FDP-E induces adipocyte inflammation and suppresses insulin-stimulated glucose disposal: effect of inflammation and obesity on fibrinogen Bβ mRNA

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Kang M, Vaughan RA, Paton CM. FDP-E induces adipocyte inflammation and suppresses insulin-stimulated glucose disposal: effect of inflammation and obesity on fibrinogen Bβ mRNA. Am J Physiol Cell Physiol 309: C767–C774, 2015. First published October 7, 2015; doi:10.1152/ajpcell.00101.2015.—Obesity is associated with increased fibrinogen production and fibrin formation, which produces fibrin degradation products (FDP-E and FDP-D). Fibrin and FDPs both contribute to inflammation, which would be expected to suppress glucose uptake and insulin signaling in adipose tissue, yet the effect of FDP-E and FDP-D on adipocyte function and glucose disposal is completely unknown. We tested the effects of FDPs on inflammation in 3T3-L1 adipocytes and primary macrophages and adipocyte glucose uptake in vitro. High-fat-fed mice increased hepatic fibrinogen mRNA expression ninefold over chow-fed mice, with concomitant increases in plasma fibrinogen protein levels. Obese mice also displayed increased fibrinogen content of epididymal fat pads. We treated cultured 3T3-L1 adipocytes and primary macrophages with FDP-E, FDP-D, or fibrinogen degradation products (FgnDP-E). FDP-D and FgnDP-E had no effect on inflammation or glucose uptake. Cytokine mRNA expression in RAW264.7 macrophage-like cells and 3T3-L1 adipocytes treated with FDP-E induced inflammation with maximal effects at 100 nM and 6 h. Insulin-stimulated 2-deoxy-[1-14C]glucose uptake was reduced by 71% in adipocytes treated with FDP-E. FDP-E, but not FDP-D or FgnDP-E, induces inflammation in macrophages and adipocytes and decreases glucose uptake in vitro. FDP-E may contribute toward obesity-associated acute inflammation and glucose intolerance, although its chronic role in obesity remains to be elucidated.

Obesity is a direct result of overnutrition and is now characterized, at least in part, as a low-grade inflammatory disease causing muscle, adipose, renal, and hepatic dysfunction (23, 25). Although the underlying mechanisms are not completely understood, it appears that activation of the systemic inflammatory response diminishes insulin sensitivity in adipose tissue, muscle, and liver. Additionally, evidence has shown that increased lipogenesis is associated with leukocyte activation and an elevated potential for thrombosis (20). Each of these factors is known to promote endothelial cell dysfunction, vascular damage, and tissue remodeling, ultimately leading to fibrosis, organ dysfunction, and thrombosis (9). After thrombi are formed in peripheral tissues, their dissolution via plasmin-mediated fibrinolysis generates FDPs, including two FDP-D fragments and one FDP-E fragment (Fig. 1) (21). Based on a previous study (13), we suspected that FDP-E content in adipose tissue may increase with obesity, which could induce macrophage activation, and thereby suppress adipocyte-mediated glucose uptake by inducing a proinflammatory phenotype.

Others have shown that fibrin and FDPs induce inflammation (6, 12), and, because inflammation in adipose tissue is a direct cause of insulin resistance, we suspected that adipose tissue deposition of FDP-E may be mediating the proinflammatory phenotype of obese adipose tissue. Notably, the FDP-E fragment is a proinflammatory molecule, which internalizes into cells and induces apoptosis (13, 15). Therefore, fibrinogen, fibrin, and FDP are associated with not only blood coagulation, but inflammation as well, which may suppress adipocyte-mediated glucose uptake. However, the role of fibrin and its degradation products are largely unknown with respect to obesity, inflammation, and insulin resistance. In this study, we examined the effect of FDP-E on inflammation in macrophages, adipocytes, and adipocyte-mediated glucose disposal. We hypothesized that FDP-E induces inflammation in macrophages and adipocytes, resulting in proinflammatory cytokine release, which decreases glucose uptake. We found that hepatic fibrinogen production and adipose tissue fibrin content were increased with obesity in high-fat (HF)-fed mice. FDP-E, but not fibrinogen degradation products (FgnDPs) or FDP-D, induced a proinflammatory phenotype in primary peritoneal macrophages, RAW264.7 cells, and mature 3T3-L1 adipocytes. Direct treatment of adipocytes with FDP-E suppressed insulin-stimulated glucose uptake in a dose-dependent manner. The results below support the hypothesis that fibrin production and FDP-E formation may be direct causes of impaired glucose disposal with obesity, possibly by inducing adipose tissue remodeling.

