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Modelos experimentales para estudiar el tejido adiposo en obesidad infantil

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Abstract

Obesity is a major public health concern that has significant repercussions on individuals' well-being and healthcare systems. It is associated with a reduced lifespan and is a risk factor for several chronic diseases, such as type 2 diabetes, coronary heart diseases, and certain types of cancers. The understanding of obesity's underlying physiological mechanisms remains limited, despite its significant impact. Childhood obesity is increasingly prevalent and, like adult obesity, it is closely linked to various metabolic diseases. Given the growing occurrence of obesity in children and its consequential clinical and physiological implications, there is a pressing need for targeted experimental research. It is crucial to employ specific cell and animal models to unravel the molecular and genetic factors that contribute to obesity in this age group. Here, we review the emerging field of childhood obesity, focusing on the different types of adipose tissue, differences between adipose tissue in adults and children, current available experimental models to study adipose tissue in children, and factors involved in the pathogenesis of childhood obesity.

Keywords: Obesity, Adipose tissue, Children, Experimental models

Childhood obesity

Obesity is a global threat. While it was previously predominant in adults, obesity is becoming more prevalent in younger generations, including children younger than 10 years old. In 2019, the World Health Organization (WHO) estimated there were 38.2 million children under the age of 5 years suffering from overweight or obesity. Data from 2016 revealed that over 340 million children and adolescents (18%) aged 5–19 years were obese or overweight. Furthermore, the WHO also reported that, while overweight and obesity were previously only considered a concern in high-income countries, they are exponentially growing in both low and middle-income countries, with a marked emphasis on urban areas (2019)[1]. Thus, efforts should be made at the research, clinical, and social levels to stop the alarming growth of obesity and its associated metabolic diseases both in adults and children.

Adipose tissue: colours and functions

Adipose tissue (AT) is a complex and highly dynamic endocrine organ with intricate metabolic activity that greatly influences the function of nearly all other organ systems through its various adipokines, lipokines, lipids, and peptides[2]. The distribution, localization, and colour of AT determines its function. AT beneath the skin is known as subcutaneous adipose tissue (SAT), whereas AT surrounding internal organs is known as visceral adipose tissue (VAT)[3]. In mammals, there are four colours of AT: white, brown, beige, and pink. In brief, the major function of white adipose tissue (WAT) is to store unused energy in the form of triglycerides and release it as fatty acids in times of systemic energy need, whereas brown adipose tissue (BAT) uses fuels, such as glucose or lipid, to maintain the core body temperature and function as a thermogenic tissue[4]. Beige adipocytes arise within WAT either due to differentiation of white adipocyte precursors or trans-differentiation of already mature white adipocytes. Beige and brown adipocytes share many functional and morphological

traits. They take up glucose and triglycerides to generate heat in response to cold exposure and exercise[5]. Finally, pink adipocytes are alveolar epithelial cells within the mammary gland that are responsible for producing and secreting milk[6]. Several models have been used to study adipose tissue in adults and children: 1) *in vitro* cell cultured lines and primary adipocytes derived from both animals and humans and 2) *in vivo* animal models (summarized in Table 1 and section "*Models to study AT*" below).

Differences in AT between children and adults

The characteristics of AT and its response to pathological inputs, like obesity, differ between children and adults[7,8]. In general, AT growth can be classified as either hypertrophic, in which the adipocyte size increases, or hyperplastic, in which the number of adipocytes increases[9]. Body fat mass expands significantly due to cell size enlargement (hypertrophy) up to 6 months of age. Nonetheless, from the age 6 months until early adolescence (around 10 years old), the fat content increases alongside cell number[10,11]. Puberty (10–18 years old) is characterized by an increase in both hypertrophy and hyperplasia. This stage is also marked by sexual dimorphism. Indeed, sexual dimorphism typically emerges during embryonic stages. However, in the case of obesity, it is during puberty that these differences become significantly pronounced and continue into adulthood. Therefore, the utilization of cell and animal experimental models becomes crucial in investigating this important aspect. Subcutaneous fat distribution differs between males and females. Around the age of 18 years, under normal weight conditions, cell number reaches a plateau, establishing the adipocyte number for the future[10,11]. In adulthood, the capacity for preadipocyte differentiation declines, and therefore, fat content increases mainly due to adipocyte hypertrophy, in contrast to childhood and adolescence[12,13]. In addition, aging has been shown to foster AT redistribution by significantly reducing SAT and simultaneously increasing VAT[13]. This rearrangement has

been strongly suggested to explain why elderly people display a worse metabolic health than younger generations[13].

