Published in final edited form as:
Int J Obes (Lond). 2010 December ; 34(12): 1695–1705. doi:10.1038/ijo.2010.107.

Secreted frizzled-related protein 1 regulates adipose tissue expansion and is dysregulated in severe obesity

Claire Lagathu[1], **Constantinos Christodoulides**[2], **Chong Yew Tan**[1], **Sam Virtue**[1], **Matthias Laudes**[1], **Mark Campbell**[1], **Ko Ishikawa**[1], **Francisco Ortega**[4], **Francisco J. Tinahones**[3], **Jose-Manuel Fernández-Real**[4], **Matej Orešič**[5], **Jaswinder K. Sethi**[1], and **Antonio Vidal-Puig**[1]

[1]Institute of Metabolic Science – Metabolic Research Laboratories, University of Cambridge, Addenbrooke's Hospital, Cambridge UK [2]Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK [3]Service of Endocrinology and Nutrition, Hospital Clinico Universitario Virgen de Victoria de Malaga, Malaga, Spain [4]Department of Diabetes, Endocrinology and Nutrition, Hospital of Girona Dr. Josep Trueta, Biomedical Research Institute and CIBEROBN Fisiopatología de la Obesidad y Nutrición CB06/03/010, Girona, Spain [5]VTT Technical Research Centre of Finland, Espoo, Finland

Abstract

Aim—The Wnt/β-catenin signalling network offers potential targets to diagnose and uncouple obesity from its metabolic complications. Here we investigate the role of the Wnt antagonist, secreted Frizzled related protein 1 (SFRP1) in promoting adipogenesis *in vitro* and adipose tissue expansion *in vivo.*

Methods—We use a combination of human and murine, *in vivo* and *in vitro* models of adipogenesis, adipose tissue expansion and obesity-related metabolic syndrome to profile the involvement of SFRP1.

Results—Secreted Frizzled related protein 1 (SFRP1) is expressed in both murine and human mature adipocytes. The expression of SFRP1 is induced during *in vitro* adipogenesis and SFRP1 is preferentially expressed in mature adipocytes in human adipose tissue. Constitutive ectopic expression of SFRP1 is proadipogenic and inhibits the Wnt/β-catenin signalling pathway. *In vivo* endogenous levels of adipose SFRP1 are regulated in line with proadipogenic states. However, in longitudinal studies of high fat diet-fed mice we observed a dynamic temporal but biphasic regulation of endogenous SFRP1. In agreement with this profile we observed that SFRP1 expression in human tissues peaks in patients with mild obesity and gradually falls in morbidly obese subjects.

Address correspondence and reprint requests to: Jaswinder K. Sethi, jks30@cam.ac.uk; Tel. +44-1223-762633; Fax. +44-1223-330598: or Antonio J. Vidal-Puig, ajv22@cam.ac.uk; Tel. +44-1223-762790; Fax. +44-1223-330598; Institute of Metabolic Science – Metabolic Research Laboratories, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

Europe PMC Funders Author Manuscripts

Conclusions—Our results suggest that SFRP1 is an endogenous modulator of Wnt/β-catenin signalling and participates in the paracrine regulation of human adipogenesis. The reduced adipose expression of SFRP1 in morbid obesity and its knock-on effect to prevent further adipose tissue expansion may contribute to the development of metabolic complications in these individuals.

Keywords

Obesity; Metabolic syndrome; Adipose tissue; Adipogenesis; Wnt Signalling

Europe PMC Funders Author Manuscripts

INTRODUCTION

Obesity is associated with a greater risk of developing chronic diseases, such as cardiovascular disease, type 2 diabetes, atherosclerosis and the so-called Metabolic Syndrome (1, 2). Despite improvements in social awareness, political measures to promote changes in life style and scientific success in unravelling the mechanisms controlling energy balance, the prevalence of obesity continues to escalate. Current predictions indicate that the epidemic of obesity will be followed by a second wave of devastating cardiovascular complications. Thus in parallel with efforts to tackle the obesity problem, a more pragmatic approach suggests that efforts should aim to uncouple obesity from its cardiometabolic complications.

Although seemingly counter intuitive, strategies that increase the capacity of adipose tissue to store lipid and therefore make individuals more obese may confer metabolic benefits. Allowing adipose tissue to store more lipid may prevent secondary metabolic complications caused by lipid being deposited in non-adipose organs. The concept of adipose tissue expansion being beneficial is supported by the apparent paradox that a paucity of adipose tissue results in lipodystrophy and this also recapitulates many features of the Metabolic Syndrome. Taken together it is likely that an optimal range exists for total body adipose tissue mass, beyond these boundaries of which metabolic dysregulation develops. In this regard the ability of adipose tissue to expand and match the storage needs of energy surplus may be a key determinant of the susceptibility to developing Metabolic Syndrome associated with obesity (1-4). Thus, understanding the signalling factors that control the titrated process of adipose tissue expansion is fundamental for a rational approach to design effective therapies to prevent and treat the Metabolic Syndrome (5).

