



OPEN

## MicroRNA Let-7 targets AMPK and impairs hepatic lipid metabolism in offspring of maternal obese pregnancies

Laís A. P. Simino<sup>1</sup>✉, Carolina Panzarin<sup>1</sup>, Marina F. Fontana<sup>1</sup>, Thais de Fante<sup>1</sup>, Murilo V. Geraldo<sup>2</sup>, Leticia M. Ignácio-Souza<sup>1</sup>, Marciane Milanski<sup>1</sup>, Marcio A. Torsoni<sup>1</sup>, Michael G. Ross<sup>3</sup>, Mina Desai<sup>3</sup> & Adriana S. Torsoni<sup>1</sup>

Nutritional status during gestation may lead to a phenomenon known as metabolic programming, which can be triggered by epigenetic mechanisms. The *Let-7* family of microRNAs were one of the first to be discovered, and are closely related to metabolic processes. Bioinformatic analysis revealed that *Prkaa2*, the gene that encodes AMPK  $\alpha 2$ , is a predicted target of *Let-7*. Here we aimed to investigate whether *Let-7* has a role in AMPK $\alpha 2$  levels in the NAFLD development in the offspring programmed by maternal obesity. *Let-7* levels were upregulated in the liver of newborn mice from obese dams, while the levels of *Prkaa2* were downregulated. *Let-7* levels strongly correlated with serum glucose, insulin and NEFA, and in vitro treatment of AML12 with glucose and NEFA lead to higher *Let-7* expression. Transfection of *Let-7a* mimic lead to downregulation of AMPK $\alpha 2$  levels, while the transfection with *Let-7a* inhibitor impaired both NEFA-mediated reduction of *Prkaa2* levels and the fat accumulation driven by NEFA. The transfection of *Let-7a* inhibitor in ex-vivo liver slices from the offspring of obese dams restored phospho-AMPK $\alpha 2$  levels. In summary, *Let-7a* appears to regulate hepatic AMPK $\alpha 2$  protein levels and lead to the early hepatic metabolic disturbances in the offspring of obese dams.

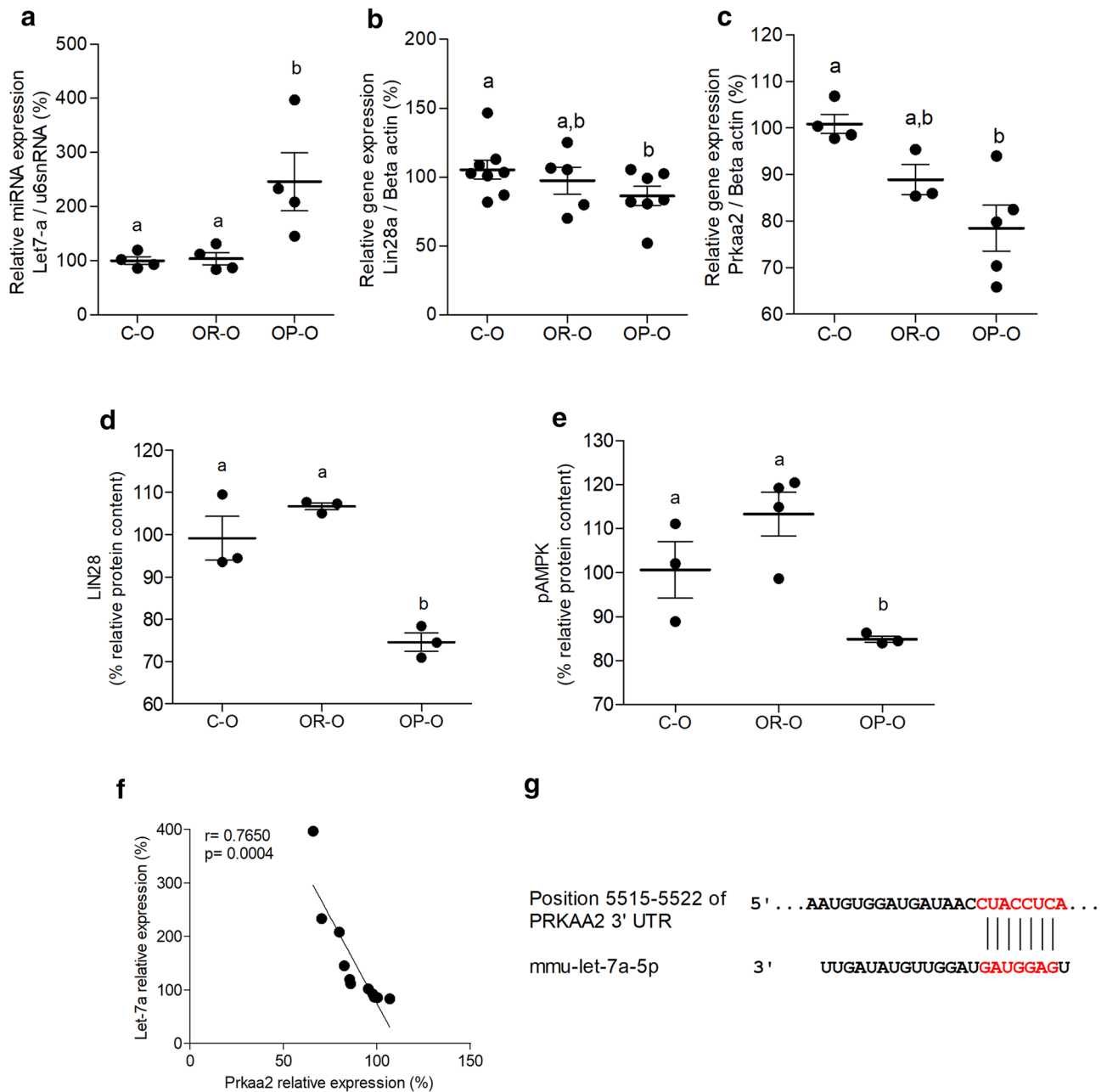
Adverse conditions during fetal and early postnatal life can have a long-term impact on health and metabolism, a phenomenon known as the *Developmental Origins of Health and Disease* (DOHaD) or metabolic programming<sup>1</sup>. Maternal obesity and high-fat diet consumption have been shown to drive deleterious effects on the offspring metabolism, predisposing them to the development of obesity, insulin resistance, dyslipidemias, and non-alcoholic fatty liver disease (NAFLD)<sup>2-6</sup>.

