The extracellular matrix protein MAGP1 supports thermogenesis and protects against obesity and diabetes through regulation of TGFβ

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ABSTRACT

Microfibril-associated glycoprotein 1 (MAGP1) is a component of extracellular matrix microfibrils. Here we show that MAGP1 expression is significantly altered in obese humans, and inactivation of the MAGP1 gene (Mfap2\(^{-/-}\)) in mice results in adipocyte hypertrophy and predisposition to metabolic dysfunction. Impaired thermoregulation was evident in Mfap2\(^{-/-}\) mice prior to changes in adiposity, suggesting a causative role for MAGP1 in the increased adiposity and predisposition to diabetes. By 5 weeks of age, Mfap2\(^{-/-}\) mice were maladaptive to cold challenge, UCP-1 expression was attenuated in the brown adipose tissue and there was reduced browning of the subcutaneous white adipose tissue. TGFβ activity was elevated in Mfap2\(^{-/-}\) adipose tissue and treatment of Mfap2\(^{-/-}\) mice with a TGFβ neutralizing antibody improved their body temperature and prevented the increased adiposity phenotype. Together, these findings indicate that MAGP1’s regulation of TGFβ is protective against the effects of metabolic stress, and its absence predisposes to metabolic dysfunction.
The extracellular matrix (ECM) has emerged as a pivotal component in cellular signaling either through direct interaction with cell-surface receptors or through the ability to regulate growth factor bioavailability. Microfibrils are abundant ECM components that impart strength to tissues and provide instructional signals that affect cellular differentiation and function (1-4). These multi-protein filaments appear in early development and are found in almost all tissues. The core components are the fibrillins, which are encoded by three genes in humans (FBN1,-2,-3) but only two functional genes in mice (Fbn1,-2). In the mouse, combined deficiency of fibrillin-1 and fibrillin-2 results in embryonic death, demonstrating that microfibrils are required for normal development and survival (5). In vertebrates, microfibril associated glycoprotein-1 and -2 (MAGP1, MAGP2) associate with fibrillin to create the functional form of the fiber (6; 7). Unlike the fibrillins that form the structural core of microfibrils, the MAGPs are modifiers of microfibril function, and not key structural elements.

A crucial function of microfibrils is their regulation of growth factor activity, particularly growth factors of the TGFβ family (8). The fibrillins covalently bind the large latent complex form of TGFβ and there is evidence that the pathomechanism associated with fibrillin-1 mutations (e.g., Marfan syndrome) is excess TGFβ activity due to an inability to sequester latent TGFβ in the ECM (8). The MAGPs also interact with TGFβ but bind the active, not latent form of the growth factor (9). Mice lacking MAGP1 have phenotypes consistent with altered TGFβ activity but they do not always overlap with those associated with fibrillin mutations, which is indicative of both overlapping and distinct functions of the two proteins (9-12). Generation of the MAGP1-deficient mouse, which has no defect in microfibril formation, provides a model to study the two distinct functions of the microfibril: providing mechanical stability to the tissue versus regulation of cell signaling pathways.
One phenotype that shows complete penetrance in MAGP1-deficient (Mfap2<sup>−/−</sup>) mice is increased adiposity. This characteristic is particularly interesting in light of studies associating obesity traits in humans to a locus on chromosome 1p36 that includes the gene for MAGP1 (MFAP2) (13-15). Further, it has been proposed that excess TGFβ is involved in the pathogenesis of metabolic diseases like obesity and diabetes (16). In humans, TGFβ-1 levels in both adipose tissue and plasma positively correlate with BMI, and elevated TGFβ-1 is a risk factor for type 2 diabetes (17-19). TGFβ has pleotropic effects on metabolic function and, in mice, inhibiting TGFβ is protective against diet-induced obesity and diabetes (19). As reviewed in Tan et al (16), TGFβ inhibits PGC-1α (peroxisome proliferative activated receptor-ɣ coactivator 1α) and UCP-1 (uncoupling protein-1), which are necessary for uncoupling respiration for heat production, and inhibits the phenotypic transition of white adipocytes to brown. The result is reduced energy expenditure and excess lipid storage. Excess TGFβ in obesity facilitates the development of metabolic syndrome by promoting inflammation and fibrosis, which impairs adipose tissue, liver, and pancreatic function.

Given the importance of the microfibril in regulating TGFβ activity, the purpose of the present study was to explore whether the increased adiposity and changes in metabolic function in MAGP1-deficient mice result from altered TGFβ activity. Here we show that Mfap2<sup>−/−</sup> mice have changes in metabolic function that lead to increased adipocyte size, ectopic lipid accumulation and insulin resistance. These changes occur secondary to reduced energy expenditure (impaired thermogenesis) associated with TGFβ-mediated suppression of PGC-1α and Ucp-1. Treating MAGP1-deficient mice with a TGFβ neutralizing antibody improved body temperature and prevented excess adiposity. Our findings establish the importance of the ECM component MAGP1 in regulating metabolic pathways associated with obesity, and identify a
mechanism whereby TGFβ’s potent effects on intracellular metabolic pathways are regulated by the microenvironment.

RESEARCH DESIGN AND METHODS

Animals and diets
Generation and genotyping of MAGP1-deficient \( (Mfap2^{-/-}) \) mice has been described (9). All mice used in this study were males on the c57Bl/6 background (Jackson Laboratories; Bar Harbor, Maine), housed in a pathogen-free animal facility, and fed standard or high fat chow \textit{ad libitum}. For caloric intake studies, food consumption of individually housed mice was measured over a consecutive 7-day period. For high-fat diet studies, mice were placed on high fat or control chow for 16 weeks [Research Diets, Inc.; New Brunswick, NJ: D12492 (HFD, 60% fat), D12450B (control, 10% fat)]. For the neutralizing anti-TGFβ antibody (\( \alpha \)-TGFβ) treatment study, mice received intraperitoneal injections of \( \alpha \)-TGFβ (clone 1D11) or control IgG1 (clone 11711) three times per week for five weeks. Antibodies were purchased from R&D Systems (Minneapolis, MN), dissolved in sterile PBS, and delivered at a dose of 1.5mg/kg. Mice described as “adults” were 5-6 months old, while “young” mice were 5-7 weeks old.