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**MATERIALS AND METHODS**

**Animals.** All experiments using mice were approved by the Texas Tech University Institutional Animal Care and Use Committee. Male C57BL/6 mice we purchased from Jackson Laboratories (Bar Harbor, ME) and kept on a 12:12-h light-dark cycle. For experiments involving diet-induced obesity, mice were either kept on 5P14-Prolab RMH 2500 standard rodent Chow (LabDiet, St. Louis, MO) or fed a HF custom research diet (TD.08500, Harlan Laboratories, Madison, WI) at 8 wk of age.

**Reagents and supplies.** Purified human fibrinogen, FDP-E, FDP-D, and FgnDP-E fragment were obtained from Aniara (Hyphen Biomed, West Chester, OH). Both FDP-E and FgnDP-E were obtained by degradation of human fibrinogen, respectively, with plasmin, and then purification with ion exchange and gel filtration chromatography. Anti-human fibrinogen Aα antibody (sc-166968) was from Santa Cruz Biotechnology (Dallas, TX). Mouse fibrinogen ELISA was from Innovative Research (IMFBGNKT, Novi, MI). Bovine insulin and 2-deoxy-β-glucose (2-DG) were from Sigma (St. Louis, MO), and 3H-labeled 2-DG was from American Radiolabeled Chemicals (St. Louis, MO).

**Primary peritoneal macrophage isolation.** Twenty-three-week-old chow-fed female C57BL/6 mice were used to collect primary peritoneal macrophages. First, mice were euthanized via isoflurane overdose, and then 5 ml of cold harvest medium (PBS without Ca2+ and Mg2+) was immediately injected through the peritoneal wall using a 26G needle and syringe. The abdomen was massaged to dislodge cells, and then the harvest medium was slowly aspirated back into the syringe. After dispensing the recovered peritoneal fluid into a 50-ml centrifuge tube, the process was repeated to collect cells with another 5 ml of cold harvest medium. The collected media containing cells was centrifuged for 10 min at 400 g, and then the supernatant was removed. The pelleted cells were resuspended in 2 ml of 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in Dulbecco’s modified Eagle’s medium (DMEM) and plated onto 12-well plates at a density of 2.6 × 104 cells/ml. After 5 h under standard conditions, cells were washed with warm PBS three times to enrich for primary macrophages, as only macrophages adhere to plastic in that time span and all other cells are removed. After 24 h, the cells were treated with 10% FBS in DMEM with 0.17 mM insulin, 10 mM glucose, 0.99 μM dexamethasone, and 0.17 μM insulin to induce differentiation. Troglitazone 1 μM, an agonist of peroxisome proliferator-activated receptor-γ, was added to ensure complete and uniform differentiation of adipocytes. Two days later, the cells were treated with 10% FBS in DMEM with 0.17 μM insulin and 1 μM troglitazone for 2 days, and then the medium was changed to 10% FBS in DMEM for the remaining 4 days.

**Assessment of M1 vs. M2 inflammation in primary macrophages.** To verify that FDP-E induces inflammation in macrophages and which phenotype (M1 vs. M2) is expressed, primary macrophages were divided into three groups: 1) nontreated control; 2) 1 μg/ml lipopolysaccharide (LPS) as a positive control; or 3) 100 nM FDP-E. After 4 h, we extracted mRNA from cells for gene expression analysis. RNA polymerase II (Pol2RA) was used for a housekeeping gene and tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 (MIP-1) were target genes for M1 phenotype. Interleukin-10 (IL-10), Ym1, and Ym2 were used for biomarker genes of M2 phenotypes. RAW 264.7 macrophage-like cells and conditioned medium. RAW 264.7 macrophage-like cells were used to verify the effect of FDP-E on inflammation and to collect conditioned medium for the glucose uptake assays. They were cultured with 10% FBS DMEM under the standard conditions, and the medium was changed every 2 days. When the cells were confluent, they were treated with regular medium or 100 nM FDP-E-supplemented medium for 24 h, and the medium was collected into one 15-ml centrifuge tube. After inverting to mix well, the conditioned medium was aliquoted and frozen at −80°C until it was used for glucose uptake assays.