Models to study AT

Studies on childhood obesity, along with its global prevalence, is a relatively recent phenomenon and, consequently, data from cell and animal models are sparse. A deeper understanding of the molecular mechanisms regulating adipocyte function and disfunction in children is necessary to plan strategies to treat and prevent obesity and related metabolic complications. Over the past few decades, several preadipocyte and mature adipocyte cell models have been used to study adipogenesis in adults. Rodent cells lines are the most widely used cell line models, although both porcine and feline primary cells have also been used to a lesser extent[14,15]. Among rodents, mouse preadipocytes including 3T3-L1, 3T3-F442A, OP9, and C3H10T1/2 stromal cells have been studied in detail over the years, with 3T3-L1 being the most established and commonly used cell line[15]. However, porcine primary preadipocytes have been suggested as a better model for obesity research due to their greater similarity to human cells[15] (Table 1).

In addition, human cell lines (ChubS7[16] and imAPAD[17] and imGPAD[17]) and primary cells have been increasingly used to study adipocyte biology and obesity-related changes. However, only one preadipocyte cell line, Simpson-Golabi-Behmel-Syndrome (SGBS), has been isolated from children[18]. This cell line was derived from the AT of an infant with SGBS, which is a quite rare syndrome characterized by macroglossia, macrosomia, renal and skeletal abnormalities, and an increased risk of embryonal cancer, distinct from polygenic or monogenic obesity. While the insights gained from studying SGBS cells are undoubtedly valuable, it is important to consider that the results may be applicable only within a specific context. A recent study has shown that these cells are metabolically distinct from primary

adipocytes[19], indicating the importance of establishing child-derived cell models from healthy and obese children for further studies (Table 1).

Table 1: **Models to study adipose tissue.** Summary of adipose *in vitro* and *in vivo* models discussed in this paper

Animal cell line models[14]	
Mouse 3T3-L1	Derived from 17- to 19-day-old Swiss 3T3 mouse embryonic fibroblasts, most used cell line model to study adipogenesis, high number of passages
Mouse 3T3-F442A	Derived from murine Swiss 3T3 cells. Able to accumulate higher fat content than 3T3-L1
Mouse OP9	Derived from the calvaria of new-born mice. Fast adipogenic differentiation (72 h). Suitable for high-throughput assays
Mouse C3H10T1/2	Derived from 14-17-day-old C3H embryonic stem cell precursors. Frequently used to study adipogenesis
Primary animal cells [13,14]	
Porcine cells	Better model for adipogenesis and adipose tissue-related complications compared to rodents due to their higher similarity towards human cells
Feline cells	Used to study adipogenesis and type 2 diabetes
Human cell line models	
ChubS7 [15]	Derived from abdominal subcutaneous adipose tissue of a 33-year old obese female, high proliferation and differentiation rate
imAPAD [16]	Derived from abdominal subcutaneous adipose tissue of a 50-year old healthy male, high proliferation and differentiation rate. Retain depot-specific function
imGPAD [16]	Derived from gluteal subcutaneous adipose tissue of a 50-year old healthy male's, high proliferation and differentiation rate. Retain depot-specific function

SGBS [17]	Derived from subcutaneous adipose tissue of a 3-month-old infant with Simpson-Golabi-Behmel syndrome. Used to study adipogenesis. Metabolically distinct from primary adipocytes
Primary human cells	
Primary Preadipocytes [14]	Primary cells, represent the most physiological model of adipose precursors. Retain their depot-of-origin and individual traits. Limited number of passages, not always easily accessible
***In vivo* animal Models**	
C. elegans [19]	Used to study factors involved in food satiety
D. melanogaster [19]	Used to study neuronal pathways implicated in fat deposition
D. rerio [19]	Frequently used model for the study of adipose tissue biology and obesity-related diseases
Zucker rat pups [20]	Used to study AT growth from suckling to puberty stages
Small litter size rodents [23]	Better model to study childhood adiposity andlateronset ofmetabolic diseases than large litter size

In vivo models are also crucial for assessing both basic and clinical assays. Small animal models, such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Danio rerio*, have displayed many advantages due to their short life cycle, known genome maps, and low maintenance cost, for studying the mechanistic underpinnings of various pathological conditions. Obesity research in *C. elegans* has uncovered factors involved in both food sensing and food satiety. Conversely, studies using *D. melanogaster* identified neuronal pathways involved in fat deposition[20]. While small animal models can be valuable for various preclinical studies, they may not be suitable for evaluating the multiorgan system effects of obesity in adults or children. Nevertheless, these animal models can contribute to understanding the molecular and neurological pathways that may be influenced or disrupted

by childhood obesity. Hence, small rodents, such as mice and rats, are widely employed as mammalian *in vivo* model systems for obesity and related disorders at the genetic, molecular, and functional levels[20]. Zucker rat pups have been used to study AT growth from suckling to puberty stages. Use of this model revealed that the highest rates of adipocyte proliferation, *de novo* lipogenesis, and outbreak of excessive fat mass occurred at the end of the suckling stage (Table 1)[21]. The premise that human metabolism and physiology differ to that of animals and that adults AT development is different between children and adults limits the insight that these studies may provide on obesity and related illnesses in children[14,15].