NAFLD is considered as a complex interaction of nutritional factors and higher susceptibility due to parental obesity programming of epigenetic mechanisms, and microRNAs (miRNAs) may play an essential role in NAFLD development, progression and diagnostics<sup>7,8</sup>. *Let-7* was one of the first miRNAs to be discovered<sup>10</sup>, and processing and maturing of *Let-7* can be inhibited by LIN28<sup>9</sup>. *Let-7*/LIN28 axis is implicated in energy metabolism, and regulates multiple aspects of glucose and lipid metabolism, while *Let-7* knockdown using antimir-based approaches appears as a potential strategy for treating metabolic diseases<sup>9,10</sup>.

NAFLD individuals commonly have decreased hepatic AMPK<sup>11,12</sup>. The maintenance of AMPK levels seems to be beneficial for the liver homeostasis, and the activation of AMPK may be of value for the prevention and treatment of metabolic disorders associated with obesity, such as type 2 diabetes mellitus and NAFLD<sup>13</sup>.

Thus, in the present study we sought to investigate whether hepatic *Let-7* would have a role in the modulation of AMPK $\alpha 2$  in the context of the NAFLD development in the offspring programmed by maternal obesity during intrauterine life.

<sup>1</sup>Laboratory of Metabolic Disorders (Labdime) – Faculty of Applied Sciences (FCA), University of Campinas (UNICAMP), 1300, Pedro Zaccaria St, Limeira, SP 13484-350, Brazil. <sup>2</sup>Institute of Biology (IB), University of Campinas (UNICAMP), Campinas, SP, Brazil. <sup>3</sup>The Lundquist Institute and David Geffen School of Medicine at Harbor-UCLA Medical Center, University of California, Los Angeles, CA, USA. ✉email: lsimino@unicamp.br



**Figure 1.** *Let-7/AMPK* potential axis is modulated in the liver of newborn male offspring from obese dams. qRT-PCR of *Let-7a* (a), *Lin28a* (b), and *Prkaa2* (c), Indirect ELISA of LIN28 (d) and pAMPK (e) in the liver of male offspring from control (C-O), obesity-resistant (OR-O) and obesity-prone (OP-O) dams at the delivery day (d0) (n = 3–8 pups/group). Pearson correlation and linear regression of hepatic levels of *Let-7a* and *Prkaa2* (n = 11) (f), predicted pairing of target region of *Prkaa2* mRNA (top) and *Let-7a* miRNA (bottom), according to TargetScan, release 7.2 (g). One-way ANOVA were used to compare groups. Bonferroni post-hoc test was used to determine a significance level of  $p \leq 0.05$ . Values are means with their standard errors represented by vertical bars. Different letters indicate statistical significance between groups.

## Results

**Let-7/AMPK axis is modulated in the liver of offspring from obese dams.** Offspring from obesity-prone HFD-fed dams (OP-O), unlike the offspring from obesity-resistant HFD-fed dams (OR-O), had lower body weight and higher serum glucose, insulin and NEFA (C-O) (Supplemental Fig. 1).

Hepatic gene expression was modulated in male OP-O, as they had higher levels of hepatic *Let-7a* (Fig. 1a), while the levels of *Lin28a* and *Prkaa2* were downregulated (Fig. 1b,c, respectively). *Let-7a* and *Prkaa2* expression were also modulated in female OP-O (Supplemental Fig. 2). Hepatic protein content of LIN28 and pAMPK were lower in OP-O (Fig. 1d,e, respectively). Altogether, *Let-7a* and *Prkaa2* mRNA levels showed a strong negative correlation (Fig. 1f). Bioinformatic analysis revealed that the gene encoding AMPK $\alpha$ 2 protein is a predicted target of *Let-7a* (Fig. 1g), thus we proceeded to investigate the mechanisms underlying this finding.

**Let-7 expression can be upregulated by NEFA, glucose and TNF $\alpha$ , and its transfection leads to the downregulation of AMPK $\alpha$ 2.** We previously showed that obesity-prone HFD-fed dams have higher serum NEFA, glucose and insulin levels<sup>14</sup>. Figure 2 (a–c) show a positive correlation between maternal serum parameters and hepatic levels of *Let-7a* in the offspring. The levels of NEFA, glucose and insulin in offspring are also correlated with their hepatic expression of *Let-7a* (Fig. 2d–f, respectively).

In AML12, the treatment with NEFA, glucose, and TNF $\alpha$  drove an upregulation of *Let-7a* similar to that observed after the transfection with *Let-7a* mimic (Fig. 2g).

