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DOI: 10.1194/jlr.M028035

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: http://doi.org/10.5167/uzh-66136

Originally published at:
Rapold, Reto A; Wueest, Stephan; Knoepfel, Adrian; Schoenle, Eugen J; Konrad, Daniel (2013). Fas activates lipolysis in a Ca2+-CaMKII-dependent manner in 3T3-L1 adipocytes. Journal of Lipid Research, 54(1):63-70. DOI: 10.1194/jlr.M028035
Fas activates lipolysis in a Ca2+-CaMKII-dependent manner in 3T3-L1 adipocytes.

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Abstract Fas (CD95) is a member of the tumor necrosis factor (TNF) receptor superfamily and plays a crucial role in the induction of apoptosis. However, like TNF, Fas can induce nonapoptotic signaling pathways. We previously demonstrated that mice lacking Fas specifically in adipocytes are partly protected from diet-induced insulin resistance, potentially via decreased delivery of FAs to the liver, as manifested by lower total liver carnitine content. In the present study, we aimed to delineate the signaling pathway involved in Fas-mediated adipocyte lipid mobilization. Treatment of differentiated 3T3-L1 adipocytes with membrane-bound Fas ligand (FasL) significantly increased lipolysis after 12 h without inducing apoptosis. In parallel, Fas activation increased phosphorylation of ERK1/2, and FasL-induced lipolysis was blunted in the presence of the ERK-inhibitor U0126 or in ERK1/2-depleted adipocytes. Furthermore, Fas activation increased phosphorylation of the Ca2+/calmodulin-dependent protein kinases II (CaMKII), and blocking of the CaM-KII-pathway (either by the Ca2+ chelator BAPTA or by the CaMKII inhibitor KN62) blunted FasL-induced ERK1/2 phosphorylation and glycerol release. In conclusion, we propose a novel role for CaMKII in promoting lipolysis in adipocytes.—Rapold, R. A., S. Wueest, A. Knoepfel, E. J. Schoenle, and D. Konrad. Fas activates lipolysis in a Ca2+-CaMKII-dependent manner in 3T3-L1 adipocytes. J. Lipid Res. 2013. 54: XXX–XXX.

Supplementary key words obesity • insulin resistance • adipose tissue inflammation

White adipose tissue (WAT) has major metabolic and endocrine functions mediated by secretion of different adipokines and fat-derived metabolites such as NEFAs. These molecules regulate food intake, energy expenditure, and glucose homeostasis (1, 2). In obesity, excess WAT accumulation is accompanied by local infiltration of macrophages and other inflammatory cells secreting different cytokines such as IL-1α, IL-1β, IL-6, IL-8, (KC) [AQ1] and MCP-1, which in turn alter the expression and secretion pattern of adipokines and cytokines and stimulate the release of FAs by elevating basal lipolysis. All of these changes contribute to the development of detrimental complications of obesity such as insulin resistance and diabetes mellitus (2, 3).

Fas (CD95, CD95L, FasL) is a member of the tumor necrosis factor (TNF) receptor superfamily (7) and is activated by Fas ligand (FasL, CD95L), a type II membrane protein (8). Fas was first described in 1989 as a surface molecule on lymphocytes that can trigger cell death (9). In the adult mouse, Fas and FasL are expressed in several tissues including fat (10, 11). Upon binding of FasL, preformed trimeric Fas complexes undergo a conformational change that results in the formation of a death-inducing signaling complex and activation of downstream pathways leading to apoptosis (9, 12). However, in addition to apoptosis, Fas can also induce nonapoptotic signaling pathways. We previously demonstrated that mice lacking Fas specifically in adipocytes are partly protected from diet-induced insulin resistance (13), suggesting that Fas is involved in regulation of fat mass and metabolism. Indeed, Fas expression is up-regulated in adipose tissue and serum levels of FasL are increased in obese humans and mouse models of obesity (14). Moreover, it has been shown that FasL induces lipolysis in 3T3-L1 adipocytes (15). However, the signaling pathways involved in Fas-mediated lipolysis have not been delineated.

