Marina Galleazzo Martins

MATERNAL HYPERGLYCEMIA AND OVERNUTRION: EFFECTS ON MATERNAL CARE AND OFFSPRING DEVELOPMENT AND BEHAVIOR ACROSS LIFE

ASSOCIAÇÃO ENTRE HIPERGLICEMIA MATERNA E SUPERNUTRIÇÃO: EFEITOS NO CUIDADO MATERNO E NO DESENVOLVIMENTO E COMPORTAMENTO DOS DESCENDENTES EM DIFERENTES FASES DA VIDA

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To the ones who came before me:
Vô Lilo, Vó Eva, Vô Henrique, and Vó Teresa

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“(...) e, eu, rio abaixo, rio a fora, rio a dentro - o rio.”

A terceira margem do rio,
João Guimarães Rosa

SUMMARY

ABSTRACT

The present study evaluated if snack intake during pregnancy and lactation could aggravate previously established maternal hyperglycemia and its consequences to maternal care, as well as offspring development, metabolism, and behavior throughout life. Our hypothesis was that snack intake during pregnancy and lactation would trigger further impairments in maternal glycemia homeostasis, resulting in changes in maternal behavior as well on offspring development, metabolism, and behavior from birth to senescence. Chapter 1 describes how snack intake altered maternal food intake and decreased glucose tolerance in STZ-treated females. Birth weight classification was normalized in the offspring from hyperglycemic dams with access to snacks, which showed a patter similar to Control offspring. Moreover, hyperglycemic dams with access to snack were less anxious and had higher maternal motivation. Chapter 2 describes short and long-term consequences of this altered maternal nutrition and metabolism to both male and female offspring, analyzing glucose metabolism, behavior, and morphometric aspects in adolescence, adulthood, and senescence. Male offspring reproductive function was impaired in adulthood, while there was an increase in anxiety-like behavior in senescence, showing that consequences may only be evident in the long-term. No further compromises on offspring metabolism were observed. In conclusion, the present study showed that snack intake during pregnancy and lactation further impaired maternal hyperglycemia, leading to disruptions on maternal motivation during lactation and reduced the incidence of macrosomia in their offspring. In adulthood, the reproductive function was disrupted, and senescent offspring showed changes on anxiety-like behavior. Future studies will describe effects on offspring learning and memory, and its possible neural substrates. The experimental model used in this study is useful to study the consequences of maternal diabetes associated with inappropriate nutrition, since the glycemic levels resemble those most observed in pregnant women diagnosed with clinical or gestational diabetes. Although snack intake aggravated the glucose intolerance of mild hyperglycemic rats, glycemic levels were still within the mild range, which might explain why this condition did not lead to major impairments for both mother and offspring. However, even this mild maternal condition was enough to change maternal and offspring outcomes, reinforcing the importance of women sustaining target glucose levels and a healthy diet during pregnancy and lactation.

Keywords: pregnancy, lactation, glucose tolerance, offspring, rats

RESUMO

O presente estudo avaliou se a ingestão de *snacks* durante a prenhez e lactação agravaria a hiperglicemia materna de ratas diabéticas e suas consequências para o cuidado materno, e o desenvolvimento, metabolismo e comportamento dos descendentes ao longo da vida. Nossa hipótese era que a ingestão de *snacks* durante a prenhez e lactação prejudicaria a homeostase glicêmica materna, resultando em alterações no comportamento materno, bem como desenvolvimento, metabolismo e comportamento dos descendentes do nascimento à senescência. O capítulo 1 descreve como a ingestão de *snacks* alterou a ingestão alimentar materna e reduziu a tolerância à glicose em fêmeas tratadas com STZ. A classificação do peso ao nascer foi normalizada nos descendentes de fêmeas hiperglicêmicas com acesso aos *snacks*, apresentando um padrão semelhante ao grupo Controle. Além disso, essas fêmeas eram menos ansiosas e apresentaram maior motivação materna. O capítulo 2 descreve consequências, a curto e longo prazo, do metabolismo e nutrição materna alterados nos descendentes machos e fêmeas, analisando o metabolismo da glicose, comportamento, e parâmetros morfométricos na adolescência, vida adulta e senescência. A função reprodutiva em descendentes machos foi alterada na vida adulta, assim como aumentaram os níveis de ansiedade na senescência, mostrando que essas consequências podem ser vistas apenas a longo prazo. Não houve comprometimento do metabolismo da glicose dos descendentes. Assim, o presente estudo mostrou que a ingestão de *snacks* durante a prenhez e lactação agrava a hiperglicemia materna, levando a alterações na motivação materna durante a lactação e reduzindo a incidência de macrossomia em seus descendentes. Na vida adulta, a função reprodutiva foi alterada, e animais idosos apresentaram maior ansiedade. Estudos futuros descreverão possíveis consequências para memória e aprendizagem dos descendentes, e as possíveis repercussões neurais. O modelo experimental utilizado nesse estudo é útil para a avaliação dos efeitos do diabete materno associado a uma nutrição inadequada, já que os níveis glicêmicos se assemelham aos observados em mulheres diagnosticadas com diabete clínico ou gestacional. Apesar dos *snacks* agravarem a hiperglicemia, os níveis glicêmicos ainda são moderados, o que poderia explicar porque não foram observadas alterações mais pronunciadas nas mães e seus descendentes. Entretanto, mesmo essa condição materna moderada foi suficiente para causar efeitos para mães e seus descendentes, reforçando a importância de mulheres manterem níveis glicêmicos adequados e uma dieta saudável durante a gestação e lactação.

