Role of fatty acid transport protein 4 in oleic acid-induced glucagon-like peptide-1 secretion from murine intestinal L cells

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Poreba MA, Dong CX, Li SK, Stahl A, Miner JH, Brubaker PL. Role of fatty acid transport protein 4 in oleic acid-induced glucagon-like peptide-1 secretion from murine intestinal L cells. Am J Physiol Endocrinol Metab 303: E899–E907, 2012. First published August 7, 2012; doi:10.1152/ajpendo.00116.2012.—The anti diabetic intestinal L cell hormone glucagon-like peptide-1 (GLP-1) enhances glucose-dependent insulin secretion and inhibits gastric emptying. GLP-1 secretion is stimulated by luminal oleic acid (OA), which crosses the cell membrane by an unknown mechanism. We hypothesized that L cell fatty acid transport proteins (FATPs) are essential for OA-induced GLP-1 release. Therefore, the murine GLUTag L cell model was used for immunoblotting, [3H]OA uptake assay, and GLP-1 secretion assay as determined by radioimmunoassay following treatment with OA ± phloretin, sulfo-N-succinimidyl oleate, or siRNA against FATP4. FATP4+/− and cluster-of-differentiation 36 (CD36)−/− mice received intraleral OA, and plasma GLP-1 was measured by sandwich immunnoassay. GLUTag cells were found to express CD36, FATP1, FATP3, and FATP4. The cells demonstrated specific [3H]OA uptake that was dose-dependently inhibited by 500 and 1,000 μM unlabeled OA (P < 0.001). Cell viability was not altered by treatment with OA. Phloretin and sulfo-N-succinimidyl oleate, inhibitors of protein-mediated transport and CD36, respectively, also decreased [3H]OA uptake, as did knockdown of FATP4 by siRNA transfection (P < 0.05–0.001). OA dose-dependently increased GLP-1 secretion at 500 and 1,000 μM (P < 0.001), whereas phloretin, sulfo-N-succinimidyl oleate, and FATP4 knockdown decreased this response (P < 0.05–0.001). FATP4−/− mice displayed higher basal GA levels (P < 0.01) but a normal response to intraepithelial OA (P < 0.05), whereas, unexpectedly, CD36−/− mice displayed higher basal GLP-1 levels (P < 0.01) but a normal response to intraepithelial OA. Together, these findings demonstrate a key role for FATP4 in OA-induced GLP-1 secretion from the murine L cell in vitro and in vivo, whereas the precise role of CD36 remains unclear.

carrier-mediated transport; cluster of differentiation 36; fatty acid uptake; GLUTag; monounsaturated fatty acid

THE INTESTINAL L CELL HORMONE glucagon-like peptide-1 (GLP-1) is secreted following nutrient ingestion, leading to glucose-dependent insulin release as well as inhibition of gastric emptying, glucagon secretion, and food intake (1, 39, 46, 48). The anti diabetic properties of this hormone have led to the use of both GLP-1 mimetics and GLP-1 degradation inhibitors in the clinic to treat patients with type 2 diabetes (33). Although nutrients such as sugars and peptones are known to stimulate L cell secretion (15, 20, 35), fats in particular are potent GLP-1 secretagogues (10, 18, 21, 23, 24). Furthermore, only fats appear to transit the intestine to the ileum (3, 24, 28), which has the highest density of L cells in the intestine (11).

Monounsaturated fatty acids (MUFAs) such as oleic acid (OA) are known to induce beneficial metabolic effects, and therefore, arguments have been made in favor of the Mediterranean diet, which is rich in OA-containing olive oil (14). Indeed, insulin-resistant patients placed on a diet enriched in MUFAs display increased plasma GLP-1 levels and improved glycemic control (34). A study in rats has also linked dietary OA to increased GLP-1 secretion and GLP-1-dependent improvements in glycemic tolerance (37). Furthermore, OA has been demonstrated to directly increase GLP-1 secretion from the intestinal L cell, as observed in the murine GLUTag (23, 37), human NCI-H716 (35), and primary fetal rat intestinal culture (23) L cell models.