In this work, we aimed to delineate the signaling pathway involved in Fas-mediated adipocyte lipid mobilization. We present evidence that Fas activation increases phosphorylation of the Ca2+/calmodulin-dependent protein kinases II (CaMKII) and downstream kinase ERK1/2, and CsA inhibited FasL-induced lipolysis. Furthermore, we show that Fas activation increases phosphorylation of the Ca2+/calmodulin-dependent protein kinases II (CaMKII), and blocking the CaM-KII-pathway (either by the Ca2+ chelator BAPTA or by the CaMKII inhibitor KN62) blunted FasL-induced ERK1/2 phosphorylation and glycerol release.

In conclusion, we propose a novel role for CaMKII in promoting lipolysis in adipocytes. This work was supported by grants from the Swiss National Science Foundation (310030-124729 and 310030-12275) and a ‘Forschungskredit’ from the University of Zurich (both to D.K.). Manuscript received 5 May 2012 and in revised form 24 September 2012. Published, JLR Papers in Press, October 21, 2012 DOI 10.1194/jlr.M028035

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Journal of Lipid Research Volume 54, 2013 1
to this well-established role of Fas in apoptosis, Fas activation contributes to nonapoptotic signaling pathways, including cell proliferation (9, 13) and the induction of inflammatory responses in different cell types (14–18). Moreover, we have recently reported that Fas is increasingly expressed in WAT of obese subjects and that Fas-deficient and adipocyte-specific Fas knockout mice are partly protected from high-fat diet-induced insulin resistance (11, 19), implicating a role for Fas in the pathogenesis of obesity-associated insulin resistance. Although the underlying mechanism remains incompletely understood, livers of adipocyte-specific Fas knockout mice had lower levels of total ceramides, which are potentially metabolized from NEFA delivered to the liver from adipose tissue. Thus, in the present study, we hypothesized that in adipocytes, Fas activation can, independently of its pro-apoptotic effects, directly induce the hydrolysis of triglycerides (i.e., increase basal lipolysis), and set-up to investigate the intracellular signaling pathway(s) involved.

MATERIALS AND METHODS

Cell culture

3T3-L1 adipocytes were cultured in DMEM (Invitrogen; Basel, Switzerland) containing 25 mM glucose (high glucose), supplemented with 10% fetal calf serum (FCS; Socochim SA, Lausanne) and antibiotics (Invitrogen). Forty-eight hours after reaching confluence (day 0, D0), cells were treated with a mixture of 500 μM methylisobutylxanthine, 1 μM dexamethasone, 1.7 μM insulin (all from Sigma; Buchs, Switzerland), and 1 μM rosiglitazone (Alexis Biochemicals) to induce differentiation. Two days later (D2) the medium was changed to high-glucose culture medium containing insulin (0.5 μM). Another 2 days later (D4), the medium was replaced by culture medium without insulin. The culture medium was replaced every other day and changed to culture medium containing 5.5 mM glucose (low glucose) after 4 days (D8). Cells were kept at least 2 days on low glucose before experiments were performed. Membrane-bound Fas ligand (Upstate; Lake Placid, NY) was added at least 2 days on low glucose before experiments were performed. Membrane-bound Fas ligand (Upstate; Lake Placid, NY) was added at least 2 days on low glucose before experiments were performed. For pretreatment experiments with rosiglitazone (Enzo Life Science; Lausen, Switzerland), mature adipocytes were incubated for 48 h with 5 μM of the compound in low-glucose medium. Thereafter, Fasl, together with rosiglitazone, was applied for another 6 h or 12 h.

For siRNA-mediated ERK1/2 knock down, mature 3T3-L1 adipocytes were treated with siRNA (target sequences for ERK1/2: 5′ ACAAGGGCATCAGTGGAGA 3′, 5′ GACCTAAAGAGAGATTAA 3′, scrambled sequences for ERK1/2: 5′ GCCACGGGACACATT 3′, 5′ GAACAAAGTGTAACGGAACA 3′) in a transfection mixture containing Lipofectamin 2000 (Invitrogen) in culture medium (low-glucose, without FCS, without p/s) according to the manufacturer’s instructions.