**Adipocyte differentiation.** 3T3-L1 preadipocytes were used to assess adipocyte inflammation and glucose uptake. The cells were cultured under the standard conditions, and DMEM containing 10% calf serum and 1% penicillin-streptomycin was used as a complete medium before confluence. The medium was changed every 2 days until confluence, after which time they were treated with 10% FBS in DMEM supplemented with 510.4 μM methylisobutylxanthine, 0.99 μM dexamethasone, and 0.17 μM insulin to induce differentiation. Troglitazone 1 μM, an agonist of peroxisome proliferator-activated receptor-γ, was added to ensure complete and uniform differentiation of adipocytes. Two days later, the cells were treated with 10% FBS in DMEM with 0.17 μM insulin and 1 μM troglitazone for 2 days, and then the medium was changed to 10% FBS in DMEM for the remaining 4 days.

**Assessment of inflammation in macrophages and adipocytes.** RAW 264.7 macrophage-like cells and mature 3T3-L1 adipocytes were treated with 100 nM FDP-E for 1, 2, 6, and 12 h to determine the pattern of response following treatment. Once we determined the optimal time for treatment, we treated cells with 100 nM FDP-E or FgnDP-E (nonthrombin activated, but plasmin digested fibrinogen) to clarify that only FDP-E and not FgnDP-E induces inflammation. We also treated cells with varying concentrations of FDP-E, including nontreated control (0 nM), 12.5, 25, 50, and 100 nM for 6 h to determine the dose-dependent effect on target gene expression. After the treatments, we extracted mRNA from cells for gene expression analysis.

**RT-PCR.** Total RNA was collected using Tri-Reagent (Molecular Research Center, Cincinnati, OH), and the RNA pellet was resuspended in RNase/DNase free ultrapure water. cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative real-time PCR was conducted using a SYBR
green PCR master mix (Applied Biosystems) with gene-specific primers for Pol2RA, TNF-α, MCP-1, MIP-1, IL-10, Ym1, and Ym2, designed to span exon-exon boundaries to prevent amplification of genomic DNA.

Adipocyte-mediated glucose disposal. Differentiated 3T3-L1 adipocytes were treated with different concentrations of FDP-E conditioned medium from the macrophage treatments described above or directly with FDP-E itself to examine the dose-dependent relationship between FDP-E and glucose uptake. For the conditioned medium experiments, dilutions of the conditioned medium from RAW 264.7 macrophage-like cells nontreated (control) or treated with 100 nM FDP-E was used to treat adipocytes (control) or treated with 100 nM FDP-E for 24 h using fresh DMEM + 10% FBS as diluent. In addition, for direct FDP-E treatment of adipocytes, cells were treated with FDP-E itself, ranging 0, 12.5, 25, 50, and 100 nM for 24 h. After treating adipocytes, cells were washed with 500 μl PBS three times and incubated in 500 μl of 1% BSA in Krebs-Ringer HEPES buffer. After 2 h in Krebs-Ringer HEPES buffer, cells were washed with 500 μl warm PBS two times, followed by the addition of 500 μl of 200 nM insulin or PBS for 30 min. Next, the cells were washed with PBS twice to completely remove insulin, followed by the addition of 500 μl of 0.1 mM 2-DG supplemented with 1 μCi 2-deoxy-α-[3H]glucose (2-[3H]2-DG) into PBS. After 5 min, the cell lysate was collected with 200 μl of 1% SDS, and [3H] levels were counted using a liquid scintillation counter.