Several G protein-coupled receptors (GPCRs), such as GPR40, GPR120, and GPR119, have been implicated as long-chain fatty acid receptors on the L cell, responding to saturated fatty acids, polyunsaturated fatty acids, and the fatty acid derivative oleoylthanolamide, respectively (10, 21, 26). In contrast, OA is known to increase GLP-1 secretion through a mechanism that is dependent on the atypical isozyme protein kinase C (PKCζ) (23, 24). Although OA can directly activate this enzyme in vitro (31), whether, and if so, how, it crosses the plasma membrane to permit direct interaction with PKCζ in the intestinal L cell is currently unknown.

Although the topic of fatty acid transport has remained controversial, it is generally believed that the predominant mechanisms underlying fatty acid uptake consist of passive diffusion and a saturable, protein-mediated process (17, 41, 43). Candidates for L cell fatty acid transport proteins include the class B scavenger receptor-cluster-of-differentiation 36 (CD36)/fatty acid translocase as well as isoforms of the fatty acid transport protein (FATP) family. CD36 is widely expressed in the body but is also involved in intestinal absorption of fatty acids, including OA in the proximal gut (8, 32, 40). The isoforms of the FATP family also demonstrate broad expression, with FATP4 being the most abundant isofrom in the small intestine (45). Previous studies have identified mRNA transcripts for CD36 as well as for FATP1, FATP3, and FATP4 in the murine GLUTag L cell line (24). The GLUTag cells have been validated extensively as an L cell model, demonstrating appropriate GLP-1 secretion in response to a wide variety of known secretagogues (4, 15, 23, 26, 37). Furthermore, the GLUTag cells have been shown to take up the OA analog C12-Bodipy-C12 (24), consistent with an ability of these cells to internalize fatty acids. Therefore, we hypothesized that one or more of the intestinal L cell...
FATPs plays a role in OA uptake and subsequent GLP-1 secretion.

METHODS

Cells. Murine GLUTag cells were grown in medium [Dulbecco’s modified Eagle’s medium (DMEM); Gibco Invitrogen, Burlington, ON, Canada] containing 25 mM glucose and 10% fetal bovine serum. Cells were plated in six- or 24-well plates coated with poly-n-lysine (Sigma Chemical, St. Louis, MO) and allowed to recover for 24 h for uptake assay or 48 h for immunoblot, transfection, and secretion experiments. Cell viability following treatment with OA was assessed by uptake of neutral red during the last hour of a 2-h incubation (36).

Small interfering RNA transfection. Preliminary attempts to knockdown FATP4 were conducted using small interfering RNA (siRNA) from Ambion (Austin, TX). However, this led to a maximum 20% reduction in FATP4 protein levels despite numerous attempts to optimize the approach (data not shown). Therefore, subsequent studies were performed by transfection of cells using SMARTpool siRNA, a mixture of four targeted FATP4 siRNA sequences (SMARTpool; Dharmacon, Lafayette, CO), or scrambled control in Opti-MEM I medium (Gibco Invitrogen) (16). The SMARTpool siRNA approach is designed to reduce “off-target” effects by ≤90% by reducing the concentration of each of the individual siRNA sequences. After optimization of the approach, based upon protein expression levels, all experiments were conducted in cells that were incubated with the siRNA (50 nM with 2.25 μM of DharmaFECT-4 transfection reagent; Dharmacon) for 5 h, washed twice, and allowed to recover for 48 h.

Immunoblot. Cells or mouse duodenum (positive control) were collected into radioimmunoprecipitation assay buffer. One-hundred microliters of protein (measured by Bradford assay; Bio-Rad, Hercules, CA) was run on a 10% gel, transferred onto a polyvinyl difluoride membrane, and probed with rabbit anti-FATP1, -3, or -4 (1:1,000; rabbit anti-CD36 (1:1,000; Cayman Chemicals, Ann Arbor, MI), and rabbit anti-actin (1:4,000; Sigma Chemical), followed by detection using hors eradise peroxidase-linked goat anti-rabbit IgG (1:2,000; Cell Signaling Technology, Beverly, MA) and electrochemiluminescence Western blotting detection reagent (Amersham GE Healthcare, Baie d’Urfe, QC, Canada).