Body composition, activity, glucose tolerance and blood parameters
Lean (protein) and fat (lipid) mass was determined on mice and tissue samples using an echoMRI 3-in-1 model instrument (Echo Medical Systems; Houston, TX). Whole body fat was also determined by Dual Energy X-Ray Absorptiometry (DEXA, PIXImus Lunar-GE; Fitchburg, WI) as described in (11). Activity of individual mice was quantified over a 24-hour period using
an infrared motion sensor (InfraMot apparatus; TSE Systems; Midland, MI) as previously
described (20). The first four hours of data (acclimation period) were not included in the
analysis. For glucose tolerance tests (GTT), mice were fasted overnight prior to 1-2g/kg
dextrose injection. For insulin sensitivity tests (ITT), mice were fasted 6 hours prior to 0.75u/kg
humulin-R insulin injection (Lilly; Indianapolis, IN). Contour meters (Bayer; Whippany, NJ)
measured tail blood glucose concentration. Serum insulin concentration following 1g/kg
dextrose injection was determined using a Singulex Erenna digital single molecule counting
platform (Singulex; St Louis, MO). Triglycerides and cholesterol concentration were determined
as previously described (21; 22). Serum leptin was assayed by ELISA using commercial reagents
(CrystalChem; Downers Grove, IL).

**Adipocyte size and number**

Adipocyte size and number was measured as previously described (23). Briefly, 50mg of
epididymal white adipose tissue (WAT) was fixed in a 0.2M collidine HCL/31 mg/mL osmium
tetraoxide solution and dissociated in a solution containing 8 M urea and 154 mM NaCl. Samples
were then analyzed on a Multisizer-3 (Beckman Coulter; Fullerton, CA) using a 400-m aperture
(dynamic linear range, 12 to 320 m). Total adipocyte number was determined for the amount of
sample analyzed (cells per mg tissue) then corrected for the total mass of the fat pad.

**Lipolysis and lipid uptake**

Assays were performed on explants of epididymal fat pads with intact ECM. Lipolysis was
determined by glycerol release following 200nM isoproterenol treatment, utilizing Sigma’s free
glycerol reagent as described previously (24). For lipid uptake, fat pads were treated with
250µM tritiated-oleic acid, with and without 10nM insulin. After incubation, fat pads were washed, homogenized in 0.1M NaOH, and radioactivity determined using a Beckman LS6000SC (25).

**Quantitative RT-PCR**

RNA was extracted from tissue with TRIzol reagent (Invitrogen; Grand Island, NY) and RNeasy columns (Qiagen; Valencia, CA). For mouse analyses, RNA was reverse transcribed using Applied Biosystem’s RT-to-cDNA kit, and then quantitative PCR was performed using a TaqMan Universal PCR Master Mix reagent kit (Applied Biosystems; Grand Island, NY). For human studies, RNA was reverse transcribed using Invitrogen’s Vilo cDNA synthesis system and qPCR performed using SYBR green. For normalization, co-amplification of the mRNA for the ribosomal protein 36B4 (mouse and human), and cyclophilin-A (mouse) was performed.

**Histology**

Tissue was fixed in 10% buffered formalin for 16 hours, dehydrated via an ethanol gradient, and stored in 70% ethanol prior to paraffin embedding. MAGP1 expression was determined by incubating tissue sections with anti-MAGP1 antibody. Inflammation and ECM deposition were evaluated by incubating tissue sections with anti-MAC-3 antibody or trichrome stain.

**Energy expenditure**

Mouse body temperature was determined by rectal probe thermometer. Adaptive thermogenesis was evaluated by 6-8 hour cold challenge. Baseline body temperature was determined then mice were placed in pre-chilled cages at 4°C. Metabolic rate was determined by indirect calorimetry.
Mice were placed in a Columbus Instruments (Columbus, OH) Oxymax chamber for 24 hours, with free access to food and water, at ambient temperature, and with normal light/dark cycle. The first four hours of data recording were considered acclimation time. Energy expenditure (Heat) was determined using the following equation: Heat=[3.815 + 1.232(V_{CO2}/V_{O2})] x V_{O2} x body weight.

**Mitochondrial content**
DNA was purified from brown adipose tissue (BAT) or seWAT using DNeasy Blood and Tissue kit (Qiagen; Valencia, CA). Quantitative PCR was performed using Syber Green (Applied Biosystems; Grand Island, NY) and primers specific for nuclear (H19) or mitochondrial DNA (CytB, ND1). Mitochondrial content was calculated as the ratio of mitochondrial DNA to nuclear DNA.

**Human subjects**
Subcutaneous abdominal adipose tissue (AT) was obtained by percutaneous biopsy from 21 obese (BMI [mean ± SD] 40.9 ± 8.0 kg/m²) and 9 lean (BMI 22.7 ± 1.9 kg/m²) men and women, after subjects fasted for ~12 h overnight, as described previously (26). No subject had any history or evidence of serious disease, took medications that can affect metabolism or the immune system, or had diabetes.

**Study approval**
All animals were treated following animal protocols approved by the Washington University’s Animal Studies Committee. For human studies, subjects gave their written informed consent
before participating in the study, which was approved by Washington University’s Human Research Protection Office.

RESULTS

MAGP1 deficiency causes excess adiposity and metabolic dysfunction

MAGP1 transcript was detectable in the adipose tissue of mice (Figure S1A), and deletion of MAGP1 in mice fed a standard chow diet resulted in significantly increased adipose tissue mass (Mfap2+/− relative to WT mice; Figure 1A-B). Although lean mass was unchanged in adult Mfap2+/− animals, whole body mass was proportionately increased relative to wild-type controls (Figure 1B-C). Elevated whole body adiposity in Mfap2+/− mice was detectable by approximately ten weeks old (Figure 1D). As expected with increased adiposity, serum leptin and WAT leptin expression were increased in Mfap2+/− mice (Table 1).

Excess adiposity in Mfap2+/− mice was the direct consequence of MAGP1 deficiency as MAGP2 and fibrillin-1 transcript expression was normal in the WAT of these animals (Figure S1B). MAGP1 deficiency in the outbred Black Swiss mouse strain also resulted in increased adiposity (Figure S1C), indicating that MAGP1-deficiency, and not genetic background, was the major determinant of fat overgrowth in these animals. Finally, excess adiposity in Mfap2+/− mice was not preceded by either increased caloric intake or reduced ambulatory activity (Figure 1E-F).

