Adipogenic Effects and Gene Expression Profiling of Firemaster® 550 Components in Human Primary Preadipocytes

Emily W.Y. Tung, Vian Peshdary, Remi Gagné, Andrea Rowan-Carroll, Carole L. Yauk, Adèle Boudreau, and Ella Atlas

Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, Canada

BACKGROUND: Exposure to flame retardants has been associated with negative health outcomes including metabolic effects. As polybrominated diphenyl ether flame retardants were pulled from commerce, human exposure to new flame retardants such as Firemaster® 550 (FM550) has increased. Although previous studies in murine systems have shown that FM550 and its main components increase adipogenesis, the effects of FM550 in human models have not been elucidated.

OBJECTIVES: The objectives of this study were to determine if FM550 and its components are active in human preadipocytes, to further investigate their mode of action.

METHODS: Human primary preadipocytes were differentiated in the presence of FM550 and its components. Differentiation was assessed by lipid accumulation and expression of peroxisome proliferator-activated receptor γ (PPARG), fatty acid binding protein (FABP) 4 and lipoprotein lipase (LPL). mRNA was collected for Poly (A) RNA sequencing and was used to identify differentially expressed genes (DEGs). Functional analysis of DEGs was undertaken in Ingenuity Pathway Analysis.

RESULTS: FM550 triphenyl phosphate (TPP) and isopropylated triphenyl phosphates (IPTP), increased adipogenesis in human primary preadipocytes as assessed by lipid accumulation and mRNA expression of regulators of adipogenesis such as PPARG, CCAAT enhancer binding protein (C/EBP) α and sterol regulatory element binding protein (SREBP) 1 as well as the adipogenic markers FABP4, LPL and perilipin. Poly (A) RNA sequencing analysis revealed potential modes of action including liver X receptor/rexinoid X receptor (LXR/RXR) activation, thyroid receptor (TR)/RXR, protein kinase A, and nuclear receptor subfamily 1 group H members activation.

CONCLUSIONS: We found that FM550, and two of its components, induced adipogenesis in human primary preadipocytes. Further, using global gene expression analysis we showed that both TPP and IPTP likely exert their effects through PPARG to induce adipogenesis. In addition, IPTP perturbed signaling pathways that were not affected by TPP. https://doi.org/10.1289/EHP1318

Introduction

Stringent flammability standards set in the state of California resulted in the widespread use of chemical flame retardants in commercial products (Dodson et al. 2012). Of these, the polybrominated diphenyl ethers (PBDEs) were among the most abundantly used; however, due to their toxicity and bioaccumulative properties, they were phased out of commerce. As such, industry was required to find alternatives such as the proprietary mixture Firemaster® 550 (FM550), which is used in commercial products including furniture, textiles, and electronics (Belcher et al. 2014; Stapleton et al. 2008).

FM550 is composed of four different compounds: bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) (8%), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) (30%), triphenyl phosphate (TPP) (17%), and isopropylated triphenyl phosphates (IPTPs) (45%) (Stapleton et al. 2008). The IPTPs consist of a mixture of mono-isopropylphenyl, diphenyl-phosphate, di-isopropylphenyl, phenyl-phosphate, and tris-isopropylphenyl-phosphate in various proportions (Phillips et al. 2016). Measurement of the FM550 metabolites diphenyl-phosphate (DPHP) and isopropyl-diphenyl-phosphate (ip-DPHP) in urine confirmed that exposure to the chemical mixture FM550 is ubiquitous (Hoffman et al. 2014). In addition, recent studies show that some of the FM550 components concentrations can reach 15,030 ng/g in house dust (Stapleton 2008) and that the metabolite ip-DPHP is ubiquitous in the urine of children at concentrations up to 24 ng/mL (Hoffman et al. 2014).

A few studies suggest that FM550 and its components have metabolic effects and act as environmental obesogens. For example, in a rodent model, perinatal and lactational exposure to FM550 induced behavioral and endocrine effects, increased adipose mass, and induced insulin resistance in the offspring (Patisaul et al. 2013). In addition, a study in murine stem cells showed that the FM550 components TPP and IPTP divert osteogenesis to the adipogenesis pathway through activation of peroxisome proliferator-activated receptor γ (PPARγ) (Pillai et al. 2014).

Although aforementioned studies (Patisaul et al. 2013; Pillai et al. 2014) suggest that FM550 is an endocrine disruptor and an environmental obesogen in murine cell cultures and animal models, little is known regarding its effects on human health and obesity. Primary human preadipocytes are a relevant tool to test the ability of chemicals to induce adipogenesis in human specimens and hence can identify a potential role for these chemicals to cause metabolic effects in humans (Boucher et al. 2014a; Boucher et al. 2014b). Previous work showed that the transcriptional cascade differs in human and murine differentiating preadipocytes (Tomlinson et al. 2006, 2010). This suggests that chemicals have potentially different specific targets in human cells compared with mouse cells. Further, human preadipocytes have different requirements for optimal differentiation compared with the mouse models (Tomlinson et al. 2006). One major difference is the requirement of clonal expansion for the murine cell model (3T3-L1) but not for the human primary preadipocytes (Janderová et al. 2003; Yeh et al. 1995). In addition, human primary preadipocytes require both dexamethasone (glucocorticoid pathway). Conversely, murine 3T3-L1 preadipocytes...
differentiate with either dexamethasone or a PPARγ agonist (Ahmed and Atlas 2016). Finally, human preadipocytes are primary cells, and therefore provide an in vitro model that is more relevant to the human condition and relevant for obesogen screening.

The purpose of this study was to determine the effects of FM550 and its individual components on adipogenesis in human primary preadipocytes, and to explore modes of action through analysis of global transcriptomic response to these chemicals. We show that FM550 and its two major components, TPP and IPTP, induce adipogenesis in human primary cells. Furthermore, global gene expression analysis revealed that pathways other than PPARγ may also be involved in the adipogenic changes induced in differentiating human cells in response to FM550 components.