The Wnt family of secreted growth factors act in a paracrine and/or autocrine manner and are known to control adipocyte differentiation (6-9). Wnt signalling relies on a sophisticated regulated network capable of providing context-dependent graded responses. Briefly, binding of specific Wnt proteins to Frizzled receptors transduces intracellular signals through either β-catenin-dependent or β-catenin-independent pathways. While both pathways may be active in preadipocytes (10), it is the former that has been best characterised and shown to potently inhibit adipogenesis both *in vitro* and *in vivo* (8, 9). In the β-catenin-dependent pathway, receptor activation leads to stabilisation and accumulation of cytosolic β-catenin. β-catenin subsequently translocates to the nucleus where it binds and activates the lymphoid enhancer-binding factor/T cell-specific transcription factor (LEF/

TCF) family of transcription factors. Wnt/TCF target genes, include *cyclin D1*, *Id2* and *c-myc* which inhibit adipogenesis (11, 12).

Constitutive activation of Wnt/β-catenin signalling in preadipocytes, inhibits differentiation, by preventing the induction of C/EBPα and PPARγ (13, 14). Conversely, inactivation of intracellular Wnt/β-catenin signalling releases the brake on adipogenesis (8, 11, 12, 15). Adipogenesis may also be enhanced by extra-cellular Wnt antagonists including secreted frizzled related proteins (SFRPs, also known as secreted apoptosis related proteins or SARPs)(16, 17). At least five structurally similar SFRPs have been identified and are characterised by a cysteine-rich (CRD) domain which resembles the Wnt ligand-binding domain found on Frizzled receptors (17). It is this domain that is required to provide modulator activities for Wnt ligands (18, 19). Consistent with this, exogenous treatment with recombinant SFRP1 and SFRP2 can disrupt Wnt/β-catenin signalling and promote adipocyte differentiation (15). Furthermore, the *Sfrp1* knockout mice also show a reduction in percent body fat (20) consistent with unopposed anti-adipogenic Wnt/β-catenin signalling. However, there is limited evidence to support a role for endogenous SFRP1 in the physiological and/or pathological development of human obesity and the metabolic syndrome. Here we report on SFRP1 expression profile studies in humans and mice and *in vitro* functional assays to examine the role and regulation of *SFRP1* during adipogenesis and in the development of human and mouse obesity.

MATERIALS and METHODS

Subjects

The characteristics of the populations studied are summarized in the online-appendix. Written informed consent was obtained from all subjects before enrolment and the appropriate Research Ethics Committees approved the studies. Four independent study populations were used: **Group A** (used in Fig 1A and 1B) comprised of samples acquired from 8 subjects undergoing elective open abdominal surgery at Addenbrooke's Hospital (6 males and 2 females, Age 66±10 years, BMI 26.2±3.8kg/m^2). All subjects were fasted for 6 hours prior to the operation. None were taking medications known to affect adipose tissue mass or metabolism (12). **Group B** (used in Fig 5A) comprised of adipose tissue obtained from subcutaneous depots during elective surgical procedure. Samples were collected from 31 female subjects with a BMI between 18 and 70 kg/m^2 who were invited to participate at the Endocrinology Service of the Hospital Universitari de Girona Dr. Josep Trueta (Girona, Spain), at the Hospital Clinico Universitario Virgen de Victoria de Malaga (Málaga, Spain) (21). **Group C** (used in Fig 5B) comprised of needle subcutaneous adipose tissues biopsies obtained from 13 monozygotic twin pairs (8 Male and 5 Female pairs) discordant for weight identified through the national population registry of Finland. One co-twin not obese (BMI <25 kg/m2), and the other one obese (BMI >30 kg/m2). The recruitment and selection process of subjects were as previously published (22). For all subjects, BMI cut-off was determine according to WHO BMI classification (http://apps.who.int/bmi/).

Murine WAT expression studies

Animals were housed in a temperature-controlled room with 12-h light/dark cycles. Food and water were available *ad libitum* unless noted. For analysis of the fed state, animals were sacrificed immediately following a dark cycle. Induction of the fasted state involves a withdrawal of feed for 12 hours, while the refeeding state involves the reintroduction of food for 12 hours following a fast. Both the murine diet studies (High Fat Diet; HFD andThiazolidinedione supplemented; TZD) were performed as previously described (23). All animal protocols used in this study were approved by the UK Home Office and the University of Cambridge.

Human WAT fractionation and expression studies

Human adipocyte and preadipocyte isolation were performed on samples obtained from Group A subjects as previously described (12, 23). Briefly, adipose tissue biopsies were placed in PBS (Sigma-Aldrich) and processed within 30 minutes. Samples were finely diced and digested in collagenase solution (Hank's balanced salt solution containing 3 mg/ml type II collagenase (Sigma-Aldrich) and 1.5% bovine serum albumin) at 37 °C for 1 hour. Subsequently, the digest was filtered through a stainless steel mesh and centrifuged at 400g for 5 minutes to separate mature adipocytes from the stromo-vascular cells.