Expansion of adipose tissue in Mfap2+/− animals was associated with poor metabolic health. Mfap2+/− mice had elevated serum TAG, tissue TAG, and serum cholesterol when compared to WT mice (Table 1). Glucose tolerance testing (GTT) demonstrated adult Mfap2+/− mice, fed standard chow diet, had impaired glucose clearance leading to elevated blood glucose,
and insulin tolerance testing (ITT) revealed reduced insulin sensitivity (Figure 2A). *Mfap2*<sup>−/−</sup> mice were also more susceptible to the adverse metabolic effects of high-fat diet (HFD). WT and *Mfap2*<sup>−/−</sup> mice show significant weight gain on the HFD; however, *Mfap2*<sup>−/−</sup> mice maintain their elevated adiposity compared to WT mice (Figure 2B). Hyperglycemia, hyperinsulinemia, and insulin resistance were significantly accentuated in *Mfap2*<sup>−/−</sup> mice relative to wild-type animals fed HFD for 12 weeks (Figure 2C-E). Further, ectopic lipid accumulation in the liver was substantially elevated in the knockout animals as compared to wild-type mice fed HFD for 14 weeks (Figure 2F). These data demonstrate that the absence of MAGP1 predisposes mice to hyperlipidemia, hyperglycemia, hyperinsulinemia, ectopic lipid accumulation, and impaired glucose metabolism; suggesting MAGP1 serves a protective role against metabolic disease.

**Mfap2**<sup>−/−</sup> adipocytes are hypertrophic

Adipocyte number and size were evaluated in standard chow-fed WT and *Mfap2*<sup>−/−</sup> mice. Adipocyte number was normal in *Mfap2*<sup>−/−</sup> WAT; however, adipocyte volume was increased. Cell number was calculated by normalizing the number of cells per milligram of digested tissue to total fat pad mass (Figure 3A). Adipocyte sizing analysis of *Mfap2*<sup>−/−</sup> WAT revealed that there were more cells with diameters between 20-50µm, fewer cells in the 60-100µm diameter range and more cells above 100µm diameter as compared to control animals (Figure 3B). As a result, the separation between the populations of small (25-50µm) and large (60-100µm) adipocytes, which was well delineated in wild-type tissue, was less so in *Mfap2*<sup>−/−</sup> WAT. Histological assessment supported larger adipocyte volume in the WAT of *Mfap2*<sup>−/−</sup> mice (Figure 3C).

To elucidate the underlying mechanisms for excess lipid content in chow-fed *Mfap2*<sup>−/−</sup> mice, the rates of fatty acid (FA) uptake and lipolysis were evaluated. Because MAGP1 is an
extracellular matrix protein, these functional assays were performed on minced WAT with intact ECM. Stimulation of FA uptake by insulin was evident in wild-type WAT (Figure 3D). Basal FA uptake in Mfap2⁻/⁻ WAT was elevated and did not respond to stimulation by 10µM insulin as observed in WT tissue indicating abnormal FA uptake in Mfap2⁻/⁻ animals (Figure 3D). Altered regulation of FA metabolism was then demonstrated in muscle, the major energy using tissue, using qPCR analysis of key lipid uptake/storage genes. HFD feeding in mice induced expression of Lpl, Cd36, Plin5, Plin2 (ADFP) and Dgat2 in both WT and Mfap2⁻/⁻ cohorts. MAGP1 deficiency, however, accentuates the expression of these genes (Figure 3E). In contrast to FA uptake, lipolysis in response to isoproterenol was normal in Mfap2⁻/⁻ WAT (Figure 3F). Further, the expression of genes that support lipid catabolism (Cpt1b, Ppara, Ppars) was not different in Mfap2⁻/⁻ tissue when compared to changes seen in WT controls (Figure 3G).

Interestingly, expression of Ppargc1a (PGC-1α) and Ucp-3, genes important to mitochondrial uncoupling, was significantly decreased in Mfap2⁻/⁻ muscle (Figure 3G). These results suggest that MAGP1 deficiency results in a defect in energy utilization and that this defect leads to excess lipid storage and adipocyte hypertrophy.

**Reduced heat production in Mfap2⁻/⁻ mice**

WAT specializes in lipid storage while the primary function of BAT is utilizing fatty acids for heat production (27; 28). To determine whether MAGP1 deficiency influences BAT function, we analyzed BAT composition and thermoregulation. On standard chow, Mfap2⁻/⁻ mice had increased BAT mass due to increased lipid accumulation (Figure 4A-B). HFD increased both BAT mass and lipid content in WT animals and accentuated the increased lipid content in Mfap2⁻/⁻ BAT (Figure 4A-B).
Measurement of body temperature during light and dark cycles found that $Mfap2^{-/-}$ mice had reduced body temperature relative to control animals during both periods (Figure 4C). To further characterize BAT functionality, adaptive thermogenesis was evaluated by exposing the mice to $4^\circ{}C$ temperature for 6 hours. Both WT and $Mfap2^{-/-}$ mice exhibited reduced body temperatures during cold exposure. However, MAGP1-deficient animals had significantly greater body temperature loss (Figure 4D). In agreement with the altered thermoregulation, $Mfap2^{-/-}$ mice were not as efficient as WT mice in up-regulating expression of the BAT-associated genes PGC-1$\alpha$ and $Ucp-1$ following cold exposure (Figure 4E).

To investigate whether impaired thermoregulation accounts for the excess adiposity of $Mfap2^{-/-}$ mice, thermoregulation was evaluated in young $Mfap2^{-/-}$ mice at an age (4 to 6-week old) before excess lipid accumulation was detectable. At 1 month, $Mfap2^{-/-}$ mice had no change in whole body adiposity, but at 5 months these mice had a significant increase in adiposity relative to WT mice (Figure 5A). At 1 month the same $Mfap2^{-/-}$ mice had reduced body temperature relative to WT mice, and this phenotype was maintained at 5 months (Figure 5A). Data from indirect calorimetry demonstrated a trend toward reduced energy expenditure in $Mfap2^{-/-}$ mice compared with WT mice, but the differences were not statistically significant (Figure 5B). Young $Mfap2^{-/-}$ mice were also maladaptive to $4^\circ{}C$ cold challenge (Figure 5C). Lipid content in $Mfap2^{-/-}$ BAT appeared slightly elevated by histology (Figure 5D), and similar to adult mice, young $Mfap2^{-/-}$ mice had reduced BAT expression of $Ucp-1$ (Figure 5E). Interestingly, $Mfap2^{-/-}$ mice had reduced thermogenesis and Ucp-1 expression despite no significant difference in mitochondrial content (Figure 5F).