[3H]oleic acid uptake assay. Cells were starved in serum-free medium overnight. [3H]OEA (3.0 μCi/ml; specific activity 1.96 × 10^5 Bq/mmol) and [14C]mannitol (0.6 μCi/ml; specific activity 2.04 × 10^4 Bq/mmol; Moravek Biochemicals, Brea, CA) were added to CaCl2-free medium containing 0.5% fatty acid-free bovine serum albumin (Sigma Chemical). In some experiments, 500 or 1,000 μM unlabeled OA (100 mM stock solution in ethanol; Sigma Chemical), 200 μM phloretin [a non-specific inhibitor of carrier-mediated transport (49)], 200 mM stock solution in ethanol; Sigma Chemical), or 400 μM sulfo-N-succinimidyl oleate [SSO; a CD36 inhibitor (6), 0.4 M stock solution in DMSO; Toronto Research Chemicals, North York, ON, Canada] was added. The maximum final concentrations of ethanol and DMSO in the medium were 1.6 and 0.1%, respectively. Finally, CaCl2 was added back to the medium to a final concentration of 1.8 mM. Cells treated with phloretin (200 μM) or SSO (400 μM) were preincubated with medium containing only phloretin or SSO, respectively, for 30 min at 37°C prior to the start of the uptake assay.

Immunocytochemistry. Cells were grown on glass coverslips until 80% confluent and then treated for 1 h with vehicle or OA, as described above. Cells were then rinsed and incubated overnight at 4°C with rabbit anti-mouse/human FATP4 antiserum (1/500; Abnova/ Cedarlane Laboratories, Burlington, ON, Canada) followed by Cy3-coupled donkey anti-rabbit IgG (1/400; Jackson ImmunoResearch/ Cedarlane Laboratories) for 1 h at 20°C, rinsed, and mounted with 4,6-diamidino-2-phenylindole for visualization using a Zeiss Axioplan microscope with Axioplan software (Carl Zeiss Canada, Don Mills, ON, Canada). Images along the z-axis were taken at 1-μm intervals.

In vivo experiments. All animal protocols were approved by the Animal Care Committee at the University of Toronto. Fatp4^−/−;Ivl-Fatp4^−/− (Fatp4-null) mice (30) were on a mixed 129/B6/CBA background. Transgenic reexpression of FATP4 in the skin of the Fatp4^−/− mice via Ivl-Fatp4^+/− is required to prevent the neonatal lethality of the whole body Fatp4 knockout. These mice have been reported to display no compensatory upregulation of other FATP isoforms in the intestine (42). Both Fatp4^−/−;Ivl-Fatp4^−/− mice and Fatp4^−/− mice were used as control mice, and the results were combined since they did not differ between genotypes (data not shown). The study was conducted using female and male littermates at 9–24 wk of age, and the results were combined. CD36^−/− mice, a generous gift from Dr. Kevin Kain (University of Toronto, Toronto, ON, Canada) and derived originally by Dr. Maria Febbraio (13), were on a C57BL/6 background. Fatp4 levels are not altered in the small intestine of these animals (42). Age-matched C57BL/6 mice (Charles River, St. Constant, QC, Canada) were used as controls. The study was conducted using both female and male mice at 8–25 wk of age, and the results were combined.

Following an overnight fast, mice were anesthetized with isoflurane, and blood samples (50–100 μl) were obtained from the saphen-
A

CD36
Actin

80 kDa
60 kDa
40 kDa
20 kDa

B

FATP1
Actin

80 kDa
60 kDa
40 kDa
20 kDa

C

FATP3
Actin

80 kDa
60 kDa
40 kDa
20 kDa

D

FATP4
Actin

80 kDa
60 kDa
40 kDa
20 kDa

Fig. 1. Expression of fatty acid transport proteins in the L cell. Immunoblot for cluster of differentiation 36 (CD36) (55 kDa: nonglycosylated intracellular form; 88 kDa: glycosylated membrane form; A), fatty acid transport protein (FATP)1 (63 kDa; B), FATP3 (72 kDa; C), and FATP4 (72 kDa; D) in murine GLUTag L cells (n = 3). Actin (42 kDa) was used as the loading control and murine duodenum as a positive (+ve) control.

RESULTS

GLUTag cells express fatty acid transport proteins. To confirm expression of the fatty acid transport proteins CD36, FATP1, FATP3, and FATP4 in the murine GLUTag L cell model, immunoblot was carried out, using mouse duodenum as a control (Fig. 1). Bands were detected consistently for all four proteins. However, interestingly, although there was a clear band of CD36 immunoreactivity at ~55 kDa, consistent with intracellular localization of CD36, little to no expression of the heavily glycosylated, high-molecular-weight cell surface form of CD36 was detected in either the cells or the tissue.

