ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 11, Issue, 11, pp. 51451-51454, November, 2021
https://doi.org/10.37118/ijdr.23118.11.2021

RESEARCH ARTICLE OPEN ACCESS

LIPID AND PROTEIN OVERLOAD SUPPORT ONLY PARTIALLY THE PREGNANCY AND LACTATION OF WISTAR RATS FEED A LOW CARB DIET

Marcia Gabriela Margato Rocha[1], Luiz Carlos dos Reis[2], Marcelo Rodrigues Pinto[2], Giuliana Cristina Marre Bruschi Thedei[2] and Geraldo Thedei Junior[2]

[1]UniversidadeFederal do Triângulo Mineiro - Uberaba, MG, Brazil
[2]Universidade de Uberaba - Uberaba, MG, Brazil

ARTICLE INFO

Article History:
Received 19th August, 2021
Received in revised form
10th September, 2021
Accepted 11th October, 2021
Published online 23rd November, 2021

Key Words:
Carbohydrate-Restricted diet,
High-fat Diet,
High-Protein Diet, Low Carb Diet.

***Corresponding author:**
Gabriela Ferreira Silva Mota

ABSTRACT

This study aimed to determine the effect of a high fat, high protein, low carb diet during pregnancy and lactation of dams and pups of Wistar rats. Female Wistar rats were fed either a control balanced diet (C) or a Low-carb, hyperproteic and hyperlipidic diet (E) during pregnancy and/or lactation period and the number, weight, adipose tissue and blod glucose from both dams (d) and pups (p) were analyzed. Diet E caused smaller litters and lean pups, without effect on dam's weight gain. Also, Diet E did not affect survival, mortality or cannibalism during lactation, but the pEE group (pups with diet E during both pregnancy and lactation) weighed more than the CC group (pups with diet C during pregnancy and lactation). No glucose alteration was observed in pups regardless of the diet consumed by the mothers. The dEE group, however, presented higher blood glucose and hepatic steatosis than the CC group. Finally, dams from the E group showed higher glycemia at the end of lactation. We concluded that protein and fat overload could only partially compensate for the lack of carbohydrates in the maternal diet.

Citation: ***Marcia Gabriela Margato Rocha, Luiz Carlos dos Reis, Marcelo Rodrigues Pinto, Giuliana Cristina Marre Bruschi Thedei, Geraldo Thedei Junior.*** "Lipid and protein overload support only partially the pregnancy and lactation of Wistar rats feed a low carb diet*", International Journal of Development Research*, 11, (11), 51451-51454.

INTRODUCTION

Obesity is a multifactorial and epidemic disease, becoming a public health problem in the world, due to the associated risk of morbidity and mortality (1–3). Maternal nutritional status during pregnancy and lactation plays an important role in determining the risk of developing metabolic diseases in adulthood, including obesity (4–7). Several studies have demonstrated that a high-fat diet and obesity during pregnancy and lactation can lead to adverse outcomes in the offspring, as the intrauterine and early postnatal environments are critical to development (8–11). The influence of a high-fat maternal diet during pregnancy and/or lactation on the offspring's growth, metabolic status, and regulation mechanisms can vary. Changes in dietary composition can have a significant impact on the nutritional status of the mother and the offspring.Recent studies suggest that a high-fat diet environment during pregnancy and lactation leads to changes in serum levels, placenta and liver gene and protein expression, and affects metabolic status in offspring at weaning (11).

The effect of a carbohydrate-restricted diet was determined in Sprague-Dawley rats by Koski and Hill (12, 13). These authors showed that a diet with minimal levels of lipid and protein (5% and 9.5%, respectively) and with up to 12% carbohydrates led to the death of 70% of the offspring by seven days after birth, suggesting that the carbohydrate level significantly affects the maintenance of an adequate lactation, as well as survival to the lactation period (12). In this sense, the increasing lipid level can at least partially replace glucose as a source of dietary energy for the lactation period, including the use of glycerol (present in the triacylglycerol) as a raw material for gluconeogenesis. Whereas there is no doubt that the presence of carbohydrates in the diet have great importance in fertility and perinatal development, the ability of lipids and protein to compensate for the carbohydrate deficiency is not clear. Despite this, low carb/ketogenic diets have been used for a variety of purposes, including weight loss. In the present work, we studied if dietetic lipid and protein overload may suppress carbohydrate deficiency during Wistar rat pregnancy and lactation, in a model of a ketogenic-like diet.