Materials and Methods

Reagents

Chemicals were purchased from the following manufacturers: human insulin (Roche Diagnostics, Indianapolis, IN, USA); 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), troglitazone, triphenyl phosphate (TPP), and dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Oakville, Ontario, Canada); 2-ethylhexyl-2,3,4,5-tetrahydrobenzoate (TBB) and bis(2-ethylhexyl)-2,3,4,5-tetramethylphthalate (Toronto Research Chemicals, Toronto, Ontario, Canada). Isopropylated triphenyl phosphate (IPTP) was a generous gift from W. Casey (National Institute of Environmental Health Sciences, National Institutes of Health in the United States). Analysis of the IPTP mixture was hampered by the lack of availability of pure standards for the various congeners suspected to be present. By comparing mass estimates of the whole molecules (from positive chemical ionization analyses) with those of molecular fragments (from electron impact mode analyses) we estimate that the majority of the material is composed of three congeners. These were triphenyl phosphate (TPP ∼18%); monoisopropylphenyl, diphenylphosphate (mITP ∼55%); and di(isopropylphenyl), phenylphosphate (dITP ∼25%). In addition, there was a small amount of tris(isoproplyphenyl)phosphate present in the test material, but at a substantially lower concentration (∼1.5%). Firemaster® 550 (FM550) was a generous gift from B. Chittam (Wellington Laboratories, Guelph, Ontario, Canada).

Culture and Differentiation of Human Primary Subcutaneous Preadipocytes

Primary human subcutaneous preadipocytes (ZenBio, Inc., Research Triangle Park, NC, USA) were obtained from female donors ages 25, 38, 40, 39, and 34 y with body mass indices of 18.8, 21.6, 23.3, 22.6, 21.5 (kg/m²) and who were of different ethnicities (one Caucasian, two Hispanic, one Asian, and one unknown) were taken from thigh, back, abdomen, and flank depots and differentiated as previously described (Boucher et al. 2016) with modifications. Briefly, human primary preadipocytes were seeded in six-well dishes in subcutaneous preadipocyte media (PM-1, Zenbio Inc.), containing 10% fetal calf serum (Wisent, Montreal, Quebec, Canada). When cells reached confluence (day 0), they were treated with 100 nM insulin (I) and 500 μM IBMX (M) until day 4, with a media change on day 2. From day 4 onward, cells received insulin with media changes on days 4 and 8. For the positive control (MIDT), where the cells require both dexamethasone and troglitazone, dexamethasone (D) (1 μM) was added from days 0 to 14 and troglitazone (T) (5 μM) from days 2 to 14 in addition to MI. Treatments with the chemicals of interest were performed as follows: a) when the chemicals were tested for their ability to replace troglitazone, the cells were treated with dexamethasone from day 0 and the test chemical (0–200 μM FM550, 0–200 μM IPTP, 0–20 μM TPP, 0–20 μM TBPH, 0–20 μM TBB) from day 2, with media replacements on days 4 and 8 (MID condition); b) when the chemicals were to replace dexamethasone, the test chemical was added from day 0, and troglitazone with the test chemical were added from day 2 onward and replaced with media changes on days 2, 4, and 8 (MIT condition). IBMX and insulin were added in all treatments as described above for the MIDT condition.

Nile Red Staining of Lipids

Primary human preadipocytes were differentiated as described above for 14 d with the indicated treatments and controls in black collagen-coated 96-well plates (Fisher Scientific, Canada). The level of differentiation was assessed using a fluorescence plate reader as follows. At day 14, cells were fixed with 4% paraformaldehyde (WVR, Canada) for 30 min followed by PBS washes. Background fluorescence was read in PBS at 485/528 for Nile red and 360/460 for DAPI. Cells were then stained with Nile red (1 μg/mL) to stain for lipid droplets and DAPI (1 μg/mL) to stain nuclei as previously described (Greenspan et al. 1985). Nile red fluorescence was read at 485/528 nm (excitation/emission) in a Synergy 2 fluorescence plate reader (BioTek Instruments, Inc., Winooski, VT, USA) and DAPI staining, nuclei staining, was measured at 360/460 nm. To calculate Nile red to DAPI ratios the background fluorescence was first subtracted from the readings of Nile red and DAPI at the respective wavelengths, and Nile red/DAPI ratios were calculated for each of the wells.

Western Blotting

Human primary preadipocytes were seeded in six-well dishes and treated according to the differentiation protocol described above. On day 14, cells were lysed in RIPA buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% sodium deoxycholate, 2% NP-40, 0.4% SDS, 10% glycerol) containing protease inhibitors (Roche Diagnostics, Laval, Quebec, Canada). Western blots were performed by probing with primary fatty acid binding protein (FABP) 4 and β-actin ACTB antibodies (Cell Signaling Technology, Danvers, MA, USA) followed by appropriate HRP-linked secondary antibodies, and developed using Clarity Western ECL Substrate (BioRad, Hercules, CA, USA). Relative optical densities were quantified using Image Lab software (BioRad), and values of terminal differentiation markers were normalized to ACTB levels.

mRNA Extraction and Real-Time Quantitative PCR (RT-qPCR)

Total RNA was extracted from differentiating human primary preadipocytes, treated as previously described. Samples were taken on days 4, 6, 9, and 12 using the RNeasy Mini kit and genomic DNA was eliminated using the RNeasy-Free DNase Kit (Qiagen, Mississauga, Ontario, Canada). RNA was reverse transcribed using iScript cDNA Synthesis Kit (BioRad). cDNA expression levels were analyzed by the CFX96-PCR Detection System using the iQSYBR SsoFast EvaGreen Supermix (BioRad). Primer sequences for each gene are summarized in Table S1. Primer efficiencies were ≥90% and specificity was confirmed by sequence blast and melting curve analysis. All target gene transcripts were normalized to ACTB expression, which was not affected by treatment. Fold inductions were calculated using time-matched, control solvent-treated samples, and the comparative CT (ΔACT) method was used for data analysis.

For RNA-seq analysis, total RNA was extracted from differentiating cells treated as described above using the RNeasy Kit and genomic DNA was eliminated using the RNeasy-Free DNase
Canonical pathways, diseases, and biological functions, using adi-

Student’s t-test was used when comparing two means. Significance was defined as \( p \leq 0.05 \). Statistical analyses were performed using SigmaPlot software version 12.5 (San Jose, CA, USA) or GraphPad Prism version 7 (La Jolla, CA, USA).

**Results**

**Effects of FM550, IPTP, and TPP on Adipogenesis in Human Primary Preadipocytes**

Previous studies in murine cell models have shown that FM550, and its component TPP, increased adipogenesis through direct activation of PPAR\( \gamma \) (Pillai et al. 2014). We investigated whether this is also true for human primary preadipocytes by assessing lipid accumulation and expression levels of adipogenic markers. Two conditions were used to establish whether FM550 and its components induce differentiation in human primary preadipocytes and if they act only through PPARG in this model system. One condition was a differentiation protocol where the chemicals of interest were added in the presence of IBMX, insulin, and dexamethasone (MID). The second condition was when the chemicals were added to human preadipocytes exposed to IBMX, insulin, and troglitazone (MIT). In positive controls, IBMX, insulin, dexamethasone, and troglitazone were added (MIDT).