Western blotting

Adipocytes were lysed in ice-cold lysis buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 1 mM EGTA, 1% NP-40, 0.25% sodium deoxycholate, 1 mM sodium vanadate, 1 mM NaF, 10 mM sodium β-glycerophosphate, 100 mM okadaic acid, 0.2 mM PMSF, and a 1:1,000 dilution of protease inhibitor cocktail (Sigma). Protein concentration was determined using a BCA assay (Pierce; Rockford, IL). Equal amounts of proteins were resolved by lithium dodecyl sulfate PAGE (4–12% gel; NuPAGE, Invitrogen) and electro-transferred onto nitrocellulose membranes (0.2 μm; BioRad, Reinach, Switzerland). Protein content on membranes was checked by Ponceau S staining. Blots were blocked in tris-buffered saline containing 0.1% Tween (TBS-T) supplemented with 5% nonfat dry milk. Primary antibody was applied in the same buffer in a dilution of 1:1,000, secondary antibody in a dilution of 1:5,000. Primary antibodies were purchased either from Cell Signaling Technology [phospho-p44/42 and total MAPKs, phosho-(Ser/Thr) PKA substrate, phospho-Ser 563, and total HSL, ATGL (MBL International; Woburn, MA); perilipin A, Millipore Actin, Fas (CD95)], Vala Sciences (phospho-Perilipin Ser 522), or Santa Cruz Biotechnology (PPARγ2, C/EBPα, pCaMKIIα (Thr 286). Signal was generated based on chemiluminescence and detected with the Fuji LAS-3000 image reader.

Measurement of lipolysis

Cells were incubated in DMEM without FCS and treated with 2 ng/ml Fasl or 1 μM isoproterenol in the presence or absence of 50 μM of the MEK inhibitor U0126 (Sigma), of 50 μM of the intracellular calcium chelator BAPTA/AM (Calbiochem), or of 50 μM of CaMKII inhibitor KN62 (Sigma), as indicated. Inhibitors or BAPTA/AM were added to the cells at the same time as Fasl. Thereafter, cells were washed in PBS, and NEFA and glycerol were collected in Krebs-Ringer-HEPES buffer supplemented with 0.1% FA-free BSA for 1 h. NEFAs in the supernatant were measured with the NEFA kit from Wako (Neuss, Germany), and glycerol with the free glycerol reagent from Sigma. Fractional re-esterification was calculated as previously described (20).

Data analysis

Data are presented as means ± SEM and were analyzed by a one-sample t-test or ANOVA with a Newman-Keuls multiple comparison test. *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS

Fas activation induces lipolysis in 3T3-L1 adipocytes

The Fas receptor (CD95) is expressed in 3T3-L1 preadipocytes and decreases during adipocyte differentiation, but is still clearly expressed in mature adipocytes (Fig. 1). Expression levels of the adipocyte-specific proteins PPARγ, C/EBPα, and perilipin reflect the respective stages of differentiation. Treatment of differentiated 3T3-L1 adipocytes with 2 ng/ml Fasl for 12 h significantly increased

![Graph](Image)

Fig. 1. The Fas receptor is expressed in 3T3-L1 cells. Lysates of 3T3-L1 preadipocytes (PreAC), differentiating (day (D) 1, 2, 4, and 6) and mature adipocytes (AC) were resolved by LDS-PAGE and immunoblotted with an antibody against the Fas receptor, PPARγ2, C/EBPα, and perilipin.
lipolysis (Fig. 2), consistent with our earlier observation (8), and without affecting their viability as assessed by TUNEL assay (see supplementary Fig. I) and MTT determination (11). Fractional reestherification did not decrease upon FasL incubation (basal: 68.0 ± 2.6%; 6 h FasL: 64.0 ± 6.0%; 12 h FasL: 63.6 ± 5.5%; P = 0.8). Such data suggest that Fas activation increases lipolysis by increased triglyceride hydrolysis rather than by decreased reestherification. Shorter incubation periods with FasL (≤ 6 h) and lower concentrations of FasL did not increase lipolysis to a significant degree (see supplementary Fig. II). Moreover, blunted Fas-stimulated lipolysis in Fas-depleted 3T3-L1 adipocytes suggests that membrane-bound Fas signals via the Fas receptor (see supplementary Fig. III).