Palavras-chave: prenhez, lactação, tolerância à glicose, descendentes, ratos

BACKGROUND

Diabetes is a chronic disease characterized by hyperglycemia, either due to reduced insulin production by the pancreas or impaired insulin action in the body [1]. If not properly treated, hyperglycemia can lead to serious and lethal consequences to the organism, followed by a considerable burden in the health system and economic loss [1]. Diabetes can be assorted into three major categories: type 1, type 2, and gestational diabetes [2]. Besides those categories, genetic syndromes, exocrine pancreas diseases, drugs, and chemicals can be the cause of specific types of diabetes, which are rare in the population. Type 1 diabetes affects between 5-10% of all diabetic patients and is defined by β-cell loss and absolute insulin deficiency. Type 2 diabetes is the most frequent type of diabetes, affecting between 90-95% of the patients, and involves insulin resistance that can lead to progressive β-cell loss. Diabetes can also be present during pregnancy in the form of clinical diabetes, when women previously diagnosed with different types of diabetes become pregnant, or gestational diabetes, when it is first diagnosed during pregnancy. Gestational diabetes is a condition in which a woman without diabetes develops high blood sugar levels during pregnancy. It can affect 5-10% of pregnancies and it is diagnosed during the second or third trimester of pregnancy [2].

Gestational diabetes results from an exacerbation of a variety of physiological adjustments that happen in pregnancy to support offspring development. During pregnancy, the maternal organism is in a state of physiologically insulin resistance, which is pathologically exacerbated in women diagnosed with gestational diabetes [3]. In healthy pregnancies, maternal metabolic homeostasis is shifted towards lipids utilization, so glucose is readily available to the growing fetus [4]. In early pregnancy, insulin sensitivity increases, allowing fat deposition in this anabolic period [5]. However, in the third trimester, there is a peak in insulin resistance, with a decrease of 50% in insulin sensitivity [6]. Furthermore, pregnant women show a positive energy balance, essential for offspring development [6]. All these factors are considerably worsened in gestational diabetes.

Even with increased information and methods of diagnosis available, diabetes in women of childbearing age is still sub-diagnosed [2]. The American Diabetes Association (ADA) recommends that pregnant women not previously diagnosed with diabetes should be screened for gestational diabetes at 24 to 28 weeks of pregnancy through a 75-g oral glucose tolerance test. A diagnosis is made when the woman has any of the following glycemia: ≥ 92 mg/dL at fasting, ≥ 180 mg/dL 1 h after glucose load, and ≥ 153 mg/dL 2 h after glucose load [2]. The diagnosis is important, so proper treatment is provided to

avoid further consequences for both mother and offspring [7]. Both gestational and clinical diabetes have consequences to the mother and offspring. Since in clinical diabetes the uterine milieu is already altered by hyperglycemia in the first stages of pregnancy, offspring of women with clinical diabetes are at higher risk of congenital anomalies. On the other hand, gestational diabetes is mostly established during the fetal stage, compromising offspring growth and development. Changes in fetal growth and development are relevant since birth weight is a major predictor of long-term health [8, 9].

Women previously diagnosed with clinical diabetes or who develop gestational diabetes have a greater risk of obstetric complications, such as preterm birth, preeclampsia, c-section, and shoulder dystocia [3, 10]. Their offspring also show a greater risk of being premature, having severe malformations, Erb's palsy, and of being classified as macrosomic at birth [3, 10, 11], usually showing increased body fat regardless of their birth weight classification [11, 12]. The offspring of the diabetic mother are also at a greater risk of developing obesity, metabolic syndrome, insulin resistance, and of having a worse lipidic panel and higher fat deposition during childhood and adolescence [6, 13-15].

In addition to metabolic impairments, the offspring from diabetic mothers also may show deficits in intelligence, motor skills, attention, and hyperactivity. Six-month-old infants born to diabetic mothers show electrophysiological differences related to visual memory [16]. During primary school, this offspring has shown poorer fine and gross motor skills and higher levels of hyperactivity [17]. Moreover, maternal hyperglycemia severity was positively correlated with poorer offspring outcomes [17]. A systematic review and meta-analysis have shown that offspring of diabetic mothers have worse cognitive and behavioral outcomes, with an increased risk of being diagnosed with attention and hyperactivity disorder as well as autism spectrum disorders [18]. These data support the relevance of sustaining adequate glucose levels throughout pregnancy, reinforcing the importance of a healthy diet during this period to prevent offspring behavioral impairment later in life.

While most women with clinical diabetes are usually already under treatment before pregnancy and thus can prevent those outcomes, gestational diabetes is often undiagnosed, leading to a late hyperglycemia management and, consequently, to higher impact for the offspring. Diabetes criteria are constantly being reviewed to determine values that would better predict or indicate poor fetal outcomes, since different levels of maternal hyperglycemia and insulin resistance lead to different adverse offspring outcomes; the greater the maternal hyperglycemia, the greater the risks to mother and offspring [19]. However, it is important to highlight that there is a positive correlation

between maternal glycemia and offspring body fat during childhood even in non-diabetic mothers [20, 21]. It has also been shown a positive correlation between offspring hyperglycemia in childhood and HbA_{1c} of mothers with no gestational diabetes [22]. Therefore, interventions during pregnancy are key to improve maternal and fetal outcomes. Notably, it has been shown that dietary interventions can improve gestational diabetes effects, resulting in a better maternal glucose management and fetal outcomes [23].

The development of clinical and gestational diabetes has been linked to several risk factors, such as ethnicity, overweight and obesity, smoking, being physical inactive, and an unhealthy diet. Dietary choices, such as an increased intake of foods rich in fat, sugar, and saturated fatty acids, are associated with an increased risk for Type 2 diabetes [24-27]. Healthy lifestyle habits during pregnancy, such as regular physical activity and adequate nutrition, are key to reduced diabetes effects both on the mother and their offspring. Notably, a healthy diet during pregnancy must be encouraged in these patients to reduce obesity and diabetes risks in future generations [28]. The impact of maternal diet independent of maternal obesity on offspring development should be addressed to promote intervention strategies that are more effective [21]. Lifestyle interventions are also crucial to separate the effects of maternal diabetes and overnutrition during pregnancy and lactation from the infant's nutritional environment in an “over-fed” house [12].

The EarlyNutrition Project recommendations highlight that pregnant women do not have to change macronutrient proportion in their diet, unless in cases of food deprivation [29]. A balanced diet should be maintained throughout pregnancy, with an increase in energy intake of no more than 10% of what should be consumed by non-pregnant women [29]. Nonetheless, poor food choices and overconsumption are related to the increase in obesity prevalence worldwide [30]. Maternal diet can also affect offspring birth weight, but studies have shown that the diet quality (proportion of macronutrients, for example) are more important than the total energy intake [31]. Researchers have shown an association between unhealthy diets during pregnancy and a higher risk of smaller birth weight, which is a predictor of long-term consequences [32-34]. Furthermore, a healthy lifestyle is associated with better hyperglycemia management during pregnancy [35, 36], while an unhealthy diet may worsen diabetes.