The transfection of AML12 with *Let-7a* mimic (Supplemental Fig. 3) led to a decrease in LIN28 and AMPK $\alpha$ 2 positive cells (Fig. 2h–i).

**Let-7a anti-miR prevents fat accumulation driven by NEFA.** The transfection of AML12 with *Let-7a* anti-miR prior to NEFA treatment was able to impair NEFA-mediated reduction of *Prkaa2* levels (Fig. 3a,b).

The treatment with NEFA led to an increase in fat deposition, however *Let-7a* anti-miR prevented fat accumulation driven by NEFA in the hepatocytes (Fig. 3c).

**Inhibition of Let-7a rescues AMPK $\alpha$ 2 levels in the ex-vivo liver slices of offspring from obese dams.** OP-O had lower basal levels of LIN28, as well as phospho-AMPK $\alpha$ 2 (Fig. 4a,b, respectively). When the liver of OP-O were transfected with *Let-7a* anti-miR, the levels of both LIN28 (Fig. 4a) and phospho-AMPK $\alpha$ 2 (Fig. 4b) were rescued.

## Discussion

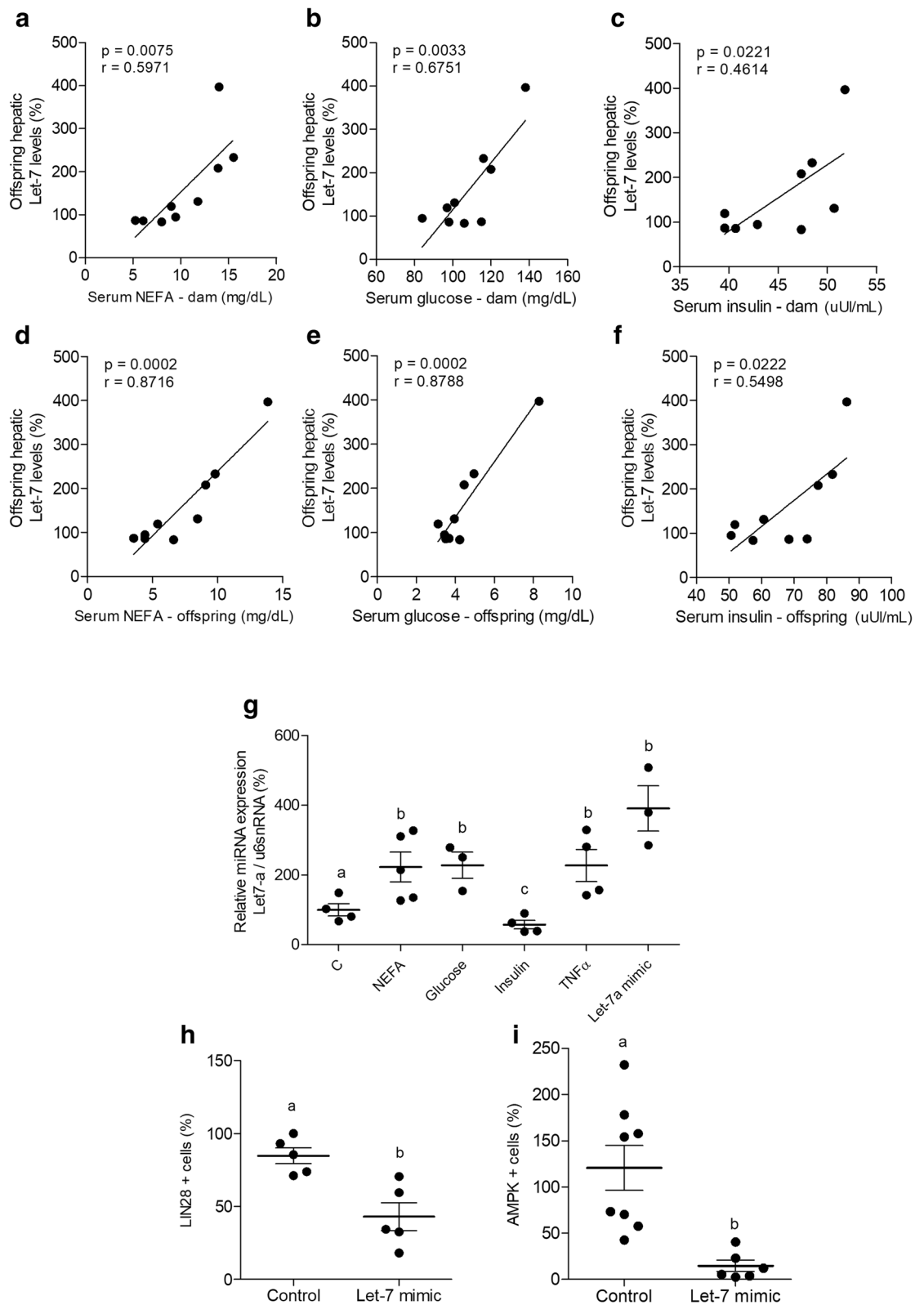
We recently showed that offspring from obesity-prone (OP-O) dams fed HFD during gestation demonstrated marked metabolic disturbances compared to offspring from obesity-resistant (OR-O) dams, with lower body weight at the delivery day (d0) being one of the main differences of OP-O from OR-O<sup>14</sup>. Here we showed that OP-O, but not OR-O, have higher hepatic levels of *Let-7a* and lower of Lin28a at d0. Interestingly, *Let-7* and Lin28 were firstly described as heterochronic regulators of developmental timing in *C. elegans*, and Shinoda and colleagues (2013) showed that Lin28 knockout mice exhibited dwarfism as early as in embryogenesis, and at birth they were 30–50% smaller than heterozygote controls<sup>15</sup>. Thus, the imbalanced levels of *Let-7/Lin28* may, at least in part, explain the lower birth weight in offspring from obese dams.