While mouse gonadal WAT had little-to-no expression of thermogenic genes such as $Ucp-1$, subcutaneous WAT (scWAT) has thermogenic potential through a process termed
“adipocyte browning” (29). Because activating the PGC-1α/UCP-1 pathway in mouse scWAT is protective against diet-induced diabetes (29-31), adipocyte browning was explored in the scWAT of \( Mfap2^{2/-} \) mice. By histology, \( Mfap2^{2/-} \) scWAT appeared to have elevated lipid content and fewer clusters of multilocular adipocytes (Figure 5G). Cold challenge induced adipocyte browning and expression of PGC-1α and \( Ucp-1 \) in WT scWAT; however, this response was significantly blunted in \( Mfap2^{2/-} \) scWAT (Figure 5H). Reduced PGC-1α and \( Ucp-1 \) expression was not due to a failure in mitochondrial biogenesis as mitochondrial content was increased appropriately in \( Mfap2^{2/-} \) scWAT following cold exposure (Figure 5I). These studies indicated MAGP1 deficiency suppressed acquisition of brown fat features in white fat, and further support a model where adiposity was increased in \( Mfap2^{2/-} \) mice due to reduced energy expenditure in the form of heat production.

**Neutralizing TGFβ activity resolves \( Mfap2^{2/-} \) phenotypes**

Microfibrils facilitate storage of TGFβ family growth factors in the ECM (8; 32), and we have shown MAGP1 functionally interacts with TGFβ (9; 10). Because TGFβ impairs thermogenesis while supporting white adipose tissue expansion and insulin resistance (16), aberrant TGFβ activity was investigated as the mechanism underlying the metabolic phenotypes in \( Mfap2^{2/-} \) mice. Shown in Figure 6A, loss of MAGP1 resulted in a significant increase in TGFβ activity in white adipose tissue as assessed by smad-2 phosphorylation. TGFβ contributes to the pathogenesis of obesity and metabolic syndrome by stimulating fibrosis and inflammation (16). Figure 6B shows that expression of the major collagens associated with the fibrotic response, collagen-1 and collagen-3, were elevated by HFD in both WT and MAGP1-deficient WAT. However, this elevation was exaggerated in the MAGP1-deficient tissue. Trichrome staining
provided further support for increased collagen deposition in HFD fed $Mfap2^{−/−}$ WAT versus WT cohorts. HFD feeding also induced expression of macrophage-associated genes in wild-type WAT (Figure 6C). As predicted, the expression of these inflammatory genes was significantly higher in the $Mfap2^{−/−}$ WAT. Increased macrophage infiltration into $Mfap2^{−/−}$ WAT relative to wild-type WAT following HFD feeding was supported by anti-Mac3 immunohistochemistry (Figure 6C). These data show that MAGP1 regulates TGFβ signaling and TGFβ-dependent processes in adipose tissue.

To determine whether dysregulation of TGFβ was responsible for the reduced thermogenesis and increased adiposity in standard chow-fed $Mfap2^{−/−}$ mice, WT and $Mfap2^{−/−}$ mice were treated with a neutralizing α-TGFβ antibody or control IgG. Before treatment, 8-week old $Mfap2^{−/−}$ mice had reduced body temperature but there was no difference in adiposity compared to WT mice (Figure 6D). Five weeks of IgG treatment failed to prevent the increase in adiposity seen in $Mfap2^{−/−}$ mice. However, adiposity in $Mfap2^{−/−}$ mice treated with the α-TGFβ was not significantly different from WT mice (Figure 6E). Importantly, treatment with the α-TGFβ antibody resulted in elevated body temperatures of $Mfap2^{−/−}$ mice to near WT levels (Figure 6E). Thus, MAGP1 supports energy expenditure and protects against excess lipid accumulation by regulating the availability of TGFβ.

**MAGP1 expression in obese humans**

To investigate the relationship between TGFβ, MAGP1 and obesity in humans, we measured TGFβ1 ($TGFB1$) and MAGP1 ($MFAP2$) gene expression in subcutaneous WAT from 30 individuals with varying body mass index (BMI). WAT expression of TGFβ1 positively correlated with obesity (Figure 7A), a finding substantiated by other laboratories (17; 19).
MAGP1 expression was also elevated in individuals with excess adiposity, and statistical significance was reached when comparing individuals considered normal weight (BMI<25) to overweight-obese individuals (BMI>25) (Figure 7B-C). Similar to human WAT, MAGP1 transcript was significantly elevated in the WAT of obese mice, following HFD feeding (Figure 7D). Immunohistochemistry using an anti-MAGP1 antibody supported increased MAGP1 expression in WAT during metabolic challenge. HFD feeding in mice did not alter expression of the microfibril molecules MAGP2 or fibrillin-1.

Collectively, the data in this manuscript demonstrate that MAGP1 supports energy expenditure by impeding TGFβ activity, excess fat accumulation is associated with increased expression of both TGFβ1 and MAGP1, and the absence of MAGP1 causes pre-disposition to obesity-associated metabolic dysfunction. Therefore altered MAGP1 expression could be considered a protective-adaptive response to obesity.

**DISCUSSION**

The adipose ECM provides a structural scaffold that defines the limits of tissue growth. Changing the physical properties of the ECM has functional consequences; fibrotic ECM restricts adipocyte expansion and function (33), while decreasing ECM rigidity results in a permissive environment that supports adipose tissue expansion (34-36). In this report, we demonstrated that ECM components contribute more than mechanical properties to adipose tissue, and identified a mechanism by which the ECM influences cellular processes involved in energy expenditure by restricting growth factor delivery. Specifically, we demonstrated that the microfibril-associated protein MAGP1 is involved in regulating thermogenesis and the browning
of white adipocytes through a TGFβ-mediated pathway. Accordingly, these data provide evidence of a novel mechanism for regulating energy metabolism by extracellular matrix proteins in the adipose tissue microenvironment. Further, our findings suggest that induction of WAT MAGP1 expression is an adaptive response that protects against excess TGFβ associated with obesity.