Statistics. ANOVA was used to analyze and determine significance between groups. The α-level was 0.05, and all data are presented as means ± SE. All experiments were conducted at least three times, regardless of the number of replicates within an experiment.

RESULTS

Fibrinogen and inflammation increase in obese mice. We first wanted to demonstrate that obese mice increase fibrinogen expression and inflammation. To achieve this, we placed wild-type male B6 mice on a HF diet for 15 wk, and we monitored body weight every 3 wk (Fig. 2A). HF-fed mice significantly increased body weight compared with chow-fed mice, and the increase in body weight was also associated with impaired glucose tolerance (Fig. 2B). At the end of 15 wk of HF feeding, we measured glucose tolerance via intraperitoneal injection of glucose (0.75 g/kg) and measured blood glucose via tail vein nick for 2 h. HF-fed mice displayed impaired glucose tolerance as expected with obesity studies. Next, we measured fibrinogen Aβ (FGB) mRNA expression in liver in chow- and HF-fed mice, where there was a ninefold increase in gene expression (Fig. 2C). FGB expression is the rate-limiting step in the assembly and secretion of the fibrinogen protein. Increases in fibrinogen Aα or fibrinogen-γ chains that are not matched by equivalent increases in FGB do not equate to increases in fibrinogen protein expression. Thus it is most appropriate to monitor changes in FGB mRNA expression to determine the extent of fibrinogen expression (which also mirrors changes in Aα and γ chains). The increase FGB mRNA expression was matched by a slight but nonsignificant increase in plasma fibrinogen protein (Fig. 2D). Chow-fed mice had 1.1 ± 0.3 vs. 1.4 ± 0.1 mg/dl in HF-fed animals (P = 0.1). Lastly, we measured inflammatory gene expression and leukocyte markers in epididymal fat of HF-fed mice (Fig. 2, E–G). As expected, HF feeding increased inflammation and leukocyte content of adipose tissue, as evidenced by increased TNF-α, IL-6, natural killer group 2D, CD8, and MCP-1 mRNA expression.

Fibrin(o)gen content of adipose tissue. Fibrinogen is a soluble, circulating blood coagulation protein produced by the liver that, when activated through thrombin-mediated cleavage, becomes insoluble and is deposited in the vasculature. Previous studies have demonstrated that fibrin, and especially its degradation product, FDP-E fragment, induces inflammation and apoptosis in various cell types. Therefore, we assessed fibrin(o)gen Aα content of epididymal and subcutaneous adipose tissue of Chow- and HF-fed mice by Western blot. The animals were perfused with PBS until the lungs and liver were blanched, then fixed by perfusion with 2% paraformaldehyde to cross-link the deposited protein in the tissue. Tissue homogenates were run on a nonreducing gel and probed using an anti-fibrinogen Aα polyclonal antibody to visualize the ~45- to 50-kDa bands that correspond (roughly) with FDP-E fragment. The Aα-protein content of the epididymal and subcutaneous fat pad was increased in obese mice with no appreciable change in liver (Fig. 2H). These results suggest that HF diet-induced obesity increases hepatic fibrinogen production and adipose tissue deposition of FDP-E, along with increased inflammation. In light of these results, we sought to determine the direct causal effect of FDP-E on adipocyte inflammation using a series of in vitro studies with purified human FDP-E.

We next tested the effect of fibrinogen treatment of premature and mature adipocytes. 3T3-L1 fibroblasts were grown to confluence and treated with 100 nM human fibrinogen or induced to differentiate into mature adipocytes before fibrinogen treatment (Fig. 3). Changes in gene expression of the fibroinotic cell surface receptor urokinase-type plasminogen-activated receptor was determined between groups with no change in preadipocytes (0.6 ± 0.04-fold P = 0.2) and a 12.6 ± 0.9-fold increase (P = 0.0002) in mature adipocytes.