Cohort studies are valuable because they can identify multiple outcomes in the population, independently of case and control groups’ formation, as well as calculate incidence rate and relative risks [37]. However, most studies showing long-term consequences of maternal diabetes and an inappropriate nutrition in the human population show some limitations. Frequently, these studies are retrospective, and

mothers show a wide range of hyperglycemia and/or glucose intolerance. Further, it is not always easy to discern between patients with clinical and gestational diabetes. In addition, it is difficult to delimitated effects related to maternal metabolism or diet intake, as well as to propose possible interventions. In this context, animal models are valuable to study manipulations during pregnancy and lactation, their effects on offspring, and their possible mechanisms [38, 39].

A variety of experimental methods have been employed to model diabetes [38]. The administration of β-cytotoxic agents, such as streptozotocin (STZ), has been widely used to develop hyperglycemia in rodents. STZ administration can induce severe or mild hyperglycemia depending on rodents' lineage and age, dose, and administration route. When STZ is giving during adulthood, animals develop severe hyperglycemia, with fasting glycemia over 300 mg/dL, and frequently above 500 mg/dL. These glucose levels are rare in diabetic patients, so alternative models of mild hyperglycemia have been developed. The neonatal STZ administration leads to mild hyperglycemia in rats, with glucose levels between 120 and 300 mg/dL, due to partial regeneration of pancreatic β-cells in the first days after birth [40, 41]. Moreover, animals will display hypoinsulinemia and glucose intolerance during adulthood [42-45]. Apart from metabolic impairments, neonatal STZ administration also leads to behavioral impairments both in dams and their offspring. STZ-treated females show reduced exploratory behavior [44], while their male offspring, but not female, show higher immobility in the open-field arena [43], indicating sex-specific effects of maternal hyperglycemia in offspring development, as previously reported [46].

Dietary manipulations during pregnancy and lactation may worsen the metabolic impairment in previously hyperglycemic females [47, 48]. Several diet manipulations have been employed to study the consequences of an unhealthy diet in animal models, like hypercaloric or high-fat diets, and cafeteria diets, high-fat and high-sugar junk foods [49]. In all these models, a variety of deleterious effects have been observed in the offspring, including hyperphagia, increased body adiposity, elevated levels of triglycerides, hyperglycemia, and insulin resistance [50, 51], as well as increased anxiety-like behavior [52-55]. Recently, a snack intake model has been proposed [56], in consonance with the increased intake of snacks between meals in the population [57]. Snack intake in addition to regular meals leads to a higher caloric intake and, consequently, body weight gain [58, 59]. Snacks composed evenly by carbohydrate and fat, like potato chips, trigger a state of hedonic hyperphagia, in which food intake is disassociated to hunger [56, 60]. These snacks contribute little to satiety and their calories are not completely compensated by a reduced caloric intake in regular meals [58, 59], resulting in an increased daily caloric intake. Since an unhealthy diet is

associated with increased risk of developing diabetes, an inappropriate nutrition during pregnancy and lactation can trigger further impairments in an already established maternal hyperglycemia in animal models, with worse consequences to mother and offspring [61].

Although maternal hyperglycemia and inappropriate nutrition effects on offspring development have been extensively explored separately, their association and its impact on maternal metabolism and offspring development and behavior throughout life is less clear. Our hypothesis is that snack intake during pregnancy and lactation will trigger further impairments in maternal glycemia homeostasis, resulting in changes in maternal behavior as well as sex-specific effects on offspring development, metabolism, and behavior from weaning to senescence.

Aligned with the Graduate Program of General Physiology research interests, this study investigated the relationship between physiological processes and the environment, particularly on how the maternal environment shapes offspring development and its survival in the long term. Furthermore, studies in animal models have the potential to be future applied to clinical settings and health improvement. The study of maternal hyperglycemia and inappropriate nutrition will be explored in chapters 1 and 2, and it may clarify window of opportunities where interventions during the perinatal period might be made to promote healthier pregnancies, that will lower the risk for the development of metabolic and cognitive disorders in the offspring throughout life. Chapter 1 describes how snack intake during pregnancy and lactation changes maternal food intake and worsens maternal hyperglycemia of STZ-treated females, as well as its impact on offspring birth weight and maternal behavior. This chapter resulted in a manuscript submitted to Physiology & Behavior in 20/04/2021 and is currently under review. Chapter 2 describes the postnatal life of male and female offspring born to those females, showing the consequences of an unbalanced maternal organism to offspring glucose metabolism, behavior, and biometric aspects in adolescence, adulthood, and senescence. This chapter has been also prepared as a manuscript according to Physiology & Behavior guideline and is yet to be submitted. Finally, a side study was conducted during an internship at the Department of Neuroscience at Carleton University that aimed to investigate the effects of maternal hyperglycemia in the offspring control of food intake after access to high-fat diet in the post-weaning period. However, these data are still under analysis and will not be presented at the moment.

CONCLUDING REMARKS

The present study evaluated if snack intake during pregnancy and lactation would aggravate previously established maternal hyperglycemia and its consequences to maternal care, as well as offspring development, metabolism, and behavior throughout life. Our hypothesis was that snack intake during pregnancy and lactation would trigger further impairments in maternal glycemia homeostasis, resulting in changes in maternal behavior as well on offspring development, metabolism, and behavior from weaning to senescence, that could be sex specific.

