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Embryonic adipose development and consequences in later life

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ABSTRACT

Adipose tissues distribute throughout the mammalian organs, though large deposits localize at specific anatomical sites, such as visceral, subcutaneous, intermuscular, and intramuscular fats. The embryonic origin of adipose tissues has been mapped using genetic mutant mice, and multiple lineages of adipose progenitor cells are recognized. We review literature for evidence of a population of embryonic adipose progenitor cells that develop in response to the abundance of caloric supplies. This adipocyte population of embryonic or juvenal origin is a likely candidate of adipocytes accounting for obesity and metabolic pathology in adults. Thus, we suggest a mechanism underlying early caloric excess impacts on obesity and metabolic problems later in adults.

Adipose tissue has physiological significance in regulation of metabolism, and its abnormal accumulation, obesity, is now a common problem associated with pathological conditions and

diseases (Kopelman, 2000). While brown adipose tissues have function in heat generation and metabolic regulation, white adipose tissues are the major adipose content in humans and the tissues for energy storage as well as the culprits in obesity and metabolic pathology. Here, we discuss the developmental aspects of the white adipose tissues, consider embryonic progenitor cells, and suggest a mechanism that a population of adipose progenitor cells present in embryonic or juvenile states influences metabolism and the risk of obesity in adults.

1. Adipose tissues

Adipose tissues, found in specific anatomical locations throughout mammals, accommodate the storage of excess calories by expanding, particularly in the context of obesity (Spiegelman & Flier, 1996; 2001; Sebo & Rodeheffer, 2019). Within these tissues, adipocytes are the primary cells responsible for fat storage. They nest within a matrix rich in collagen, accompanied by progenitor cells, blood vessels, fibroblasts, and immune cells. The primary role of White Adipose Tissues (WAT) is to serve as the primary lipid reservoir in mammals (Varga et al., 2011). These lipid-storing cells amass and can be identified in various anatomical sites, including craniofacial regions, visceral areas, and subcutaneous, intermuscular, and intramuscular depots. The visceral and subcutaneous depots hold significant importance for mammals. While mouse models offer valuable insights into the study of adipose biology and often serve as a basis for understanding human conditions, it's imperative to recognize the substantial anatomical differences in adipose tissue distribution between mice and humans. Such differences are depicted in **Fig. 1** (Börgeson et al., 2022). Consequently, any extrapolation of findings from mouse models to human situations necessitates cautious interpretation.

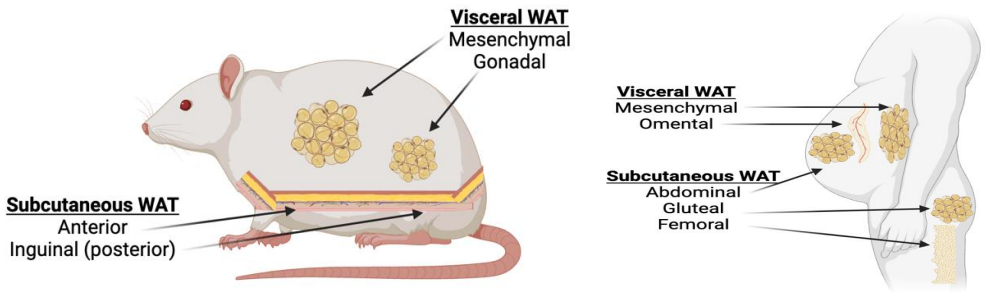

Fig. 1: illustration of fat depots in mammals comparing mouse and human adipose depots. The white adipose tissues (WAT) are the main lipid/fat storages in mammals. The adipocytes, the lipid storing cells of the tissues, aggregate in masses and distributes in several anatomical sites: such as the craniofacial/facial, the visceral, subcutaneous, intermuscular, intramuscular depots. The visceral and subcutaneous fats are the two key depots in mammals. There are significant

differences between mouse models and humans in their adipose anatomical arrangements, as illustrated. The illustration was based on a review article (Börgeson et al., 2022).