Lipid accumulation was measured on day 14 by Nile red fluorescence normalized to DAPI fluorescence. Human primary preadipocytes treated with the FM550 mixture exhibited a dose-dependent increase in lipid accumulation, with statistical significance achieved in the MID condition at 200 \( \mu M \) FM550, where a 4-fold increase as compared with MID alone was achieved (Figure 1A). TPP also induced a 4-fold increase in lipid accumulation relative to MID controls at 20 \( \mu M \) (Figure 1B). An increasing trend was also seen with TPP treatment in the MIT condition, but this was not statistically significant. IPTP treatment induced a significant ~4-fold increase in lipid accumulation by Nile red staining at both 10 and 20 \( \mu M \), as compared with the MID condition (Figure 1C). An increasing trend was also observed in the MIT condition; however, this increase did not reach statistical significance (Figure 1C). TBB and TBPH did not increase lipid accumulation, although an increasing trend was observed in the TBB dose–response curve (Figure 1D,E). As expected, the positive control MIDT increased lipid accumulation (see Figure S1).

**Effects of FM550, IPTP, and TPP on FABP4 Protein Expression in the Presence of Dexamethasone**

To further assess the extent of adipocyte differentiation and to delineate whether in human preadipocytes FM550 and its components up-regulate the expression of known PPARG targets, protein levels of the mature adipocyte marker, FABP4, were measured by Western blotting. In the presence of MID, 200 \( \mu M \) FM550 significantly increased FABP4 expression by ~4-fold compared with MID controls; however, this effect was not observed when the mixture was added in the presence of MIT (Figure 2A). This may be partially due to the higher background in the presence of troglitazone given that FABP4 is a known target of PPARG. Similarly, 20 \( \mu M \) TPP induced a 6-fold increase in FABP4 expression relative to MID (Figure 2B), although increasing trends were observed at lower concentrations but did not reach statistical significance. Again, this effect was not observed in the presence of MIT (Figure 2B). Remarkably, IPTP treatment induced an increase not only at 20 \( \mu M \) (10-fold), but
Effects of TPP and IPTP on the mRNA Expression of Transcriptional Regulators of Adipogenesis and Adipogenic Markers in Human Primary Preadipocytes

Our initial findings indicated that the MID condition was optimal for FM550-induced human adipocyte differentiation and that the FM550 components TPP and IPTP-containing 18% TPP were adipogenic. Therefore, the temporal changes in the mRNA levels of transcription factors and differentiation markers of adipogenesis were assessed in response to 20 μM TPP and 20 μM IPTP relative to MID controls.

First we assessed the mRNA levels of master regulators of adipogenesis, PPARG and CCAT enhancing binding protein α (CEBPA). PPARG expression was increased through the course of differentiation in TPP- and IPTP-treated cells relative to MID controls (Figure 3A). CEBPA levels were also increased following treatment with both TPP and IPTP during adipogenesis compared with MID controls (Figure 3B). In the TPP-treated cells, this increase was observed earlier in differentiation; however, similar levels were attained by both chemicals by the end of differentiation (Figure 3E). For the mature adipocyte markers, both FABP4 and lipoprotein lipase (LPL) mRNA expression levels were increased with adipogenesis in response to both TPP and IPTP relative to MID control. Again, although the increase with IPTP was delayed relative to TPP, both components led to similar levels by the end of differentiation (Figure 3C, D). Perilipin (PLIN) mRNA expression, indicative of lipid droplet formation, was also up-regulated by TPP and IPTP during adipogenesis as compared with MID control. TPP increased PLIN levels as early as day 6, and the levels were maintained throughout the differentiation process. However, the increase by IPTP was to a lower extent than in TPP-treated cells and reached statistical significance only at day 9 (Figure 2E). We also measured the mRNA levels of the lipogenic transcription factor, sterol regulatory element binding transcription factor 1 (SREBF1), and found that both TPP and IPTP increased its expression during differentiation as compared with MID (Figure 3F). As expected, the positive control (i.e., MID) increased the expression of PPARG, CEBPA, FABP4, LPL, PLIN, and SREBF1 throughout the differentiation process as illustrated in Figure S2.

Transcriptional Profiling of Human Primary Preadipocytes Treated with TPP, IPTP, and Troglitazone

To further investigate the effects of TPP and IPTP on the adipogenic pathway, human primary preadipocytes were differentiated in the presence of MID supplemented with 20 μM TPP, 20 μM IPTP, or 5 μM troglitazone. At day 6 of differentiation, RNA was collected from five donors and used for RNA-seq. Overall, TPP and IPTP treatments resulted in 380 and 713 DEGs, respectively, whereas troglitazone treatment resulted in 3,277 (Figure 4). Of the DEGs affected by TPP, only 5.8% were distinct; in contrast, 29% of DEGs affected by IPTP were unique. Most of the genes up-regulated by TPP (84%) were in common with troglitazone; whereas only 65% of the IPTP affected genes were also affected by troglitazone. Hierarchical cluster analysis of the fold changes for all DEGs from IPTP, troglitazone, and TPP shows that TPP- and IPTP-induced expression profiles cluster together and apart from troglitazone, indicating that they more closely resemble each other than the troglitazone-treatment group (Figure 4B). In addition, pairwise analysis using fold changes of the common genes shows that TPP-induced expression profiles are more strongly correlated with troglitazone and IPTP (correlation = 0.87 and 0.86, respectively), than IPTP is correlated with troglitazone (correlation = 0.75). All correlations are significant with a p-value of <0.00001. Overall, this analysis demonstrates that the DEGs in common across the treatments are highly correlated, and that IPTP is less similar to troglitazone than TPP. The top 10 up- and down-regulated DEGs affected by TPP, IPTP, and troglitazone are listed in Table 1. Table 1 shows that 45% of the top DEGs were common between TPP and IPTP, 20% of the top DEGs in TPP-treated cells were in common with troglitazone, and only 5% of the top DEGs in IPTP-treated cultures were in common with troglitazone.
Validation of Select Differentially Expressed Genes by RT-qPCR

We validated some of the DEGs found in the RNA-seq analysis by RT-qPCR. Of the up-regulated genes, FABP5, PLIN4, and phosphoenolpyruvate carboxykinase 1 (PCK1) were increased in both TPP- and IPTP-treated cells. However, only TPP significantly increased the mRNA levels of the above genes (Figure 5A–C). By contrast, other genes including ATP-binding cassette subfamily G member 1 (ABCG1), solute carrier organic anion transporter family, member 4C1 (SLCO4C1), and FABP3 were significantly increased by IPTP and not TPP (Figure 5D–F). It is possible that by adding more repeats we would have reached statistical significance for FABP5, PCK1, PLIN4, ABCG1, and SLCO4C1 for both treatments. Some genes were equally increased by both treatments (Figure 5G–I). All of these genes were up-regulated by troglitazone control (see Figure S3). Interestingly, lipopolysaccharide binding protein (LBP) levels were down-regulated in IPTP-treated cells, not affected by TPP exposure, and up-regulated in troglitazone positive controls (Figure 5J; see also Figure S3). Further, keratin 18 (KRT18) levels were decreased by both TPP and IPTP; however, the expression of this gene was not changed in response to troglitazone (Figure 5K; see also Figure S3).