**Fas activation increases phosphorylation of HSL**

Beta adrenergic receptor agonists such as catecholamines stimulate lipolysis via adenylate cyclase-dependent activation of PKA and consecutive activation of HSL, perilipin 1, and ATGL. Inhibiting such effect, insulin activates phosphodiesterase 3, which converts cAMP to 5′-AMP, thereby diminishing cAMP-mediated PKA activity, which results in inhibition of lipolysis. To examine whether Fas-mediated lipolysis comprises activation of PKA, phosphorylation of PKA substrates was determined in 3T3-L1 adipocytes. As expected, the β12 receptor agonist isoproterenol increased phosphorylation of PKA substrates significantly. In contrast, treatment with FasL had no effect on the abundance of phosphorylated PKA substrates (Fig. 3A). However, even though not detected by the PKA substrate antibody, incubation of 3T3-L1 adipocytes with FasL for 6 h and 12 h significantly increased phosphorylation of HSL at Ser563 (Fig. 3B), whereas it had no effect on total HSL protein levels (see supplementary Fig. IV) and phosphorylation of perilipin (Fig. 3B). In addition, ATGL protein levels were slightly but not significantly upregulated upon Fas incubation (Fig. 3C). Thus, Fas-mediated lipolysis may depend on activation of HSL and/or ATGL.

**Fas-mediated lipolysis is ERK dependent**

An alternative signaling pathway to activate lipolysis in adipocytes involves the p44/42 MAP kinases (ERK1/2), as was shown for TNFα (21). Because Fas belongs to the TNF receptor superfamily, we postulated that FasL-induced lipolysis is mediated via ERK1/2 activation. Incubation of mature 3T3-L1 adipocytes with 2 ng/ml FasL increased phosphorylation of ERK1/2 significantly after 6 h and 12 h, whereas total protein concentration of ERK1/2 was not affected (Fig. 4A). To exclude the possibility that the effects of FasL treatment were due to a FasL-mediated increase in TNFα secretion and, thus, to a paracrine regulatory loop mediated by this cytokine, TNFα concentration was determined in the supernatant. As depicted (see supplementary Fig. V), incubation with 2 ng/ml FasL for 12 h did not lead to increased TNFα secretion to the medium. We therefore concluded that Fas-mediated ERK1/2 activation was independent of TNFα.

To assess whether Fas-induced lipolysis is dependent on ERK1/2 activation, we incubated 3T3-L1 cells in the presence or absence of the MEK1/2 inhibitor U0126. Inhibition of the MAP kinase pathway with U0126 (50 μM) completely blocked Fas-induced ERK1/2 phosphorylation and lipolysis (Fig. 4B). To further strengthen a role of the p44/42 MAP kinase pathway in Fas-mediated lipolysis, 3T3-L1 adipocytes were treated with targeted or scrambled siRNA against ERK1/2, respectively. Compared with scrambled siRNA control, siRNA targeted toward ERK1/2 decreased ERK1/2 protein by about 50%, an effect that was associated with a significant blunting of Fas-stimulated lipolysis (Fig. 4C). Thus, Fas-stimulated lipolysis is dependent on ERK1/2 activation.

To further corroborate a Fas-ERK1/2-lipolysis pathway in adipocytes, we tested whether PPARγ agonists such as thiazolidinediones (TZDs), which were demonstrated to inhibit whole-body lipolysis in patients with type 2 diabetes (22), could inhibit Fas-induced lipolysis and if so, whether activation of ERK1/2 was also diminished. As shown in Fig. 4D, FasL-stimulated ERK1/2 phosphorylation was reduced in the presence of rosiglitazone without affecting total ERK1/2 protein content. Correspondingly, rosiglitazone also reduced FasL-mediated lipolysis (Fig. 4D).