MATERIAL AND METHODS

Animals: Wistar rats were provided by the Central Bioteriumat the University of Uberaba. During all experiments, the animals received a diet and filtered water *ad libitum*.The temperature was kept between 22°C and 26°C, on a 12-hour light and 12-hour dark cycle. Before the experiment, the animals were acclimated to these conditions during 1 week. This study was approved by the Ethic Committee (protocol nr. CEEA 005/2008) and was performed in accordance with the principles outlined by the Brazilian College for Animal Experimentation (COBEA).

Diets: The control diet (C) was a balanced standard diet for rats from Labcil® (São Paulo, Brazil) comprised of calcium carbonate, whole corn flour, rice bran, soybean meal, wheat bran, dicalcium phosphate, cane syrup, sodium chloride, soybean oil, a vitamin and mineral complex providing 290 kcal/100g, according to the manufacturer's information. The experimental diet (E) used is the AIN93G(14), modified to reduce carbohydrates (3.3% of calories)and enhance protein (29.7% of calories) and lipid(67% of calories), thus becoming hyperproteic, hyperlipidic and hypoglycemic, with an energy value of 605 kcal/100g as previously described (15). Briefly, it was prepared homogenizing the following ingredients (g/100 g of diet): casein (45g), commercial lard (45g), sucrose (5g), microcrystalline cellulose (0.16 g), vitamin mix AIN93 (1g), mineral mix AIN93G (3.5g), L-cystine (0.3g), choline bitartrate (0.25g) and cholesterol (0.12g).

Experimental design: Thirty two virgin female Wistar rats were divided equally and randomly into the control group ("C", n=16; average weight 223.62 ± 15.93 g) and experimental group ("E", n=16; average weight of 225.44 ± 16.58 g), according to the diet to be received during gestation (C or E, respectively). For mating, 11 male adult Wistar rats (average weight 296.5 ± 21.5 g) were used at the proportion of 1 male to every 3 females. They were housed in plastic cages for 1 week. After visualizing the vaginal plug, males were removed from the cages and each female was housed in an individual cage. Immediately after the birth of the pups, eight dams from the control group were switched to the Experimental diet and 8 dams from Experimental group were switched to thecontrol diet, forming four subgroups in the lactation period: dCC (dams fed diet C during gestation and lactation), dCE (dams fed diet C during gestation and diet E during lactation), dEE (dams fed diet E during gestation and lactation) and dEC (dams feed diet E during gestation and diet C during lactation). During the lactation period, each mother fed only 8 pups since the exceeding number was randomly euthanized less than 12 hours after birth. Pups were named accordingly to dams, as pCC, pCE, pEE and pEC and euthanasia occurred at day 21 of lactation (see below).

Effects of diet

On the number of pups at birth and during lactation period: During the period expected for the birth of litters (20-21 days after mating), dams were observed twice daily (morning and early evening), to count the number of offspring as soon as they were born. The count of the pups was performed twice per week throughout the lactation period (21 days). We considered it "death" when dead pups were observed inside the cage. We considered it "cannibalism" when the number of pups was smaller than that observed in the previous count, without dead animals inside the cage, or when only parts of pups were found.

On the weight of animals and adipose tissue: The dams were weighed weekly during the period of pregnancy and lactation on a digital scale with an accuracy of 0.5 g. The weight of the pups was monitored weekly from day 1 of birth until the 21st day of life using a digital scale with an accuracy of 0.02g. For mothers and pups, the "absolute weight gain" was determined, expressed as the difference between current weight and previous weight.

After thelactation period, litters and their mothers were euthanized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) followed by laparotomy, rupture of the diaphragm and removal of organs and adipose tissue. The weight of adipose tissue was expressed as grams of tissue/100g of body weight. At the time of euthanasia, a sample of blood was collected from the renal vein, centrifuged (12000xg, 15 min) to obtain a serum for glucose measurement.