OA is taken up by GLUTag cells and stimulates GLP-1 secretion. GLUTag cells demonstrated uptake of [3H]OA for ≥60 min (Fig. 2A). No significant uptake of the cell integrity control [14C]mannitol was observed in any of the treatment groups. Uptake of [3H]OA was competitively inhibited in a dose-dependent manner by 500 and 1,000 μM unlabeled OA to 60 ± 2 and 37 ± 2%, respectively, of control levels at t = 60

A

CPM/Protein (µg)

Time (min)

B

GLP-1 Secretion (% of control)

Control OA H2O2

0.00 0.03 0.06 0.09

OD (540 nm)

Control H2O2 OA

0.0

150 µM 355 µM 500 µM 1000 µM

Oleic acid

C

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Fig. 2. Oleic acid (OA) uptake in the L cell and the effect of OA on glucagon-like peptide-1 (GLP-1) secretion. A: GLUTag cells were incubated with [3H]OA and treated with vehicle control (solid line) or 500 (dashed line) or 1,000 µM (dotted line) unlabeled OA, followed by determination of [3H]OA uptake (○). [14C]mannitol was used as a cell integrity control in each treatment group (●). Counts per minute (cpm) were normalized to total protein (inset: expanded scale) (n = 6). B: GLUTag cells were treated with vehicle (control) or increasing concentrations of OA for 2 h, and secretion of GLP-1 was determined by radioimmunoassay (n = 6–11). Basal secretion was 8.6 ± 0.8% of total cell content. C: GLUTag cells were treated for 2 h with vehicle alone (control), 5 mM H2O2, or 1,000 µM OA for 2 h, followed by determination of viability using neutral red uptake, as assessed by optical density (OD) at 540 nm (n = 8). *P < 0.05, **P < 0.01, and ***P < 0.001 vs. control; ##P < 0.01 and ###P < 0.001 for 500 vs. 1,000 µM unlabeled OA or as indicated.
min ($P < 0.001$ vs. control and $P < 0.01$ vs. each other). Independent experiments that included an additional time point between 45 and 60 min (i.e., $t = 52$ min) confirmed the linearity of the response between 45 and 60 min ($r^2 = 0.999$, $n = 8$; data not shown). The absolute uptake of $[^3H]OA$ by the GLUTag cells over 60 min was $3.4 \times 10^{-12}$ mmol-min$^{-1}$-cell$^{-1}$.

Furthermore, a combination of the vehicle-only (control) data from multiple experiments (including the data shown in Figs. 2A, 3A, and 4A, as well as additional studies to make $n = 7$, with each experiment conducted in at least triplicate) revealed that the slope of the line of the $[^3H]OA$ uptake curve increased at $t = 45$ min from $0.52 \pm 0.06$ (at $t = 0–45$ min) to $1.60 \pm 0.22$ (at $t = 45–60$ min, $P < 0.01$). This increase in slope was not observed in paired cells treated with 1,000 $\mu$M unlabeled OA, which demonstrated a straight line from $t = 0–60$ min (slope $= 0.27 \pm 0.03$; $r^2 = 0.99$).

Treatment of GLUTag cells with increasing concentrations of OA also led to a dose-dependent increase in GLP-1 secretion such that 500 and 1,000 $\mu$M OA increased GLP-1 release to $124 \pm 9$ and $159 \pm 9$% of control cells, respectively ($P < 0.05–0.001$ vs. control and $P < 0.01$ vs. each other; Fig. 2B). The positive control PMA increased GLP-1 secretion to $154 \pm 12$% of controls ($P < 0.001$). Importantly, cell viability assay demonstrated no effect of incubation with the highest concentration of OA for 2 h (Fig. 2C).