**Fas-mediated lipolysis is CaMKII dependent**

CaMKII was previously shown to mediate magnolol-triggered lipolysis in sterol ester-loaded 3T3-L1 preadipocytes in an ERK1/2-dependent fashion (23). We therefore
postulated that FasL-induced lipolysis may be dependent on intracellular changes in calcium levels, because it is well accepted that Fas activation can increase intracellular calcium levels (24). Indeed, preincubation of 3T3-L1 adipocytes with the intracellular calcium chelator BAPTA/AM prevented both FasL-induced ERK activation and lipolysis (Fig. 5A), suggesting that both effects of Fas activation are dependent on an intracellular calcium rise. In response to the latter, the developing calcium/calmodulin complex may activate CaMKII, leading to intramolecular autophosphorylation at several sites, including Thr286, Thr305, and Thr306 (25). As depicted in Fig. 5B, incubation of mature 3T3-L1 adipocytes with 2 ng/ml FasL increased phosphorylation of CaMKII at Thr286 significantly after 6 h and 12 h. Moreover, pretreatment with the CaMKII inhibitor KN62 reduced FasL-mediated ERK1/2 activation as

Fig. 3. Fas activation increases phosphorylation of HSL. Fully differentiated 3T3-L1 adipocytes were treated with FasL (2 ng/ml) for the indicated time periods or isoproterenol (1 µM) for 30 min. Lysates were resolved by LDS-PAGE and immunoblotted with phospho-(Ser/Thr) PKA substrate (A), phospho-HSL (Ser563), and phospho-perilipin (B) or ATGL (C) antibodies. Shown are representative blots and densitometry analyses of four to eight independent experiments. *P<0.05, **P<0.01.
Fig. 4. Fas-mediated lipolysis is ERK dependent. A: Fully differentiated 3T3-L1 adipocytes were treated with FasL (2 ng/ml) as indicated. Lysates were resolved by LDS-PAGE and immunoblotted with phosphorylated (Thr202/Tyr204) and total ERK1/2 antibodies. Shown are representative blots and quantification of seven independent experiments. B: 3T3-L1 adipocytes were treated with 2 ng/ml FasL and different concentrations of the MEK inhibitor U0126. Shown are representative blots in the upper panel. For the determination of lipolysis, cells were incubated with FasL for 12 h in the presence (white bars) or absence (black bars) of 50 µM U0126, and glycerol release was determined. Results are the means ± SEM of six independent experiments. C: (Left panel) siRNA-treated cells [scrambled (scr, black bars) or target (white bars)] were lysed 72 h after transfection. Total cell lysates were resolved by LDS-PAGE and immunoblotted with total ERK1/2 and actin antibodies. Shown are representative blots (upper panel) and densitometry analyses of four independent experiments (lower panel). Right panel: Glycerol release was determined 72 h after siRNA-mediated ERK1/2 depletion. Results are the means ± SEM of six independent experiments. D: Cells were incubated with or without 5 µM rosiglitazone for 60 h. During the last 6 h or 12 h of such incubation, cells were treated with FasL. Left panel: total cell lysates were resolved by LDS-PAGE and immunoblotted with anti-phospho-ERK1/2 or anti-ERK1/2 antibodies. Shown are representative blots and quantification of four independent experiments. Right panel: glycerol release was determined. Results are the means ± SEM of four independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001.
characterized by an elevated rate of basal lipolysis (30), which might be at least partly mediated by self-production of inflammatory cytokines acting in an autocrine manner, possibly as a self-protective cellular mechanism against excessive cellular over-growth. Third, because insulin is the major anti-lipolytic hormone, insulin resistance at the adipocyte level results in increased lipolysis, creating a vicious cycle between hypertrophy, inflammation, lipolysis, and insulin resistance. Fas may be a key component of such dysregulation: Fas is activated by FasL, which can be produced by inflammatory cells infiltrating adipose tissue in obesity. Moreover, expression of Fas is increased in adipose tissue of obese humans and in isolated adipocytes of obese and diabetic mice, and intriguingly, its protein expression correlates with adipocyte size and is therefore increased in hypertrophic adipocytes (11).