Canonical Pathways and Upstream Regulators Identified in IPA’s Knowledge Base in Human Primary Preadipocytes Treated with TPP and IPTP

The DEGs obtained from the RNA-seq analysis of TPP, IPTP, and troglitazone treatments were analyzed using IPA to identify
The enrichment of canonical pathways and upstream regulators. The top canonical pathways for each treatment identified in IPA are summarized in Table 2. As expected, the adipogenesis pathway was significantly enriched in TPP-, IPTP-, and troglitazone-treatment groups. In addition, there were several common pathways between TPP and IPTP treatments, such as liver X receptor (LXR)/retinoid X receptor (RXR) activation, cholesterol biosynthesis I, and lipopolysaccharide (LPS)/interleukin-1 (IL-1)–mediated inhibition of RXR function (Table 2A,B). Of these pathways, only LPS/IL-1–mediated inhibition of RXR function
The pathway was also in the top 20 pathways affected by troglitazone (Table 2C). Only 65% of the canonical pathways were common to both TPP and IPTP treatments. Some of the distinct pathways identified in TPP-treated cells were protein kinase A signaling, AMP-activated protein kinase (AMPK) signaling, and type II diabetes mellitus signaling. In the IPTP treatment, unique pathways included caveolar-mediated endocytosis signaling, clathrin-mediated endocytosis signaling, and acute phase response signaling. Of the top 20 pathways affected by IPTP and TPP treatment, several pathways overlapped and included superpathway of cholesterol biosynthesis, cholesterol biosynthesis (I, II, III), LXR/RXR activation (Table 2A,B). Troglitazone treatment was also associated with effects on pathways such as cAMP-mediated signaling and PXR/RXR activation, in addition to mitochondrial dysfunction and oxidative phosphorylation pathways (Table 2C).

Using IPA we also identified potential upstream regulators of the DEGs. As expected, PPARγ was identified as a common top upstream regulator in an activated state for all treatments. In addition, SREBF1, another adipogenic transcriptional regulator, was found among the top upstream regulators. Accordingly, the insulin-induced gene (INSIG) 1 and 2, proteins known to be involved in the negative regulation of SREBF1 function, were predicted to be inhibited in all treatments. The membrane-bound transcription factor site-1 protease (MBTPS1) (also known as serine protease 1: S1P), a serine protease involved in SREBF1 activation, was predicted to be activated in all treatments as expected although not always in the top 20 (Inoue et al. 2001). By contrast, the transcription factor Krüppel-like factor (KLF) 15, known to be involved in adipocyte differentiation (Mori et al. 2005), was predicted to be active only in TPP and troglitazone treatments but not IPTP (Table 3). Furthermore, CEBPA, another important transcription factor in adipogenesis (Lane et al. 1996) was found as an upstream regulator in TPP and troglitazone treatments (not in the top 20), but not IPTP. Finally, lamin B1 (LMNB1), a matrix protein involved in cytoskeletal organization and adipogenesis (Verstraeten et al. 2011), was predicted to be inhibited by IPTP but not TPP or troglitazone treatment.

Using the common and unique DEGs to the three treatments identified by the Venn diagram (Figure 4) upstream regulators were identified in IPA. Table 4 shows the top upstream regulators using the common DEGs and Table 5 lists the upstream regulators based on the unique DEGs for TPP, IPTP, and troglitazone. As expected, PPARγ was the top common upstream regulator for all treatments. Interestingly, based on the unique DEGs for TPP there were only few upstream regulators identified (Table 5A). IPTP had many more upstream regulators identified in IPA from which the top ones based on the number of molecules involved are being listed (Table 5B). As expected, troglitazone had the most upstream regulators identified in IPA based on a much larger number of unique DEGs, and the top 10 are listed in Table 5C.