On the biochemical parameters: Glucose was quantified from serum using Bioplus 2000® semi automatized system (Barueri, São Paulo, Brazil), with enzymatic kits (Gold Analysa, São Paulo, Brazil), according to instructions from the supplier.

On the Steatosis: After euthanasia, fragments of the hepatic upper lobe were subjected to the standard hematoxylin and eosin (HE) staining procedure to evaluate hepatic steatosis in a common light microscope in a semi-quantitative way(16).

Statistical analysis: The results were organized into an electronic worksheet and used for statistical analysis with GraphPad Prism. All variables were tested for normal distribution (Komolgorov-Smirnov test) and variance with Bartlett's test (homogeneity). For comparisons between two groups (gestational period), the Student's t-test was used. For comparison between different subgroups (lactation period), One-Way ANOVA analysis of variance was used followed by the Bonferroni post-test (results expressed as mean±standard deviation) when satisfactory homogeneity and normality was observed. We used non-parametric analysis of variance when normality and/or homogeneity was unsatisfactory, with the Kruskal-Wallis test followed by Dunn's multiple comparison test. In this case, results were expressed as medians with minimum and maximum values. Survival was analyzed with the Logrank test for trend and with the Gehan-Breslow-Wilcoxon test. For analysis of steatosis, the Fisher Exact Test was used. For all comparisons, the level of significance was 5%.

RESULTS

The control group showed an average of 14.36±1.12 pups per litter, a value significantly higher ($p<0.0001$) than the experimental group (11.08±4.25 pups per litter). In addition, the pups from dams fed the experimental diet showed an average birth weight significantly smaller (5.61±1.10 g) than the pups from control dams (6.41±0.77 g), as shown in Table 1. Throughout the lactation period, we did not detect statistically significant differences in the survival, mortality or cannibalism among the pups from the four experimental groups (data not shown). At the end of lactation period, pups from subgroup pEC showed an absolute weight gain (54.89g; 35.39g/86.39g) significantly higher ($p<0.05$) than subgroup pCE (37.59g; 30.59g/67.59g). There was no difference between pCC and other groups. Concerning the adipose tissue, we observed a significant increase ($p<0.05$) of pEE when compared to the pCC and pCE groups (Table 1). No significant differences for glycemic values were shown between the pup groups at the end of lactation (Table 1). Concerning the weight gain of dams during the gestational period, there was no significant difference between groups C and E (97.92g±25.56g and 88.23g±23.70g, respectively). During lactation, the absolute weight gain of dams from the EC group (20.5g; -20g/30.0g) was higher ($p<0.05$) than those from the CE subgroup (-21g; -104.0g/-15g) (Table 1). The weight of the adipose tissue from the dams fed the experimental diet during gestation and lactation was higher than the other groups, whereas without statistical significance (Table 1). Additionally, Table 1 shows that mothers fed HLP diet during pregnancy and lactation (dEE) had significantly higher blood glucose than those fed a standard diet in the same periods. Histopathological analysis at the end of lactation showed that dams from EE, CE and EC had a 100% incidence of steatosis, against 25% steatosis in the dCC subgroup ($p<0.05$). For pups, we observed 100% and 50% occurrence of hepatic steatosis from the pEE and pEC groups, respectively, and no steatosis in the CC and CE groups.

Table 1. Effect of a low carb, hyperlipidic and hyperproteic diet on birth weight, absolute weight gain, adipose tissue and glycemia of Wistar rat pups