This hypothesis was explored in chapters 1 and 2. Chapter 1 described how snack intake during pregnancy and lactation altered maternal food intake and changed the glucose tolerance of STZ-treated females, as well as its impact on maternal behavior and offspring birth weight. We have shown that snack intake aggravates glucose intolerance in hyperglycemic females. This maternal metabolic impairment led to changes in offspring birth weight and altered maternal motivation during lactation. Birth weight classification was normalized in the offspring from hyperglycemic dams with access to snacks, which showed a patter similar to Control offspring. Corroborating previous studies, offspring from hyperglycemic dams were more frequently classified as large por pregnancy age, while offspring from snack-fed dams were more frequently classified as small. Interestingly, birth weight is a strong predictor of long-term consequences to the offspring, being related to an increased risk of developing obesity and metabolic impairments later in life.

On this way, some findings may not be a direct consequence of maternal manipulations themselves, but they may occur as a reflection of these manipulations on offspring birth weight, which is a strong predictor of future outcomes. Mechanisms related to this growth impairment could also be investigated in future studies. In the study conducted during the internship at Carleton University placentas were collected that could help to elucidate how maternal hyperglycemia and snack intake may change nutrients' flow during pregnancy, which might explain offspring outcomes later in life.

Short and long-term consequences to the offspring were then explored in Chapter 2, which described the effects of the association of an impaired maternal metabolism and altered nutrition to offspring glucose metabolism, behavior, and biometric aspects in adolescence, adulthood, and senescence. While offspring metabolism was not further compromised, changes were observed on offspring reproductive function and general behavior in adulthood and senescence, respectively, showing that consequences may only be evident in the long-term. Although no major

impairments were observed on the behavioral tasks evaluated, it is known that both maternal diabetes and inappropriate nutrition are linked to offspring cognitive impairments [49, 62]. For that reason, object recognition and spatial memory in the Morris water maze were also evaluated in the offspring of hyperglycemic dams with access to snacks, which may help to elucidate these behavioral impairments in all age groups. However, given the complexity of this evaluation, the analysis of the videos from this behavioral task is still under way. Results will be assembled in a manuscript addressing impacts of maternal hyperglycemia and snack intake on offspring learning. Additionally, besides morphometric analysis, offspring from both sexes and from all age groups were perfused, and brains were collected for future evaluation. Brain analysis will be planned in order to correlate with possible learning impairments.

Despite not reproducing all aspects of the diabetic syndrome, the experimental model used in this study is useful to study the consequences of maternal hyperglycemia associated with an inappropriate nutrition, since the glycemic levels observed resemble those found in pregnant women diagnosed with clinical or gestational diabetes. Another positive aspect of this study is the analysis of both male and female offspring outcomes. Studying both sexes is extremely important since sex-specific differences related to maternal metabolism have already been described and could help to delineate future experimental and clinical research. Furthermore, offspring outcomes are rarely followed until senescence. Frequently, experimental studies evaluate offspring up until earl adulthood, while most clinical studies still follow only children and adolescents. Therefore, this study broadens the analysis of offspring outcomes, both related to animals' sex and age.

As the experimental model of snack intake described in this study was proven effective to further impair the glucose tolerance of hyperglycemic rats, new research questions emerged, aiming to better understand the impact of this maternal manipulation on other offspring outcomes, such as food intake control and preference. This resulted in a research grant submission in which I'm a collaborator that was granted and is currently under way (Impact of maternal diabetes and snack consumption on male and female offspring control of food intake, FAPESP 2019/01306-2). This project provided the basis for two Master's thesis (Effects of chronic central leptin infusion on food intake of offspring of rats with mild hyperglycemia, FAPESP 2019/06974-3; Effects of chronic central leptin infusion on sexual behavior and reproductive tract of offspring of rats with mild hyperglycemia, CNPq) and an undergraduate thesis (Impact of maternal diabetes and snack consumption on offspring food preference, FAPESP 2020/03604-8) that are also under way with my collaboration. Plus, I was co-advisor in an undergraduate thesis

(Maternal hyperglycemia and overnutrition: effects on anxiety-like behavior of Wistar rats during lactation).

The experimental model employed in the present study was adjust to better suit the new projects. A change in rat lineage, from Wistar to Sprague-Dawley rats, was judged more adequate to the new proposed aims. In addition, as we started using Sprague-Dawley rats, we observed that the experimental model of neonatal STZ injection, which worked well on Wistar rats, was not the most adequate for this lineage. Thus, a modified protocol of STZ administration during pregnancy was employed with success. Although the protocol for inducing hyperglycemia and rat lineage was changed, the aggravated glucose tolerance phenotype was similar to the one described in the present study, reinforcing the validity and consistence of the experimental model.

In conclusion, the present study showed that snack intake during pregnancy and lactation further impaired maternal hyperglycemia, leading to disruptions in offspring birth weight and maternal motivation during lactation, as well as to impaired offspring reproductive function in adulthood and changes in anxiety-live behavior in senescence. Future studies will describe effects on offspring learning and memory, and its possible neural substrates. Although snack intake aggravated the glucose intolerance of mild hyperglycemic rats, glycemic levels were still within the mild range (120 – 300 mg/dL), which might explain why this condition did not lead to major impairments for both mother and offspring. However, the experimental model used in this study is useful to study the consequences of maternal diabetes associated with inappropriate nutrition, since the glycemic levels observed resemble those most observed in pregnant women diagnosed with clinical or gestational diabetes. On the other hand, this experimental model may not be the most suitable to evaluate intervention strategies during pregnancy and lactation that might improve the consequences of this maternal metabolic impairment, because maternal and fetal impairments are limited. However, it should be considered that even this mild maternal condition was enough to change maternal and offspring outcomes, reinforcing the importance of women sustaining target glucose levels and a healthy diet during pregnancy and lactation.

REFERENCES

Aerts, L. and F.A. Van Assche, Animal evidence for the transgenerational development of diabetes mellitus. Int J Biochem Cell Biol, 2006. 38(5-6): p. 894-903.

Agmo, A., Male rat sexual behavior. Brain Res Brain Res Protoc, 1997. 1(2): p. 203-9.

Ainge, H., et al., A systematic review on animal models of maternal high fat feeding and offspring glycaemic control. Int J Obes (Lond), 2011. 35(3): p. 325-35.

Akyol, A., S.C. Langley-Evans, and S. McMullen, Obesity induced by cafeteria feeding and pregnancy outcome in the rat. Br J Nutr, 2009. 102(11): p. 1601-10.