Phloretin decreases OA uptake and GLP-1 secretion by GLUTag cells. Treatment of GLUTag cells with the nonspecific inhibitor of carrier-mediated transport phloretin decreased the uptake of $[^3H]OA$ by $38 \pm 4$% at $t = 15$ min ($P < 0.001$) and by $14 \pm 4$% at $t = 60$ min ($P < 0.05$; Fig. 3A). As in previous uptake assays, unlabeled OA (1,000 $\mu$M) decreased $[^3H]OA$ uptake at $t = 5–60$ min ($P < 0.01–0.001$), whereas no significant uptake of $[^14C]$mannitol was observed in any of the treatment groups. As found previously, incubation of the cells with OA (1,000 $\mu$M) increased GLP-1 secretion by $137 \pm 21$% (Fig. 3B). Pre- and coinubation with phloretin (200 $\mu$M) markedly reduced OA-induced GLP-1 secretion by $67 \pm 14$% but did not abrogate the effect of OA ($P < 0.01$). Basal secretion in the presence of phloretin alone was $85.3 \pm 10.2$% of control values ($P = $ not significant (NS)). Control experiments demonstrated no effect of phloretin treatment on PMA-induced GLP-1 release (secretion was $104.9 \pm 22.7$% of that found in the absence of phloretin, $P = $ NS; data not shown).

CD36 plays a role in the L cell in vitro but not in vivo. Treatment of GLUTag cells with the CD36 inhibitor SSO reduced $[^3H]OA$ uptake by $36 \pm 8$% at $t = 60$ min ($P < 0.001$; Fig. 4A). As observed previously, unlabeled OA (1,000 $\mu$M) decreased $[^3H]OA$ uptake at $t = 15–60$ min ($P < 0.05–0.001$), and no significant uptake of $[^14C]$mannitol was observed in any of the treatment groups. Basal secretion in the presence of SSO alone was increased to $221.9 \pm 43.5$% of control ($P < 0.05$). However, SSO treatment decreased OA-induced GLP-1 secretion by GLUTag cells from $243 \pm 37$% of control values to $151 \pm 15$% ($P < 0.05$; Fig. 4B). Control experiments demonstrated no effect of SSO treatment on PMA-induced GLP-1 release (secretion was $80.7 \pm 12.5$%, which was found in the absence of SSO; $P = $ NS). Therefore, the role of CD36 was examined in vivo using the CD36-null mouse. Immunoblotting confirmed the absence of CD36 in the ileum of CD36$^{-/-}$ mice (Fig. 4C). To determine the effect of OA on plasma GLP-1 levels, 125 mM OA was injected directly into the ileum of anesthetized control and CD36-null mice, and blood samples were collected at $t = 0$ and 15 min or at $t = 0$ and 60 min in a paired fashion for determination of total plasma GLP-1 levels. The CD36-null mice were found to have increased basal GLP-1 plasma levels by $61.3 \pm 19.4$% compared with control animals ($P < 0.01$; Fig. 4D). However, no differences in GLP-1 plasma levels were observed between the two groups of
mice at \( t = 15 \) or 60 min following intraileal injection of OA, when the absolute values were compared (Fig. 4D), or following determination of the change from basal levels (data not shown).

**FATP4 plays a role in the L cell in vitro and in vivo.** Because there is no specific inhibitor for FATP4, this protein was knocked down in the GLUTag cells using FATP4-targeting siRNA, leading to a maximum 27 \( \pm \) 6\% reduction in protein levels (\( P < 0.05 \); Fig. 5A). Nonetheless, knockdown of FATP4 reduced \([^{3}H]OA\) uptake at \( t = 60 \) min by 28 \( \pm \) 7\% (\( P < 0.05 \); Fig. 5B). Basal GLP-1 secretion in the presence of FATP4 siRNA alone was not different from control values (146.1 \( \pm \) 21.3\% of control, \( P > 0.05 \)). In contrast, FATP4 knockdown completely abrogated OA-induced GLP-1 secretion, decreasing release from 220 \( \pm \) 29 to 121 \( \pm \) 21\% of control values (\( P < 0.05 \); Fig. 5C). Control experiments showed no effect of FATP4 knockdown on PMA-induced GLP-1 release (secretion was 101.9 \( \pm \) 7.8\% of that found for PMA with the scrambled control, \( P = NS \); data not shown). Immunocytochemistry for FATP4 immunoreactivity revealed a lack of membrane localization, with the majority of the staining appearing in the cytoplasm and/or perinuclear area of both vehicle- and OA-treated cells (Fig. 5D).