Primary preadipocyte cultures

For human preadipocyte isolation, human adipose tissue samples were obtained from Group A subjects. Murine primary preadipocytes were isolated from epididymal WAT of 6 weeks old male C57B/6 as described previously (24). Human and murine primary culture were induced to differentiate three days post confluence, by adding DMEM/Hams F12 with 33 μmol/L Biotin, 17 μmol/L panthothenic acid, 10μg/ml apotransferin, 0.2 nmol/L triiodothyronine, 100 nmol/L cortisol, 500 nmol/L insulin). For the first three days of culture, 0.25 mmol/L 1-methyl-3-isobutylxanthine (IBMX) was also added to the medium. Differentiation medium was replaced after 3 days on the first occasion and thereafter every 2 days.

Generation, culture and differentiation of 3T3-L1 preadipocyte cell lines

SFRP1 3T3-L1 cell lines (or control) were generated with the pBabe-Puro retroviral vector system as previously described (11). The retroviral expression construct encoding Human SFRP1 cDNA was cloned by excision from Human image clone # 5265277 (NCBI BC036503) with Xma1 and Not1 digest, followed by Klenow fill-in and blunt-end-ligation into the SnaB1 site of the retroviral expression vector pBabe-puro. Correct orientation was confirmed by at least three different diagnostic digests and sequencing. 3T3-L1 cells were cultured, differentiated into adipocytes and stained for Oil Red O as described previously (11). Briefly, 3T3-L1 preadipocytes were differentiated 2 days post confluence in DMEM with 10% fortified bovine serum (Cosmic Calf Serum, Hyclone) supplemented with 5nmol/L insulin, 0.25 μmol/L dexamethasone, and 0.5 mmol/L IBMX (for full induction cocktail; MDI). IBMX was omitted from submaximal induction cocktail (DI). The medium was then supplemented only with insulin for 2 days. Differentiated cells were only used when at least 95% of the cells showed an adipocyte phenotype by accumulation of lipid

droplets by Day 8. Cells were analysed by phase contrast microscopy. All retrovirally transfected 3T3-L1 cell lines were kept in puromycin-containing medium throughout culture and differentiation procedures.

RNA isolation and quantitative RT-PCR

RNA preparation, reverse transcription and conditions for TaqMan real-time reverse transcription (RT)-PCR were performed as previously described; (12) and (23) for **Groups A**; (21) for **Group B**; and (22) for **Group C**. Primers and probes were either purchased from Perkin Elmer or designed using Primer Express software (Applied Biosystems) and sequences from the GenBank database.

Protein extraction and Western blotting

Before protein analysis by Western blotting, medium was removed and cell monolayers were washed with ice-cold PBS and then frozen in liquid nitrogen. These were thawed on ice and scraped into lysis buffer as previously described (23) at 4°C. Proteins were electroblotted onto nitrocelluose membrane (Amersham Biosciences, Piscataway, NJ, USA). Specific proteins were detected by incubation with the appropriate primary and horseradish-peroxidase-conjugated secondary antibodies. Immune complexes were detected by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA).

Wnt-TCF4/LEF reporter assay

For TCF4 reporter assay, SFRP1 and EV 3T3-L1 cells grown to confluence and Topflash promoter-reporter assays were performed as previously described (12).

Statistical analysis

Data from densitometrical analysis, luciferase assays and quantitative real-time PCR are presented as mean ± SEM of at least three independent experiments. Statistical significance was determined with Student's t-test (*P<0.05, **P<0.01, ***P<0.001). Analysis of variance was determined using single factor ANOVA on SPSS 16.0.

RESULTS

SFRP1 gene expression increases during human and murine adipogenesis

Our previous microarray profiling studies of human preadipocytes identified numerous novel genes regulated early during adipogenesis (25). These included SFRP1, a secreted Wnt antagonist, which was induced 3-fold, 48 hours after onset of differentiation. This observation was first validated by measuring *SFRP1* gene expression during differentiation of subcutaneous preadipocytes from human subjects using Real Time PCR (Fig 1A). Our results indicate that *SFRP1* mRNA can be detected in confluent preadipocytes and that its expression increases following induction of adipocyte differentiation, being four-fold higher at 48h hours compared to baseline. This is consistent with the greater relative expression found in mature human adipocytes relative to stromavascular the fraction (Fig 1B). The adipogenic profile is also not restricted to human adipogenesis, since *Sfrp1* mRNA was also increased following induction of differentiation in both, mouse primary SVF cultures (Fig.

1C) and in the murine preadipocyte cell line, 3T3-L1 (Fig. 1D). SFRP1 protein levels closely followed the pattern of RNA expression (Fig. 1E). These results also indicate that the 3T3-L1 cell model could be a useful tool to further characterise the regulation and functional role of SFRP1 during adipogenesis.