Elevated TGFβ levels correlate with obesity in humans and mice (17; 19), and suggest a mechanistic link between MAGP1 and metabolism. A study by Yadav et al. (19) demonstrated that, in mice, down-regulation of the TGFβ signaling pathway through deletion of a TGFβ signaling mediator, smad3, resulted in a phenotype nearly opposite to that of the *Mfap2*+/− mouse. Smad3-knockout mice are lean, have improved glucose metabolism, are protected from diet-induced obesity, and thermogenesis is improved. They also found that systemic blockade of TGFβ signaling protects mice from obesity, diabetes, and hepatic steatosis. MAGP1 binds active TGFβ and controls its bioavailability through sequestration in the ECM. In the absence of MAGP1, there is less sequestered and, hence, more active TGFβ. The mechanistic pathway whereby elevated TGFβ signaling associated with MAGP1 deficiency influences energy metabolism is through reduced energy dissipation and transcriptional regulation of PGC-1α and UCP-1. TGFβ-induced smad phosphorylation allows smad to bind to and inhibit PRDM16 function. PRDM16 is a transcription co-regulator that is essential to BAT development and drives expression PGC-1α, which is a transcription co-regulator of itself and the thermogenic gene UCP-1. Smad also inhibits the function of PPARβ/δ. PPARβ/δ supports energy dissipation through fatty acid oxidation and PGC-1α expression. The metabolic phenotypes found in *Mfap2*−/− mice are supportive of excess TGFβ signaling. *Mfap2*+/− mice have increased smad phosphorylation, impaired transcription of both PGC-1α and UCP-1, and reduced energy
expenditure (thermogenesis). These changes contribute to an accumulation of body fat mass, adipose tissue inflammation, ectopic lipid accumulation, and predisposition to metabolic dysfunction.

Several studies have associated obesity traits in humans to a locus around chromosome 1p36 that includes the gene for MAGP1 (MFAP2) (13-15). In a study of human WAT from normal, overweight and obese individuals, we found MAGP1 to be elevated in the WAT from individuals with BMIs greater than 25. While it remains to be determined in humans what role the increase in MAGP1 plays in obesity-associated metabolic dysfunction, we demonstrate in this report that the inability to increase MAGP1 expression during diet-induced obesity results in exacerbated metabolic disease in mice; suggesting that modulation of MAGP1 expression is a protective-adaptive response to metabolic challenge. Figure 7E is a graphic representation of the proposed function of MAGP1. In adipose tissue, MAGP1 supports sequestration of active TGFβ in the ECM. MAGP1’s capacity to limit free TGFβ facilitates homeothermy and adaptive thermogenesis by allowing transcription of PGC-1α and Ucp-1. In pathologic conditions where TGFβ secretion is excessive, such as obesity, we hypothesize that MAGP1 expression is induced as a protective-adaptive response to sequester excess active TGFβ, thereby facilitating energy dissipation and protecting against inflammation and fibrosis.

Exemplifying the distinct functions of the ECM (mechanical vs cell signaling) is the change in adiposity associated with Marfan syndrome (fibrillin-1 mutation). Marfan syndrome has been attributed to disrupted assembly of microfibrils and subsequently the inability to sequester the latent TGFβ complex (8; 32). Given TGFβ’s positive effect on adipocyte hypertrophy and negative effect on thermoregulation it would be expected that individuals with Marfan syndrome would be predisposed to obesity and diabetes, similar to Mfap2−/− mice.
However, most individuals with Marfan syndrome have reduced adiposity (32; 37; 38). It is somewhat surprising that mutations in two proteins of the same extracellular fiber can result in such contrasting phenotypes. However, MAGP1 is not a structural protein of the microfibril but a modifier of fibrillin function. MAGP1’s deletion leaves the core microfibril intact. In contrast, mutation of fibrillin, the structural backbone of microfibrils can disrupt the mechanical integrity of microfibrils and thus alter the physical properties of the adipose ECM. It is plausible that individuals with mutant fibrillin-1 have adipose ECM that constrains adipocyte growth, resulting in smaller adipocytes.

In summary, this study demonstrates that the ECM component MAGP1 has the capacity to regulate growth factor availability that is important for maintaining normal metabolic function, and provides further support for TGFβ’s role in the etiology of obesity-associated metabolic disease. Our results also highlight the contribution that accessory proteins, like MAGP1, provide to overall microfibril function and tissue homeostasis.

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REFERENCES

1. Corson GM, Charbonneau NL, Keene DR, Sakai LY: Differential expression of fibrillin-3 adds to microfibril variety in human and avian, but not rodent, connective tissues. Genomics 2004;83:461-472

2. Sakai LY, Keene DR, Engvall E: Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986;103:2499-2509

3. Zhang H, Apfelroth SD, Hu W, Davis EC, Sanguineti C, Bonadio J, Mecham RP, Ramirez F: Structure and expression of fibrillin-2, a novel microfibrillar component preferentially located in elastic matrices. J Cell Biol 1994;124:855-863

4. Zhang H, Hu W, Ramirez F: Developmental expression of fibrillin genes suggests heterogeneity of extracellular microfibrils. J Cell Biol 1995;129:1165-1176

5. Carta L, Pereira L, Arteaga-Solis E, Lee-Arteaga SY, Lenart B, Starcher B, Merkel CA, Sukoyan M, Kerkis A, Hazeki N, Keene DR, Sakai LY, Ramirez F: Fibrillins 1 and 2 perform partially overlapping functions during aortic development. J Biol Chem 2006;281:8016-8023

6. Cleary EG, Gibson MA: Elastin-associated microfibrils and microfibrillar proteins. Int Rev Connect Tissue Res 1983;10:97-209

7. Segade F: Functional evolution of the microfibril-associated glycoproteins. Gene 2009;439:43-54

8. Ramirez F, Carta L, Lee-Arteaga S, Liu C, Nistala H, Smaldone S: Fibrillin-rich microfibrils - structural and instructive determinants of mammalian development and physiology. Connect Tissue Res 2008;49:1-6

9. Weinbaum JS, Broekelmann TJ, Pierce RA, Werneck CC, Segade F, Craft CS, Knutsen RH, Mecham RP: Deficiency in microfibril-associated glycoprotein-1 leads to complex phenotypes in multiple organ systems. J Biol Chem 2008;283:25533-25543

10. Craft CS, Broekelmann TJ, Zou W, Chappel JC, Teitelbaum SL, Mecham RP: Oophorectomy-induced bone loss is attenuated in MAGP1-deficient mice. J Cell Biochem 2012;113:93-99

11. Craft CS, Zou W, Watkins M, Grimston S, Brodt MD, Broekelmann TJ, Weinbaum JS, Teitelbaum SL, Pierce RA, Civitelli R, Silva MJ, Mecham RP: Microfibril-associated glycoprotein-1, an extracellular matrix regulator of bone remodeling. J Biol Chem 2010;285:23858-23867
12. Werneck CC, Vicente CP, Weinberg JS, Shifren A, Pierce RA, Broekelmann TJ, Tollefsen DM, Mecham RP: Mice lacking the extracellular matrix protein MAGP1 display delayed thrombotic occlusion following vessel injury. Blood 2008;111:4137-4144