**Discussion**

We found that FM550, and its components TPP and IPTP, induce adipogenesis in human primary preadipocytes as demonstrated...
| Gene symbol | Gene name | GO biological process | Fold change | FDR (p-value) |
|-------------|-----------|-----------------------|-------------|---------------|
| **ITIH1**   | Inter-alpha-trypsin inhibitor heavy chain 1 | Hyaluronan metabolic process | 7.9 | <0.0001 |
| **PKC1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 7.1 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 6.6 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 5.7 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 5.6 | <0.0001 |
| **SLCO4C1** | Solute carrier organic anion transporter family, member 4C1 | Cell differentiation | 5.3 | <0.0001 |
| **FND5**    | Fibronectin type III domain-containing protein 5 | Response to muscle activity | 4.6 | <0.0001 |
| **ADIPOQ**  | Adiponectin, C1Q, and collagen domain containing | Adiponectin-activated signaling pathway | 4.5 | <0.0001 |
| **CDH1**    | Cadherin-related family member 1 | Homophilic cell adhesion via plasma membrane adhesion molecules | 4.3 | <0.0001 |
| **ITIH5**   | Inter-alpha (globulin) inhibitor H5 | Negative regulation of peptidase activity | 4.3 | <0.0001 |
| **PCK1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 4.2 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 4.2 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 4.2 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 4.2 | <0.0001 |
| **SLCO4C1** | Solute carrier organic anion transporter family, member 4C1 | Cell differentiation | 4.2 | <0.0001 |
| **FND5**    | Fibronectin type III domain-containing protein 5 | Response to muscle activity | 4.2 | <0.0001 |
| **ADIPOQ**  | Adiponectin, C1Q, and collagen domain containing | Adiponectin-activated signaling pathway | 4.2 | <0.0001 |
| **CDH1**    | Cadherin-related family member 1 | Homophilic cell adhesion via plasma membrane adhesion molecules | 4.2 | <0.0001 |
| **ITIH5**   | Inter-alpha (globulin) inhibitor H5 | Negative regulation of peptidase activity | 4.2 | <0.0001 |
| **PCK1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 4.2 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 4.2 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 4.2 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 4.2 | <0.0001 |
| **SLCO4C1** | Solute carrier organic anion transporter family, member 4C1 | Cell differentiation | 4.2 | <0.0001 |
| **FND5**    | Fibronectin type III domain-containing protein 5 | Response to muscle activity | 4.2 | <0.0001 |
| **ADIPOQ**  | Adiponectin, C1Q, and collagen domain containing | Adiponectin-activated signaling pathway | 4.2 | <0.0001 |
| **CDH1**    | Cadherin-related family member 1 | Homophilic cell adhesion via plasma membrane adhesion molecules | 4.2 | <0.0001 |
| **ITIH5**   | Inter-alpha (globulin) inhibitor H5 | Negative regulation of peptidase activity | 4.2 | <0.0001 |
| **PCK1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 4.2 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 4.2 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 4.2 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 4.2 | <0.0001 |
| **SLCO4C1** | Solute carrier organic anion transporter family, member 4C1 | Cell differentiation | 4.2 | <0.0001 |
| **FND5**    | Fibronectin type III domain-containing protein 5 | Response to muscle activity | 4.2 | <0.0001 |
| **ADIPOQ**  | Adiponectin, C1Q, and collagen domain containing | Adiponectin-activated signaling pathway | 4.2 | <0.0001 |
| **CDH1**    | Cadherin-related family member 1 | Homophilic cell adhesion via plasma membrane adhesion molecules | 4.2 | <0.0001 |
| **ITIH5**   | Inter-alpha (globulin) inhibitor H5 | Negative regulation of peptidase activity | 4.2 | <0.0001 |
| **PCK1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 4.2 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 4.2 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 4.2 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 4.2 | <0.0001 |
| **SLCO4C1** | Solute carrier organic anion transporter family, member 4C1 | Cell differentiation | 4.2 | <0.0001 |
| **FND5**    | Fibronectin type III domain-containing protein 5 | Response to muscle activity | 4.2 | <0.0001 |
| **ADIPOQ**  | Adiponectin, C1Q, and collagen domain containing | Adiponectin-activated signaling pathway | 4.2 | <0.0001 |
| **CDH1**    | Cadherin-related family member 1 | Homophilic cell adhesion via plasma membrane adhesion molecules | 4.2 | <0.0001 |
| **ITIH5**   | Inter-alpha (globulin) inhibitor H5 | Negative regulation of peptidase activity | 4.2 | <0.0001 |
| **PCK1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 4.2 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 4.2 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 4.2 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 4.2 | <0.0001 |
| **SLCO4C1** | Solute carrier organic anion transporter family, member 4C1 | Cell differentiation | 4.2 | <0.0001 |
| **FND5**    | Fibronectin type III domain-containing protein 5 | Response to muscle activity | 4.2 | <0.0001 |
| **ADIPOQ**  | Adiponectin, C1Q, and collagen domain containing | Adiponectin-activated signaling pathway | 4.2 | <0.0001 |
| **CDH1**    | Cadherin-related family member 1 | Homophilic cell adhesion via plasma membrane adhesion molecules | 4.2 | <0.0001 |
| **ITIH5**   | Inter-alpha (globulin) inhibitor H5 | Negative regulation of peptidase activity | 4.2 | <0.0001 |
| **PCK1**    | Phosphoenolpyruvate carboxykinase 1 | Glucose metabolic process | 4.2 | <0.0001 |
| **FABP4**   | Fatty acid binding protein 4 | Cholesterol homeostasis | 4.2 | <0.0001 |
| **GPBAR1**  | G-protein-coupled bile acid receptor 1 | Cell surface bile acid receptor signaling pathway | 4.2 | <0.0001 |
| **PPP1R1A** | Protein phosphatase 1 regulatory inhibitor subunit 1A | Glycogen metabolic process | 4.2 | <0.0001 |
by lipid accumulation and expression of adipogenic markers. Further, we found that IPTP was able to increase lipid accumulation and FABP4 protein expression at lower concentrations than TPP. Our findings are in agreement with a previous study showing that treatment of murine pluripotent cells with the FM550 components TPP and IPTP diverted the cells to an adipogenic fate, as assessed by lipid accumulation and perilipin levels (Pillai et al. 2014). However, (Pillai et al. 2014) used an IPTP mixture containing 40% TPP and concluded that the main adipogenic component in FM550 was likely TPP. Here we show in human primary preadipocytes that an IPTP mixture containing only 18% TPP was more potent than pure TPP at inducing lipid accumulation and FABP4 expression.

The human preadipocyte differentiation model system allowed us to explore the mode of action of the chemicals of interest leading to adipogenesis and, potentially, obesity whether through PPARG activation or glucocorticoid pathways. Chemicals are tested for their ability to activate PPARG when replacing troglitazone in the differentiation medium and via the glucocorticoid pathway when chemicals are replacing dexamethasone. FM550 and its components, TPP and IPTP, are able to increase lipid accumulation in the presence of dexamethasone in human preadipocytes, although a positive trend, which did not reach statistical significance, was also observed in the presence of troglitazone for this end point in our study. It is very likely that statistical significance would have been achieved if more donors were used for the experiments. Using a murine model, others have shown that TPP and IPTP increased lipid accumulation; however, it is unclear which nuclear receptor these chemicals are targeting because murine cell models require either dexamethasone or troglitazone for differentiation leading to adipogenesis and, potentially, obesity whether through PPARG activation or glucocorticoid pathways.