SFRP1 overexpression promotes adipogenesis in 3T3-L1 preadipocytes through the inhibition of Wnt/β-catenin dependent signalling

To assess the functional consequences of increased SFRP1 expression on the process of adipocyte differentiation we generated stable preadipocyte cell lines constitutively over-expressing human SFRP1. *SFRP1* expressing 3T3-L1 preadipocytes displayed a 10-fold increase in *SFRP1* mRNA compared to empty vector (EV) expressing 3T3-L1 cells (Fig. 2A). Following adipogenesis, significantly greater levels of SFRP1 expression were maintained in day 8 adipocytes (Fig. 2A). A similar expression pattern was reflected in the protein levels of sFRP1 (Fig. 2B). To examine whether SFRP1 could modulate Wnt/β-catenin signalling, EV and SFRP1 expressing preadipocytes were induced to differentiate and cytosolic protein extracts were screened for β-catenin levels (Fig. 2C). When compared to control EV preadipocytes, ectopic expression of human SFRP1 resulted in decreased cytosolic β-catenin levels in 3T3-L1 preadipocytes. This reduction was also consistent with reduced expression of CyclinD1 (*Ccnd1*), a Wnt/β-catenin target gene during adipogenesis (fig. 2D). Furthermore, in promoter assays, SFRP1 expressing cells also displayed reduced TCF/LEF activity (Fig 2E), and appeared to be less responsive to exogenous Wnt3a treatment (Fig. 2E). Hence, SFRP1 can inhibit Wnt/β-catenin-dependent signalling in stably transfected 3T3-L1 preadipocytes.

To evaluate the impact of increased SFRP1 levels on adipogenic potential, lipid accumulation was assessed in EV and SFRP1 over expressing preadipocytes following adipogenic induction. As illustrated in Figure 3A, although SFRP1 3T3-L1 cells and EV 3T3-L1 cells accumulated lipids to a similar extent when stimulated with the full induction cocktail (MDI), sub-maximal stimulation (DI) revealed that SFRP1 3T3-L1 cells have greater adipogenic potential. This was consistent with increased expression levels of the adipogenic/lipogenic markers aP2 (*Fabp4*) and PPARγ (*Pparg*) in DI-treated SFRP1 3T3-L1 cells (Fig. 3B and 3C respectively). Thus, constitutive expression of SFRP1 in 3T3-L1 preadipocytes promotes adipogenesis and lipid accumulation. This suggests that in vivo endogenous SFRP1 is a good candidate to facilitate adipogenesis and that it is not simply induced as a consequence of differentiation.

Sfrp1 is regulated in vivo by nutritional and metabolic status

We next sought to determine whether *Sfrp1* mRNA levels were physiologically regulated *in vivo* by nutritional status. Fasting and refeeding experimental conditions were selected as opposing physiological paradigms with respect to acute fuel availability and requirement for storage in adipose tissue. We evaluated *Sfrp1* mRNA expression in whole white adipose tissue (WAT) and isolated mature adipocytes. In response to 24hr fasting, there was a non-significant trend toward reduced *Sfrp1* levels relative to whole adipose tissue collected from fed mice (p=0.07). However, in response to 24hr-refeeding, *Sfrp1* mRNA levels were markedly increased beyond the levels found in the fed state (Fig. 4A) suggesting WAT

SFRP1 is regulated during changes in nutritional flux. The same profile was observed in mature adipocytes isolated from a separate group of mice subjected to the same manipulation (Fig. 4B).

To further investigate the physiological regulation of *Sfrp1* in the context of sustained positive energy balance, we analysed the expression of *Sfrp1* in adipose tissue of mice fed a high fat diet. We observed that following short-term exposure (3 days) to a high fat diet (HFD), *Sfrp1* expression was significantly induced (Fig. 4C). Intriguingly, with longer term HFD exposure (i.e. 4 weeks and 6 months), adipose tissue expansion plateaus (Fig 4E) and becomes increasingly dysfunctional as assessed by Glut4 expression (Fig. 4D). During this time, the relative levels of *Sfrp1* progressively fall and by 6 months, they drop below the levels observed in lean chow fed mice (Fig 4C).

To further test the correlation between fat deposition and endogenous SFRP1 expression, we used rosiglitazone (TZD) treatment as a pharmacological approach to stimulate adipogenesis and lipid accumulation *in vivo*. Mice fed a TZD supplemented diet showed increased expression of adipogenic markers in their adipose tissue such as aP2 (23). More importantly this increase in adipose tissue expandability was associated with significantly increased *Sfrp1* levels (Fig. 4F).

Taken together, these observations suggest that *in vivo*, WAT *Sfrp1* mRNA level is increased when there is a demand for fat storage and adipose expansion; a finding consistent with its *in vitro* role in promoting recruitment of new adipocytes and adipocyte maturation. However, this increase in *Sfrp1* appears not to be sustainable despite the maintenance of a positive caloric balance and fat storage demand. This suggests that SFRP1 may be a useful marker, and supports the existence of an upper physiological limit to which adipose depot can expand and accumulate lipid. Interestingly, the fall in *Sfrp1* levels at 6 months of HFD is associated with the development of metabolic complications.