2. Embryonic origin of adipose tissues

Adipocytes are believed to differentiate from precursor cells, yet the biology and regulation of these precursor cells remain elusive (Gesta et al., 2007; Louveau et al., 2016; Sanchez-Gurmaches & Guertin, 2014). It's theorized that these precursor cells are housed within the vascular structures of adipose tissues, but definitive markers and signals for their differentiation have yet to be clearly established (Louveau et al., 2016).

Through mouse models and genetic tracing methods, the embryonic origins of adipose depots have been studied (Billon & Dani, 2012; Sanchez-Gurmaches & Guertin, 2014). These tissues emerge from mesenchymal stem cells during embryonic development and undergo intricate morphogenetic and differentiation processes (Sebo et al., 2018; Sebo & Rodeheffer, 2019). Lineage tracing indicates that the embryonic mesoderm is the primary source for most adipose tissues, notably the visceral (VAT) and subcutaneous (SAT) adipose tissue. In contrast, the embryonic neuron ectoderm is responsible for forming craniofacial adipose regions (C/FAT). These origins and distributions are illustrated in **Fig. 2** (Majka, et al., 2011; Sanchez-Gurmaches & Guertin, 2014; Sebo et al., 2018; Sebo & Rodeheffer, 2019).

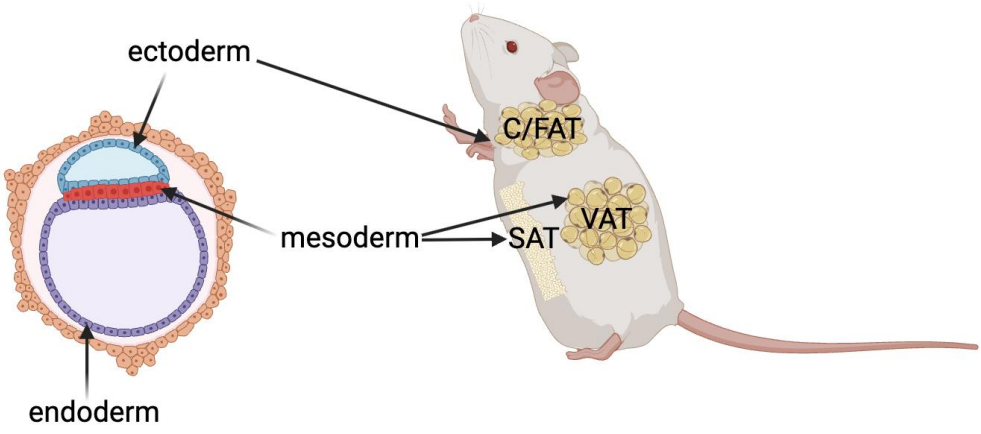

Fig. 2: illustration of adipose tissue embryonic origin. Mouse models and genetic approaches are used to study and trace the embryonic origin of adipose depots. The results suggest that the majority of adipose tissues including the visceral adipose tissue (VAT) and the subcutaneous adipose tissue (SAT) are originated from the embryonic mesoderm. The embryonic neuron ectoderm contributes to adipose in craniofacial/facial (C/FAT) regions. The illustration is based on a reference (Majka, et al., 2011).

3. Adipocyte progenitor cells --- differentiation, properties, and regulation of systematic metabolism

White adipose tissues (WAT) distributed in multiple anatomical locations comprise the largest energy storage and endocrine organ in the body. This WAT, the "fat tissue", plays a prominent role in regulating appetite, glucose metabolism, insulin sensitivity, inflammation, and tissue repair, and metabolic homeostasis is critically regulated and dependent on healthy adipose tissue (Wang et al., 2013). In humans, adipocytes continuously undergo turnover, though adipose tissue maintains a relatively stable number of adipocytes even in adulthood. Radiocarbon dating methods were used to determine the half-life or turnover rate of adipocytes in humans, providing valuable insight into adipose tissue dynamics and the regulation of body weight. By analyzing the incorporation of Carbon-14 (a radioactive isotope released into the atmosphere during nuclear bomb tests) in the DNA of adipocytes, a study estimated that approximately 8% of fat cells are renewed annually in adults, irrespective of the individual's weight status or sex (Spalding et al., 2008; Arner & Spalding, 2010). The adipocyte has a slower turnover rate than previously thought, though the turnover rate was higher in individuals with a higher body mass index, suggesting a slightly higher rate of adipocyte replacement in obesity. Interestingly, the total number of fat cells in the body remains relatively constant in adulthood, even after significant weight loss (Spalding et al., 2008; Arner & Spalding, 2010), suggesting that the number of adipocytes is determined during childhood and adolescence. When it comes to obesity, the size of the adipocytes increases (hypertrophy), and in some cases, the number of adipocytes can also increase (fat cell hyperplasia) (Jo et al., 2009). Weight loss can lead to a decrease in the size of the adipocytes (Stenkula & Erlanson-Albertsson, 2018), but, as mentioned before, the total number of adipocytes tends to remain stable (Ye et al., 2022).