Global gene expression analysis to evaluate the mode of action of the chemicals of interest leading to adipogenesis and, potentially, obesity whether through PPARG activation or glucocorticoid pathways. Chemicals are tested for their ability to activate PPARG when replacing troglitazone in the differentiation medium and via the glucocorticoid pathway when chemicals are replacing dexamethasone. FM550 and its components, TPP and IPTP, are able to increase lipid accumulation in the presence of dexamethasone in human preadipocytes, although a positive trend, which did not reach statistical significance, was also observed in the presence of troglitazone for this end point in our study. It is very likely that statistical significance would have been achieved if more donors were used for the experiments. Using a murine model, others have shown that TPP and IPTP increased lipid accumulation; however, it is unclear which nuclear receptor these chemicals are targeting because murine cell models require either dexamethasone or troglitazone for differentiation (Gimble et al. 1990). Our lipid accumulation data suggest that FM550, TPP, and IPTP may have other effects in addition to PPARG activation. Further, we have shown that in the murine 3T3-L1 model, the lipogenesis (lipid accumulation) mediated by dexamethasone and IPTP was not inhibited by the PPARG antagonist GW9662, whereas troglitazone-mediated lipogenesis was (Tung et al. 2017). Moreover, in our study the test chemicals were added in the presence of 5 μM troglitazone, a concentration by which PPARG is likely to be saturated (Nagai et al. 2011) and unlikely to be further activated, supporting that mechanisms beyond PPARG activation are at work for lipogenesis. Therefore one may hypothesize that the glucocorticoid receptor may be a target. However, TPP and IPTP were not able to activate the glucocorticoid receptor in luciferase reporter assays (data not shown). Of further note, Pillai et al. (2014) showed a modest increase in PPAR transactivation by TPP and IPTP relative to solvent control, and also showed that TPP was more potent than IPTP in mediating this effect. Therefore, these authors concluded that TPP was the adipogenic component in FM550 (Pillai et al. 2014). Interestingly, we observed a higher PPARG transactivation in IPTP treatments compared with TPP treatments in luciferase assays (data not shown). Of importance, our mixture of isoproprylated compounds (IPTP) contained only 18% TPP by analysis, whereas Pillai et al. (2014) used a mixture containing 40%. Therefore, one may not conclude that TPP is the main PPARG activator in the FM550 mixture or that this is the only mechanism of action of the chemicals.

In addition to using a human model to assess the effects of FM550 components in inducing adipogenesis, we also performed global gene expression analysis to evaluate the mode of action of these chemicals. To the best of our knowledge, this study is the first to compare global transcriptomic changes in response to FM550 components in differentiating human preadipocyte or any other system. Interestingly, we found that IPTP treatment resulted in twice the number of affected DEGs as TPP. About half of the
DEGs a

ff

ected by IPTP were distinct (i.e., were not shared with
either TPP or troglitazone). In contrast, most of the TPP-a
ffected DEGs were in common with troglitazone treatment. This sug-
gests that TPP acts mainly through PPARG activation, whereas
IPTP perturbed a variety of regulatory pathways beyond just
PPARG. Indeed, ∼84% of the DEGs induced by TPP treatment
were in common with DEGs induced by the PPARG agonist tro-
glitazone, as opposed to 64% DEGs in common between IPTP
and troglitazone.

When we assessed the transcript levels of select DEGs by
RT-qPCR, we found striking di
r
erences between the three treat-
ments. The fold change in the expression of some DEGs was
larger in the TPP treatment versus the IPTP treatment, and
vice
r
ersa.
For example, PCK1, PLIN4, and FABP5 were all increased
to a greater extent by TPP. All these genes are involved in lipogen-
esis, an integral part of adipocyte differentiation (Ducharme and
Bickel 2008). Interestingly, IPTP treatment induced higher expres-
sion levels of transporters, such as ABCG1 and SLCO4C1 than

TPP. ABCG1 expression is positively correlated with triglyceride
accumulation during adipogenesis by increasing fatty acid influx
(Frisdal et al. 2015). Fatty acids are known to act as endogenous
PPARG agonists (Krey et al. 1997), and therefore this may be a
plausible mechanism by which these chemicals indirectly activate
PPARG. SLCO4C1 is a member of the transporter superfamily
mediating the transport of thyroid hormones, T3 and T4 (van der
Deure et al. 2010). It has been shown that when preadipocytes are
transduced with an inactive thyroid hormone receptor mutant a
decrease in the expression of adipogenic markers is observed (Liu
et al. 2015). This suggests that increased intracellular thyroid hor-
mone levels, which may be mediated by SLCO4C1, enhance
human adipogenesis.

The distinct effects observed in the TPP and IPTP treatments
are also apparent in the canonical pathways enriched in IPA. For
TPP treatment, many of the pathways were in common with the
troglitazone treatment, as expected, although some were unique.
Of those, protein kinase A signaling is relevant, as it is elevated

Figure 5. Validation of selected differentially expressed genes (DEGs) by RT-qPCR. Human primary preadipocytes were differentiated in the presence of MID supplemented with 20 μM TPP or 20 μM IPTP. At day 6 of differentiation, RNA was collected for RNA-seq analysis. The mRNA levels of select DEGs from RNA-seq analysis were quantified by RT-qPCR. Levels were normalized to endogenous ACTB mRNA, and expressed as a fold over the control condition (MID) for each treatment. Results from five separate donor samples are graphically presented as means ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 for TPP- and IPTP- treated samples compared with control; #p < 0.05, ##p < 0.01, and ###p < 0.001 for TPP-treated compared with IPTP-treated samples, as assessed by one-way ANOVA with Tukey’s post hoc tests.
early in adipogenesis (Klemm et al. 1998). During a standard differentiation protocol, IBMX is used to increase intracellular cAMP concentrations, which in turn activates PKA (Reusch et al. 2000). Because all treatments contained IBMX, a phosphodiesterase inhibitor, it is remarkable that only TPP further increased this process. Another relevant pathway that was enriched in TPP was the LXR/RXR activation pathway.

