Differential regulation of placental amino acid transport by saturated and unsaturated fatty acids

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Lager S, Jansson T, Powell TL. Differential regulation of placental amino acid transport by saturated and unsaturated fatty acids. Am J Physiol Cell Physiol 307: C738–C744, 2014. First published August 20, 2014; doi:10.1152/ajpcell.00196.2014.—Fatty acids are critical for fetal development. They are essential structural components of cellular membranes and precursors of bioactive compounds, with particularly large amounts being deposited in the fetal brain (13). Fatty acids can also influence cellular signaling through a multitude of mechanisms, with effects dependent on their level of saturation. For instance, saturated and polyunsaturated fatty acids have distinct effects on inflammatory signaling, for instance, palmitic acid (PA) but not OA reduces IκBα expression. We hypothesized that saturated and unsaturated fatty acids differentially affect trophoblast amino acid transport and cellular signaling. To test this hypothesis, PHTs were cultured in docosahexaenoic acid (DHA; 50 μM), OA (100 μM), or PA (100 μM). DHA and OA were also combined to test whether DHA could counteract the OA stimulatory effect on amino acid transport. The effects of fatty acids were compared against a vehicle control. Amino acid transport was measured by isotope-labeled tracers. Activation of inflammatory-related signaling pathways and the mechanistic target of rapamycin (mTOR) pathway were determined by Western blot analysis. Exposure of PHTs to DHA for 24 h reduced amino acid transport and phosphorylation of pS6 (23). In contrast, OA increased amino acid transport and phosphorylation of ERK, mTOR, S6 kinase 1, and rpS6. The combination of DHA with OA increased amino acid transport and rpS6 phosphorylation. PA did not affect amino acid transport but reduced IκBα expression. In conclusion, these fatty acids differentially regulated placental amino acid transport and cellular signaling. Taken together, these findings suggest that dietary fatty acids could alter the intrauterine environment by modifying placental function, thereby having long-lasting effects on the developing fetus.

FATTY ACIDS are critical for fetal development. They are essential structural components of cellular membranes and precursors of bioactive compounds, with particularly large amounts being deposited in the fetal brain (13). Fatty acids can also influence cellular signaling through a multitude of mechanisms, with effects dependent on their level of saturation. For instance, saturated and polyunsaturated fatty acids differentially modulate Toll-like receptor (TLR)4 signaling (38). The polyunsaturated ω-3 fatty acid docosahexaenoic acid (DHA; 22:6 n-3) has been shown to exert anti-inflammatory actions, partly mediated via the membrane-bound G protein-coupled receptor 120 (GPR120) (25). Both TLR4 (6) and GPR120 (21) are expressed on the syncytiotrophoblast microvillus plasma membrane in the human-term placenta, consistent with the possibility that maternal fatty acids modulate placental function (10, 24, 26, 33) through interactions with these receptors.

In a variety of tissues, it has been shown that the fatty acids DHA, oleic acid (OA; 18:1 n-9), and palmitic acid (PA; 16:0) have distinct effects on inflammatory signaling pathways. For instance, DHA inhibits, whereas PA stimulates, activity of the IκB/NF-κB signaling pathway (7, 25, 31). Other inflammatory-related pathways responding to fatty acids in other tissues include STAT3 (19) as well as MAPK pathways (ERK, JNK, and p38 MAPK) (2, 19, 25, 39).

In addition to inflammatory pathways, fatty acids can also influence mechanistic target of rapamycin (mTOR) (2, 17, 31), a serine/threonine kinase regulating protein translation, cell growth, and metabolism (23). Activation of this kinase results in the phosphorylation of eukaryotic initiation factor 4E-binding protein 1, S6 kinase (S6K)1, and ribosomal protein (rp)S6 (23). In the context of placental function, we have shown that the mTOR pathway is a positive regulator of trophoblast amino acid transport (27–29).