Alfaradhi, M.Z. and S.E. Ozanne, Developmental programming in response to maternal overnutrition. Front Genet, 2011. 2: p. 27.

American Diabetes, A., 14. Management of Diabetes in Pregnancy: Standards of Medical Care in Diabetes-2021. Diabetes Care, 2021. 44(Suppl 1): p. S200-S210.

American Diabetes, A., 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care, 2021. 44(Suppl 1): p. S15-S33.

Andreollo, N.A., et al., Rat's age versus human's age: what is the relationship? Arq Bras Cir Dig, 2012. 25(1): p. 49-51.

Baglietto-Vargas, D., et al., Diabetes and Alzheimer's disease crosstalk. Neurosci Biobehav Rev, 2016. 64: p. 272-87.

Bannon, A.W., et al., Multiple behavioral effects of cocaine- and amphetamine-regulated transcript (CART) peptides in mice: CART 42-89 and CART 49-89 differ in potency and activity. J Pharmacol Exp Ther, 2001. 299(3): p. 1021-6.

Baso, A.C., et al., Effects of maternal exposure to picrotoxin during lactation on physical and reflex development, square crossing and sexual behavior of rat offspring. Pharmacol Biochem Behav, 2003. 75(4): p. 733-40.

Battaglia, F.C. and G. Meschia, Principal substrates of fetal metabolism. Physiol Rev, 1978. 58(2): p. 499-527.

Bellisle, F., Meals and snacking, diet quality and energy balance. Physiol Behav, 2014. 134: p. 38-43.

Benhalima, K., et al., Characteristics and pregnancy outcomes across gestational diabetes mellitus subtypes based on insulin resistance. Diabetologia, 2019. 62(11): p. 2118-2128.

Berends, L.M., et al., Programming of central and peripheral insulin resistance by low birthweight and postnatal catch-up growth in male mice. Diabetologia, 2018. 61(10): p. 2225-2234.

Broadhurst, P.L., The place of animal psychology in the development of psychosomatic research. Fortschr Psychosom Med, 1960. 1: p. 63-9.

Buchanan, T.A., A.H. Xiang, and K.A. Page, Gestational diabetes mellitus: risks and management during and after pregnancy. Nat Rev Endocrinol, 2012. 8(11): p. 639-49.
Bueno, A., et al., Evaluation of placental glycogen storage in mild diabetic rats. Acta Cir Bras, 2010. 25(2): p. 132-6.
Bult, A. and C.B. Lynch, Nesting and fitness: lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behavior. Behav Genet, 1997. 27(3): p. 231-40.
Carobrez, A.P. and L.J. Bertoglio, Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci Biobehav Rev, 2005. 29(8): p. 1193-205.
Catalano, P. and S.H. deMouzon, Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. Int J Obes (Lond), 2015. 39(4): p. 642-9.
Catalano, P.M., et al., Increased fetal adiposity: a very sensitive marker of abnormal in utero development. Am J Obstet Gynecol, 2003. 189(6): p. 1698-704.
Chapelot, D., The role of snacking in energy balance: a biobehavioral approach. J Nutr, 2011. 141(1): p. 158-62.
Chavatte-Palmer, P., A. Tarrade, and D. Rousseau-Ralliard, Diet before and during Pregnancy and Offspring Health: The Importance of Animal Models and What Can Be Learned from Them. Int J Environ Res Public Health, 2016. 13(6).
Clawson, R.C., et al., Continuous access to snacks from weaning onwards in female rats causes weight gain, insulin insensitivity, and sustained leptin resistance in adulthood. Physiol Behav, 2019. 201: p. 165-174.
Connor, K.L., et al., Nature, nurture or nutrition? Impact of maternal nutrition on maternal care, offspring development and reproductive function. J Physiol, 2012. 590(9): p. 2167-80.
Cooper, J.A., A.R. Arulpragasam, and M.T. Treadway, Anhedonia in depression: biological mechanisms and computational models. Curr Opin Behav Sci, 2018. 22: p. 128-135.
Cunha Fda, S., et al., Both food restriction and high-fat diet during gestation induce low birth weight and altered physical activity in adult rat offspring: the "Similarities in the Inequalities" model. PLoS One, 2015. 10(3): p. e0118586.
Damasceno, D.C., et al., Maternal-fetal outcome, lipid profile and oxidative stress of diabetic rats neonatally exposed to streptozotocin. Exp Clin Endocrinol Diabetes, 2011. 119(7): p. 408-13.
Damasceno, D.C., et al., Mild diabetes models and their maternal-fetal repercussions. J Diabetes Res, 2013. 2013: p. 473575.

Dean, A. and R.M. Sharpe, Clinical review: Anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders. J Clin Endocrinol Metab, 2013. 98(6): p. 2230-8.

Dearden, L. and S.E. Ozanne, Early life origins of metabolic disease: Developmental programming of hypothalamic pathways controlling energy homeostasis. Front Neuroendocrinol, 2015. 39: p. 3-16.

Dearden, L., S.G. Bouret, and S.E. Ozanne, Nutritional and developmental programming effects of insulin. J Neuroendocrinol, 2021: p. e12933.

Denis, R.G., et al., Palatability Can Drive Feeding Independent of AgRP Neurons. Cell Metab, 2015. 22(4): p. 646-57.

Dennedy, M.C. and F. Dunne, The maternal and fetal impacts of obesity and gestational diabetes on pregnancy outcome. Best Pract Res Clin Endocrinol Metab, 2010. 24(4): p. 573-89.

Dudley, K.J., et al., Offspring of mothers fed a high fat diet display hepatic cell cycle inhibition and associated changes in gene expression and DNA methylation. PLoS One, 2011. 6(7): p. e21662.

Englund-Ogge, L., et al., Associations between maternal dietary patterns and infant birth weight, small and large for gestational age in the Norwegian Mother and Child Cohort Study. Eur J Clin Nutr, 2019. 73(9): p. 1270-1282.

Fadl, H.E., et al., Maternal and neonatal outcomes and time trends of gestational diabetes mellitus in Sweden from 1991 to 2003. Diabet Med, 2010. 27(4): p. 436-41.