FDP-E increases inflammation in macrophages. To test the direct effect of FDP-E on inflammation, we isolated nonstimulated primary peritoneal macrophages from C57BL/6 mice. Cells were then cultured to determine whether FDP-E induces either the pro- (M1) or anti-inflammatory (M2) phenotype. A separate set of cells was cultured and treated with LPS as a positive control. Both FDP-E and LPS significantly increased proinflammatory (M1) cytokine expression (Fig. 4, A and C), but not anti-inflammatory expression (M2) (Fig. 4, B and D). FDP-E increased TNF-α 59.9 ± 8.9-fold (P = 0.0005), MCP-1 55.9 ± 7.1-fold (P = 0.0002), and MIP-1 89.3 ± 8.9-fold (P < 0.0001) (fold change vs. vehicle control). LPS increased TNF-α 747.6 ± 59.7-fold (P < 0.0001), MCP-1 1679.3 ± 751.7-fold (P = 0.04), and MIP-1 942.9 ± 200.6-fold (P = 0.003) vs. control as well. Although the effect of FDP-E is much smaller than the LPS-positive control, it seems sufficient to induce inflammation based on the increased proinflammatory cytokine expression. It is also likely that the lower level of inflammation observed from FDP-E falls within a physiologically relevant range and may be tolerated for longer periods of time. These results suggest that FDP-E induces inflammation via the M1 phenotype more than the M2 phenotype inducing proinflammatory responses, we used RAW 264.7 macrophage-like cells as an in vitro model. To determine the necessity of thrombin activation, we tested the effect of FgnDP-E fragment (plasmin digested...
but not thrombin activated) and compared it to FDP-E (plasmin digested and thrombin activated) (Fig. 5A). The results demonstrated an increase in MCP-1 expression with FDP-E (2.1 ± 0.3-fold, \( P < 0.04 \)) and not with FgnDP-E treatment (1.3 ± 0.1-fold, \( P < 0.5 \)). This suggests that thrombin-mediated activation of fibrin (from fibrinogen) is required for its proinflammatory effects and to induce inflammation or apoptosis (10, 13).

Physiological levels of FDP-E can range from 0 to 2 μM, depending on health status, and >1 μM is sufficient to induce apoptosis in vitro, although levels at that concentration are seen under conditions such as sepsis or disseminated intravascular coagulation. We wanted to test the effect of FDP-E at low physiological concentrations (~nM) in an attempt to capture the effect of heightened chronic fibrin deposition and examine the response of both macrophages and adipocytes. RAW264.7 cells were treated with increasing concentrations of FDP-E, ranging from 0- to 100 nM for 6 h, and MCP-1 mRNA expression was measured (Fig. 5B). Expression of MCP-1 mRNA increased 1.9 ± 0.5-fold \( (P < 0.07) \), 1.9 ± 0.1-fold \( (P < 0.0001) \), 5.6 ± 2-fold \( (P = 0.04) \), and 3.9 ± 0.7-fold \( (P = 0.009) \), with increasing concentrations of FDP-E, from 0 to 12.5, 25, 50, and 100 nM. There was a slight decrease at 100 nM compared with 50 nM, but no significant difference between them \( (P = 0.3) \).

We also determined the time course of FDP-E treatment on inflammation with 100 nM FDP-E for different times, from 0 to 12 h (Fig. 5C). FDP-E treatment increased MCP-1 6.4 ± 0.5-fold \( (P < 0.0001) \), 13.4 ± 3.4-fold \( (P = 0.004) \), 29.5 ± 9.8-fold \( (P = 0.02) \), and 40.6 ± 15.6-fold \( (P = 0.02) \) over
control at 1, 2, 6, and 12 h, respectively. The results showed that MCP-1 gene expression increased steadily until 6 h and slightly at 12 h, although the difference between 6 and 12 h was not significant ($P = 0.5$).