To further explore the role of FATP4 in OA-induced GLP-1 secretion, the FATP4-null mouse model was utilized. Immunoblotting confirmed the absence of FATP4 in the ileum of FATP4-null mice (Fig. 5E). As for the CD36-null mice, 125 mM OA was injected directly into the ileum of anesthetized wild-type and FATP4-null mice. Although no differences were seen between the two groups of animals at \( t = 0 \) and 15 min, plasma GLP-1 levels were markedly lower in the FATP4-null mice at \( t = 60 \) min by 72.3 \( \pm \) 4.8\% compared with control animals (\( P < 0.05 \); Fig. 5F).

**DISCUSSION.** The antidiabetic hormone GLP-1 is released from the intestinal L cell upon nutrient ingestion. Fatty acids, and OA in particular, are potent GLP-1 secretagogues, exerting direct effects on the intestinal L cell in vitro as well as increasing GLP-1 release in both humans and rodents (2, 23, 24, 35, 37). However, the mechanism underlying OA-induced GLP-1 secretion has not been fully elucidated, with the only essential component identified to date being the isozyme PKC\( \zeta \) (23, 24). The results of the current study demonstrate that the L cell specifically takes up OA via a carrier-mediated process and that FATP4 plays a key role in OA-induced GLP-1 secretion both in vitro and in vivo.

In keeping with a requirement for FATP4 in OA uptake by the intestinal L cell, FATP4 mRNA (24) and protein are expressed in GLUTag cells. Furthermore, both phloretin treatment and a 27\% knockdown of FATP4 reduced OA uptake by up to 28\%, consistent with reports of parallel decreases in protein levels and OA uptake in enterocytes after FATP4 knockdown (45). However, our findings in the GLUTag cells differ from those made in endothelial cells, in which a 50\% knockdown of FATP4 completely abrogated the ability of vascular endothelial growth factor B to induce OA uptake (16), and from brain microvessel endothelial cells, in which 50\% knockdown did not alter OA uptake at all (29a). Hence, the response to modulation of FATP4 expression appears to be highly cell specific, with the intestinal L cell behaving in a fashion similar to the gut absorptive cells rather than to the unrelated adipocytes and endothelial cells.

Consistent with a role for FATP4 in the regulation of OA-induced GLP-1 secretion, both phloretin treatment and FATP4 knockdown markedly reduced stimulated GLP-1 release in vitro, and knockout of FATP4 completely abrogated
the intestinal L cell secretory response to OA in vivo. However, somewhat unexpectedly, the effects of both phloretin treatment and FATP4 knockdown to reduce OA uptake by the GLUTag cells were relatively modest compared with their ability to prevent OA-induced GLP-1 release. A similar dissociation between OA uptake and insulin secretion has been noted in mouse insulinoma MIN6 cells such that knockdown of the adipose differentiation-related protein that coats lipid droplets decreases OA uptake by 17% but reduces insulin secretion by more than 50% (12). This discrepancy was attributed to impaired lipid metabolism, although the exact mechanisms were not investigated. We have also observed in GLUTag cells that, despite only a 23% knockdown of the receptor GPR119, there was a much greater effect (e.g., 45% decrease) on GLP-1 secretion induced by the OA derivative oleoylethanolamide reported plasma membrane expression (45), FATP4 also localizes to the endoplasmic reticulum, where its expression drives fatty acid uptake through its ability to act as long-chain acyl-CoA synthetase (29). Hence, intracellular compartmentalization of this enzyme in the L cell could explain the relatively late effect of FATP4 knockdown on fatty acid uptake (e.g., at 60 min only) compared with that of phloretin (e.g., at both 5–30 and 60 min). Finally, the absolute uptake of \[^{[3}\text{H}]\text{OA} \] by the GLUTag cells over 60 min was found to be 3.4 \times 10^{-12} \text{nmol-min}^{-1}\text{-cell}^{-1}. Although markedly lower than the uptake of OA reported for enterocytes (1.2 \times 10^{-7} \text{nmol-min}^{-1}\text{-cell}^{-1}),
GLP-1 secretion was found in vitro but not in vivo. Hence, the likely through delivery of the fatty acid to its effector, PKCε, plays a key role in both OA uptake and OA-induced GLP-1 secretion in the enteroendocrine L cell in vitro and in vivo likely through delivery of the fatty acid to its effector, PKCε. In contrast to the findings on FATP4, a role for CD36 in GLP-1 secretion was found in vitro but not in vivo. Hence, the CD36 inhibitor SSO decreased both [3H]OA uptake and OA-induced GLP-1 release in the GLUTag cells, whereas CD36 knockdown did not prevent the effects of OA on GLP-1 secretion in vivo. SSO has been reported to be a specific CD36 inhibitor, reducing fatty acid uptake by up to 70% in a wide variety of tissues (6). However, a recent report showing that SSO also inhibits complex III of the mitochondrial respiratory chain has called the specificity of SSO into question (7). Such a role for CD36 would be consistent with the finding of only the lower-molecular-weight, intracellular form of this protein (22) in the GLUTag cells. Hence, the finding that CD36-null mice exhibit a normal GLP-1 secretory response to intraluminal OA suggests that CD36 does not play an essential role in the effects of OA on the intestinal L cell, whereas it appears to be relatively more important in the immortalized GLUTag cells. Nonetheless, the finding of higher basal GLP-1 levels in the null animals, compared with control mice, implies either that CD36 plays a minor role in the regulation of GLP-1 release under fasting conditions, which seems unlikely, or that the mice have undergone compensatory responses to the global loss of CD36.