At the end of pregnancy, OA and PA together constitute ~60% of the nonesterified fatty acid (NEFA) species in the maternal circulation (37), and NEFA levels correlate positively with maternal body mass index (19). We have previously reported that OA stimulates placental amino acid transport in primary human trophoblasts (PHTs) (19). This effect is dependent on TLR4 (19); however, the cellular signaling pathways mediating the activation of amino acid transport by OA remain to be fully established. In addition, regulation of trophoblast amino acid transport by DHA and PA has not previously been studied. Therefore, the aim of the present study was to determine the effects of DHA, OA, and PA on amino acid transport activity and cellular signaling in cultured PHTs. We tested the hypothesis that these fatty acids differentially regulate trophoblast amino acid transport and cellular signaling.

MATERIALS AND METHODS

Ethics. Human placental tissue samples from term pregnancies (≥37 ± 0 wk of gestation) were collected after written informed consent. Placental samples and medical information were added to a tissue/data repository. The protocol was approved by the Institutional Review Board of the University of Texas Health Science Center (San Antonio, TX, HSC20100262H). De-identified tissue samples were available for the present study.

Isolation and maintenance of trophoblast cells. Villous cytotrophoblasts were isolated as previously described (4, 20). Trophoblast cells were separated by DNase/trypsin digestion and purified on a Percoll gradient. Cells were plated at an approximate density of 150,000 cells/cm². Cell culture media (equal volumes of Ham’s F-12 and high-glucose DMEM) contained 10% FBS (S11550, Atlanta Biologicals, Lawrenceville, GA) and antibiotics (gentamicin, penicillin, and
streptomycin). Media were changed daily. Starting at 66 h after being plated, cells were cultured in media containing 1% FBS supplemented with BSA-bound fatty acids (DHA: 25 or 50 μM, OA: 100 μM, PA: 100 μM), and each fatty acid treatment had its own control (containing cell culture media with an equal amount of fatty acid-free BSA stock buffer). Twenty-four hours later (at 90 h after cell plating), amino acid uptake was measured, cell viability was assessed, or samples were collected for protein expression analysis.

Characterization and viability of isolated cells. As an indicator of biochemical differentiation, the release of human chorionic gonadotropin into the cell culture media was measured using a commercial ELISA (IBL-America, Minneapolis, MN). Cell viability was determined by the ability of the cultured cells to metabolize MTT (thiazolyl blue tetrazolium bromide, Sigma-Aldrich, St Louis, MO), and cleaved caspase-3 was used to measure apoptosis.

Harvesting cells. Trophoblast cells were harvested by the addition of RIPA buffer containing phosphatase inhibitor cocktails 1 and 2 and protease inhibitor cocktail (final dilution: 1:100, Sigma-Aldrich) and collected after being scraped with a spatula. Cells were stored at −80°C until further analysis. Protein concentrations were determined by the bicinchoninic acid assay method (Thermo Fisher Scientific, Waltham, MA).

Measurement of amino acid uptake. System A amino acid transporter activity was measured as Na⁺-dependent [14C]methylaminobutyric acid (MABA) uptake, and system L transporter activity was measured by 2-amino-2-norbornanecarboxylic acid (BCH)-inhibitable uptake of [3H]leucine (final concentration: 12.5 nM), as previously described (4, 19). Transporter-mediated uptakes were calculated by subtracting uptake in Na⁺-free/BCH buffer (nonmediated uptake) from uptake in Na⁺-containing buffer (total uptake). Protein concentrations were measured with the Lowry method. Uptakes were expressed as picomoles of amino acid taken up per milligram of protein per minute. The mean uptake of controls was arbitrarily assigned a value of 1.0 to facilitate comparisons between groups.

