and increased resistance to infection (Musselman and Kühllein, 2018). This supports a model in which insulin signalling and the immune response negatively regulate each other to maintain the energy balance.

**ORIGIN AND DEVELOPMENT/ DIFFERENTIATION OF ADULT FAT BODY**

The origin of the adult FB, in invertebrate, as in mammals, remains elusive and unexplored due to the difficulty in its manipulation (The mesoderm and its derivatives and BM, 1993; Hoshizaki et al., 1995; Berry et al., 2013). Although both larval and adult FBs play a role as energy storage organs and nutrient availability sensing, they show different features. For example, contrary to the larval FB, adult FB is able to expand by increasing the number of adipocytes. Moreover, they might not share a common origin: while larval FB derives from the nine embryonic bilateral primordia, the origin of the adult FB has not been identified (Hoshizaki et al., 1994; Hoshizaki et al., 1995; Aguila et al., 2007).

During metamorphosis, unlike most larval tissues that undergo histolysis, some of the larval FB cells persist and are found in the newly eclosed adult, free floating as single cells or small clusters. These larval fat cells are refractive to the autophagic cell death that
removes most of the larval cells during metamorphosis. It has been shown that these larval adipocytes, now dissociated, are a source of nutrients during the non-feeding stage of adulthood, approximately the first 3 days after eclosion (Agui et al., 2007). Three to five days after eclosion these cells are replaced by the adult fat adipocytes (Johnson and Butterworth, 1985), which accumulate lipid reserves through feeding and de novo lipid synthesis during those days. The myokine Pvf1 (PDGF- and VEGF-related factor 1) represses lipid synthesis at the end of the adult FB lipid build-up phase by activating TOR pathway synthesis during those days. The myokine Pvf1 (PDGF- and VEGF-related factor 1) represses lipid synthesis at the end of the adult FB lipid build-up phase by activating TOR pathway

The development of adult FB might require the expression of genes driven by a number of enhancers that are identified through enhancer traps (Table 4). Most of them, except for 29D and RD61, drive the expression in larval FB as well as in adult one 3-76a, X8-157a and l(3)2E2 driving the expression in fat cells of all stages. As l(3)2E2 is an enhancer of the srp gene, the activity of Srp might be also involved in fat cell decision or/and differentiation programmes at adult stage (Tables 3, 4) (Hoshizaki et al., 1995).

Although the cells that give rise to the adult fat cells have not been identified, two fundamentally different mechanisms have been suggested to explain how the adult FB arises: 1) cell remodelling, a process in which larval FB tissue is dissociated and simultaneous synthesis of adult FB from undifferentiated ASC (Haunerland and Shirk, 1995).

Potential Adipose Stem Cell Population

It has been shown that adult FB derives from the mesoderm (Lawrence and Johnston, 1986). However, the ASC population that maintain the adult FB has not been identified. Hoshizaki et al. suggested that a subset of adip epithelial cells, precursors of adult thoracic muscles, might be as well the precursors of adult adipocytes (Hoshizaki et al., 1995). Adip epithelial cells are in fact a plausible source of ASCs, since two of the fat cells-specific enhancer traps mentioned above, 3-76a and X8-157a, are also active in the adip epithelial cells. This suggests a possible lineage connexion for fat cells from embryo to adult, including the adip epithelial cells during larval stage. Furthermore, adip epithelial cells are the precursors of adult muscles (Holz et al., 1997) and a subset of these cells expressing Breathless are the precursors of the adult tracheal air sacs (Sato and Kornberg, 2002). This might suggest that adip epithelial cells could potentially be the pluripotent stem cell population in the adult stage.

ROLE OF ADULT FAT BODY

In spite of the fact that most of the functional studies in Drosophila are conducted at larval stages, there are enough evidences to ensure that the adult FB carries out liver, adipose, and immune functions (Hotamisligil, 2017; Arrese and Soulages, 2010). Oenocytes, specialized hepatocyte-like cells, are closely associated to adipocytes, specifically at the subcuticular FB (Figure 2) (Gutierrez et al., 2007). In fact, very recently, the role of oenocytes regulating lipid synthesis and content in the adipose tissue has been described, a role-played by hepatocytes in mammals. Furthermore, loss of function of TOR pathway in adult oenocytes leads to obesity (Ghosh et al., 2020).

Although further studies would be necessary to prove the equivalence of these organs, there is a subcuticular FB that is extended through the whole Drosophila adult body (Figure 2), and also a FB wrapping some organs such as the heart, intestine, spermatheca and brain, and these could be the equivalent to mammalian subcutaneous and visceral WAT, respectively.