Fernandez-Twinn, D.S. and S.E. Ozanne, Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. Physiol Behav, 2006. 88(3): p. 234-43.

Fernandez-Twinn, D.S., et al., Intrauterine programming of obesity and type 2 diabetes. Diabetologia, 2019. 62(10): p. 1789-1801.

Ford, S.P. and N.M. Long, Evidence for similar changes in offspring phenotype following either maternal undernutrition or overnutrition: potential impact on fetal epigenetic mechanisms. Reprod Fertil Dev, 2011. 24(1): p. 105-11.

Francis, E.C., et al., Maternal blood glucose level and offspring glucose-insulin homeostasis: what is the role of offspring adiposity? Diabetologia, 2021. 64(1): p. 83-94.

Friedman, M.I. and I. Ramirez, Food intake in diabetic rats: relationship to metabolic effects of insulin treatment. Physiol Behav, 1994. 56(2): p. 373-8.

Gawlinska, K., et al., Maternal dietary patterns are associated with susceptibility to a depressive-like phenotype in rat offspring. Dev Cogn Neurosci, 2021. 47: p. 100879.

Gerardin, D.C., et al., Neuroendocrine and reproductive aspects of adult male rats exposed neonatally to an antiestrogen. Pharmacol Biochem Behav, 2006. 83(4): p. 618-23.
Han, J., et al., Rat maternal diabetes impairs pancreatic beta-cell function in the offspring. Am J Physiol Endocrinol Metab, 2007. 293(1): p. E228-36.
Hillier, T.A., et al., Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. Diabetes Care, 2007. 30(9): p. 2287-92.
Hoch, T., et al., Fat/carbohydrate ratio but not energy density determines snack food intake and activates brain reward areas. Sci Rep, 2015. 5: p. 10041.
Hoch, T., M. Pischetsrieder, and A. Hess, Snack food intake in ad libitum fed rats is triggered by the combination of fat and carbohydrates. Front Psychol, 2014. 5: p. 250.
Howie, G.J., et al., Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. J Physiol, 2009. 587(Pt 4): p. 905-15.
Huang, Y., et al., Maternal high-fat diet during pregnancy and lactation affects hepatic lipid metabolism in early life of offspring rat. J Biosci, 2017. 42(2): p. 311-319.
Hussain, Y., S.K. Jain, and P.K. Samaiya, Short-term westernized (HFFD) diet fed in adolescent rats: Effect on glucose homeostasis, hippocampal insulin signaling, apoptosis and related cognitive and recognition memory function. Behav Brain Res, 2018. 361: p. 113-121.
Iessi, I.L., et al., Evaluation of neonatally-induced mild diabetes in rats: Maternal and fetal repercussions. Diabetol Metab Syndr, 2010. 2(1): p. 37.
Imamura, F., et al., Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. BMJ, 2015. 351: p. h3576.
InterAct, C., et al., Consumption of sweet beverages and type 2 diabetes incidence in European adults: results from EPIC-InterAct. Diabetologia, 2013. 56(7): p. 1520-30.
Johansson, B., B. Meyerson, and U.J. Eriksson, Behavioral effects of an intrauterine or neonatal diabetic environment in the rat. Biol Neonate, 1991. 59(4): p. 226-35.
Kamana, K.C., S. Shakya, and H. Zhang, Gestational diabetes mellitus and macrosomia: a literature review. Ann Nutr Metab, 2015. 66 Suppl 2: p. 14-20.
Kedia, S. and S. Chattarji, Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice. J Neurosci Methods, 2014. 233: p. 150-4.
Kinney, B.A., et al., Maternal hyperglycemia leads to gender-dependent deficits in learning and memory in offspring. Exp Biol Med (Maywood), 2003. 228(2): p. 152-9.
Kiss, A.C., et al., Animal models for clinical and gestational diabetes: maternal and fetal outcomes. Diabetol Metab Syndr, 2009. 1(1): p. 21.

Kiss, A.C.I., et al. Effects of chronicle central insulin infusion during lactation on maternal food intake and pup growth. in 5th Parental Brain Conference. 2013. Regensburg.
Kiss, A.C.I., et al., Impact of maternal mild hyperglycemia on maternal care and offspring development and behavior of Wistar rats. Physiol Behav, 2012. 107(3): p. 292-300.
Kiss, A.C.I., et al., Neonatally induced mild diabetes: influence on development, behavior and reproductive function of female Wistar rats. Diabetol Metab Syndr, 2013. 5(1): p. 61.
Kitzmiller, J.L., et al., Managing preexisting diabetes for pregnancy: summary of evidence and consensus recommendations for care. Diabetes Care, 2008. 31(5): p. 1060-79.
Klein, M.O., Dieta hiperlipídica materna: influências sobre o comportamento maternal e o desenvolvimento da prole, in Farmacologia. 2016, Universidade de São Paulo: São Paulo.
Kleinert, M., et al., Animal models of obesity and diabetes mellitus. Nat Rev Endocrinol, 2018. 14(3): p. 140-162.
Klimas, J.E., Oral glucose tolerance during the life-span of a colony of rats. J Gerontol, 1968. 23(1): p. 31-4.
Knudsen, V.K., et al., Major dietary patterns in pregnancy and fetal growth. Eur J Clin Nutr, 2008. 62(4): p. 463-70.
Koletzko, B., et al., Nutrition During Pregnancy, Lactation and Early Childhood and its Implications for Maternal and Long-Term Child Health: The Early Nutrition Project Recommendations. Ann Nutr Metab, 2019. 74(2): p. 93-106.
Lawlor, D.A., P. Lichtenstein, and N. Langstrom, Association of maternal diabetes mellitus in pregnancy with offspring adiposity into early adulthood: sibling study in a prospective cohort of 280,866 men from 248,293 families. Circulation, 2011. 123(3): p. 258-65.
Leng, G., et al., The determinants of food choice. Proc Nutr Soc, 2017. 76(3): p. 316-327.
Leshner, A.I., H.I. Siegel, and G. Collier, Dietary self-selection by pregnant and lactating rats. Physiol Behav, 1972. 8(1): p. 151-4.
Ley, S.H., et al., Prevention and management of type 2 diabetes: dietary components and nutritional strategies. Lancet, 2014. 383(9933): p. 1999-2007.
Londei, T., A.M. Valentini, and V.G. Leone, Investigative burying by laboratory mice may involve non-functional, compulsive, behaviour. Behav Brain Res, 1998. 94(2): p. 249-54.
Lucion, A.B. and M.C. Bortolini, Mother-pup interactions: rodents and humans. Front Endocrinol (Lausanne), 2014. 5: p. 17.
Macleod, D.J., et al., Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl, 2010. 33(2): p. 279-87.