Adipocytes are believed to originate from precursor cells. While their biology and regulation remain enigmatic, it's theorized that these precursors reside within the vascular structures of adipose tissues, awaiting signals for differentiation (Louveau et al., 2016).

Adipocyte differentiation has been extensively studied through cultured cells and knockout mice. Central to this process is the transcription factor, peroxisome proliferator-activated receptor (PPAR) (Lowell, 1999). Recognized as the linchpin of adipocyte lineage, PPAR activates the expression of numerous genes, driving the creation of new adipocytes and determining their size and number (**Fig. 3**) (Evans et al., 2004; Floyd & Stephens, 2012).

Its absence in mice results in an inability to develop adipose tissue, underscoring its vital role in adipogenesis (Jones et al., 2005; Hinds Jr et al., 2021).

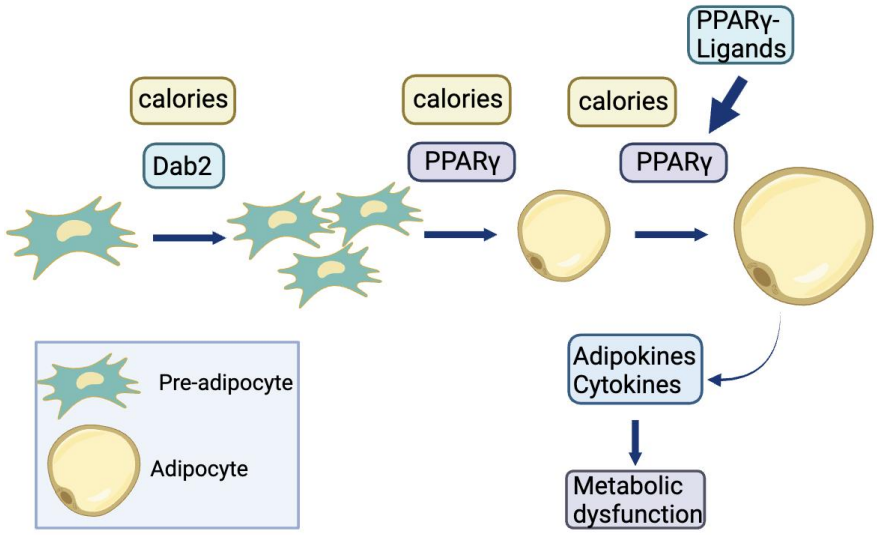

Fig. 3: Illustration of adipocyte differentiation and cell size expansion, regulation by Dab2/MAPK pathway, PPAR , and caloric intake. A population of pre-adipocytes are established during embryonic development and early/juvenile growth. Dab2 and caloric exposure regulate the expansion of the number of adipocyte progenitor cells. PPAR , is the master transcription factor for adipocyte differentiation, and together with caloric intakes, determine adipocyte differentiation and hypotrophy (expansion of lipid storage). PPAR is activated by endogenous lipid ligands, as well as to be a target of pharmaceutical drugs. The transcription programming of mature adipocytes is fully activated by the cooperation between PPAR , C/EBP, and RXR. Hypertrophic adipocytes secrete excessive inflammatory adipokines and cytokines, which regulate metabolism and may cause metabolic dysfunction.