Groups	Birth weigth[1] (g)	Absolute weight gain[2] (g)	Adipose tissue[3] (g/100g body weight)	Glucose[3] (mg/dL)
pCC	6.41± 0.77^{A}	48.59 (36.59; 69.59)^{A,B}	0.80 ± 0.27^{A}	173.38 ± 64.23^{A}
pCE		37.59 (30.59; 67.59)^{A}	0.75 ± 0.23^{A}	162.00 ± 57.10^{A}
pEE	5.61 ± 1.10^{B}	49.89 (0.39; 70.39)^{A,B}	1.08 ± 0.45^{B}	210.82 ± 34.0^{A}
pEC		54,89 (35,39; 86,39)^{B}	0.90 ± 0.31^{AB}	181.50 ± 97.28^{A}
dCC	-	4 (-20.0; 21.0)^{A,B}	1.67 ± 0.76	56.00 ± 4.24^{A}
dCE	-	-21.0 (-104.0; -15.0)^{A}	1.89 ± 1.29	206.5 ± 30.40^{A,B}
dEE	-	9.0 (-91.0; 30.0)^{A,B}	4.66 ± 2.20	222.43 ± 70.04^{B}
dEC	-	20.5 (-20.0; 30.0)^{B}	2,41 ± 1.01	182.50 ± 3.53^{A,B}

[1] *Student's t-test*. Capital letters in the same column indicate p <0.0001.
[2]*Kruskal-Wallis test and Dunn's multiple comparison post-test. Values expressed in median, minimum and maximum.* Capital letters in the same column indicate p<0.05.
[3]*One Way ANOVA and Bonferroni's post-test*. Values expressed as Mean ± SD. Capital letters in the same column indicate p<0.05.

DISCUSSION

The present study shows that a low-carbohydrate diet associated with an overload of lipids and proteins impairs pregnancy, resulting in a smaller litter and low birth weight. This dietallows, however, the pregnancy to occur and the pups to survive the lactation period, unlike low carb diets with a minimum amount of proteins and fats. According to Koski et al(13), carbohydrate-restricted diets altered milk production and/or composition, reducing growth and increasing mortality in the rat pups. Moreover, studies show that a rich lipid diet offered to female rats during pregnancy reduces their reproductive capacity (17) and promotes reduced pup weight in litters (18). In contrast, rats fed a high saturated fat diet during pregnancy exhibited increased placental transport and significant fetal overgrowth related with an mTOR and eIF2alpha signaling (19). Zhao et al.(11) did not observe a statistically significant difference in birth weight of rats fed a high fat diet. However, these authors used a diet with a significant carbohydrate level (45% of calories). This fact points out that the carbohydrate content was a determinant of the low birth weight of the offspring and that the protein and fat excess was not enough to compensate the carbohydrate restriction. Our data show that offspring pEC had a higher weight compared to offspring pCE (Table 1), indicating that a nutritionally adequate diet during lactation can promote a recovery in body mass gain after a nutritionally unbalanced pregnancy. In addition, at the end of lactation, no significant difference was observed when comparing the weight of the pCC group to the other groups.

Fetal development is dependent on a constant supply of nutrients, including glucose and amino acids, and inadequate nutrition can have a considerable effect on maternal and fetal metabolism.Our results suggest that an overload of lipids and protein do not supply the necessary glucose requirements during pregnancy and lactation.Pups did not show glycemic changes at the end of lactation period, regardless of maternal diet. However, we did demonstrate that dams fed the HLP diet during pregnancy and lactation had higher glycemia at the end of lactation, suggesting that the high content of lipids and proteins present in the HLP diet reduced insulin sensitivity when compared to rats fed a balanced diet. The dams that received the HLP diet only during pregnancy (dEC) or during lactation (dCE) also had an increase in glycemia, although not significant, which suggests that an unbalanced diet may affect glycemic control, as pointed out earlier (20). During lactation, no significant mortality or cannibalism was observed among the pup subgroups. These data differ from the findings described previously (12), where these events occurred for offspring whose mothers received a carbohydrate restricted diet throughout pregnancy and/or lactation. According to our data, the HLP diet offered to the dams is adequate in terms of energy necessary for the lactation period. In this case, the breast milk contributed to the deposition of fat in puppies (pEE) whose mother was fed HLP diet during pregnancy and lactation, suggesting that carbohydrate restriction during the gestational period may result in a biochemical adaptation to better use energy resources when they were made available, as previously described (21).