Malik, V.S., et al., Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: a meta-analysis. Diabetes Care, 2010. 33(11): p. 2477-83.
Maresch, C.C., et al., Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. Hum Reprod Update, 2018. 24(1): p. 86-105.
Martins, M.G., et al., Snack intake further impairs maternal glucose tolerance and reduces the incidence of macrosomia in the offspring of mild hyperglycemic rats. Submitted, 2021.
Mendonca, M., A. Bilgin, and D. Wolke, Association of Preterm Birth and Low Birth Weight With Romantic Partnership, Sexual Intercourse, and Parenthood in Adulthood: A Systematic Review and Meta-analysis. JAMA Netw Open, 2019. 2(7): p. e196961.
Merzouk, H., et al., Time course of changes in serum glucose, insulin, lipids and tissue lipase activities in macrosomic offspring of rats with streptozotocin-induced diabetes. Clin Sci (Lond), 2000. 98(1): p. 21-30.
Morris, M.J., et al., Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. Neurosci Biobehav Rev, 2015. 58: p. 36-45.
Naismith, D.J., D.P. Richardson, and A.E. Pritchard, The utilization of protein and energy during lactation in the rat, with particular regard to the use of fat accumulated in pregnancy. Br J Nutr, 1982. 48(2): p. 433-41.
Nelson, C.A., et al., Neurocognitive sequelae of infants of diabetic mothers. Behav Neurosci, 2000. 114(5): p. 950-6.
Njung'e, K. and S.L. Handley, Evaluation of marble-burying behavior as a model of anxiety. Pharmacol Biochem Behav, 1991. 38(1): p. 63-7.
Nolan, C.J. and J. Proietto, The feto-placental glucose steal phenomenon is a major cause of maternal metabolic adaptation during late pregnancy in the rat. Diabetologia, 1994. 37(10): p. 976-984.
Numan, M. and B. Woodside, Maternity: neural mechanisms, motivational processes, and physiological adaptations. Behav Neurosci, 2010. 124(6): p. 715-41.
Oh, W., N.L. Gelardi, and C.J. Cha, Maternal hyperglycemia in pregnant rats: its effect on growth and carbohydrate metabolism in the offspring. Metabolism, 1988. 37(12): p. 1146-51.
Okubo, H., et al., Maternal dietary patterns in pregnancy and fetal growth in Japan: the Osaka Maternal and Child Health Study. Br J Nutr, 2012. 107(10): p. 1526-33.
Olaiya, M.T., et al., Birthweight and early-onset type 2 diabetes in American Indians: differential effects in adolescents and young adults and additive effects of genotype, BMI and maternal diabetes. Diabetologia, 2019. 62(9): p. 1628-1637.

Ong, K.K., et al., Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ, 2000. 320(7240): p. 967-71.
Ornoy, A., et al., Diabetes during Pregnancy: A Maternal Disease Complicating the Course of Pregnancy with Long-Term Deleterious Effects on the Offspring. A Clinical Review. Int J Mol Sci, 2021. 22(6).
Ornoy, A., et al., School-age children born to diabetic mothers and to mothers with gestational diabetes exhibit a high rate of inattention and fine and gross motor impairment. J Pediatr Endocrinol Metab, 2001. 14 Suppl 1: p. 681-9.
Paredes, R.G. and A. Agmo, Has dopamine a physiological role in the control of sexual behavior? A critical review of the evidence. Prog Neurobiol, 2004. 73(3): p. 179-226.
Peić, D. and V. Hernández. Countee. 2016 14 de abril de 2016 [cited 2016 02 de agosto de 2016]; 1.0.4 [Available from: https://www.counteeapp.com/.
Peleg-Raibstein, D., E. Luca, and C. Wolfrum, Maternal high-fat diet in mice programs emotional behavior in adulthood. Behav Brain Res, 2012. 233(2): p. 398-404.
Pereira, M.R.F., et al., Can maternal exposure to paracetamol impair reproductive parameters of male rat offspring? Reprod Toxicol, 2020. 93: p. 68-74.
Perez-Perez, A., et al., Leptin and Nutrition in Gestational Diabetes. Nutrients, 2020. 12(7).
Perng, W., et al., A prospective study of associations between in utero exposure to gestational diabetes mellitus and metabolomic profiles during late childhood and adolescence. Diabetologia, 2020. 63(2): p. 296-312.
Powe, C.E., et al., Augmented insulin secretory response in early pregnancy. Diabetologia, 2019. 62(8): p. 1445-1452.
Ramanathan, M., A.K. Jaiswal, and S.K. Bhattacharya, Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats. Psychopharmacology (Berl), 1998. 135(4): p. 361-7.
Ramanathan, M., A.K. Jaiswal, and S.K. Bhattacharya, Hyperglycaemia in pregnancy: effects on the offspring behaviour with special reference to anxiety paradigms. Indian J Exp Biol, 2000. 38(3): p. 231-6.
Ribaroff, G.A., et al., Animal models of maternal high fat diet exposure and effects on metabolism in offspring: a meta-regression analysis. Obes Rev, 2017. 18(6): p. 673-686.
Saito, F.H., et al., Heat shock protein production and immunity and altered fetal development in diabetic pregnant rats. Cell Stress Chaperones, 2013. 18(1): p. 25-33.
Saito, F.H., et al., Repercussions of mild diabetes on pregnancy in Wistar rats and on the fetal development. Diabetol Metab Syndr, 2010. 2(1): p. 26.