Moreover, recent studies have shown that the balance in the *Let-7/Lin28* axis also has a major role in the energy homeostasis, especially in alterations related to glucose and insulin signaling<sup>10,16</sup>. On the other hand, AMPK is known as a cellular energy sensor, and its expression and activation have been largely studied as key mechanisms to prevent and treat metabolic abnormalities related to glucose and lipid homeostasis<sup>11,12</sup>. We found no reports that have linked the AMPK levels with the *Let-7* modulation in the context of lipid homeostasis and NAFLD development. However, in 2012, McCarty speculated that metformin could act as an antagonist of Lin28, thus leading to *Let-7* upregulation<sup>17</sup>. Zhong and colleagues (2016) explored the molecular mechanisms underlying the antitumorigenic properties of metformin and they identified that the treatment of cancer cells with both metformin and AICAR, AMPK-activating agent, drove an upregulation of *Let-7* levels<sup>18</sup>. However, it is possible that there may be a feedback regulation among AMPK and *Let-7* levels. We identified *Prkaa2*, the gene that encodes the  $\alpha$ 2 subunit of AMPK protein, as a predicted target of *Let-7* family by computational analysis of miRNA/mRNA interaction. The inversely correlated expression of *Let-7a* and *Prkaa2* in the liver of mice acutely and chronically exposed to HFD is another evidence of their interaction.

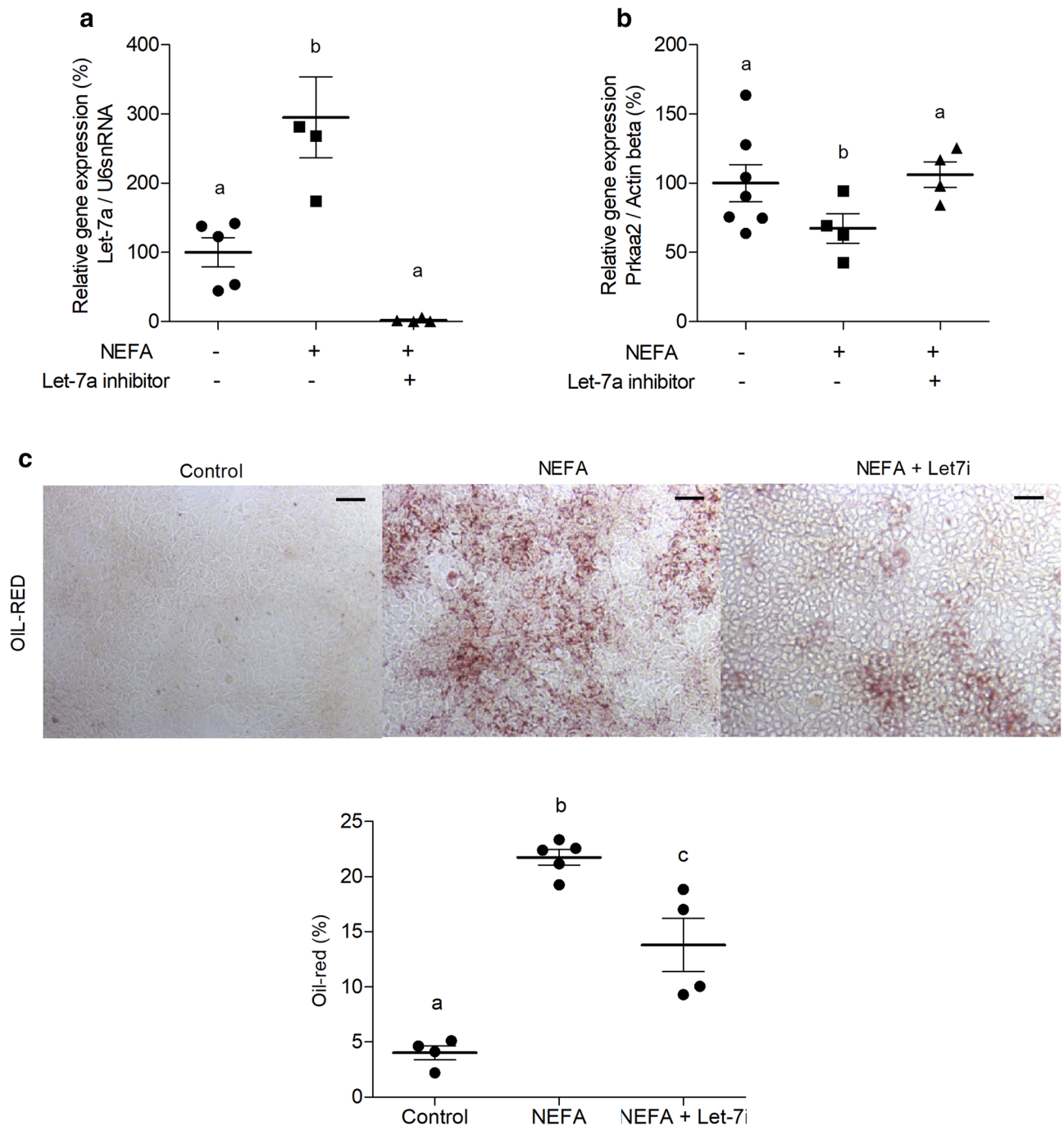
Male newborns from obese dams had lower *Prkaa2* transcript levels and AMPK $\alpha$ 2 proteins levels in the liver. At normal conditions, AMPK is activated by high levels of AMP, and triggers catabolic while inhibiting anabolic processes to restore cellular energy homeostasis<sup>13</sup>. In the liver, the activation of AMPK blocks the synthesis of fatty acids, TG, cholesterol, and proteins while activating oxidative processes<sup>13,19,20</sup>, and it has been shown that obese, diabetic, or non-alcoholic fatty liver disease (NAFLD) individuals have decreased hepatic AMPK<sup>11,12</sup>. We previously reported that male OP-O have higher hepatic TG content, and upregulated *Srebf1* expression at birth, while they present higher hepatic TG and cholesterol levels, and upregulation in *Fasn* and *Srebf1* expression after weaning<sup>14</sup>. These findings are consistent with the lower *Prkaa2/AMPK $\alpha$ 2* levels in the liver. We showed here that *Let-7* anti-miR transfection in hepatocytes can prevent fat accumulation induced by NEFA. This is consistent with the study from Frost and Olson (2011) which showed that mice fed a HFD but treated with a *Let-7* inhibitor prevented excessive fat storage in the liver<sup>9</sup>. In another study the constitutive expression of *Let-7* was sufficient to induce ectopic lipid accumulation in the liver<sup>21</sup>. Thus, the disruption in the hepatic lipid homeostasis in the offspring of obese dams may be driven by *Let-7*-induced AMPK $\alpha$ 2 depletion.