In obesity contexts, heightened PPAR activity promotes adipogenesis, especially when existing adipocytes reach their fat-storage capacity (Kubota et al., 1999). Furthermore, PPAR was identified early on as a master regulator of adipogenesis, with both *in vivo* and *in vitro* studies solidifying its central role (Kubota et al., 1999; Grygiel-Górniak, 2014). Tissue-specific gene knockout investigations, using both aP2-Cre and Adipoq–Cre mouse lines, demonstrated that the adipose-specific loss of PPAR causes drastic adipose tissue atrophy and hampers adipokine secretion (Floyd & Stephens, 2012). One of its pivotal downstream effects is the activation of the transcription factor C/EBP during adipocyte differentiation (Madsen et al., 2014).

Endogenous ligands, including prostaglandin J2 and oxidized fatty acids, activate PPAR , and there are synthetic ligands, like thiazolidinediones, employed in type 2 diabetes treatment. Once activated, PPAR modulates several pathways, enhancing insulin signaling and suppressing inflammation (Forman et al., 1995; Greenfield & Chisholm, 2004; Leonardini et al., 2009; Scirpo et al., 2015; Lebovitz, 2019). Dysfunctional adipose tissue can lead to

systemic inflammation and insulin resistance. Adipocyte progenitors are established during embryonic development, with factors like Dab2 and calorie exposure influencing their numbers (Tao et al., 2016a).

In summary, PPAR is the central transcription factor for adipocyte differentiation. Along with caloric intake, it determines adipocyte differentiation and hypertrophy. Mature adipocyte transcription is a combined effort of PPAR , C/EBP, and RXR. Dysfunctional adipocytes can release inflammatory substances, potentially leading to metabolic issues (Floyd & Stephens, 2012).

4. Metabolic functions and regulation of adipose tissues

White adipose tissue (WAT), an essential endocrine organ, plays a pivotal role in maintaining energy balance in our bodies, chiefly by producing vital hormones such as leptin (Martínez-Sánchez, 2020). The production and secretion of leptin by adipocytes are influenced by a variety of factors including the nutritional status, hormonal inputs, and various intracellular signaling pathways (Russell et al., 1998; Sweeney et al., 2001; Cohen, et al., 1979; Meek & Morton, 2012; Klok, et al., 2007; Chan, et al., 2003). As a general rule, when the lipid storage in adipocytes expands, reflecting a surplus of energy reserves, there is a corresponding increase in the production of Leptin (Longo et al., 2019).

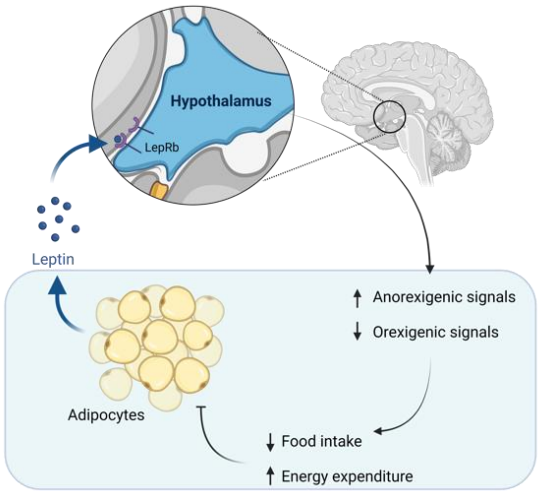

Fig. 4: Feedback regulation of adipocytes by Leptin. Illustration of the metabolic regulation of adipose tissues by Leptin. Leptin is mainly produced and secreted by adipocytes. High circulating leptin level produced from abundant adipocytes facilitate a feedback loop by signaling to brain. A high number of Leptin receptors are present in hypothalamus, which produces neuronal activity and peptides to increase anorexigenic signals and to reduce orexigenic signals. The resulted suppression of appetite leads to reduced caloric intakes and limit adipose expansion. Often, defective Leptin signaling loop lads to excessive caloric intakes, metabolic pathology, and obesity.