Sampey, B.P., et al., Cafeteria diet is a robust model of human metabolic syndrome with liver and adipose inflammation: comparison to high-fat diet. Obesity (Silver Spring), 2011. 19(6): p. 1109-17.
Sanchez-Alavez, M., et al., Insulin causes hyperthermia by direct inhibition of warm-sensitive neurons. Diabetes, 2010. 59(1): p. 43-50.
Sasaki, A., et al., Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. Neuroscience, 2014. 272: p. 92-101.
Sasaki, A., et al., Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. Neuroscience, 2013. 240: p. 1-12.
Seidell, J.C. and J. Halberstadt, Obesity: The obesity epidemic in the USA - no end in sight? Nat Rev Endocrinol, 2016. 12(9): p. 499-500.
Sharma, S., M.F. Fernandes, and S. Fulton, Adaptations in brain reward circuitry underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. Int J Obes (Lond), 2013. 37(9): p. 1183-91.
Sinzato, Y.K., et al., Comparison of streptozotocin-induced diabetes at different moments of the life of female rats for translational studies. Lab Anim, 2021: p. 236772211001895.
Sinzato, Y.K., et al., Neonatally induced mild diabetes in rats and its effect on maternal, placental, and fetal parameters. Exp Diabetes Res, 2012. 2012: p. 108163.
Slining, M.M., K.C. Mathias, and B.M. Popkin, Trends in food and beverage sources among US children and adolescents: 1989-2010. J Acad Nutr Diet, 2013. 113(12): p. 1683-94.
Smith, U. and B.B. Kahn, Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids. J Intern Med, 2016. 280(5): p. 465-475.
Song, J.W. and K.C. Chung, Observational studies: cohort and case-control studies. Plast Reconstr Surg, 2010. 126(6): p. 2234-2242.
Soulimane-Mokhtari, N.A., et al., Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. Clin Sci (Lond), 2005. 109(3): p. 287-95.
Sousa, R.A.L., et al., Consequences of gestational diabetes to the brain and behavior of the offspring. An Acad Bras Cienc, 2018. 90(2 suppl 1): p. 2279-2291.
Steger, R.W., M. Rabe, and T. Bucher. Altered sex behavior in the male offsrping of diabet- ic rats may be secondary to alterations in fetal testosterone levels. in 81st Annual meeting of the endocrine society. 1999.
Tai, M.M., A mathematical model for the determination of total area under glucose tolerance and other metabolic curves. Diabetes Care, 1994. 17(2): p. 152-4.

Thompson, J.M., et al., Maternal dietary patterns in pregnancy and the association with small-for-gestational-age infants. Br J Nutr, 2010. 103(11): p. 1665-73.
Thyssen, S., E. Arany, and D.J. Hill, Ontogeny of regeneration of beta-cells in the neonatal rat after treatment with streptozotocin. Endocrinology, 2006. 147(5): p. 2346-56.
Triadou, N., et al., Experimental chemical diabetes and pregnancy in the rat. Evolution of glucose tolerance and insulin response. Diabetes, 1982. 31(1): p. 75-9.
Tsan, L., et al., Western Diet Consumption During Development: Setting the Stage for Neurocognitive Dysfunction. Front Neurosci, 2021. 15: p. 632312.
Tsuji, K., et al., Characteristic features of insulin secretion in the streptozotocin-induced NIDDM rat model. Metabolism, 1988. 37(11): p. 1040-4.
Vieira, M.L., et al., Lactational exposure to sulpiride: assessment of maternal care and reproductive and behavioral parameters of male rat pups. Physiol Behav, 2013. 122: p. 76-83.
Vucetic, Z., et al., Maternal high-fat diet alters methylation and gene expression of dopamine and opioid-related genes. Endocrinology, 2010. 151(10): p. 4756-64.
Walf, A.A. and C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc, 2007. 2(2): p. 322-8.
Wang, R.N., L. Bouwens, and G. Kloppel, Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. Diabetologia, 1994. 37(11): p. 1088-96.
Weltens, N., D. Zhao, and L. Van Oudenhove, Where is the comfort in comfort foods? Mechanisms linking fat signaling, reward, and emotion. Neurogastroenterol Motil, 2014. 26(3): p. 303-15.
WHO, W.H.O., Diet, Nutrition and the Prevention of Chronic Diseases, W.H.O. WHO, Editor. 2003, World Health Organization: Geneva.
WHO, W.H.O., Global Report on Diabetes, WHO, Editor. 2016, World Health Organization: France.
Whybrow, S., et al., Effects of two weeks' mandatory snack consumption on energy intake and energy balance. Obesity (Silver Spring), 2007. 15(3): p. 673-85.
Woodside, B., et al., Many mouths to feed: the control of food intake during lactation. Front Neuroendocrinol, 2012. 33(3): p. 301-14.
Wright, T., S.C. Langley-Evans, and J.P. Voigt, The impact of maternal cafeteria diet on anxiety-related behaviour and exploration in the offspring. Physiol Behav, 2011. 103(2): p. 164-72.
Yamamoto, J.M., et al., Gestational Diabetes Mellitus and Diet: A Systematic Review and Meta-analysis of Randomized Controlled Trials Examining the Impact of Modified

Dietary Interventions on Maternal Glucose Control and Neonatal Birth Weight. Diabetes Care, 2018. 41(7): p. 1346-1361.
Yamamoto, J.M., et al., Neurocognitive and behavioural outcomes in offspring exposed to maternal pre-existing diabetes: a systematic review and meta-analysis. Diabetologia, 2019. 62(9): p. 1561-1574.
Zemdegs, J., et al., High-fat diet-induced metabolic disorders impairs 5-HT function and anxiety-like behavior in mice. Br J Pharmacol, 2016. 173(13): p. 2095-110.
Zheng, J., et al., Maternal high-fat diet regulates glucose metabolism and pancreatic beta cell phenotype in mouse offspring at weaning. PeerJ, 2020. 8: p. e9407.
Zhu, Y. and C. Zhang, Prevalence of Gestational Diabetes and Risk of Progression to Type 2 Diabetes: a Global Perspective. Curr Diab Rep, 2016. 16(1): p. 7.