SFRP1 expression in human adipose tissue

We next investigated whether the results observed in rodents could be extrapolated to obesity and metabolic dysregulation in humans. First we profiled *SFRP1* expression in adipose tissue in a cohort of 31 non-diabetic white European female subjects across a range of body mass indices (BMI) (Table 1 supplement). Based on our previous observations of murine *Sfrp1* expression during diet-induced obesity (Fig. 4C), we postulated that *SFRP1* expression in human adipose tissue would also vary in a manner that would be associated with a) the degree of adiposity (i.e. BMI), b) the remaining potential of adipose tissue for further expansion and/or c) the metabolic status (insulin sensitivity). As shown in Figure 5A the profile of *SFRP1* expression in human WAT tends to rise in states of mild obesity, peaking at a BMI of 30-35, and gradually falls back to baseline levels in individuals with higher BMIs. Although the limited sample size in the 30-35kg/m^2 BMI category (n=3 subjects) prevented ANOVA analysis from achieving statistical significance ($p = 0.059$), a second independent cohort of subjects with BMI between 25 and 30 further substantiated this observation (fig. 5B). Specifically, microarray expression analysis was performed on subcutaneous adipose tissue from 13 pairs (8 male pairs and 6 female pairs) of monozygotic twins discordant for weight (22). The average BMI was 25.17 kg/m^2 (95% CI: 24.5-25.9

kg/m^2) and 30.57 kg/m^2 (95% CI: 29.5-31.5 kg/m^2) for lean and obese twins respectively ($p<0.001$). This degree of obesity falls in the region where the previous data would suggest a correlation between a rise in *SFRP1* levels and BMI. Despite the mean difference in BMI (between the lean and obese twin within each pair) being as little as 5.39kg/m^2, *SFRP1* levels in WAT showed a significantly higher expression in the obese twins ($p=0.005$) (Fig. 5B). These findings are consistent with the data generated from our rodent models and support the hypothesis that *SFRP1* expression increases when BMI rises, but with sustained positive energy balance and despite marked adipose expansion, the adipose tissue is no longer able to maintain a high level of expression and the levels of *SFRP1* gradually fall.

DISCUSSION

Our group, together with others, have previously shown that the Wnt/β-catenin signalling pathway can regulate adipogenesis both *in vitro* and *in vivo* (reviewed in (8, 9)). Many of these studies have focused on genetic and/or pharmacological manipulation of Wnt/β-catenin signalling components in murine models. However, recently we have sought to extend these investigations to better understand the role of endogenous Wnt signalling in adipose tissue plasticity in rodents and humans. Recently, we reported on a nutritionally regulated, preadipocyte gene, Dact1, which promotes adipogenesis through coordinated effects on gene expression. This results in the selective alteration of both intracellular and paracrine/autocrine components of the Wnt/β-catenin signaling pathway (23). Furthermore, the expression and activity of Wnt/β-catenin signaling network appears to be context and species dependent, indeed some components such as Dkk1, appear to be expressed in human adipose tissue but not in murine models (12).

Here we demonstrate that SFRP1, may also be an important extracellular modulator of Wnt/β-catenin signalling and is itself regulated during adipogenesis and adiposity. We have shown that SFRP1 is dynamically regulated during both human and murine adipogenesis and also promotes adipocyte differentiation *in vitro*. A proadipogenic role for SFRP1 is consistent with the reduced adiposity of SFRP1-null mice(20). More importantly, we also present evidence that endogenous SFRP1 is acutely upregulated in response to re-feeding and short-term HFD *in vivo*, in WAT. This places SFRP1 as a strong candidate that contributes to the titrated adipose tissue response to short term nutritional challenges. However, our data also supports the concept that the same homeostatic system that regulates adipose tissue expansion and function is limited and can fail under chronic nutritional surplus, as found in morbid obesity. Beyond a set limit of adipose tissue mass, the capacity for further adipose tissue expansion is exhausted, which we believe results in the metabolic complications associated with obesity.

Our results from mouse models indicate that *Sfrp1* is up regulated *in vivo* during anabolic states leading to fat deposition; such as re-feeding and activation of the proadipogenic programme by thiazolidinedione treatment. Induction of *Sfrp1* expression is also a feature of the early stages of HFD-induced obesity, particularly when fat accretion rate is at its peak and the rate of adipose expansion, with respect to total adipose mass, is at its highest. However, just as increase in fat mass expansion reaches a plateau despite continued positive energy balance, the program of adipogenesis *in vivo* is unlikely to be sustained indefinitely.

Intriguingly, it is under these circumstances that we observed a relative down regulation of *Sfrp1* expression (after 6 months of exposure to HFD). These observations suggest that *Sfrp1* exerts a modulatory role on Wnt signalling in immature adipocytes and in preadipocytes, which may ensure the timely recruitment and development of adipose tissue to accommodate a surplus of energy in the early phases of the evolution towards obesity. Moreover, the biphasic profile ending in marked down regulation of SFRP1 when the adipose tissue mass is no longer expanding (but interestingly is also more metabolically compromised) suggests that the subsequent down regulation of *Sfrp1* with sustained caloric excess may reflect the fact that this adipose tissue has reached its physiological limit of expansion. To date, only Dact1 has been reported to exhibit such a profile, albeit slightly delayed. In contrast, the expression of closely related Sfrp5 in the same tissues continues to increase as nutritional surplus continues (23). Taken together these data suggest that the biphasic phenomenon is restricted to specific components of the Wnt/β-catenin signalling.