Leptin, primarily produced by adipocytes, plays an integral role in the regulation of body weight and energy balance (Martínez-Sánchez, 2020) (**Fig. 4)**. It acts as a key regulator of hunger and energy expenditure, contributing to the maintenance of energy homeostasis. An increase in Leptin levels signals the body to decrease appetite and increase energy expenditure, while a decrease in leptin levels incites the opposite response, stimulating hunger and reducing energy usage (Picó et al., 2022; Russell et al., 1998). However, despite Leptin's integral role in energy balance and adipose tissue homeostasis, leptin signaling can become dysregulated, leading to leptin resistance (Ceddia et al., 2002; Shimabukuro, 2017). This condition is marked by the ineffectiveness of Leptin in reducing appetite and stimulating energy expenditure despite high circulating leptin levels, contributing to the difficulty in achieving sustained weight loss in obesity (El-Haschimi et al., 2000). Often, defective Leptin signaling loop leads to excessive caloric intakes, metabolic pathology, and obesity (Klok et al., 2007). In summary, Leptin is a central figure in the regulation of energy homeostasis (**Fig. 4**).

Leptin carries out its functions through its interaction with the leptin receptor (LepR) (Gorska et al., 2010). There are six known LepR isoforms, and their expression varies among tissues. The long isoform, LepRb, is primarily expressed in the central nervous system, particularly in the hypothalamus (Kielar et al., 1998). It plays a pivotal role in leptin action and energy homeostasis. Short LepR isoforms like LepRe and LepRa also have essential roles. LepRe acts as a leptin-binding protein that neutralizes leptin, thereby regulating energy homeostasis (Ramos-Lobo & Donato Jr, 2017). LepRa is expressed in various organs including the kidney and lung and may play a crucial role in the transport of leptin through the blood-brain barrier to the brain (Di Spiezio et al., 2018).

Once Leptin is released into the bloodstream, it performs its principal action within the brain, specifically the hypothalamus (Sweeney et al., 2001) (**Fig. 4**). This is facilitated by Leptin binding to its respective receptors located on the surface of hypothalamic neurons (Cohen et al., 1996). The binding of Leptin initiates the changes in the transcription of target genes. This results in an increase in the production of anorexigenic peptides that suppress appetite, and a reduction in the production of orexigenic peptides that stimulate appetite (Gorska et al., 2010; Kielar et al., 1998). Consequently, there is an overall decrease in food intake and an increase in energy expenditure. Consequently, Leptin plays an instrumental role in energy homeostasis through its signaling mechanism. It communicates the body's energy status to the brain. In a scenario where there is an expansion of adipose tissue (as seen in overeating or obesity), there is increased production and secretion of leptin, which signals to the brain to

suppress appetite and increase energy expenditure (Halaas et al., 1995; Ceddia et al., 2002). Conversely, when adipose tissue shrinks (as in fasting or weight loss), there is a reduction in leptin production, leading to increased appetite and decreased energy expenditure (Wang et al., 2011; Garcia-Galiano, et al., 2019) (**Fig. 4**).

However, a major hurdle in the action of Leptin arises in cases of obesity (Klok et al., 2007; Ceddia et al., 2002; Ramos-Lobo & Donato Jr, 2017). Despite the high levels of circulating Leptin, the brain appears to be unresponsive or resistant to its effects, a condition known as Leptin resistance (Ramos-Lobo & Donato Jr, 2017), which poses a significant challenge when considering the use of leptin or its analogs as a therapeutic strategy for obesity. The precise mechanisms behind Leptin resistance are intricate and not completely understood, but they may involve defects in Leptin transport, signaling, and function of target neurons. Later in life, deterioration and aging of adipose tissues, and abnormal level of adipose tissues accumulated due to life styles will present pathological conditions affecting the overall health of individual.

5. Epidemiological evidence for early caloric intakes impacting metabolism in adults

As anecdotal observations suggest the existence of large variations of individual in their susceptibility to obesity, we consider factors that may contribute to these varietiers, either genetics or non-genetic determinants. One interesting question is the influence of embryonic and early exposure to calories on adipose tissues and obesity later in adult life.