Western blot analysis. Western blots were performed on precast gels from Bio-Rad (Hercules, CA). After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (overnight, 4°C). The following primary antibodies were purchased from Cell Signaling Technology (Davers, MA): 4EBP1 (no. 9452), phosphorylated (P)-4EBP1 (Thr37/46, no. 9459), AMP-activated protein kinase (AMPK; no. 2532), P-AMPK (Thr172, no. 2535), caspase-3 (no. 9662), ERK (no. 4695), P-ERK (Thr202/Tyr204, no. 4370), IncBrx (no. 4812), JNK (no. 9252), P-JNK (Thr183/Tyr185, no. 4668), mTOR (no. 2983), P-mTOR (Ser2448, no. 5536), p38 MAPK (no. 9212), S6K1 (no. 9202), P-S6K1 (Thr389, no. 9205), rpS6 (no. 2217), P-rpS6 (Ser235/236, no. 4858), STAT3 (no. 9139), and P-STAT3 (Tyr705, no. 9145). The primary antibody targeting P-p38 MAPK (Thr180/Tyr182) was purchased from Abcam (ab32557, Cambridge, UK). Primary antibodies were diluted in Tris-buffered saline containing 1–3% BSA, and incubations were carried out overnight (4°C). After incubation with the appropriate secondary peroxidase-labeled IgG antibody (1 h, room temperature), immunolabeling was detected using SuperSignal Dura West (Thermo Fisher Scientific) in a G:Box ChemiXL1.4 (Syngene, Cambridge, UK). The relative density of bands was measured by densitometry (GeneTools, Syngene), and protein expression was adjusted for amount of total protein (amido black stain). The mean value of controls was arbitrarily assigned a value of 1.0 to facilitate comparisons between groups.
Data presentation and statistics. The number of experiments (n) represents the number of placentas studied. Data are presented as means ± SE. Statistical analysis was carried out with GraphPad Prism 5 (version 5.04, GraphPad Software, La Jolla, CA). Differences between two groups were evaluated statistically by paired t-test and for multiple groups by repeated-measures ANOVA followed by Tukey’s multiple-comparison test. P values of <0.05 were considered significant.

RESULTS

Characterization and viability of isolated trophoblast cells. The secretion of human chorionic gonadotropin into cell culture media was significantly higher after 66 and 90 h of culture compared with 18 and 42 h of culture, indicating biochemical differentiation (n = 12, P < 0.01; Fig. 1A). Twenty-four-hour treatments with fatty acids (DHA: 50 μM, OA: 100 μM, PA: 100 μM) did not affect cell viability (n = 4; Fig. 1B), nor did it increase apoptosis (n = 4–7; Fig. 1C).

Amino acid transport. Mean amino acid transport activity in control PHTs (system A activity: 10 ± 1.3 pmol·mg⁻¹·min⁻¹; system L activity: 0.1 ± 0.01 pmol·mg⁻¹·min⁻¹) was similar to what we have previously reported (4). Activity of the system A amino acid transporter was markedly decreased after exposure to 25 or 50 μM DHA (n = 10, P < 0.05; Fig. 2A). In contrast, incubation in 100 μM OA stimulated system A activity (n = 7, P < 0.01; Fig. 2A). Cotreatment of trophoblast cells with DHA (50 μM) and OA (100 μM) did not prevent OA stimulation of system A activity (n = 7, P < 0.01; Fig. 2A). PA (100 μM) did not alter trophoblast system A amino acid transport activity (n = 7; Fig. 2A).

Uptake of amino acids by the system L amino acid transporter was significantly reduced by 25 or 50 μM DHA (n = 10, P < 0.05; Fig. 2B). System L amino acid transport activity was not affected by 100 μM OA or 100 μM PA treatment (n = 7; Fig. 2B). The decrease in system L activity in response to 50 μM DHA was prevented by cotreatment with 100 μM OA (n = 7; Fig. 2B).

Inflammatory signaling. DHA or PA alone did not alter the phosphorylation of ERK. In contrast, ERK phosphorylation was significantly increased by OA (n = 7, P < 0.05; Fig. 3A). Activation of the ERK signaling pathway by OA was prevented by coinubation with DHA (Fig. 3A). Expression of IkBa was decreased by PA treatment (n = 7, P < 0.05; Fig. 3B), whereas it was unaffected by DHA and/or OA. JNK phosphorylation was not altered by any of the fatty acids studied (Fig. 3C). DHA inhibited p38 MAPK and STAT3 signaling (n = 9, P < 0.05; Fig. 3, D and E), whereas there was a trend toward increased STAT3 phosphorylation in response to OA (P = 0.07). PA or co-treatment with DHA and OA had no significant effect on p38 MAPK or STAT3 inflammatory signaling pathways. Total protein expression of ERK, JNK, and p38 MAPK was not affected by the fatty acid treatments, whereas total STAT3 was significantly decreased by exposure to DHA (P < 0.05; data not shown).