Curiously, offspring from HFD females that did not develop the obese phenotype (obesity-resistant) were somehow protected from major metabolic disturbances and had no alterations in hepatic *Let-7/Lin28* axis. Therefore, we hypothesized that there might be some metabolic particularity in obesity-prone dams that led to the modulation of the hepatic *Let-7* in their offspring. Accordingly, we found that some serum parameters of the dams, such as NEFA, glucose and insulin, positively correlate with hepatic levels of *Let-7a* of the offspring. Indeed, cultured hepatocytes revealed that NEFA, glucose, and TNF $\alpha$  treatments lead to an upregulation of *Let-7a* levels, although the correlation with insulin levels has not been observed. Katayama and colleagues (2015) have shown that *Let-7* levels can be directly regulated by glucose and TNF $\alpha$  in HEK293 cells, while insulin was unable to activate the *Let-7* promoter region<sup>22</sup>.

Despite TNF $\alpha$  had been able to drive *Let-7* upregulation, there is a lack of consensus as to whether that maternal cytokines can be transported to the fetus during pregnancy. Glucose and NEFA, on the other hand, have specific transporters in the placenta, GLUTs and FATPs, respectively, and are also the major energy substrates for fetal development<sup>23,24</sup>. Thus, we believe that the adverse conditions of obese pregnant dams, e.g. the



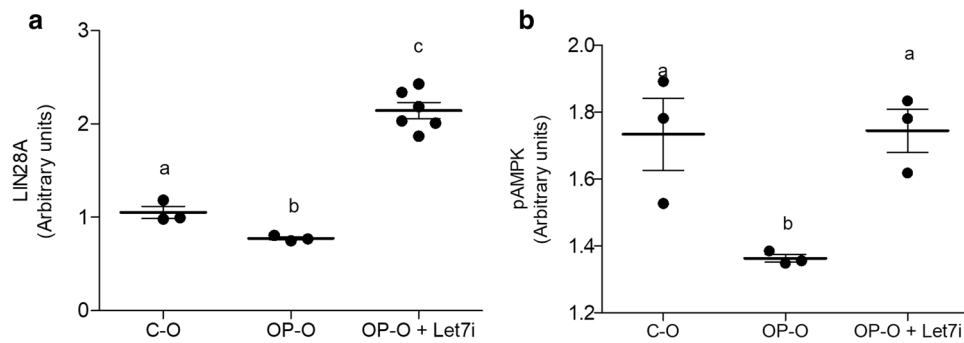
**Figure 2.** NEFA, glucose and TNF $\alpha$  drives *Let-7a* upregulation, which direct downregulates AMPK $\alpha$ 2 levels. Pearson correlation of hepatic *Let-7a* in male offspring with maternal NEFA (a), glucose (b), and insulin (c), and Pearson correlation of hepatic *Let-7a* in male offspring with offspring's NEFA (d), glucose (e), and insulin (f) (n = 9); qRT-PCR of *Let-7a* in AML12 cell extract treated with NEFA (500  $\mu$ M), glucose (20 mM), insulin (120 nM), TNF $\alpha$  (40 ng/mL) or *Let-7a* mimic (10 nM) for 24 h (g); confocal immunofluorescence quantification of LIN28 (h) and AMPK $\alpha$ 2 (i) positive cells of AML12 cell extract transfected with negative control or *Let-7a* mimic (10 nM) for 24 h. The correlations were constructed by using all experimental groups (control, HFD-fed prone to obesity and HFD-fed resistant to obesity). Experiments with the cell line were performed in quadruplicates and repeated twice. Student's T test were used to compare the results at confocal immunofluorescence analysis. One-way ANOVA were used in the qRT-PCR analysis to compare all groups. Bonferroni post-hoc test was used to determine a significance level of  $p \leq 0.05$ . Values are means with their standard errors represented by vertical bars. Different letters indicate statistical significance between groups.