In modern dates, overweight and obesity in children is an alarming epidemic (Deckelbaum & Williams, 2001). The significant increase in childhood obesity frequency in the population is partially attributed by an increased number of obese women entering pregnancy (Tarantal & Berglund, 2014). Some speculate that offspring of obese mothers are subject to an increased risk of obesity due to an unfavorable fetal environment that affects the development of adipose tissues, and the impact of maternal obesity extends beyond neonatal life to adolescence and adulthood (Tenenbaum-Gavish & Hod, 2013; Tarantal & Berglund, 2014). Epidemiological data suggest that obesity during adolescence and adulthood can be traced back to fetal and early childhood exposures (Muhlhausler & Smith, 2009). Longitudinal studies have found that most overweight/obese children would become overweight and obese adults (Efrat et al., 2013). Emerging data suggest associations between

the influence of maternal and fetal factors and caloric exposure in the early years of life, on risk of later development of adult obesity and its comorbidities (Deckelbaum & Williams, 2001; Muhlhausler & Smith, 2009). The evidence that childhood overweight and obesity are strong predictors for severe obesity over the whole life course leads to a scientific paradigm that the approximate number of adipocytes is set for life at an early age (Muhlhausler & Smith, 2009; Efrat et al., 2013).

In summary, adipose tissue is the main site of energy storage, in the form of lipid droplets. In response to excessive dietary caloric intake or starvation, the adipocytes can expand or condense in both cell size and number (Muhlhausler & Smith, 2009; Efrat et al., 2013). Excessive fat also leads to pathological conditions, and obesity is a concern of modern society (Efrat et al., 2013). Epidemiological evidence indicates that obesity at an early age leads to persistent health problems in adults (Deckelbaum & Williams, 2001; Muhlhausler & Smith, 2009). Additionally, childhood obesity is an especially strong predictor of adult obesity and health issues (Deckelbaum & Williams, 2001).

6. Evidence from lab feeding studies of rodent models

Decades ago, laboratory studies using rodents to model the caloric impacts were performed to seek support of the epidemiological data and ideas that early caloric intakes impact metabolic pathology later in adult life (Knittle & Hirsch, 1968; Tsujikawa & Kimura, 1980).

Their observations generally indicate that early nutritional exposure could determine permanent characteristics of fat cells in size and number in adult animals. These studies highlighted the importance of early nutritional experiences, and proposed that similar alterations in cellularity and metabolism caused in early life may be the etiology of obesity in humans (Knittle & Hirsch, 1968).

Additional experiments found that the impacts also found in embryonic stages when maternal rats were given a restricted or free excessive diet during pregnancy, leading to the conclusion that cellular effects of early feeding on later fat cell size and number depended on the phase of growth in the rat (Tsujikawa & Kimura, 1980).

In reviewing the various studies of farm animals, it was concluded that irrespective of species, white adipose tissue has a large capacity to expand postnatally, and early nutrition during fetal and perinatal periods can pre-determinate later growth of adipose tissue (Louveau et al., 2016).

7. Clues from Dab2 null mice for a population of embryonic/Juvenile adipocyte progenitor cells

Dab2 is an endocytosis adaptor protein and also found to modulate cellular signaling (Tao et al., 2016b). The LDL receptor is a receptor bound by Dab2 to be recruited into clatherin-coated vesicles, and Dab2 together with Arh (autosomal recessive hypercholesterolemia) mediate LDL endocytosis and cholesterol homeostasis (Tao et al., 2016c).

Dab2 gene knockout is embryonic lethal, but Dab2 null mice can be produced using conditional deletion to bypass early embryonic requirements (Moore et al., 2013). One interesting observation was that Dab2 null mice do not gain significant weight when given a high fat/high caloric diet (Tao et al., 2016a). Analyses in cells and knockout mice suggest that Dab2 promotes adipocyte differentiation by enabling PPAR nuclear entry through suppressing Ras/MAPK activity (Tao et al., 2016a), which otherwise phosphorylate PPAR and thus preventing its nuclear entry to facilitate adipognsis (Hu et al., 1996; Adams et al., 1997; Bost et al., 2005; Burgermeister & Seger, 2007).