AMPK and mTOR signaling. The cellular energy state, as measured by AMPK phosphorylation, was not affected by the fatty acid treatments (n = 6–7; Fig. 4A). Phosphorylation of mTOR was reduced by DHA and increased by OA treatment (n = 9, P < 0.05; Fig. 4B). Downstream of mTOR, phosphorylation of 4EBP1 and rpS6 was lower in PHTs exposed to DHA (n = 9, P < 0.05; Fig. 4, C and E). OA increased the phosphorylation of S6K1 and rpS6 (n = 9, P < 0.05; Fig. 4, D and E) but not 4EBP1. Co-treatment of cells with DHA and OA resulted in unaltered phosphorylation levels of 4EBP1 and S6K1, whereas rpS6 phosphorylation was increased compared with control (n = 9, P < 0.05; Fig. 4E). Total protein expression of AMPK, mTOR, 4EBP1, and S6K1 was not affected by the fatty acid treatments, whereas total rpS6 was significantly decreased by DHA and increased by OA (P < 0.05; data not shown).

DISCUSSION

In the present report, we show, for the first time, that the fatty acids DHA, OA, and PA differentially regulate trophoblast amino acid transport. Similarly, these fatty acids had diverse effects on inflammatory and mTOR signaling pathways in cultured PHTs.
DHA inhibited both trophoblast cellular signaling (p38 MAPK, STAT3, and mTOR) and amino acid transport activity. This may reflect a cause-and-effect relationship. We have previously shown that p38 MAPK (3), STAT3 (18), and mTOR signaling (27, 28) are positive regulators of trophoblast amino acid transport. However, further studies are required to definitely establish through which of these pathways DHA affects amino acid transport. We have recently shown that placental p38 MAPK and STAT3 phosphorylation positively correlate with maternal body mass index (5), with obese mothers having an increased risk of delivering a large baby (34). Because placental amino acid transporter expression positively correlates with birth weight (15), increasing DHA levels could represent a mechanism to prevent fetal overgrowth by alleviating excessive placental uptake of amino acids. Consistent with this hypothesis, preliminary data from our laboratory suggest that supplementation of DHA to obese pregnant women normalizes placental amino acid transport capacity (Lager et al., unpublished observations).

We have previously reported that 400 μM OA stimulates trophoblast system A amino acid transporter activity (19). In the present report, we extended these observations by showing that OA also increases system A activity at 100 μM, which is a more physiological concentration (37). Furthermore, in our

![Fig. 3. Inflammatory signaling pathways after fatty acid stimulation. A–E: phosphorylation of ERK (A), expression of IκBα (B), phosphorylation of JNK (C), p38 MAPK (D), and STAT3 (E) in cultured primary human trophoblast cells after exposure to fatty acids for 24 h (DHA: 50 μM, OA: 100 μM, PA: 100 μM) compared with vehicle controls. F: representative Western blot. P-, phosphorylated form. Data are means ± SE. *P < 0.05 (paired t-test).](http://ajpcell.physiology.org/)
previous study (19), we showed that OA (400 μM) activated JNK and STAT3 while having no effect on the mTOR pathway. In the present study, OA (100 μM) had a major impact on mTOR signaling, whereas STAT3 phosphorylation only tended to be increased. This discrepancy is likely due to the different fatty acid concentrations used and highlights that not only fatty acid species but also concentration will impact cellular signaling. A similar observation has previously been reported by Arous and coworkers (2); they showed that low and high doses of OA differentially regulated mTOR signaling in HepG2 cells.

Individually, DHA and OA had distinct effects on PHT amino acid transport and cellular signaling. However, in vivo, the syncytiotrophoblast is exposed to a combination of different fatty acids from maternal blood. The combination of DHA and OA resulted in increased system A activity and phosphorylation of rpS6. All other effects on cellular signaling by the fatty acids individually were counteracted in the combination experiments. Because mTOR signaling is a powerful regulator of trophoblast amino acid transporters (27–29), these findings suggest the effect of fatty acids on system A amino acid transport is mediated by the mTOR signaling pathway. Furthermore, when DHA and OA were combined, the reduction in system L amino acid transport activity observed in response to DHA alone was prevented. Taken together, these findings highlight the importance of not only studying fatty acids individually but also fatty acids in combination.