**Figure 3.** Inhibition of *Let-7a* rescues NEFA-induced alterations in hepatocytes. qRT-PCR of *Let-7a* (a) and *Prkaa2* (b); representative Oil-red images (scale-bar = 10  $\mu$ m), and quantification (c) of AML12 cell extract treated with NEFA (500  $\mu$ M) or NEFA (500  $\mu$ M) plus *Let-7a* inhibitor transfection (10 nM) for 24 h. Experiments were performed in quadruplicates and repeated twice. One-way ANOVA were used in the qRT-PCR analysis to compare all groups. Bonferroni post-hoc test was used to determine a significance level of  $p \leq 0.05$ . Values are means with their standard errors represented by vertical bars. Different letters indicate statistical significance between groups.

excessive serum glucose and NEFA, may be related to the hepatic upregulation of *Let-7* and to the consequent metabolic disturbances of the offspring.

Metformin, an antidiabetic drug that have been effectively used to treat not only diabetes but related conditions, such as body weight management and NAFLD, functions primarily by activating AMPK<sup>25</sup>. Interestingly, here we showed that inhibition of *Let-7* may exert similar effects, leading to AMPK activation, since the *ex-vivo* transfection with *Let-7a* anti-miR rescued LIN28 and phospho-AMPK $\alpha$ 2 levels in the liver of the newborn offspring of obese dams. Further studies are necessary in order to investigate the translational impact of the present



**Figure 4.** Ex-vivo manipulation of hepatic *Let-7a* rescues pAMPK $\alpha$ 2 levels. LIN28A (a) and pAMPK $\alpha$ 2 (b) levels of ex-vivo liver transfection of newborn male offspring with *Let-7a* inhibitor (10 nM) for 24 h by indirect ELISA. One-way ANOVA were used in the analysis to compare all groups. Values are means (n = 3–6/group) with their standard errors represented by vertical bars. Bonferroni post-hoc test was used to determine a significance level of  $p \leq 0.05$ . Different letters indicate statistical significance between groups.

results. However, based on our findings we believe that *Let-7* anti-miR may exert a potential therapy to prevent the fetal metabolic programming effects from obese mothers.

In summary, our data showed strong evidence that *Let-7a* may regulate hepatic AMPK $\alpha$ 2 protein levels. Furthermore, we suggest that maternal obesity, but not maternal HFD apart from the obese phenotype, leads to hepatic modulation of the potential *Let-7*/AMPK axis, and may be related to the metabolic disturbances presented by the newborn offspring from obese dams.

## Methods

**Experimental animals and diets.** All of the experimental procedures were performed in accordance with the ARRIVE guidelines, and the guidelines of the Brazilian Society of Science in Laboratory Animals and were approved by the local Ethics Committee for Animal Use (ID protocols 4349-1, and 3963-1) of the University of Campinas (UNICAMP). All experimental animals were obtained from Animal Breeding Center at the University of Campinas (CEMIB) and were maintained in individual polypropylene micro-isolators at  $22 \pm 1$  °C and lights on from 06:00 to 18:00 h.

Twelve five-week old female Swiss mice (*Mus musculus*) were fed a standard chow diet (Nuvilab CR-1, Nuvital, PR—Brazil, C; 3.5 kcal/g, 9.5% fat) or a high-fat diet as previously described<sup>5</sup> (HFD: 4.6 kcal/g, 45% fat) for an adaptation period of 4 weeks before mating. At the end of the adaptation period, HFD females were classified as obesity-prone or obesity-resistant, as described by Simino et al., 2020<sup>14</sup>, and they were mated with control male mice. One male for two female mice were housed together to mate. During pregnancy, female mice were fed the same diet of the adaptation period. At the delivery day (d0), newborns were euthanized and liver was dissected and immediately sectioned to *ex-vivo* analysis or frozen in liquid N<sub>2</sub> followed by -80 °C storage to qPCR and immunofluorescence.

**In silico analysis of miRNA potential targets.** The *Let-7*/mRNAs target prediction was performed using MiRWalk 2.0 platform (<http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/>), accessing a total of 12 algorithms. Interactions were considered valid when predicted by TargetScan algorithm, and at least 5 other algorithms.

**Quantitative real time PCR (qPCR).** Total RNA and microRNA were extracted from liver (~150 mg) or cells using RNazol RT (Molecular Research Center, MRC, Cincinnati, OH—USA) according to the manufacturer's recommendations, and quantified using NanoDrop ND-2000. Reverse transcription was performed with 3  $\mu$ g of total RNA or miRNA by specific reverse transcription kits (Thermo Fisher Scientific, Waltham, Massachusetts—USA). The relative expression of mRNAs (Prkaa2 ID Mm01264789\_m1, Lin28a ID Mm00524077\_m1) and microRNAs (*Let-7a* ID 000377, U6srRNA ID 001973) was determined using a Taqman detection system (Thermo Fisher Scientific, Waltham, Massachusetts—USA). qPCR was performed on an ABI Prism 7500 Fast platform, and data were expressed as relative values determined by the comparative threshold cycle (Ct) method ( $2^{-\Delta\Delta Ct}$ ).