Our study showed a greater weight gain for pEC group, as well as previous findings (22), suggesting that a high fat diet during gestation may cause an adaptation in the pups' metabolism affecting the lactation period, according to the Barker hypothesis (23). Additionally, dams from the dCE group showed more body weight reduction during lactation than the dEC group, suggesting that the ketogenic diet is more harmful if offered during lactation than during pregnancy. It is surprising that a ketogenic diet can maintain pregnancy and lactation, showing that the protein and/or fat overload can, at least in part, supply the energy demand needed during this period. In fact, it was reported that a high-fat diet before and during pregnancy increases the transport of nutrients from the placenta. This transport reflected positively on fetal growth in C57/BL6 mice (24), although it was observed that a ketogenic diet during gestation can result in alterations to embryonic organs (25). It is clear from the analysis of the results presented, that the overload of protein and fat compensates for the lack of carbohydrates, but only partially, as it allows pregnancy to be carried out. However, there is a loss in the development of the puppies, expressed by the smaller litter number and lower birth weight. We propose that the carbohydrate restriction during the gestational period may result in biochemical adaptations to better use of energy resources when they were made available, as previously described (23, 26, 27).

Conclusion

The present investigation demonstrated that the HPL diet during pregnancy promotes weight gain and increases glycemic levels, being able to interfere in the number of births, and also to promote higher weight recovery of pups during lactation. In conclusion, a protein and fat overload could only partially compensate for the lack of carbohydrates in the maternal diet due to the low birth weight at weaning and the risk of maternal glucose unbalance and excess fat deposition in the pups.

Acknowledgment

The authors thank FAPEMIG (Grant numberCDS 1285/05 and number CDS APQ 2542-4.08-07), CNPq and the University of Uberaba (Grant number2006/051) for the continuous support tothis research. They also thank the Mário Palmério University Hospital for the biochemical analysis.

REFERENCES

Axen K V., Harper MA, Kuo YF, Axen K. Very low-carbohydrate, high-fat, weight reduction diet decreases hepatic gene response to glucose in obese rats. Nutr Metab 2018;15:1–13. https://doi.org/10.1186/s12986-018-0284-9.

Barker DJ. Childhood causes of adult diseases. Arch Dis Child 1988;63:867–9. https://doi.org/10.1136/adc.63.7.867.

Bouret SG. Early life origins of obesity: Role of hypothalamic programming. J Pediatr Gastroenterol Nutr 2009;48. https://doi.org/10.1097/MPG.0b013e3181977375.

Bringhenti I, Moraes-Teixeira JA, Cunha MR, Ornellas F, Mandarim-de-Lacerda CA, Aguila MB. Maternal Obesity during the Preconception and Early Life Periods Alters Pancreatic Development in Early and Adult Life in Male Mouse Offspring. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0055711.

Chapman C, Morgan LM, Murphy MC. Maternal and Early Dietary Fatty Acid Intake: Changes in Lipid Metabolism and Liver Enzymes in Adult Rats. J Nutr 2000;130:146–51. https://doi.org/10.1093/jn/130.2.146.

de Borba AJ, Rocha MGM, da Silva MF, Tibúrcio DT de S, Pereira SA de L, dos Reis LC, et al. Dieta hiperlipídico-proteica utilizada para emagrecimento induz obesidade em ratos. Rev Nutr 2011;24:519–28. https://doi.org/10.1590/s1415-52732011000400001.

Dudley KJ, Sloboda DM, Connor KL, Beltrand J, Vickers MH. 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. https://doi.org/10.1371/journal.pone.0021662.

Feakins RM. Obesity and metabolic syndrome: pathological effects on the gastrointestinal tract. Histopathology 2016;68:630–40. https://doi.org/10.1111/his.12907.

Fernandes FS, Sardinha FLC, Badia-Villanueva M, Carulla P, Herrera E, Tavares do Carmo MG. Dietary lipids during early pregnancy differently influence adipose tissue metabolism and fatty acid composition in pregnant rats with repercussions on pup's development. Prostaglandins, Leukot Essent Fat Acids 2012;86:167–74. https://doi.org/10.1016/j.plefa.2012.03.001.

Gaccioli F, White V, Capobianco E, Powell TL, Jawerbaum A, Jansson T. Maternal Overweight Induced by a Diet with High Content of Saturated Fat Activates Placental mTOR and eIF2alpha Signaling and Increases Fetal Growth in Rats1. Biol Reprod 2013;89. https://doi.org/10.1095/biolreprod.113.109702.