Interestingly, the effects of both FATP4 knockdown and CD36 inhibition on OA uptake by the GLUTag cells were found to occur at t = 60 min only, compared with the inhibition of uptake caused by phloretin as well as by unlabeled OA, at early (t = 5–30/45 min) as well as late (t = 60 min) time points. These findings are also consistent with the observation of a change in the rate of OA uptake at t = 45–60 min, although studies of a longer duration may be useful in examining this phenomenon further. Nonetheless, these findings support the notion that multiple uptake mechanisms may be taking place in the L cell over the course of the 60-min assay, including possible roles for FATP1 and -3. Additionally, transport proteins, including CD36 and FATP1, are known to translocate to the plasma membrane from subcellular locations (5, 44). Therefore, it is possible that there is an upregulation of plasma membrane fatty acid transport proteins after t = 45 min, which would explain the increased rate of OA uptake at this time point. Further studies will clearly be necessary to elucidate the specific roles of all of these proteins in the intestinal L cell.

Finally, increasing concentrations of OA increased GLP-1 secretion from the murine GLUTag L cell model in a dose-dependent manner. Although there is evidence that accumulation of free fatty acids in tissues can lead to lipotoxicity and cell dysfunction (38), the highest dose used in this current study (1,000 µM OA) is well below the physiological concentration of OA reached in the ileum (~105 mM), as determined by measurement of the OA concentration in chyme following oral gavage of olive oil (24). Furthermore, exposure of the GLUTag cells to 1,000 µM OA had no effect on cell viability, consistent with our previous findings using 500 µM OA (23).

However, because the luminal concentration of fat, as well as the aboral distance transited by ingested fat, is dependent upon the load of fat ingested (28), the absolute concentration of OA to which the intestinal L cell is exposed will vary depending upon the meal.

The findings of this study indicate a role for fatty acid transport proteins, and specifically FATP4, in OA-induced GLP-1 secretion by the intestinal L cell. Endogenous GLP-1 production has been shown to be elevated upon stimulation with MUFA such as OA (24, 37) and has been implicated in the improved glycemic control observed in insulin-resistant patients placed on a MUFA-rich diet (34). Although FATP4 plays a role in mediating the effects of OA on the L cell, FATP4 is not likely to be a therapeutic target due to its widespread distribution throughout the body, including the enterocytes and skin (13, 45). Instead, this signaling pathway, including the essential isozyme PKCε (23, 24), should be explored further to identify suitable therapeutic targets that could be manipulated to increase endogenous GLP-1 secretion in patients with type 2 diabetes. Finally, mutations in FATP4 were described recently in patients with ichthyosis prematurity syndrome, a condition characterized by premature birth with the infant covered in thick, caseous skin and having respiratory complications, followed by lifelong dry, thick skin (25). Whether these patients exhibit reduced GLP-1 release and a subsequent impairment in glycemic control has not been explored. Nonetheless, an essential role for FATP4 has been established in the skin in these patients as well as in FATP4-null mice, and it now appears that FATP4 additionally plays a key role in mediating OA-induced GLP-1 secretion from the intestinal L cell.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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ROLE OF FATP4 IN GLP-1 SECRETION


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