**Immunofluorescence.** Liver fragments from newborn male offspring was embedded in Tissue-Tek (Sakura, Torrance, CA—USA), frozen and sectioned into 12- $\mu$ m-thick sections. AML12 cells were plated in round slides and treated as described below. Liver slices and AML12 cells were blocked with 3% albumin for 120 min. After, they were incubated with specific primary antibodies (AMPK $\alpha$ 2 (1:50 dilution), Santa Cruz Biotechnology, CA—USA, or LIN28 (1:250 dilution), Abcam, Cambridge, MA—USA) overnight and secondary antibodies (Donkey anti-mouse FITC conjugated (1:250 dilution), Abcam, Cambridge, MA—USA). IMMU-Mount medium with DAPI was used to cover the slides (Vectashield, Vector Laboratories, Burlingame, CA—

USA). Slices were visualized and captured by TCS SP5 II Leica confocal microscopy (Leica Microsystems, Wetzlar, Hesse—Germany). The number of AMPK $\alpha$ 2<sup>+</sup> and LIN28<sup>+</sup> cells was counted using ImageJ software.

**Cell culture and transfection.** In vitro analysis were performed using AML12 mouse hepatocyte cell line (ATCC CRL-2254). Cells were maintained in DMEM:HAM-F12 medium (1:1, 3.15 g/L glucose) (Sigma Aldrich), with 10% FBS, 100U/mL penicillin, 0.1 mg/mL streptomycin, 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml de selenium and 40 ng/mL dexamethasone, and incubated at 37 °C in 5% CO<sub>2</sub>. Experiments were performed between passages 10 and 20. Cells were grown as monolayers and after seeding they were treated with glucose (20 mM), insulin (120 nM), TNF $\alpha$  (40 ng/mL), non-esterified fatty-acids (NEFA—500  $\mu$ M), or transfected with *Let-7a* mimic (10 nM, Ambion) and Lipofectamine RNAiMax (Invitrogen), in serum free culture medium, for 24 h. Cells were harvested to qPCR analysis as described above. Next, cells were seeded above round slides and transfected with *Let-7a* mimic (10 nM, Ambion) and Lipofectamine RNAiMax (Invitrogen), in serum free culture medium, for 24 h. Slides were submitted to immunofluorescence analysis as described above. Further, reverse transfection of *Let-7a* anti-miR (10 nM, Ambion) and Lipofectamine RNAiMax (Invitrogen) were performed and 24 h after seeding, cells were treated with NEFA (500  $\mu$ M) for 24 h. Cells were then harvested for qPCR analysis or Oil-Red (Sigma Aldrich) staining, as described by Mehlem et al. (2013)<sup>26</sup>.

**Ex vivo analysis.** The liver of the offspring from control and obese dams (C-O and OP-O, respectively) were extracted at the delivery day, manually sectioned into ~2 mm slices and immediately incubated in Krebs–Henseleit buffer (KHB, 5 mM NaCl, 118 mM KCl, 1.1 mM MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, 2.5 mM CaCl<sub>2</sub>·2H<sub>2</sub>O, 25 mM D-Glucose, 9 mM HEPES) at 4 °C. After, slices were transferred to culture plates with high-glucose DMEM medium (Sigma Aldrich) with 100U/mL penicillin, 0.1 mg/mL streptomycin, and incubated at 37 °C in 5% CO<sub>2</sub>. After 2 h, slices were transferred to new plates with culture medium and negative control or *Let-7a* anti-miR (20 nM, Ambion) and Lipofectamine RNAiMax (Invitrogen). After 24 h, slices were harvested to indirect ELISA analysis of phospho-AMPK $\alpha$ 2 (1:50, Santa Cruz Biotechnology, CA – USA) and LIN28 (1:250, Abcam, Cambridge, MA—USA).

**Statistical analysis.** Results are expressed as means and their standard errors. Student's T test was used to compare two groups. Analysis of variance (ANOVA) was assessed for multiple comparisons, and Bonferroni's post-test was used to determine the significance level of  $p \leq 0.05$ .