There are not many evidences indicating the existence of a BAT or beige adipocytes tissue in Drosophila. However, a set of genes coding for Uncouple proteins, including UCP1 that is a marker for BAT in mammalian systems, are conserved in Drosophila (Harms and Seale, 2013). Similarly to UCP1, Drosophila Ucp4C has been involved into the dissipation of energy in the mitochondria (Cannon and Nedergaard, 2004; Da-Re et al., 2014).

Metabolism

Adult FB has an important role in physiology, longevity as well as disease, e.g., cancer (Figure 4).

Metabolic Organ

Similarly to the larval one, the adult FB is the central metabolic organ involved in the accumulation of fat and glycogen from caloric overload and in the mobilization of the stored fat during starvation or egg production (Lee and Park, 2004; Parra-Peralbo and Culi, 2011; Musselman et al., 2013; Mattila and Hietakangas, 2017; Zhao and Karpac, 2017; Weaver and Drummond-Barbosa, 2019). Not surprisingly, in females the FB has higher proportion of lipids than that in males (Johnson and Butterworth, 1985). In contrast to larval FB there are evidences suggesting the ability of adult FB to grow in order to accumulate lipids, in obese flies (DiAngelo and Birnbaum, 2009).

In the adult FB, Akh/AkhR signalling activates cAMP-responsive element binding (CREB) transcription factor (Song et al., 2017). CREB downregulation was shown to promote overeating and obesity in adult flies (Iijima et al., 2009). In addition, Akh/AkhR signalling modulates TAG content in adult FB through the store-operated calcium entry (SOCE) (Baumbach et al., 2014). Under short-term fasting conditions, Akh/AkhR signalling promotes lipase bmm gene expression by reducing Lkb1-Sik3 (Salt-inducible kinase 3)-HDAC4 (Histone deacetylase 4) signalling axis, probably through Foxo (Wang et al., 2011; Choi et al., 2015). Under long-term fasting conditions, however, the reduction of the Lkb1-Sik3 pathway to induce the lipolytic response is independent of Akh/AkhR. Conversely, insulin pathway induces Sik3 activity under feeding conditions, independently of Lkb1 (Choi et al., 2015) (Figure 4).

Recently, Relish known to be part of Imd pathway, as mentioned above, has been identified as a repressor of bmm gene expression through FOXO, by fasting-dependent histone deacetylation, during metabolic adaptation to fasting (Molaei et al., 2019).
Crosstalk in Inter-Organ Communication: Links to Fat Body

Recently, an intestinal/neuronal/FB inter-organ communication has been described in adults to preserve energy homeostasis. In response to nutrients, enteroendocrine cells secrete systemically the hormone Bursicon α (Burs) binds to its neural receptor DLgr2. Burs/ DLgr2 signalling regulates energy metabolism through a neuronal relay that represses AKH production and, therefore, the subsequent modulation of AKHR signalling within the FB. The reduction of systemic Burs/DLgr2 signalling leads to exacerbated glucose oxidation, strong lipodystrophy and depletion of energy stores with the consequent reduced organismal resistance to nutrient deprivation conditions (Scopelliti et al., 2019). Therefore, Bursicon inhibits the mobilization of glycogen storage under nutrient availability (Figure 4).

Aging and Longevity

The overexpression in the adult FB of the gene foxo, encoding for the key target of the IIS pathway, leads to increased life span (Giannakou et al., 2004). Similarly, overexpression of the gene Sir2 in adult FB increases longevity in both sexes. It also modulates the composition of the LD proteome, a plausible mechanism underlying extended longevity by Sir2 as LDs regulate aging processes (Goldberg et al., 2009; Hoffmann et al., 2013). All these evidences point to a role of the adult AT in controlling longevity.