13. Hoffmann K, Mattheisen M, Dahm S, Nurnberg P, Roe C, Johnson J, Cox NJ, Wichmann HE, Wienker TF, Schulze J, Schwarz PE, Lindner TH: A German genome-wide linkage scan for type 2 diabetes supports the existence of a metabolic syndrome locus on chromosome 1p36.13 and a type 2 diabetes locus on chromosome 16p12.2. Diabetologia 2007;50:1418-1422

14. Liu YJ, Xu FH, Shen H, Liu YZ, Deng HY, Zhao LJ, Huang QY, Dvornyk V, Conway T, Davies KM, Li JL, Recker RR, Deng HW: A follow-up linkage study for quantitative trait loci contributing to obesity-related phenotypes. J Clin Endocrinol Metab 2004;89:875-882

15. Pausova Z, Gaudet D, Gossard F, Bernard M, Kaldunski ML, Jomphe M, Tremblay J, Hudson TJ, Bouchard G, Kotchen TA, Cowley AW, Hamet P: Genome-wide scan for linkage to obesity-associated hypertension in French Canadians. Hypertension 2005;46:1280-1285

16. Tan CK, Chong HC, Tan EHP, Tan NS: Getting 'smad' about obesity and diabetes. Nutrition and Diabetes 2012;2:1-13

17. Alessi MC, Bastelica D, Morange P, Berthet B, Leduc I, Verdier M, Geel O, Juhan-Vague I: Plasminogen activator inhibitor 1, transforming growth factor-beta1, and BMI are closely associated in human adipose tissue during morbid obesity. Diabetes 2000;49:1374-1380

18. Herder C, Zierer A, Koenig W, Roden M, Meisinger C, Thorand B: Transforming growth factor-beta1 and incident type 2 diabetes: results from the MONICA/KORA case-cohort study, 1984-2002. Diabetes Care 2009;32:1921-1923

19. Yadav H, Quijano C, Kamaraju AK, Gavrilova O, Malek R, Chen W, Zerfas P, Zhigang D, Wright EC, Stuelten C, Sun P, Lonning S, Skarulis M, Sumner AE, Finkel T, Rane SG: Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. Cell Metab 2011;14:67-79

20. Chakravarthy MV, Zhu Y, Lopez M, Yin L, Wozniak DF, Coleman T, Hu Z, Wolfgang M, Vidal-Puig A, Lane MD, Semenkovich CF: Brain fatty acid synthase activates PPARalpha to maintain energy homeostasis. J Clin Invest 2007;117:2539-2552

21. Li B, Nolte LA, Ju JS, Han DH, Coleman T, Holloszy JO, Semenkovich CF: Skeletal muscle respiratory uncoupling prevents diet-induced obesity and insulin resistance in mice. Nat Med 2000;6:1115-1120

22. Marshall BA, Tordjman K, Host HH, Ensor NJ, Kwon G, Marshall CA, Coleman T, McDaniel ML, Semenkovich CF: Relative hypoglycemia and hyperinsulinemia in mice with heterozygous lipoprotein lipase (LPL) deficiency. Islet LPL regulates insulin secretion. J Biol Chem 1999;274:27426-27432
23. Harris RB, Martin RJ: Metabolic response to a specific lipid-depleting factor in parabiotic rats. Am J Physiol 1986;250:R276-286

24. Liu Y, Zhou D, Abumrad NA, Su X: ADP-ribosylation factor 6 modulates adrenergic stimulated lipolysis in adipocytes. Am J Physiol Cell Physiol 2010;298:C921-928

25. Nassir F, Wilson B, Han X, Gross RW, Abumrad NA: CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. J Biol Chem 2007;282:19493-19501

26. Bradley D, Conte C, Mittendorfer B, Eagon JC, Varela JE, Fabbrini E, Gastaldelli A, Chambers KT, Su X, Okunade A, Patterson BW, Klein S: Gastric bypass and banding equally improve insulin sensitivity and beta cell function. J Clin Invest 2012;122:4667-4674

27. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmuller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J: Brown adipose tissue activity controls triglyceride clearance. Nat Med 2011;17:200-205

28. Saely CH, Geiger K, Drexel H: Brown versus white adipose tissue: a mini-review. Gerontology 2012;58:15-23

29. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaar T, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM: Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 2012;150:366-376

30. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonenkov A, Flier JS, Maratos-Flier E, Spiegelman BM: FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. Genes Dev 2012;26:271-281

31. Koncarevic A, Kajimura S, Cornwall-Brady M, Andreucci A, Pullen A, Sako D, Kumar R, Grinberg AV, Liharska K, Ucran JA, Howard E, Spiegelman BM, Seehra J, Lachey J: A novel therapeutic approach to treating obesity through modulation of TGFbeta signaling. Endocrinology 2012;153:3133-3146

32. Judge DP, Dietz HC: Marfan's syndrome. Lancet 2005;366:1965-1976

33. Chun TH, Hotary KB, Sabeh F, Saltiel AR, Allen ED, Weiss SJ: A pericellular collagenase directs the 3-dimensional development of white adipose tissue. Cell 2006;125:577-591

34. Bradshaw AD, Graves DC, Motamed K, Sage EH: SPARC-null mice exhibit increased adiposity without significant differences in overall body weight. Proc Natl Acad Sci U S A 2003;100:6045-6050
35. Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, Zhang BB, Bonaldo P, Chua S, Scherer PE: Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. Mol Cell Biol 2009;29:1575-1591

36. Kos K, Wilding JP: SPARC: a key player in the pathologies associated with obesity and diabetes. Nat Rev Endocrinol 2010;6:225-235

37. Graul-Neumann LM, Kienitz T, Robinson PN, Baasanjav S, Karow B, Gillessen-Kaesbach G, Fahsold R, Schmidt H, Hoffmann K, Passarge E: Marfan syndrome with neonatal progeroid syndrome-like lipodystrophy associated with a novel frameshift mutation at the 3' terminus of the FBN1-gene. Am J Med Genet A 2010;152A:2749-2755