Received: 5 January 2021; Accepted: 7 April 2021

Published online: 26 April 2021

## References

- Fernandez-Twinn, D. S., Constância, M. & Ozanne, S. E. Intergenerational epigenetic inheritance in models of developmental programming of adult disease. *Semin. Cell. Dev. Biol.* **43**, 85–95. <https://doi.org/10.1016/j.semcdb.2015.06.006> (2015).
- Melo, A. M. et al. Hypothalamic endoplasmic reticulum stress and insulin resistance in offspring of mice dams fed high-fat diet during pregnancy and lactation. *Metabolism* **63**(5), 682–692 (2014).
- Reginato, A. et al. Autophagy proteins are modulated in the liver and hypothalamus of the offspring of mice with diet-induced obesity. *J. Nutr. Biochem.* **34**, 30–41 (2016).
- de Fante, T. et al. Diet-induced maternal obesity alters insulin signalling in male mice offspring rechallenged with a high-fat diet in adulthood. *PLoS ONE* **11**(8), e0160184. <https://doi.org/10.1371/journal.pone.0160184> (2016).
- de Paula Simino, L. A. et al. Lipid overload during gestation and lactation can independently alter lipid homeostasis in offspring and promote metabolic impairment after new challenge to high-fat diet. *Nutr. Metab. (Lond)*. **14**(1), 16 (2017).
- Lemes, S. F. et al. Maternal consumption of high-fat diet in mice alters hypothalamic notch pathway, NPY cell population and food intake in offspring. *Neuroscience* **371**, 1–15 (2018).
- Ceccarelli, S., Panera, N., Gnani, D. & Nobili, V. Dual role of microRNAs in NAFLD. *Int J Mol Sci.* **14**(4), 8437–8455 (2013).
- Thompson, M. D. Developmental programming of NAFLD by parental obesity. *Hepatol. Commun.* **4**(10), 1392–1403 (2020).
- Frost, R. J. & Olson, E. N. Control of glucose homeostasis and insulin sensitivity by the Let-7 family of microRNAs. *Proc. Natl. Acad. Sci.* **108**(52), 21075–80 (2011).
- Zhu, H. et al. The Lin28/let-7 axis regulates glucose metabolism. *Cell* **147**(1), 81–94 (2011).
- Garcia, D., Hellberg, K., Chaiz, A., Wallace, M. & Shaw, R. J. Genetic liver-specific AMPK activation protects against diet-induced obesity and NAFLD. *Physiol. Behav.* **176**(3), 139–148 (2019).
- Nagata, D. & Hirata, Y. The role of AMP-activated protein kinase in the cardiovascular system. *Hypertens. Res.* **33**(1), 22–28 (2010).
- Day, E. A., Ford, R. J. & Steinberg, G. R. AMPK as a therapeutic target for treating metabolic diseases. *Trends Endocrinol. Metab.* **28**(8), 545–560. <https://doi.org/10.1016/j.tem.2017.05.004> (2017).
- Simino, L. A. P. et al. Maternal resistance to diet-induced obesity partially protects newborn and post-weaning male mice offspring from metabolic disturbances. *J. Dev. Orig. Health Dis.* <https://doi.org/10.1017/S204017442000094X> (2020).
- Shinoda, G. et al. Fetal deficiency of Lin28 programs life-long aberrations in growth and glucose metabolism. *Stem Cells* **31**(8), 1563–73 (2013).
- Thornton, J. E. & Gregory, R. I. How does Lin28 let-7 control development and disease?. *Trends Cell Biol.* **22**(9), 474–482. <https://doi.org/10.1016/j.tcb.2012.06.001> (2012).
- McCarty, M. F. Metformin may antagonize Lin28 and/or Lin28B activity, thereby boosting let-7 levels and antagonizing cancer progression. *Med. Hypotheses* **78**(2), 262–269. <https://doi.org/10.1016/j.mehy.2011.10.041> (2012).
- Zhong, T. et al. Metformin alters DNA methylation genome-wide via the H19/SAHH axis. *Oncogene* **36**(17), 2345–2354 (2017).
- Smith, B. K. et al. Treatment of nonalcoholic fatty liver disease: Role of AMPK. *Am. J. Physiol. Endocrinol. Metab.* **311**(4), E730–E740 (2016).
- Fullerton, M. D. AMP-activated protein kinase and its multifaceted regulation of hepatic metabolism. *Curr. Opin. Lipidol.* **27**(2), 172–180 (2016).
- Wu, L. et al. Precise let-7 expression levels balance organ regeneration against tumor suppression. *Elife* **4**, e09431 (2015).

22. Katayama, M., Sjögren, R. J., Egan, B. & Krook, A. miRNA let-7 expression is regulated by glucose and TNF- $\alpha$  by a remote upstream promoter. *Biochem. J.* **472**(2), 147–156 (2015).
23. Lager, S. *et al.* Effect of IL-6 and TNF- $\alpha$  on fatty acid uptake in cultured human primary trophoblast cells. *Placenta* **32**(2), 121–127. <https://doi.org/10.1016/j.placenta.2010.10.012> (2011).
24. Perazzolo, S. *et al.* The influence of placental metabolism on fatty acid transfer to the fetus. *J. Lipid. Res.* **58**(2), 443–454 (2017).
25. Prakash, S., Rai, U., Kosuru, R., Tiwari, V. & Singh, S. Amelioration of diet-induced metabolic syndrome and fatty liver with Sitagliptin via regulation of adipose tissue inflammation and hepatic Adiponectin/AMPK levels in mice. *Biochimie* **168**, 198–209. <https://doi.org/10.1016/j.biochi.2019.11.005> (2019).
26. Mehlem, A., Hagberg, C. E., Muhl, L., Eriksson, U. & Falkevall, A. Imaging of neutral lipids by oil red O for analyzing the metabolic status in health and disease. *Nat. Protocols* **8**(6), 1149–1154 (2013).

## Acknowledgements

This work was supported by the grants from the *Coordination for the Improvement of Higher Education Personnel* (CAPES), *National Council for Scientific and Technological Development* (CNPq, AST Grant #303209/2019-4), *São Paulo Research Foundation* (FAPESP, LAPS Grant #2015/01947-7 and AST grant #2019-03351-5), and *National Institutes of Health* (Grant # 1R01HD099813-01A1). The Laboratory of Metabolic Disorders (Labdime) belongs to the Obesity and Comorbidities Research Center (OCRC).

## Author contributions

A.S.T. and L.A.P.S. performed the experimental design. L.A.P.S., C.P., M.F.F. and T.F. were involved in the data acquisition and analyses. A.S.T., L.A.P.S., M.A.T., L.M.I.S., M.M., M.V.G., M.D. and M.G.R. were involved in the data interpretation, manuscript writing, and critical revision. A.S.T., M.A.T., L.M.I.S. and M.M. provided the funding for the study. All authors approved the final version and declare no conflicts of interest.

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1038/s41598-021-88518-8>.

**Correspondence** and requests for materials should be addressed to L.A.P.S.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021