Factors involved in the pathogenesis of childhood obesity

In terms of socioeconomic and environmental factors, a child's body mass index (BMI) and its mother's BMI and education status have been reported to be associated with risk of becoming obese[22]. These three risk factors are particularly noteworthy from the age of 6 years onwards. Overweight and obesity were markedly prevalent among children who previously displayed a cluster of all three, providing both easy and quick evaluations and further prevention assessments at clinical encounters[22].

Furthermore, some studies have attempted to uncover epigenetic patterns directly linked to emerging childhood obesity. These transcriptional modifications may offer insight into predisposition as they switch in response to environmental factors, such as physical activity or nutrition, and differ between different ethnicities. An example of this is the epigenetic modification of hypothalamic feeding circuits in offspring when subjected to stress during pregnancy or postnatal periods. This alteration leads to changes in the expression of neuropeptides, affecting their regulation of feeding behaviour[23, 24]. As a result, energy metabolism is altered in the offspring increasing their susceptibility to develop obesity and metabolic-like syndromes in adulthood[23]. These epigenetic modifications include mutations

in gene promoters associated with both appetite regulation and stress. Additionally, adverse experiences during early childhood can lead to DNA methylations in genes associated with obesity[23,25].

DNA methylation of the nuclear respiratory factor 1 (NRF1) gene at a crucial CpG dinucleotide was strongly correlated with obesity emergence at age 3-5–years in nonobese Hispanic children. However, data on other ethnicities has not been reported[26].

While the influence of mothers on the transmission of obesity risk to their offspring has been extensively studied[23,24,27,28], only few studies have evaluated the potential contribution of fathers to their children's obesity development[27,29]. However, recent investigations indicate that interventions aimed at reducing obesity in men, such as exercise and weight loss, can have a positive impact on sperm quality and alter patterns of DNA methylation in sperm[27]. In order to gain a comprehensive understanding of the underlying intergenerational mechanisms involved in early human exposure, it is crucial for future research to address both maternal and paternal epigenetic and epidemiological studies. Studying all these factors in detail will benefit from the establishment of reliable models to study childhood obesity, including child-derived cell models, and animal models.

Conclusions and future perspectives

Obesity and its associated metabolic diseases, such as diabetes, insulin resistance, cardiovascular disease, asthma, and some types of cancer, are becoming more prevalent worldwide. This is especially worrisome at younger ages. Importantly, research into obesity has largely focused on adults and overlooked in children. *In vivo* and *in vitro* models to study childhood obesity are still very limited. Thus, experimental models to study AT in childhood obesity are required to elucidate accurate early prediction markers and, ultimately, appropriate prevention strategies.

References

1. Obesity and overweight. Accessed February 21, 2023. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

2. Kershaw EE, Flier JS. Adipose Tissue as an Endocrine Organ. Published online 2004. doi:10.1210/jc.2004-0395

3. Von Bank H, Kirsh C, Simcox J. Aging Adipose: Depot Location Dictates Age-Associated Expansion and Dysfunction. doi:10.1016/j.arr.2021.101259

4. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004;84(1):277-359. doi:10.1152/PHYSREV.00015.2003

5. Cheng L, Wang J, Dai H, et al. Brown and beige adipose tissue: a novel therapeutic strategy for obesity and type 2 diabetes mellitus. *Adipocyte*. 2021;10(1):48-65. doi:10.1080/21623945.2020.1870060

6. Giordano A, Smorlesi A, Frontini A, Barbatelli G, Cint S. MECHANISMS IN ENDOCRINOLOGY: White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ. *Eur J Endocrinol*. 2014;170(5):R159-R171. doi:10.1530/EJE-13-0945

7. Reilly JJ. Obesity in childhood and adolescence: evidence based clinical and public health perspectives. *Postgrad Med J*. 2006;82:429-437. doi:10.1136/pgmj.2005.043836

8. Takahashi H, Menendez A, Wanczyk H, et al. Obesity and Adipose Tissue Dysfunction: From Pediatrics to Adults. Published online 2022. doi:10.3390/genes13101866

9. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. *Front Endocrinol (Lausanne)*. 2016;7(APR). doi:10.3389/FENDO.2016.00030

10. Knittle JL, Timmers K, Ginsberg-Fellner F, Brown RE, Katz DP. *The Growth of Adipose Tissue in Children and Adolescents CROSS-SECTIONAL AND LONGITUDINAL STUDIES OF ADIPOSE CELL NUMBER AND SIZE*.