Despite the well established role for Wnt/β-catenin signalling during adipogenesis *in vitro* and in *vivo*, an important aspect that remains less well characterised is whether the same signals play a role in regulating human adipose tissue mass. We show that endogenous *SFRP1* is also regulated in human adipose tissue and that it is predominantly expressed in adipocytes. Furthermore, our cross sectional studies suggest that SFRP1 expression may exhibit a biphasic profile in human adipose tissue, similar to that observed in rodents. Specifically, human adipose *SFRP1* expression rises in individuals showing mild obesity (BMI between 25kg/m2 and 30kg/m2) and progressively falls thereafter during the evolution towards morbid obesity. Clearly complete validation of this model in humans would require a prospective study, which currently is not feasible.

We have already demonstrated that WNT signalling components found in adipose tissue are not the same in mice and men (12). Indeed, this may be pertinent here since human and murine SFRP1 share only 55% identity at the amino acid level and, we find that unlike the data from our murine models of diet-induced obesity, the relative *SFRP1* levels in WAT from morbidly obese individuals (BMI >40kg/m2) do not fall below that of lean individuals (BMI <25kg/m2). However, a more careful interrogation reveals that both experiments may not be directly comparable. In fact a key difference is that after 6 months of HFD, C57Bl6 mice become severely insulin resistant and have impaired glucose tolerance, while the cross sectional human study recruited individuals with normal glucose tolerance. Given the impaired glucose tolerance of the 6 month HFD-fed mice a better comparison between the human and murine obese groups may be made between the 4 week HFD-fed mice and the obese individuals (Fig 4C vs Fig 5A). Although a complete metabolic profile is not available for this human data set and a small number of female subjects only, were studied, it is clear that this human data can not be used alone to represent the general population. However, the findings from this first study are consistent with a) the in vitro data, b) the murine data and c) the human twin study data. Therefore, we believe that the observed trend to be supportive of the role of SFRP1 in adipose tissue expansion. Taken together it provides a compelling basis for the focus of future studies to further analyse/explore these novel observations.

In obese insulin resistant states, localised adipose tissue inflammation occurs and may contribute to limiting further adipose tissue expansion. Indeed, we and others have

demonstrated that pro-inflammatory cytokines found in insulin resistant states can impair adipogenesis by converging with Wnt/β-catenin signalling (11, 26). There is also evidence of impaired adipogenic capacity in human obesity (27-29). The elevated inflammatory cytokines found in insulin resistant states of obesity may provide an explanation for the reduced levels of SFRP1 found in the C57Bl6 mice fed a HFD for 6 months. To confirm this relationship in human WAT, it would be necessary to have access to adipose tissue from severely obese patients with untreated and/or uncontrolled diabetes. That being said this does not preclude the possibility that the apparently “normal” levels of SFRP1 observed in morbid obesity should be considered inappropriately low for the degree of adipose expansion of these individuals. In this respect this inappropriate levels of SFRP1 may reflect the biological limit to which these individuals can expand their fat depot.

Overall, our results suggest a model of adipose tissue expansion characterised by upregulation of *SFRP1* in early stages of obesity. This elevation of SFRP1, could inhibit Wnt signalling and therefore facilitate the adipose tissue expansion, allowing nutrient storage demands to be met. Further weight gain results in sFRP1 levels falling back to the same values as lean subjects. These low levels of sFRP1 in more obese subjects could be considered as inappropriately low for their adipose tissue mass. Low levels of sFRP1 observed in obesity may limit adipogenesis through increased Wnt/β-catenin signalling, compromising further adipose tissue expansion (Fig. 6). We postulate that this failure to further accommodate surplus energy may by default facilitate ectopic lipid accumulation and promote metabolic complications. We also speculate that changes in the levels of SFRP1 may alter the balance between the processes of preadipocyte recruitment/growth and potentially have a direct impact on hypertrophic and hyperplastic patterns of adipose tissue expansion. Given the increased metabolic complications found in hypertrophic obesity compared to hyperplastic obesity, increasing adipose tissue cellularity through increased SFRP1 levels is an attractive therapeutic strategy.