Indirect effect of FDP-E on glucose uptake in adipocytes. There are two possible ways in which FDP-E could affect glucose uptake in adipocytes: 1) directly, by altering adipocyte function, or 2) indirectly, via cytokines secreted from macrophages acting as secondary agents. Since we observed a significant increase in inflammation from FDP-E-treated macrophages, we collected conditioned medium collected from RAW 264.7 cells treated with 100 nM FDP-E for 24 h. Fully differentiated 3T3-L1 adipocytes were divided into two groups to assess 2-DG uptake: one group without and the other with insulin. Each group was treated with diluted conditioned medium from 1:100 up to 1:10 dilution for basal glucose uptake (Fig. 6A) and 1:100 to 1:1 for insulin-stimulated uptake (Fig. 6B). After 24 h of treatment with conditioned medium, we measured glucose uptake using $[^{3}H]2$-DG. There was no significant effect of FDP-E on $[^{3}H]2$-DG uptake in either the noninsulin- or insulin-treated cells. Concentrations of $[^{3}H]2$-DG in noninsulin-treated cells did not change with the addition of conditioned media. Additionally, insulin-stimulated $[^{3}H]2$-DG was not affected with 1:100, 1:10, or 1:1 diluted conditioned medium. While FDP-E can induce inflammation in primary macrophages and RAW264.7 cells, it does not appear that conditioned medium from the latter negatively impacts adipocyte-mediated glucose disposal.

FDP-E increases inflammation in adipocytes. We performed similar experiments in 3T3-L1 adipocytes as those done in RAW macrophage-like cells. Mature adipocytes were used to determine the direct effect of FDP-E on the proinflammatory response in adipocytes and its effect on glucose uptake. As we observed in RAW264.7 cells, only FDP-E (7.8 ± 2.3 fold, $P = 0.001$) and not FgnDP-E (1.1 ± 0.1, $P = 0.2$) resulted in increased MCP-1 expression (Fig. 7A). Additionally, MCP-1 mRNA expression increased 1.3 ± 0.09-fold ($P = 0.01$), 2.4 ± 0.4-fold ($P = 0.04$), 2.3 ± 0.4-fold ($P = 0.03$), and 8.5 ± 2.5-fold ($P = 0.01$) with increasing concentrations of FDP-E (0 vs. 12.5, 25, 50, and 100 nM, respectively) (Fig. 7B). The time course pattern of FDP-E treatment increased MCP-1 expression from 2.4 ± 0.1-fold ($P < 0.0001$), 8.9 ± 0.1-fold ($P < 0.0001$), 11.3 ± 3.1-fold ($P = 0.01$), and 8.6 ± 3.1-fold ($P = 0.04$) at 1, 2, 6, and 12 h, respectively (Fig. 7C). The response of RAW264.7 cells and adipocytes was similar in that 6–12 h
of treatment with 100 nM FDP-E, but not FgnDP-E, induced inflammation.

Unlike FDP-E-conditioned medium treatment, differentiated 3T3-L1 cells treated with increasing concentrations of FDP-E displayed impaired insulin-stimulated glucose disposal. Non-insulin-stimulated cells displayed no impairment in [3H]2-DG uptake (Fig. 8A), whereas the insulin-treated cells displayed reduced glucose uptake from 998 ± 28 (for control) to 845 ± 65 (P = 0.03), 919 ± 18 (P = 0.02), 829 ± 38 (P = 0.003), and 707 ± 24 counts/min (P < 0.0001) with increasing concentrations of FDP-E from 12.5 nM to 100 nM (Fig. 8B). We also compared glucose disposal between cells treated with 100 nM FDP-E or FgnDP-E with insulin, and only FDP-E decreased the capacity of glucose uptake significantly (707 ± 24 vs. 1,004 ± 41 counts/min for FgnDP-E, P < 0.0001). In vitro, FDP-E increases inflammation in macrophages and adipocytes and is responsible for impaired insulin-stimulated glucose disposal in adipocytes. Since insulin-stimulated glucose uptake was inhibited, but not basal uptake, we measured glucose transporter (GLUT)-4 mRNA expression in 3T3-L1 adipocytes that were treated with insulin ± 100 nM FDP-E. After 18 h of treatment with FDP-E, GLUT-4 mRNA was reduced to 32 ± 0.1% vs. control-treated cells (P = 0.02) (Fig. 8C).