Heerwagen MJR, Miller MR, Barbour LA, Friedman JE. Maternal obesity and fetal metabolic programming: A fertile epigenetic soil. Am J Physiol - Regul Integr Comp Physiol 2010;299. https://doi.org/10.1152/ajpregu.00310.2010.

Howie GJ, Sloboda DM, Kamal T, Vickers MH. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. J Physiol 2009;587:905–15. https://doi.org/10.1113/jphysiol.2008.163477.

Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics 2015;33:673–89. https://doi.org/10.1007/s40273-014-0243-x.

Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T. High□fat diet before and during pregnancy causes marked up□regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. FASEB J 2009;23:271–8. https://doi.org/10.1096/fj.08-116889.

Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21. https://doi.org/10.1002/hep.20701.

Kopelman PG. Obesity as a medical problem. Nature 2000;404:635–43. https://doi.org/10.1038/35007508.

Koski KG, Hill FW, Lönnerdal B. Altered Lactational Performance in Rats Fed Low Carbohydrate Diets and Its Effect on Growth of Neonatal Rat Pups. J Nutr 1990;120:1028–36. https://doi.org/10.1093/jn/120.9.1028.

Koski KG, Hill FW. Effect of Low Carbohydrate Diets during Pregnancy on Parturition and Postnatal Survival of the Newborn Rat Pup. J Nutr 1986;116:1938–48. https://doi.org/10.1093/jn/116.10.1938.

MacPherson REK, Castelli LM, Miotto PM, Frendo-Cumbo S, Milburn A, Roy BD, et al. A Maternal High Fat Diet Has Long-Lasting Effects on Skeletal Muscle Lipid and PLIN Protein Content in Rat Offspring at Young Adulthood. Lipids 2015;50:205–17. https://doi.org/10.1007/s11745-014-3985-5.

Mathias JR, Dodd ME, Walters KB, Yoo SK, Erik A, Huttenlocher A. Rat Maternal Obesity and High Fat Diet Program Offspring Metabolic Syndrome. Am J Obstet Gynecol 2014;211:1–13. https://doi.org/10.1016/j.dci.2009.07.003.Characterization.

Reeves PG, Nielsen FH, Fahey GC. AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. J Nutr 1993;123:1939–51. https://doi.org/10.1093/jn/123.11.1939.

Shaw MA, Rasmussen KM, Myers TR. Consumption of a High Fat Diet Impairs Reproductive Performance in Sprague-Dawley Rats. J Nutr 1997;127:64–9. https://doi.org/10.1093/jn/127.1.64.

Sussman D, van Eede M, Wong MD, Adamson SL, Henkelman M. Effects of a ketogenic diet during pregnancy on embryonic growth in the mouse. BMC Pregnancy Childbirth 2013;13:109. https://doi.org/10.1186/1471-2393-13-109.

Vido DS, Nejm MB, Silva NR, Silva SMA, Cravo SL, Luz J. Maternal obesity and late effects on offspring metabolism. Arq Bras Endocrinol Metabol 2014;58:301–7. https://doi.org/10.1590/0004-2730000003043.

Ware S, Voigt J-P, Langley-Evans SC. Body composition and behaviour in adult rats are influenced by maternal diet, maternal age and high-fat feeding. J Nutr Sci 2015;4:e3. https://doi.org/10.1017/jns.2014.64.

Zhang J, Zhang F, Didelot X, Bruce KD, Cagampang FR, Vatish M, et al. Maternal high fat diet during pregnancy and lactation alters hepatic expression of insulin like growth factor-2 and key microRNAs in the adult offspring. BMC Genomics 2009;10:478. https://doi.org/10.1186/1471-2164-10-478.

Zhao M, Li Y, Yao H, Dou L, Zhang S, Zhao Q, et al. Sex-specific Alterations in Serology and the Expression of Liver FATP4 Protein in Offspring Exposed to High-Fat Diet during Pregnancy and/or Lactation. Lipids 2018;53:301–11. https://doi.org/10.1002/lipd.12029.

****