### Table 2. Top 20 significant canonical pathways identified by IPA.

| IPA canonical pathway                                                                 | p-Value    | Ratio |
|--------------------------------------------------------------------------------------|------------|-------|
| **TPP**                                                                             | <0.0001    | 0.227 |
| LXR/RXR activation                                                                 | <0.0001    | 0.099 |
| LPS/IL-1–mediated inhibition of RXR function                                        | <0.0001    | 0.177 |
| Superpathway of cholesterol biosynthesis                                            | <0.0001    | 0.177 |
| TR/RXR activation                                                                   | <0.0001    | 0.177 |
| Protein kinase A signaling                                                          | 0.009      | 0.0526|
| Tight junction signaling                                                            | 0.0002     | 0.0909|
| AMPK signaling                                                                      | 0.001      | 0.0758|
| Adipogenesis pathway                                                                | 0.0003     | 0.0947|
| Hepatic fibrosis/hepatic stellate cell activation                                   | 0.001      | 0.0796|
| ILK signaling                                                                       | 0.002      | 0.0769|
| Cholesterol biosynthesis I                                                           | <0.0001    | 0.889 |
| Cholesterol biosynthesis II (via 24,25-dihydrolanosterol)                           | <0.0001    | 0.889 |
| Cholesterol biosynthesis III (via Desmosterol)                                      | <0.0001    | 0.889 |
| Cellular effects of sildenafil (Viagra)                                              | <0.0001    | 0.133 |
| Type II diabetes mellitus signaling                                                  | 0.0008     | 0.093 |
| G-protein–coupled receptor signaling                                                | 0.019      | 0.0552|
| Cardiac β-adrenergic signaling                                                      | 0.001      | 0.0972|
| cAMP-mediated signaling                                                             | 0.01       | 0.0631|
| Stearate biosynthesis I (animals)                                                    | <0.0001    | 0.231 |
| FXR/RXR activation                                                                  | 0.003      | 0.0968|
| **IPTP**                                                                            | <0.0001    | 0.288 |
| LXR/RXR activation                                                                 | <0.0001    | 0.159 |
| Hepatic fibrosis/hepatic stellate cell activation                                   | <0.0001    | 0.124 |
| LPS/IL-1–mediated inhibition of RXR function                                        | <0.0001    | 0.0917|
| Clathrin-mediated endocytosis signaling                                             | 0.003      | 0.625 |
| Superpathway of cholesterol biosynthesis                                            | <0.0001    | 0.0962|
| Acute phase response signaling                                                      | 0.003      | 0.0909|
| Tight junction signaling                                                            | 0.0005     | 0.0855|
| ILK signaling                                                                       | 0.008      | 0.145 |
| FXR/RXR activation                                                                  | 0.0003     | 0.145 |
| TR/RXR activation                                                                   | 0.0003     | 0.145 |
| Agranulocyte adhesion and diapedesis                                                | 0.005      | 0.0978|
| Cellular effects of sildenafil (Viagra)                                              | 0.001      | 0.133 |
| Cholesterol biosynthesis I                                                           | <0.0001    | 0.778 |
| Cholesterol biosynthesis II (via 24,25-dihydrolanosterol)                           | <0.0001    | 0.778 |
| Cholesterol biosynthesis III (via Desmosterol)                                      | <0.0001    | 0.778 |
| Atherosclerosis signaling                                                            | 0.01       | 0.101 |
| Hepatic cholestasis                                                                 | 0.04       | 0.0761|
| Adipogenesis pathway                                                                | 0.05       | 0.0737|
| Caveolar-mediated endocytosis signaling                                             | 0.008      | 0.118 |
| Paxillin signaling                                                                  | 0.03       | 0.0896|
| Troglitazone                                                                        |            |       |
| Role of macrophages, fibroblasts, and endothelial cells in rheumatoid arthritis      | 0.017      | 0.236 |
| Mitochondrial dysfunction                                                           | <0.0001    | 0.425 |
| Noradrenaline and adrenaline degradation                                            | 0.001      | 0.51  |
| Mitochondrial L-carnitine shuttle pathway                                            | 0.005      | 0.545 |
| LPS/IL-1–mediated inhibition of RXR function                                        | <0.0001    | 0.38  |
| cAMP-mediated signaling                                                             | 0.003      | 0.279 |
| Fatty acid β-oxidation I                                                            | <0.0001    | 0.625 |
| Cardiac β-adrenergic signaling                                                      | 0.001      | 0.319 |
| PXR/RXR activation                                                                  | 0.028      | 0.295 |
| Agranulocyte adhesion and diapedesis                                                | 0.029      | 0.261 |
| Melanocyte development and pigmentation signaling                                   | 0.005      | 0.316 |
| Glutaryl-CoA degradation                                                             | <0.0001    | 0.9   |
| PPARα/β/γ/RXRα activation                                                           | <0.0001    | 0.316 |
| p38 MAPK signaling                                                                  | 0.011      | 0.295 |
| Sperm motility                                                                      | 0.011      | 0.295 |
| Oleate biosynthesis II (animals)                                                    | 0.019      | 0.571 |
| Endothelin-1 signaling                                                              | 0.028      | 0.25  |
| Myc-mediated apoptosis signaling                                                    | 0.039      | 0.289 |
| Regulation of the epithelial–mesenchymal transition pathway                         | 0.02       | 0.252 |
| Glutathione-mediated detoxification                                                 | 0.017      | 0.412 |

Note: Human primary preadipocytes were differentiated in the presence of MID supplemented with 20 μM TPP, 20 μM IPTP, or 5 μM troglitazone. At day 6 of differentiation, RNA was collected from five donors and used for RNA-seq. Genes that had ≥ +1.5 or ≤ −1.5 a fold change were uploaded in to IPA for analysis using the adipose tissue as the target organ. The pathways were sorted by number of molecules involved and the top 20 are shown.
was collected from five donors and used for RNA-seq. Genes that had endocytosis signaling and clathrin-mediated endocytosis signaling, both of which are involved in insulin receptor and solute carrier family 2 (facilitated glucose transporter), member 4 treatment was AMPK Signaling, which is involved in energy metabolism and fatty acid oxidation (Hardie et al. 2006).

Table 3. Top 20 upstream regulators identified by IPA.