DISCUSSION

It is well known that obesity and chronic inflammation are interrelated as well as an increased risk for thrombosis. It is thought that obese individuals have high levels of cytokines because of adipose tissue remodeling during hypercaloric-induced adipocyte hypertrophy and hyperplasia (19, 28), but how this relates to the coagulation system is not known. Recently, it was reported that tissue factor (TF)-factor VIIa signaling via protease activated receptor-2 induces adipocyte inflammation (2), with the TF/factor VIIa/protease activated receptor-2 signaling complex well-known to be involved in angiogenesis and wound healing. Furthermore, increased plasma cytokines are known to induce hepatic fibrinogen production, which further exacerbates inflammation, especially following fibrinolytic cleavage into FDP-D and FDP-E (8, 16, 26).

Obesity has been shown in numerous clinical (22), cross-sectional (23, 24), and mechanistic studies to be associated with increased fibrinogen expression (18, 25, 26). What is most surprising is the almost complete lack of information concerning the role that fibrinogen plays in obesity-related morbidities. Inflammation has been shown to increase the acute phase production of fibrinogen (5, 22) and is likely to play a major role in promoting, enhancing, and/or prolonging obesity-induced fibrinogen production. Once inflamed adipocytes begin secreting proinflammatory cytokines, they will increase the potential for thrombosis, largely through activation of cell surface TF expression and activity. The increase in TF activity increases thrombin activation, which will then increase the rate of fibrinogen conversion into fibrin. Fibrin deposition has been shown to further increase inflammation, and, based on our

Fig. 5. FDP, not fibrinogen degradation products (FgnDPs), induce inflammation in RAW264.7 cells. A: RAW264.7 macrophage-like cells were treated with FgnDP fragment-E (FgnDP-E) or FDP-E for 6 h. Cells were treated with increasing concentrations of FDP-E, ranging from 0 to 100 nM for 6 h (B) or increasing time from 0 to 12 h (C), and MCP-1 mRNA expression was measured. MCP-1 gene expression increased steadily until 6 h, and the difference between 6 and 12 h was not significant (P = 0.4). Values are means ± SE; n = 4 replicates/group. *P < 0.05 vs. control.

Fig. 6. No effect of conditioned medium from FDP-E-treated RAW264.7 macrophages on glucose uptake in adipocytes. Differentiated 3T3-L1 cells were divided into two groups: without insulin (A) or with insulin (B). Each group was treated with various dilutions of the conditioned medium collected from RAW264.7 cells treated with 100 nM FDP-E or nontreated for 24 h. Basal and insulin-stimulated 2-deoxy-D-[3H]glucose ([3H]2-DG) uptake was measured with no significant differences between groups. This suggests that FDP-E does not indirectly suppress glucose uptake in adipocytes. Values are means ± SE; n = 3 per group. CPM, counts/min.
results, FDP-E may be an integral component in the activation of adipocyte inflammation and monocyte recruitment. In our studies, HF-fed mice increased body weight and glucose intolerance, and we found that hepatic fibrinogen production was increased alongside obesity and inflammation. The plasma levels of fibrinogen were not significantly different; however, with such a large reservoir (>1 mg/dl), it is not surprising that a 22% increase failed to reach significance. Adipose tissue fibrin content was increased in obese mice, and, based on the mechanistic data, we can conclude that fibrin deposition may be responsible for local and systemic inflammation.

Exposure of endothelial cells, leukocytes, and fibroblasts to fibrin has been shown to activate NF-κB and JNK signaling with subsequent increases in MCP-1 expression and secretion (15–17). Fibrinogen and fibrin have been shown to bind to various integrins (αVβ3 or αaβ2), adhesion molecules (ICAM or VCAM), and cell surface receptors (Toll-like receptor-4), all of which have been shown to activate inflammation and induce leukocyte migration. While it is unknown which receptor FDP-E engages to induce inflammation, we are certain that it does in fact activate cytokine production in primary macrophages and RAW264.7 cells. Furthermore, the present studies demonstrate that fibrinogen expression and fibrin deposition increase with obesity and may suggest that coagulation and impaired glucose tolerance could be linked via proinflammatory mechanisms.