38. Summers KM, Nataatmadja M, Xu D, West MJ, McGill JJ, Whight C, Colley A, Ades LC: Histopathology and fibrillin-1 distribution in severe early onset Marfan syndrome. Am J Med Genet A 2005;139:2-8
Table 1: Body composition, biochemical and hormonal characteristics
(5-month old male mice, n=5-10)

|                          | Mean ± S.E.M                  | P-value |
|--------------------------|-------------------------------|---------|
| Serum TAG (mg/dL)        |                               |         |
| WT                       | 68.95 ± 2.83                  | 0.023   |
|                          | 86.86 ± 7.27                  |         |
| L/L                      |                               |         |
| Liver TAG (µg/mg)        |                               |         |
| WT                       | 2.31 ± 0.287                  | 0.019   |
|                          | 3.37 ± 0.279                  |         |
| L/L                      |                               |         |
| Heart TAG (µg/mg)        |                               |         |
| WT                       | 0.46 ± 0.033                  | 0.035   |
|                          | 0.62 ± 0.064                  |         |
| L/L                      |                               |         |
| Muscle TAG (µg/mg)       |                               |         |
| WT                       | 3.34 ± 0.398                  | 0.194   |
|                          | 4.60 ± 0.886                  |         |
| L/L                      |                               |         |
| Serum Cholesterol (mg/dL)|                               |         |
| WT                       | 110.32 ± 3.90                 | 0.17    |
|                          | 119.21 ± 5.29                 |         |
| L/L                      |                               |         |
| Blood Glucose (mg/dL)    |                               |         |
| WT                       | 118.0 ± 7.87                  | 0.0009  |
|                          | 160.3 ± 6.92                  |         |
| L/L                      |                               |         |
| Serum Leptin (ng/mL)     |                               |         |
| WT                       | 1.5 ± 0.21                    | 0.0008  |
|                          | 3.7 ± 0.44                    |         |
| L/L                      |                               |         |
| WAT Leptin mRNA (2^ΔCT)  |                               |         |
| WT                       | 0.567 ± 0.141                 | 0.03    |
|                          | 0.972 ± 0.101                 |         |
| L/L                      |                               |         |
FIGURE LEGENDS

Figure 1: MAGP1 deficiency causes excess adiposity in mice.  A) *Mfap2<sup>−/−</sup>* mice have significantly more WAT. Mass and differential volume (photo) of epididymal fat pads from 5 month old WT and *Mfap2<sup>−/−</sup>* mice (mean±s.e.m.; n=9,8).  B) Whole body fat and lean content determined by EchoMRI on 5 month old mice (mean±s.e.m.; n=9,8).  C) Body weight of 5 month old mice (mean±s.e.m.; n=9,8).  D) Increased adiposity due to MAGP1 deletion is apparent by 9-10 weeks of age. Longitudinal echoMRI study of whole body adiposity from 5 weeks of age to 15 weeks (mean±s.e.m.; n=10,8).  E) Daily food consumption was measured in 7-week old mice, prior to changes in adiposity. Food intake was determined per mouse, over 7 days (mean±s.e.m; n=5,5).  F) Infrared-based activity measured during light (day) and dark (night) cycles (mean±s.e.m; n=9,8). Student’s t-test was used for single comparisons (*=p-value ≤0.05).

Figure 2: Adipose tissue expansion in *Mfap2<sup>−/−</sup>* mice is associated with metabolic dysfunction.  A) Glucose (GTT) and insulin (ITT) tolerance test in 5-6 month old mice, following 12-hour overnight fast and 1g/kg dextrose injection or 6-hour fast and 0.75U/kg insulin injection (n=7-10).  B) High fat feeding exacerbates the metabolic dysfunction associated with MAGP1 deficiency. Whole-body fat content in response to control chow and high-fat diet (HFD, 60% fat) was determined by EchoMRI (mean±s.e.m., n=8-10).  C) 6-hour fast blood glucose in WT and *Mfap2<sup>−/−</sup>* mice fed chow or high fat diet for 12 weeks (mean±s.e.m.; n=8-10).  D) Serum insulin concentration in response to 1g/kg dextrose injection; WT and *Mfap2<sup>−/−</sup>* mice were fed chow or high fat diet for 12 weeks (mean±s.e.m.; n=7-10).  E) ITT following 6-hour
fast and 0.75U/kg insulin injection on WT and Mfap2−/− mice consuming chow or HFD for 13 weeks (n=8-10 per group). F) Ectopic lipid accumulation is enhanced in MAGP1- deficient tissue. Biopsy EchoMRI and histology [hematoxylin/eosin (H&E), scale bar = 400µm] was performed on liver from WT and Mfap2−/− mice that consumed HFD for 14 weeks (mean±s.e.m.; n=5-6). Student’s t-test was used for single comparisons, *P<0.05.

**Figure 3: Lipid storage is enhanced in Mfap2−/− WAT.** A) Total adipocyte number in epididymal WAT (number of adipocytes per mg of tissue multiplied by total fat pad mass, mean±s.e.m.; n=5). B) White adipocyte size distribution was determined using a Beckman Multisizer, lines represent average of all samples (left). The distribution curves were used to determine the average volume of small and large adipocytes (right), (mean±s.e.m.; n=10,8). C) A pictomicrograph of epididymal WAT from 5 month old WT and Mfap2−/− mice (scale bar = 100µm, image color was inverted to accentuate adipocyte cell borders). D,F) Fatty acid uptake, not lipolysis, is enhanced in Mfap2−/− WAT. Assays were performed on intact WAT explants. D) Fatty acid uptake, determined by uptake of tritiated oleic acid, in the presence/absence of 10µM insulin (mean±s.e.m.; n=5-6). F) Lipolysis, determined by glycerol release following 10µM isoproterenol addition (mean±s.e.m.; n=4-5). E,G) Differential expression of lipid metabolism-associated genes in Mfap2−/− muscle tissue. RT-qPCR was performed on RNA extracted from the muscle of WT and Mfap2−/− mice fed control chow or HFD (mean±s.e.m.; n=5-6). Gene targets included: Lpl (lipoprotein lipase), Cd36 (FA translocase), Plin5 (perilipin 5), Plin2 (ADFP, perilipin 2), Dgat2 (diacylglycerol acyltransferase 2), Cpt1b (carnitine palmitoyl transferase 1b), Ppara (peroxisome proliferator activated-receptor alpha), Pparδ (peroxisome proliferator
activated-receptor delta), Ppargc1a (PGC-1α, PPARγ coactivator 1 alpha), Ucp-3 (uncoupling protein-3). Student’s t-test was used for single comparisons (*=p-value ≤0.05).