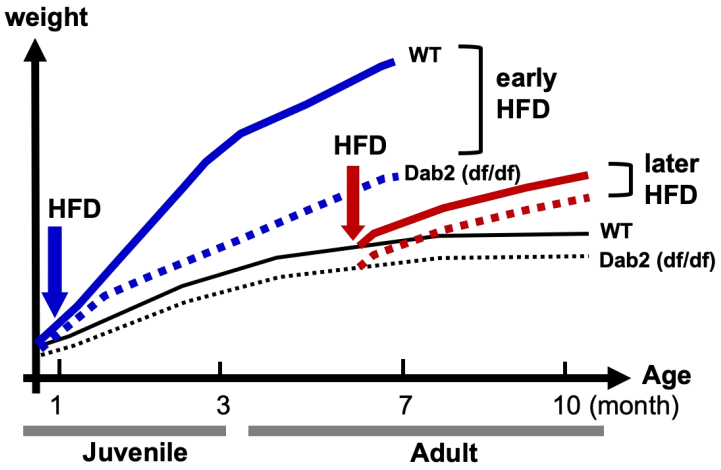

Fig. 5: Evidence of a population of Dab2-regulated adipose progenitor cells from growth curve of Dab2 null mice. Illustration of weight curves of control wildtype (WT) and Dab2 null mice fed with normal and high fat diet are illustrated based on a publication (Tao et al., 2016a). Dab2 null mice have a much reduced ability to expend adipose tissue and gain weight comparing to wildtype (WT) control mice, when feeding of high fat/high caloric diet started at a young (3 weeks) of age. However, the differences in weight gain are absent when the feeding started at an older age (6 months). The differences in weight gain were determined to be accounted for by adipocyte number rather than cell size. The proposal that Dab2 is required for the caloric induced differentiation of a population of pre-adipocyte present in early but not in adult stages is proposed to explain the results.

As shown in the illustration (**Fig. 5**), Dab2 null mice have a much reduced ability to expend adipose tissue and gain weight comparing to wildtype (WT) control mice, when feeding of high fat/high caloric diet started at a young (3 weeks) of age. However, the

differences in weight gain are absent when the feeding started at an older age (6 months). The differences in weight gain were determined to be accounted for by adipocyte number rather than cell size. The proposal that Dab2 is required for the caloric induced differentiation of a population of pre-adipocyte present in early but not in adult stages is proposed to explain the results (Tao et al., 2016a).

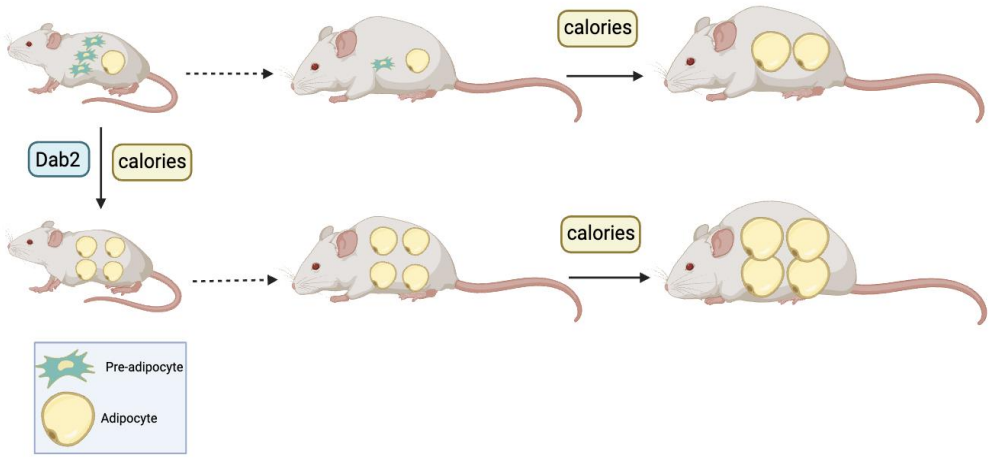

Fig. 6: Model: a population of pre-adipocyte progenitor cells present in embryonic/early stages determines adipose cell number and contributes to obesity later in life. Illustration of an embryonic adipose progenitor cell population is presented, based on a publication (Tao et al., 2016b). The pre-adipocyte progenitor cells are present in juvenile animals, and are absent or reduced in matured adults. When the animals are exposed to high calories, the abundant progenitor cells in juveniles are differentiated into adipocytes in a Dab2-depedent mechanism. As a consequence, the animals exposed to high calories during juvenile stages have a larger number of adipocytes, which give the animals a higher capacity to gain weight in adult stage when high calories are given. Thus, early caloric exposure endows the animal a higher number of adipocytes, which make the animal a higher possibility of developing severe obesity in later adult life when it encouters again high caloric diet.