In agreement with such notion, we show herein that chronic stimulation of the death receptor Fas induced well as lipolysis (Fig. 5C). These data strongly suggest that Ca-mediated activation of CaMKII is a proximal signaling response to Fas activation, which is propagated further downstream via ERK1/2 to induce basal lipolysis.

DISCUSSION
In obesity, higher basal lipolysis rate resulting in increased release of NEFAs into the circulation contributes to the development of hepatic and total body insulin resistance (26). Several factors contribute to such increase in lipolysis. First, obesity is associated with a persistent low-grade inflammation of adipose tissue, as manifested by an increased production and secretion of proinflammatory cytokines such as TNFα and IL-6 (27). The latter in turn can directly stimulate adipocyte lipolysis, even as isolated factors (21, 28, 29). Second, hypertrophic adipocytes are characterized by an elevated rate of basal lipolysis (30), which might be at least partly mediated by self-production of inflammatory cytokines acting in an autocrine manner, possibly as a self-protective cellular mechanism against excessive cellular over-growth. Third, because insulin is the major anti-lipolytic hormone, insulin resistance at the adipocyte level results in increased lipolysis, creating a vicious cycle between hypertrophy, inflammation, lipolysis, and insulin resistance. Fas may be a key component of such dysregulation: Fas is activated by FasL, which can be produced by inflammatory cells infiltrating adipose tissue in obesity. Moreover, expression of Fas is increased in adipose tissue of obese humans and in isolated adipocytes of obese and diabetic mice, and intriguingly, its protein expression correlates with adipocyte size and is therefore increased in hypertrophic adipocytes (11).
lipolysis in 3T3-L1 adipocytes. Importantly, this effect occurred under conditions that did not induce apoptosis (11). Like TNFα, FasL stimulates lipolysis through activation of the ERK1/2 MAP kinases, because Fas activation led to increased phosphorylation of ERK1/2 and pretreatment with the MEKI/1 inhibitor U0126 or siRNA-mediated downregulation of ERK1/2 blocked Fas-induced lipolysis. In the case of TNFα, it was proposed that ERK1/2-dependent downregulation of the lipid droplet coating protein perilipin is responsible for the increase in lipolysis (21). We also observed a downregulation of perilipin in cells treated with FasL for 12 h. However, in contrast to TNFα, such effect was not mediated by ERK1/2, because FasL-induced decrease in perilipin expression was not prevented by ERK inhibition (see supplementary Fig. VI). Moreover, 6 h of FasL incubation did not decrease perilipin protein content (data not shown) but increased FFA and glycerol release. All these results suggest that Fas-mediated lipolysis is independent of a decrease in perilipin expression.