11. Poissonnet CM, LaVelle M, Burdi AR. Growth and development of adipose tissue. *J Pediatr*. 1988;113(1 PART 1):1-9. doi:10.1016/S0022-3476(88)80520-1

12. Frigolet ME, Gutiérrez-Aguilar R. The colors of adipose tissue. *Gac Med Mex*. 2020;156(2):142-149. doi:10.24875/GMM.M20000356

13. Reyes-Farias M, Fos-Domenech J, Serra D, Herrero L, Sánchez-Infantes D. White adipose tissue dysfunction in obesity and aging. *Biochem Pharmacol*. 2021;192. doi:10.1016/J.BCP.2021.114723

14. Lee MJ, Fried SK. Optimal Protocol for the Differentiation and Metabolic Analysis of Human Adipose Stromal Cells. Published online 2014. doi:10.1016/B978-0-12-800280-3.00004-9

15. Ruiz-Ojeda FJ, Rupérez AI, Gomez-Llorente C, Gil A, Aguilera CM. Molecular Sciences Cell Models and Their Application for Studying Adipogenic Differentiation in Relation to Obesity: A Review. doi:10.3390/ijms17071040

16. Darimont C, Zbinden I, Avanti O, et al. Reconstitution of telomerase activity combined with HPV-E7 expression allow human preadipocytes to preserve their differentiation capacity after immortalization. *Cell Death Differ*. 2003;10:1025-1031. doi:10.1038/sj.cdd.4401273

17. Todor Cevi C M, Hilton C, Mcneil C, et al. A cellular model for the investigation of depot specific human adipocyte biology. Published online 2017. doi:10.1080/21623945.2016.1277052

18. Fischer-Posovszky P, Newell FS, Wabitsch M, Tornqvist HE. Human SGBS Cells – a Unique Tool for Studies of Human Fat Cell Biology. *Obes Facts*. 2008;1(4):184-189. doi:10.1159/000145784

19. Yeo CR, Agrawal M, Hoon S, et al. SGBS cells as a model of human adipocyte browning: A comprehensive comparative study with primary human white subcutaneous adipocytes OPEN. doi:10.1038/s41598-017-04369-2

20. Dey SK, Senapati S. In Vivo Models for Obesity and Obesity Related Carcinogenesis. *Obesity and Cancer*. Published online 2021:279-300. doi:10.1007/978-981-16-1846-8_14

21. Pouteau E, Turner S, Aprikian O, et al. Time course and dynamics of adipose tissue development in obese and lean Zucker rat pups. *Int J Obes (Lond)*. 2008;32(4):648-657. doi:10.1038/SJ.IJO.0803787

22. Gulyaeva O, Dempersmier J, Sul HS. Genetic and Epigenetic Control of Adipose Development. doi:10.1016/j.bbalip

23. López-Taboada I, González-Pardo H, Conejo NM. Western Diet: Implications for Brain Function and Behavior. *Front Psychol*. 2020;11. doi:10.3389/FPSYG.2020.564413/FULL

24. Miller AL, Lumeng JC. Pathways of Association from Stress to Obesity in Early Childhood. *Obesity*. 2018;26:1117-1124. doi:10.1002/oby.22155

25. Rushing A, Sommer EC, Zhao S, Po'e EK, Barkin SL. Salivary epigenetic biomarkers as predictors of emerging childhood obesity. doi:10.1186/s12881-020-0968-7

26. Rushing A, Sommer EC, Zhao S, Po'e EK, Barkin SL. Salivary epigenetic biomarkers as predictors of emerging childhood obesity. doi:10.1186/s12881-020-0968-7

27. Lin J, Gu W, Huang H. Effects of Paternal Obesity on Fetal Development and Pregnancy Complications: A Prospective Clinical Cohort Study. *Front Endocrinol (Lausanne)*. 2022;13. doi:10.3389/FENDO.2022.826665/FULL

28. Entringer S, Buss C, Swanson JM, et al. Fetal Programming of Body Composition, Obesity, and Metabolic Function: The Role of Intrauterine Stress and Stress Biology. *J Nutr Metab*. 2012;2012:16. doi:10.1155/2012/632548

29. Shawe J, Delbaere I, Ekstrand M, et al. Preconception care policy, guidelines, recommendations and services across six European countries: Belgium (Flanders), Denmark, Italy, the Netherlands, Sweden and the United Kingdom. *Eur J Contracept Reprod Health Care*. 2015;20(2):77-87. doi:10.3109/13625187.2014.990088