Cancer-Associated Cachexia

Tumors and their microenvironment can produce different circulating factors that cause cachexia, the wasting syndrome observed in advance cancer patients which is characterized by a general metabolic dysfunction that includes systemic inflammation, increased catabolism and lipolysis or proteolysis in muscles and AT (Ebadi and Mazurak, 2014). In Drosophila, two main models of cachexia have been described which show similarities with human patients (Figueroa-Clarevega and Bilder, 2015; Kwon et al., 2015; Song et al., 2019). One of the models is induced by activation of Yorkie (Yki), the Yap1 oncogen ortholog, in intestine stem cells that secrete a PDGF- and VEGF-related factor 1 (PvF1) ligand. Pvf1 leads to the pathological activation of ERK/MAPK signalling in peripheral tissues and induce wasting of muscles and AT (Song et al., 2019). The other model consists of the transplantation, in adult flies, of clones of eye disc cells mutant for the polarity gene scribble and ectopically expressing an activated form of Ras (V12, RasV12, scrib−/−). Interestingly, both tumor models secrete high levels of ImpL2 (Figueroa-Clarevega and Bilder, 2015; Kwon et al., 2015). Increased levels of circulating ImpL2 reduce systemic insulin signalling, which leads to reduction of nutrients uptake by muscle and adipose tissue, driving organ wasting. The RasV12, scrib−/− tumors also induce a systemic autophagy stress response in muscles and AT that mediates organ wasting (Katheder et al., 2017; Khezri et al., 2021). Recently, another wasting model in Drosophila, relates the FB remodelling and muscle detachment to the tumor-secreted matrix metalloproteinase 1 (Mmp1). Mmp1 can modulate TGFβ signalling in the FB and disrupts the basement membrane/extracellular matrix in FB and muscle (Lodge et al., 2021). All these studies show that the conservation of the signalling pathways and the existing
genetic tools, make of Drosophila an important model to study the process of organ wasting and to identify new molecular mechanisms involved in this process.

**Immunity and Xenobiotic Response**

The FB acts as a detoxifying tissue based on the expression of members of the Cytochrome P450 (Cyp450) superfamily of monoxygenases. These are enzymes involved in metabolizing foreign substances and drugs implicated in resistance to insecticides (Feyereisen, 1999; Chung et al., 20097; Terhzaz et al., 2015).

Recently, Weaver and Drummond-Barbosa showed that the nuclear receptor Svp regulates a number of factors involved in immunity and xenobiotic detoxification responses in adult female FB (Weaver and Drummond-Barbosa, 2020). Specifically, Svp would acts as the first line of defence against infections, regulating genes involved in the capture and elimination of foreign pathogens. Svp also regulates the expression of genes encoding members of the CYP450 family involved in the initiation of phase I of the xenobiotic detoxification response. Reduction ofsvp expression results in the upregulation of genes encoding Metallothionein A and B (MtnA and MtnB) (Weaver and Drummond-Barbosa, 2020). MtnA and B are enzymes involved in heavy metal detoxification and protection against free radicals and have been involved in the response to xenobiotic and immune stress (Bonneton et al., 1996). It has been suggested that a reduced activity of Svp could lead to a toxic scenario, which would need MtnA and B activity to eliminate this toxicity (Weaver and Drummond-Barbosa, 2020) (Figure 4).

**CONCLUDING REMARKS AND FUTURE PERSPECTIVES**

The AT is a central organ, which regulates metabolism and immune responses, as inflammation, so that it has a major impact on human physiology. AT dysfunction associates to metabolic diseases such as: obesity, diabetes, lipodystrophies and cancer-associated cachexia.

Despite of the advance in the knowledge in the last years, still there are many open questions that need to address about the functions and development of AT.

However, the knowledge at this moment can only be obtained through the studies of animal models. Drosophila can be a good model for the study of AT based on the possibility of the genetics analysis that can be performed in vivo, the lower complexity of the tissue and the functional conservation of this tissue along the evolution.

Further studies focused on tracing the cell lineages expressing the transcription factors Svp, Srp and Kr, involved in the determination and differentiation and maintenance of fat cells during embryonic and larval stages, would shed light on how those processes develop and what are the actors involved. Similarly, it would be very interesting to trace the cells showing expression driven by the enhancer traps I(3)2E2, 3-76a and X8-157, which show expression in embryonic, larval and adult fat cells.

Adipocytic cells could potentially represent the ASC population of adult FB in Drosophila. Future characterization of the gene expression profile of this population will help to understand the origin and cellular differentiation of adult adipocytes. Also, it will reveal the mechanisms leading to the different adipocyte fates as well as putative fate-switching factors.

Most of the studies that shed light on the functions carried out by FB were conducted at larval stages. Therefore, further studies are needed to characterize and identify potentially new functions of the adult AT related to regulation of energy homeostasis and immunity that may be conserved in mammals. The identification of adult FB-secreted derived signals would drive to a comprehensive understanding of the roles that this organ is playing in inter-organ communication and in AT wasting, which would help to understand human cancer-associated cachexia and other diseases like obesity and DMT2.

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EP-P conceived the idea, wrote the manuscript and elaborated the figures and tables. AT wrote the manuscript and reviewed the figures and tables. RB wrote/reviewed the manuscript, reviewed the figure and reviewed/elaborated the tables.

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