Herein, we present evidence for calcium-triggered activation of ERK in Fas activation-mediated lipolysis. Fas activation in cells was previously shown to raise intracellular free Ca\(^{2+}\) levels (24). Similarly, increased intracellular Ca\(^{2+}\) levels induced by endoplasmic reticulum (ER) stress were shown to induce lipolysis in adipocytes ERK1/2 independently (31). Hence, Fas-induced ERK activation may be mediated by ER stress-triggered Ca\(^{2+}\) release. However, as shown in supplementary Fig. VII, FasL stimulation of 3T3-L1 adipocytes did not provoke ER stress. Moreover, the extracellular Ca\(^{2+}\)-chelator EDTA blunted Fas-induced ERK activation and lipolysis similar to the intracellular chelator BAPTA (see supplementary Fig. VIII). Such data suggest that extracellular Ca\(^{2+}\) influx rather than Ca\(^{2+}\) release from ER is involved in Fas-induced lipolysis. Increased intracellular Ca\(^{2+}\) is bound by the calcium-binding protein calmodulin (CaM) forming a complex. The latter then binds to and thereby activates CaMKII. Activation of CaMKII by the Ca\(^{2+}\)/CaM complex allows intramolecular autophosphorylation at several sites, including Thr286. This generates calcium-independent activity that persists after dissociation of calcium/CaM, allowing transient calcium elevation to promote prolonged kinase activation (25). We found that FasL treatment increased phosphorylation at Thr286 of CaMKII. Moreover, Fas activation-induced lipolysis was prevented in the presence of the CaMKII inhibitor as well as of the Ca\(^{2+}\) chelator, BAPTA. Thus, we postulate that Fas activation in adipocytes increases intracellular Ca\(^{2+}\) levels, leading to activation of CaMKII, which, in turn, activates ERK1/2 and, thus, lipolysis. Accordingly, it was previously reported that trans-10, cis-12-conjugated linoleic acid-induced ERK1/2 activation in adipocytes is dependent on a rise in intracellular free Ca\(^{2+}\) levels and consecutive activation of CaMKII (32). Unfortunately, lipolysis was not addressed in this study. The data presented herein may suggest a novel pathway for lipolysis in adipocytes via CaMKII-dependent activation of ERK. Interestingly, such a pathway may be very ancient and evolutionarily preserved, because CaMKII-mediated release of FFAs was recently demonstrated to play a role in pheromone biosynthesis in insects (33).

Interestingly, the TZD rosiglitazone reduced both Fas-mediated ERK1/2 phosphorylation and lipolysis. PPARγ agonists have profound effects on adipocyte metabolism and were shown to improve insulin sensitivity in patients with type 2 diabetes. Moreover, in a recent paper, treatment with another TZD, pioglitazone, was able to reduce whole-body lipolysis in type 2 diabetic patients (22). We previously described a potential role for Fas activation in obesity-associated insulin resistance (11) and show herein that Fas activation leads to increased lipolysis. Thus, our experiments may point to a role of TZDs in counteracting Fas-induced metabolic changes in adipocytes and further underscore the importance of ERK activation for FasL-induced lipolysis. Similarly, TZDs were previously found to antagonize the effects of TNFα on adipocytes (34).

We recently showed that treatment of 3T3-L1 adipocytes with 2 ng/ml FasL for 12 h reduced protein levels of Akt/PKB (protein kinase B) (19). Moreover, a recent publication reported that deletion of rictor, an essential component of the Akt kinase mammalian target of rapamycin complex 2 (mTORC2), increases basal lipolysis as well as phosphorylation of HSL at Ser563 in adipocytes (35). Hence, it is conceivable that Fas-mediated reduction in Akt protein levels contributed to FasL-induced lipolysis, potentially via increased phosphorylation of HSL at serine residue 563. Moreover, ERK1/2-mediated phosphorylation of HSL at Ser600 (36) might contribute to Fas-induced lipolysis. However, such a corresponding anti-phospho-HSLSer600 antibody is not commercially available and, thus, not available to us for evaluating a potential role of HSLSer600 phosphorylation in this study. Besides activation of HSL, we cannot exclude the involvement of ATGL to Fas activation-induced lipolysis, because ATGL protein levels were slight, albeit not significantly increased, after FasL treatment.

In summary, activation of the Fas receptor alters the metabolism of adipocytes and leads to ERK1/2-mediated lipolysis, which may be triggered by a Fas-increased increase in intracellular calcium levels and hence, autophosphorylation of the Ca\(^{2+}\)/calmodulin-dependent protein kinases II. Thus, our findings suggest an important role of the Fas receptor in the development of adipose tissue dysfunction in the context of obesity (37).

The authors thank Dr. Assaf Rudich from the Department of Clinical Biochemistry and the S. Daniel Centre for Health and Nutrition, Ben-Gurion University, Beer-Sheva, Israel, for helpful discussion.

**REFERENCES**


Author Queries

[AQ1]: What does KC indicate?

[AQ2]: Please clarify “set-up to investigate.” Is “prepared to investigate” meant?

[AQ3]: Please spell out p/s.

[AQ4]: Please spell out BCA.

[AQ5]: Please spell out MEK.

[AQ6]: Please spell out TUNEL and MTT.

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