| Upstream regulator | Molecule type         | Predicted activation state | Activation z-score | p-Value |
|--------------------|-----------------------|-----------------------------|---------------------|---------|
| TPP                |                       |                             |                     |         |
| PPARG              | Ligand-dependent nuclear receptor | Activated | 5.895 | <0.0001 |
| SREBF1             | Transcription regulator | Activated | 5.424 | <0.0001 |
| SCAP               | Other                 | Activated | 4.618 | <0.0001 |
| CEBPA              | Transcription regulator | Activated | 3.652 | <0.0001 |
| NR1H3              | Ligand-dependent nuclear receptor | Activated | 3.546 | <0.0001 |
| PPARGC1A           | Transcription regulator | Activated | 3.465 | <0.0001 |
| ATP7B              | Transporter           | Activated | 3.464 | <0.0001 |
| PPARGC1B           | Transcription regulator | Activated | 3.248 | <0.0001 |
| KLIF5              | Transcription regulator | Activated | 2.905 | <0.0001 |
| NR1H2              | Ligand-dependent nuclear receptor | Activated | 2.865 | <0.0001 |
| INSI1G2            | Other                 | Activated | 4.967 | <0.0001 |
| POR                | Enzyme                | Inhibited | −3.069 | <0.0001 |
| ELOVL5             | Enzyme                | Inhibited | −2.976 | <0.0001 |
| EPAS1              | Transcription regulator | Activated | −2.969 | <0.0001 |
| INSI1G2            | Other                 | Inhibited | −2.934 | <0.0001 |
| ASXL1              | Transcription regulator | Activated | −2.646 | <0.0001 |
| TNF                | Cytokine              | Inhibited | −2.620 | <0.0001 |
| MKL1               | Transcription regulator | Activated | −2.591 | <0.0001 |
| LEP                | Growth factor         | Inhibited | −2.480 | <0.0001 |
| PML                | Transcription regulator | Activated | −2.462 | <0.0001 |
| IPTP               |                       |                             |                     |         |
| SREBF1             | Transcription regulator | Activated | 4.43  | <0.0001 |
| PPARG              | Ligand-dependent nuclear receptor | Activated | 4.20  | <0.0001 |
| SCAP               | Other                 | Activated | 4.05  | <0.0001 |
| NR1H3              | Ligand-dependent nuclear receptor | Activated | 3.70  | <0.0001 |
| ATP7B              | Transporter           | Activated | 3.32  | <0.0001 |
| PPARGC1B           | Transcription regulator | Activated | 2.93  | <0.0001 |
| MBTPS1             | Peptidase             | Activated | 2.43  | <0.0001 |
| PPARD              | Ligand-dependent nuclear receptor | Activated | 2.38  | <0.0001 |
| FAS                | Transmembrane receptor | Activated | 2.35  | <0.0001 |
| NR1H2              | Ligand-dependent nuclear receptor | Activated | 2.23  | <0.0001 |
| INSI1G1            | Other                 | Inhibited | −4.64 | <0.0001 |
| EPAS1              | Transcription regulator | Activated | −3.08 | <0.0001 |
| ELOVL5             | Enzyme                | Inhibited | −2.98 | <0.0001 |
| MKL1               | Transcription regulator | Activated | −2.94 | <0.0001 |
| IKBKB              | Kinase                | Inhibited | −2.84 | <0.0001 |
| TGFBI              | Growth factor         | Inhibited | −2.83 | <0.0001 |
| INSI1G2            | Other                 | Inhibited | −2.76 | <0.0001 |
| POR                | Enzyme                | Inhibited | −2.75 | <0.0001 |
| LEP                | Growth factor         | Inhibited | −2.48 | <0.0001 |
| LMNB1              | Other                 | Inhibited | −2.43 | <0.0001 |

Trogilitazone

| Upstream regulator | Molecule type         | Predicted activation state | Activation z-score | p-Value |
|--------------------|-----------------------|-----------------------------|---------------------|---------|
| PPARG              | Ligand-dependent nuclear receptor | Activated | 6.706 | <0.0001 |
| PPARA              | Ligand-dependent nuclear receptor | Activated | 6.302 | <0.0001 |
| PPARGC1A           | Transcription regulator | Activated | 6.283 | <0.0001 |
| SREBF1             | Transcription regulator | Activated | 5.048 | <0.0001 |
| SCAP               | Other                 | Activated | 4.942 | <0.0001 |
| RB1                | Transcription regulator | Activated | 4.767 | <0.0001 |
| KLF15              | Transcription regulator | Activated | 4.712 | <0.0001 |
| INSR               | Kinase                | Activated | 4.667 | <0.0001 |
| PPARGC1B           | Transcription regulator | Activated | 4.223 | <0.0001 |
| PNPLA2             | Enzyme                | Activated | 3.763 | <0.0001 |
| RICTOR             | Other                 | Activated | −5.182 | <0.0001 |
| INSI1G1            | Other                 | Activated | −5.099 | <0.0001 |
| TNF                | Cytokine              | Activated | −4.972 | <0.0001 |
| KDM5A              | Transcription regulator | Activated | −4.494 | <0.0001 |
| TWIST1             | Transcription regulator | Activated | −3.656 | <0.0001 |
| F2R                | G-protein-coupled receptor | Activated | −3.359 | <0.0001 |
| TGFBI              | Growth factor         | Activated | −3.189 | <0.0001 |
| INSI1G2            | Other                 | Activated | −3.088 | <0.0001 |
| Aldosterone        | Chemical-endogenous mammalian | Activated | −3.084 | <0.0001 |
| HSD17B4            | Enzyme                | Activated | −2.985 | <0.0001 |

Note: Human primary preadipocytes were differentiated in the presence of MID supplemented with 20 μM TPP, 20 μM IPTP, or 5 μM troglitazone. At day 6 of differentiation, RNA was collected from five donors and used for RNA-seq. Genes that had ≥ 1.5 or ≤ −1.5 a fold change were uploaded into IPA for analysis using the adipose tissue as the target organ. The upstream regulators were sorted by z-score and the top 20 are shown.
In conclusion, we demonstrated that FM550 and its components, TPP and IPTP, induced adipogenesis in human primary preadipocytes.

Conclusion
In conclusion, we demonstrated that FM550 and its components, TPP and IPTP, induced adipogenesis in human primary preadipocytes.
Boucher JG, Husain M, Rowan-Carroll A, Williams A, Yauk CL, Atlas E. 2014b. Janderová L, McNeil M, Murrell AN, Mynatt RL, Smith SR. 2003. Human mesenchymal cell aggregates: a model for human adipogenesis. Obes Res 11(1):65–74, PMID:12574087, https://doi.org/10.1038/obes.2003.113.

Kawaguchi N, Sundberg C, Kivelborg M, Moghadaszadeh B, Asmar M, Dietrich N, et al. 2003. ADAM12 induces actin cytoskeleton and extracellular matrix reorganization during early adipocyte differentiation by regulating β1 integrin function. J Cell Sci 116(pt 19):3893–3904, PMID:12915587, https://doi.org/10.1242/jcs.00699.

Klemm DJ, Roessler WV, Boras T, Colton LA, Felder K, Reusch JE. 1998. Insulin stimulates cAMP-response element binding protein activity in HepG2 and 3T3-L1 cell lines. J Biol Chem 273(2):917–923, PMID:9545879, https://doi.org/10.1074/jbc.273.2.917.

Krey G, Braissant O, L’Horter F, Kalkhoven E, Perroud M, Parker MG, et al. 1997. Human adipocytes: a new cell type expressing the glucose transporter GLUT4. J Biol Chem 272(15):9001–9007, PMID:9171241, https://doi.org/10.1074/jbc.272.15.9001.

Lam JC, Lau RK, Murphy MB, Lam PK. 2009. Temporal trends of hexabromocyclododecanes (HBCDs) and polybrominated diphenyl ethers (PBDEs) and detection of two novel flame retardants in marine mammals from Hong Kong, South China. Environ Sci Technol 43(18):6944–6949, PMID:19866725.

Lane MD, Lin FT, MacDoughal DA, Vasseur-Cognet M. 1998. Control of adipocyte differentiation by CCAAT/enhancer binding protein alpha (CEBP alpha). Int J Obes Relat Metab Disord 20(suppl 3):S91–S96, PMID:9604845.