Fig. 4. Mechanistic target of rapamycin (mTOR) signaling after fatty acid stimulation. A–E: phosphorylation of AMP-activated protein kinase (AMPK; A), mTOR (B), eukaryotic initiation factor 4E-binding protein (4EBP)1 (C), S6 kinase (S6K)1 (D), and ribosomal protein (rp)S6 (E) in cultured primary human trophoblast cells after exposure to fatty acids for 24 h (DHA: 50 μM, OA: 100 μM, PA: 100 μM) compared with vehicle controls. F: representative Western blot. Data are means ± SE. *P < 0.05 (paired t-test).
PA has been reported to inhibit system A activity in rat muscle (14). In contrast, PA did not affect trophoblast amino acid transport in the present study. The effects of exposure to PA were limited to decreased expression of IkBa. Reduced IkBa may suggest, but does not conclusively prove, increased activation of NF-kB, a transcription factor associated with inflammation. Our findings are in agreement with previously published work demonstrating that PA reduces IkBa expression in skeletal muscle (31). The activation of inflammatory pathways in PHTs by PA appears specific to IkBa because no additional signaling pathways were affected. In agreement with our findings, PA does not alter the activity of ERK, JNK, or p38 MAPK signaling pathways in BeWo cells (a trophoblast-like choriocarcinoma cell line) (30).

During pregnancy, maternal NEFA concentrations are less affected by fasting/postprandial cycles than in nonpregnant individuals, resulting in more constant levels of circulating NEFAs (13). Furthermore, maternal triglyceride and NEFA levels continue to increase as pregnancy progresses (1, 9). To mimic this chronic fatty acid exposure, PHTs were incubated in fatty acids for 24 h. However, it cannot be excluded that the effects of fatty acids on trophoblast cell signaling and amino acid transport may be different after acute exposure. In the present study, 100 μM OA and 100 μM PA were selected because they are within the span of observed concentrations in the maternal circulation at the end of pregnancy (37). DHA at 50 μM is, however, above the physiological range, and the effect on trophoblast amino acid transport was therefore confirmed with a lower dose (25 μM DHA).

In the present report, we did not investigate the specific receptors or alternative mechanisms linking fatty acids to the signaling pathways and amino acid transporter systems under study. It is likely that TLR4 is involved in mediating the effects of OA, as we have previously shown (19). An alternative possibility for the effects of DHA could be activation of the GPR120 pathway. Membrane-bound GPR120 is expressed in the human placenta (21). GPR120 signaling interferes with inflammatory pathways by preventing formation of the complex between transforming growth factor-β-activated kinase (TAK1) and TAK1-binding protein 1 (25), a complex that is upstream of IkB, JNK, and p38 MAPK (8). Hence, activation of GPR120 signaling may explain the reduced p38 MAPK phosphorylation seen with DHA exposure. Fatty acids can also affect signaling through intracellular receptors, such as peroxisome proliferator-activated receptors (11), and by affecting lipid rafts in the cell membranes (36). The precise mechanisms by which DHA, OA, and PA differentially modulate cellular signaling in PHTs remain to be established.

Altered fatty acid content in the maternal diet has been shown to affect fetal growth (12) and placental efficiency (22) as well as resulting in developmental programming of offspring (32) in animal models. The possible role of the placenta in developmental programming has been highlighted in recent reviews (16, 35). In the present study, we show that the fatty acids DHA, OA, and PA have a differential effect on placental amino acid transport and cellular signaling pathways. Taken together, these findings suggest that fatty acids could alter the intraterine environment by modifying placental function, thereby having long-term health effects upon the developing fetus.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: S.L., T.J., and T.L.P. conception and design of research; S.L. performed experiments; S.L. analyzed data; S.L., T.J., and T.L.P. interpreted results of experiments; S.L. prepared figures; S.L. drafted manuscript; S.L., T.J., and T.L.P. edited and revised manuscript; S.L., T.J., and T.L.P. approved final version of manuscript.

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FATTY ACIDS AND PLACENTAL FUNCTION