The study of Dab2 null mice provided evidence for the presence of an adipocyte progenitor cell population present only in juvenile but lost in adults (**Fig. 6**). The observation was that when fed a high caloric diet, the Dab2 null mice did not gain significant weight and develop obesity compared to those of wildtype mice when the feeding started at a young age (Tao et al., 2016a). The Dab2 null adipocytes were found as enlarged as those of wildtypes, but the smaller fat depots are accounted for by a fewer number of fat cells. However, when feeding of a high caloric diet started at an older age (6 months of older), the differences in fat generation were absent between wildtype and Dab2 null mice. The implication is that this pre-adipocyte population accounts for a mechanism for influence of early caloric intake on propensity of obesity risk later in adults (Tao et al., 2016a). The result suggests that Dab2 is essential for differentiation of an adipocyte precursor population that is present in juveniles but depleted in mature mice (Tao et al., 2016a).

These observations support that the number of adipocyte progenitor cells can be significantly expanded in early life (Dab2 dependent), but fat progenitor cell number are fewer in mature animals (Tao et al., 2016b).

Based on both epidemiological findings and laboratory analyses using rodent feeding studies, a model has been proposed by (Tao et al., 2016b) as illustrated in **Fig. 6**. This model posits that an early-life caloric surplus triggers the recruitment of additional progenitor cells that differentiate and form adipocytes. Interestingly, these precursor cells are prevalent during youth but diminish or are absent in adulthood. Consequently, juvenile animals exposed to high-caloric diets have an abundance of these progenitor cells, which, through a Dab2-dependent mechanism, differentiate into adipocytes. This leads to these animals having a greater number of adipocytes. As a result, when these animals reach adulthood, they possess an enhanced capacity to gain weight when subjected to high-caloric diets, potentially predisposing them to obesity and related metabolic conditions.

Summary and Prospective

Adipose tissues predominantly stem from the mesodermal layer of the embryonic germinal layer (Sebo et al., 2018). Research suggests that exposure to calories during the early juvenile phase plays a pivotal role in determining the count of pre-adipocyte progenitors (Muhlhausler & Smith, 2009; Efrat et al., 2013). Once matured, an individual's adipocyte count stabilizes and remains fairly constant. Surplus caloric intake in the context of obesity leads to an expansion in the size of these adipocytes rather than an increase in their number (Muhlhausler & Smith, 2009; Efrat et al., 2013). Hence, early-life caloric exposure primes juveniles with an abundance of pre-adipocytes, predisposing them to a heightened risk of severe obesity upon encountering excessive caloric intake later in life.

Studies utilizing mouse models offer deeper insights into the regulation and characteristics of pre-adipocyte populations. These studies also offer a foundational understanding that aligns with epidemiological research, correlating early-life dietary patterns to obesity trends observed in adult and adolescent humans (Deckelbaum & Williams, 2001; Efrat et al., 2013). Our proposed model in this article specifically highlights the mechanistic pathway from early caloric exposure to the development of obesity via adipocyte size expansion and pre-adipocyte proliferation. We anticipate that this model will not only inspire

further research and validation but also pave the way for targeted interventions to address the growing societal challenge of obesity.

Abbreviations:

AP2,	Adaptin protein 2
aP2,	adipocyte protein2
Arh,	autosomal recessive hypercholesterolemia
C/EBPs,	CCAAT/enhancer binding proteins
C/FAT,	craniofacial/facial
Dab2,	Disabled-2
EGF,	Epidermal Growth Factor
LDL,	Low density liproprotein
PPAR,	peroxisome proliferator-activated receptor
RXR,	retinoid X receptor
SAT,	subcutaneous adipose tissue
TGF-beta,	transforming growth factor beta
VAT,	visceral adipose tissue
WAT,	White adipose tissue

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Author Contributions

As part of a graduate course, all authors participated in an extensive literature review and discussion of the topic (adipocyte development). All authors participated in drafting of one or more sections of the article and making of figures, and especially JMS contributed to many parts in the article. XXu assembled and prepared the first draft, and all authors edited and agreed to the final version.

Ethical and Conflict of Interests:

The authors declaim no financial involvement and no conflict of interest of the content in the article.

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