Primary macrophages activated by FDP-E expressed an M1-like proinflammatory phenotype, and we suspected that cytokines in the conditioned medium from FDP-E-activated RAW264.7 macrophages would inhibit glucose uptake in adipocytes. There was no significant difference in glucose uptake between control and FDP-E-treated conditioned medium groups, suggesting that the impact of FDP-E in macrophage inflammation may be below the level of physiological significance. However, we employed RAW264.7 cells, which may not be the most appropriate cells to assess the indirect effect of FDP-E-mediated inflammation on adipocyte glucose disposal. RAW264.7 may not be as sensitive to nanomolar concentrations of FDP-E as primary macrophages. It may also be true that FDP-E does not affect glucose uptake in adipocytes indirectly (i.e., via macrophage activation). However, the direct effect of FDP-E on adipocyte inflammation and glucose disposal in adipocytes was clear. Insulin-stimulated glucose uptake showed a decreased ability with increasing concentrations of FDP-E. These results suggest that FDP-E inhibits insulin-stimulated glucose disposal. GLUT-4 is a major glucose transporter in adipocytes, and it is activated by insulin. We suspect that FDP-E reduces glucose uptake through suppression of GLUT-4. There are two possible mechanisms to explain why: one is by decreased gene and/or protein expression of GLUT-4 and another via decreased GLUT-4 translocation.

Fig. 7. FDPs, not FgnDPs, induce inflammation in 3T3-L1 adipocytes. A: mature 3T3-L1 adipocytes were treated with FgnDP-E or FDP-E for 6 h. Cells were treated with increasing concentrations of FDP-E, ranging from 0 to 100 nM for 6 h (B) or increasing time from 0 to 12 h (C), and MCP-1 mRNA expression was measured. Values are means ± SE; n = 4 replicates/group. *P < 0.05 vs. control.

Fig. 8. FDP-E suppresses glucose uptake in adipocytes. Differentiated 3T3-L1 cells were divided into two groups: without insulin (A) or with insulin (B). Each group was treated with increasing concentrations of FDP-E from 0 to 100 nM for 24 h, and [3H]2-DG uptake was measured. FDP-E directly suppressed glucose uptake in adipocytes (the difference between blank and 0 represents basal glucose uptake). C: glucose transporter (GLUT)-4 mRNA expression was significantly reduced with FDP-E treatment. Values are means ± SE; n = 5 replicates/group. *P < 0.05 vs. control.
In addition to its role in hemostasis, the coagulation system, including fibrinogen, fibrin, and FDPs, is known to be involved in tissue remodeling (6, 7, 10, 12, 27). The provisional fibrin matrix, which involves collagen, platelets, and fibrin, serves as the initial step in wound healing, a process that is thought to be essential in matrix remodeling. Within the current understanding of adipose tissue expansion, the extracellular matrix, and inflammation (17, 29), there has been almost a complete lack of attention to the role, cause, and consequence of increased fibrin deposition. Obesity is associated with increased fibrin production, yet the effect of fibrin on adipocyte-mediated glucose disposal was completely unknown before the present study. Now it is clear that localized fibrin deposition may enhance adipocyte inflammation; however, further studies are needed to fully elucidate its role in systemic glucose disposal and insulin sensitivity. We suspect that the endogenous role of FDP-E is to provide an acute, localized signal within the adipose tissue to increase either blood flow, remodeling, or both. The coagulation system is intrinsically linked to the angiogenic system via proinflammatory mechanisms (3, 7, 10, 24), and it is possible that FDP-E could be an early signal for obesity-induced tissue remodeling and/or expansion. However, we are limited in our speculation in that we did not assess tissue remodeling, yet further studies into this aspect would be warranted.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: M.K., R.A.V., and C.M.P. performed experiments; M.K., R.A.V., and C.M.P. analyzed data; M.K., R.A.V., and C.M.P. interpreted results of experiments; M.K., R.A.V., and C.M.P. prepared figures; M.K. and C.M.P. drafted manuscript; M.K., R.A.V., and C.M.P. edited and revised manuscript; M.K., R.A.V., and C.M.P. approved final version of manuscript; R.A.V. and C.M.P. conception and design of research.

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