**Figure 4: Impaired heat production in Mfap2<sup>−/−</sup> mice.** A) BAT mass is elevated in adult WT and Mfap2<sup>−/−</sup> mice fed either control chow or high-fat diet (HFD) for 16 weeks (mean±s.e.m.; n=7-10). B) Increased BAT mass is due to increased lipid content. BAT lipid to protein content was determined by biopsy EchoMRI (mean±s.e.m.; n=5-6). C) Rectal temperatures of adult mice during day (12pm) and night (12am), (mean±s.e.m.; n=5-6). D) Rectal body temperature in adult mice during a 4°C cold challenge (mean±s.e.m.; n=4-5). E) RT-qPCR of thermogenesis-related genes in BAT from adult mice kept at room temperature (RT) or 4°C for 6 hours. Data represents the averages of samples from two independent studies, (mean±s.e.m.; n=7).

**Figure 5: Increased adiposity in Mfap2<sup>−/−</sup> mice is preceded by impaired energy expenditure.** A) Longitudinal comparison of adiposity (left) and temperature (right). Adiposity (Echo-MRI) and rectal temperature were determined at 1 month then 5 months of age (mean±s.e.m.; n=8-10). B) Energy expenditure was determined by indirect calorimetry in ~7 week old mice. **B-Left** Heat production (EE) curves represent the average of 3 WT or 4 Mfap2<sup>−/−</sup> experimental groups, each group consisted of 3-4 mice. **B-Right** EE is presented as the daytime (1pm-7pm) and nighttime (8pm-7am) values. C) Rectal temperature in young (6 week) mice during 4°C cold challenge (mean±s.e.m.; n=5). D) H&E stained BAT sections from 6-week old WT and Mfap2<sup>−/−</sup> mice, scale bar =100µm. E) Transcript expression of Ucp-1 in the BAT of 6-week old WT and
Mfap2−/− mice (RT-qPCR, mean±s.e.m.; n=5). F) Normal mitochondrial copy number in Mfap2−/− BAT. Copy number is ratio of mitochondrial (Cytb, Nd1) to nuclear (H19) DNA (qPCR, mean±s.e.m.; n=4). G) H&E stained scWAT sections from 6-week old WT and Mfap2−/− mice, scale bar =100µm. H) PGC-1α and Ucp-1 transcript expression in ~6-week old mouse scWAT during 8 hour cold challenge (mean±s.e.m.; n=3-5 per genotype per timepoint). I) Normal mitochondrial copy number in Mfap2−/− scWAT. Copy number is ratio of mitochondrial (Cytb, cytochrome b; Nd1, NADH dehydrogenase subunit 1) to nuclear (H19,) DNA (qPCR, mean±s.e.m.; n=3-5). Student’s t-test was used for single comparisons (*=p-value ≤0.05).

Figure 6: Abberrent TGFβ activity causes impaired thermoregulation and excess adiposity in Mfap2−/− mice. A) Immunoblot of WAT lysate using antibodies to phosphorylated-smad2 (p-smad2), total-smad2 (t-smad2) and gapdh. Shown are lysates from 2 mice per genotype. B-C) WAT from mice on chow or HFD for 16 weeks. B) ECM deposition in WAT was assessed by RT-qPCR of collagen-1 (Col1) and collagen-3 (Col3) and by trichrome staining of tissue. C) WAT macrophage infiltration was assessed by RT-qPCR of macrophage-associated genes [Tnfa (Tnfa), IL6, Itgax (CD11c), Arg1 (arginase-1) and IL10] and immunohistochemistry using an anti-MAC-3 antibody. B-C) Bar graphs are presented as mean±s.e.m, n=5-6 for RT-qPCR. Histology scale bars represent 100µm. D-E) Neutralizing TGFβ antibody (α-TGFβ) treatment prevents excess adiposity and improves body temperature in Mfap2−/− mice. Adiposity (EchoMRI) and rectal temperature were determined before (D) and after (E) 5 weeks of treatment with control IgG or α-TGFβ (mean±s.e.m.; n=4-5 per treatment group).
Figure 7: MAPG1 correlates with TGFβ1 expression in obese humans. A-C) TGFβ1 and MAGP1 transcript expression in human scWAT was determined and compared to each other and the individual’s BMI. Quantitative RT-PCR was used to determine TGFβ1 (TGFB1) and MAGP1 (MFAP2) expression in human scWAT (n=29). A-B) TGFβ1 expression in WAT positively correlates with BMI and MAGP1 expression. C) Human MFAP2 (MAGP1) transcript expression was plotted against the individual’s BMI (n=29). D) Positive correlation between MAGP1 expression in WAT and obesity is confirmed in mice D-top) MAGP1 (Mfap2), not MAGP2 (Mfap5) or fibrillin-1 (Fbn1), correlates with obesity in mice. Transcripts were measured in WAT of mice fed standard chow diet (chow) or high fat diet (HFD). Data was plotted as mean±s.e.m. of HFD WAT expression relative to chow WAT expression (n=5,5). D-bottom) Immunohistochemistry with anti-MAGP1 antibody of WAT from control chow and HFD fed WT mice. E) Proposed model for the mechanism by which MAGP1 regulates energy expenditure in mice. MAGP1 is a sink for TGFβ thereby supporting lipid catabolism and thermogenesis. When active TGFβ concentration becomes excessive (i.e. MAGP1 saturation or MAGP1 deficiency), free TGFβ reduces PPAR and PRDM-16 co-activation of PGC-1α, which in turn prevents activation of UCP-1 transcription and thus, thermogenesis. The imbalance of TGFβ and MAGP1 not only leads to reduced thermogenesis but also to excess lipid accumulation, fibrosis/inflammation, and eventually to features of metabolic syndrome.
Figure 4

A  

B  

C

D  

E

200x256mm (300 x 300 DPI)
Figure 6

A

p-smad2

l-smad2

gapdh

B

C

D

E

WT

Mfap2Δ

WT

Mfap2Δ

WT

Mfap2Δ

WT

Mfap2Δ

201x257mm (300 x 300 DPI)
Supplemental Figure 1: Microfibril components are present in adipose tissue. A) MAGP1 transcript expression in BAT, WAT and epididymal WAT (epWAT) of WT mice, determined by RT-qPCR (mean+s.e.m., n=6). B) MAGP1 deficiency does not alter mRNA transcript expression of other microfibril-associated genes. MAGP1, MAGP2, fibrillin-1 (Fbn1) transcript were quantified by RT-qPCR. RNA was purified from WT and Mipap2⁻/⁻ epWAT (mean+s.e.m., n=10,8). C) MAGP1 deletion in the outbred Black Swiss mouse strain results in increased adiposity. Percent whole body fat was determined by DEXA (mean+s.e.m.; n=5,8). Student’s t-test was used for single comparisons (*P<0.05).