In summary, we have identified and characterised the role of SFRP1, a secreted inhibitor of Wnt signalling, as a determinant of adipose tissue expandability. Future studies using larger cohorts may provide evidence of its usefulness as a marker that heralds the risk of obesity-associated metabolic complications. Collectively our data support the concept of a maximum capacity of adipose tissue expansion and further implicates Wnt signalling as an important modulator of adipose tissue expandability with potential therapeutic use in the treatment of obesity-associated metabolic complications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank the Addenbrooke’s hospital surgeons for performing the adipose tissue biopsies. This research is funded by the Biotechnology and Biological Science Research Council (BBSRC), Medical Research Council (MRC), and the European Union Sixth Framework Programme on Hepatic and Adipose tissue functions in the Metabolic Syndrome (EU-FP6 HEPADIP) (A.V.P. and J.K.S.). Personal support is also acknowledged as follows: ALFEDIAM and Marie Curie Postdoctoral fellowship (C.L.), MRC Doctoral Studentships (C.C. and S.V.), MRC Career Establishment Award (A.V-P.) and BBSRC David Phillips Fellowship (J.K.S.).

REFERENCES

1. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature. 2006; 444:881–887. [PubMed: 17167477]
2. Sethi JK, Vidal-Puig AJ. Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. J Lipid Res. 2007; 48:1253–1262. [PubMed: 17374880]
3. Virtue S, Vidal-Puig A. It's not how fat you are, it's what you do with it that counts. PLoS Biol. 2008; 6:e237. [PubMed: 18816166]
4. Tan CY, Vidal-Puig A. Adipose tissue expandability: the metabolic problems of obesity may arise from the inability to become more obese. Biochem Soc Trans. 2008; 36:935–940. [PubMed: 18793164]
5. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008; 9:367–377. [PubMed: 18401346]
6. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol. 1998; 14:59–88. [PubMed: 9891778]
7. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 2004; 20:781–810. [PubMed: 15473860]
8. Prestwich TC, Macdougald OA. Wnt/beta-catenin signaling in adipogenesis and metabolism. Curr Opin Cell Biol. 2007; 19:612–617. [PubMed: 17997088]
9. Christodoulides C, Lagathu C, Sethi JK, Vidal-Puig A. Adipogenesis and WNT signalling. Trends Endocrinol Metab. 2009; 20:16–24. [PubMed: 19008118]
10. Kennell JA, MacDougald OA. Wnt signaling inhibits adipogenesis through beta-catenin-dependent and -independent mechanisms. J Biol Chem. 2005; 280:24004–24010. [PubMed: 15849360]
11. Cawthorn WP, Heyd F, Hegyi K, Sethi JK. Tumour necrosis factor-alpha inhibits adipogenesis via a beta-catenin/TCF4(TCF7L2)-dependent pathway. Cell Death Differ. 2007; 14:1361–1373. [PubMed: 17464333]
12. Christodoulides C, Laudes M, Cawthorn WP, Schinner S, Soos M, O'Rahilly S, et al. The Wnt antagonist Dickkopf-1 and its receptors are coordinately regulated during early human adipogenesis. J Cell Sci. 2006; 119:2613–2620. [PubMed: 16763196]
13. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab. 2006; 4:263–273. [PubMed: 17011499]
14. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 2006; 7:885–896. [PubMed: 17139329]
15. Bennett CN, Ross SE, Longo KA, Bajnok L, Hemati N, Johnson KW, et al. Regulation of Wnt signaling during adipogenesis. J Biol Chem. 2002; 277:30998–31004. [PubMed: 12055200]
16. Jones SE, Jomary C. Secreted Frizzled-related proteins: searching for relationships and patterns. Bioessays. 2002; 24:811–820. [PubMed: 12210517]
17. Kawano Y, Kypta R. Secreted antagonists of the Wnt signalling pathway. J Cell Sci. 2003; 116:2627–2634. [PubMed: 12775774]
18. Dennis S, Aikawa M, Szeto W, d'Amore PA, Papkoff J. A secreted frizzled related protein, FrzA, selectively associates with Wnt-1 protein and regulates wnt-1 signaling. J Cell Sci. 1999; 112(Pt 21):3815–3820. [PubMed: 10523516]
19. Galli LM, Barnes T, Cheng T, Acosta L, Anglade A, Willert K, et al. Differential inhibition of Wnt-3a by Sfrp-1, Sfrp-2, and Sfrp-3. Dev Dyn. 2006; 235:681–690. [PubMed: 16425220]
20. Bodine PV, Zhao W, Kharode YP, Bex FJ, Lambert AJ, Goad MB, et al. The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol. 2004; 18:1222–1237. [PubMed: 14976225]
21. Ortega FJ, Mayas D, Moreno-Navarrete JM, Catalan V, Gomez-Ambrosi J, Esteve E, et al. The Gene Expression of the Main Lipogenic Enzymes is Downregulated in Visceral Adipose Tissue of Obese Subjects. Obesity (Silver Spring). 2009
22. Pietilainen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, Keranen H, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. PLoS Med. 2008; 5:e51. [PubMed: 18336063]

23. Lagathu C, Christodoulides C, Virtue S, Cawthorn WP, Franzin C, Kimber WA, et al. Dact1, a nutritionally regulated preadipocyte gene, controls adipogenesis by coordinating the Wnt/beta-catenin signaling network. Diabetes. 2009; 58:609–619. [PubMed: 19073771]

24. Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, et al. The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-gamma2 isoform. Diabetes. 2005; 54:1706–1716. [PubMed: 15919792]

25. Laudes M, Christodoulides C, Sewter C, Rochford JJ, Considine RV, Sethi JK, et al. Role of the POZ zinc finger transcription factor FBI-1 in human and murine adipogenesis. J Biol Chem. 2004; 279:11711–11718. [PubMed: 14701838]

26. Isakson P, Hammarstedt A, Gustafson B, Smith U. Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor-alpha, and inflammation. Diabetes. 2009; 58:1550–1557. [PubMed: 19351711]

27. Yang X, Jansson PA, Nagaev I, Jack MM, Carvalho E, Sunnerhagen KS, et al. Evidence of impaired adipogenesis in insulin resistance. Biochem Biophys Res Commun. 2004; 317:1045–1051. [PubMed: 15094374]

28. Permana PA, Nair S, Lee YH, Luczy-Bachman G, Vozarova De Courten B, Tataranni PA. Subcutaneous abdominal preadipocyte differentiation in vitro inversely correlates with central obesity. Am J Physiol Endocrinol Metab. 2004; 286:E958–962. [PubMed: 14970008]

29. Tchoukalova Y, Koutsari C, Jensen M. Committed subcutaneous preadipocytes are reduced in human obesity. Diabetologia. 2007; 50:151–157. [PubMed: 17096115]

Europe PMC Funders Author Manuscripts

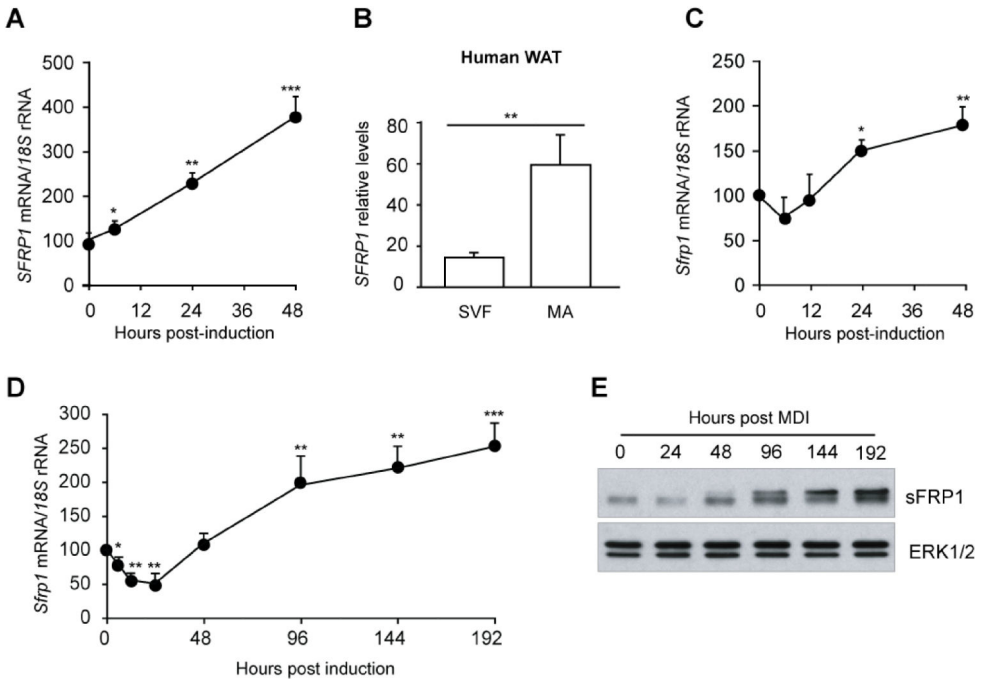

Figure 1. SFRP1 expression during human and mouse adipogenesis

(A) Human *SFRP1* mRNA levels, normalized to 18S rRNA levels, were measured using real time RT PCR at the indicated hours of differentiation of primary human (Group A) SVF cultures. *P<0.05, **P<0.01, ***P<0.001 versus Time 0. **(B)** SFRP1 mRNA levels, normalized to 18S rRNA, were measured in stroma-vascular cells (SVF) and mature adipocytes (MA) from human subcutaneous white adipose tissue (WAT from Group A, n=8). **P<0.01. **(C)** Mouse *Sfrp1* mRNA levels, normalized to 18S rRNA levels, were measured using real time RT PCR at the indicated hours of differentiation of primary mouse SVF cultures. *P<0.05, **P<0.01 versus Time 0. **(D)** *Sfrp1* mRNA levels, normalized to 18S rRNA levels, were measured at the indicated hours post-induction (MDI) of 3T3-L1 preadipocyte differentiation. *P<0.05, **P<0.01, ***P<0.001 versus Time 0. **(E)** Whole-cell protein lysates were extracted at the indicated days of differentiation of 3T3 L1 cells and analysed by immunoblotting. Representative immunoblots of Sfrp1 and ERK1/2 (loading control) are shown.

Europe PMC Funders Author Manuscripts

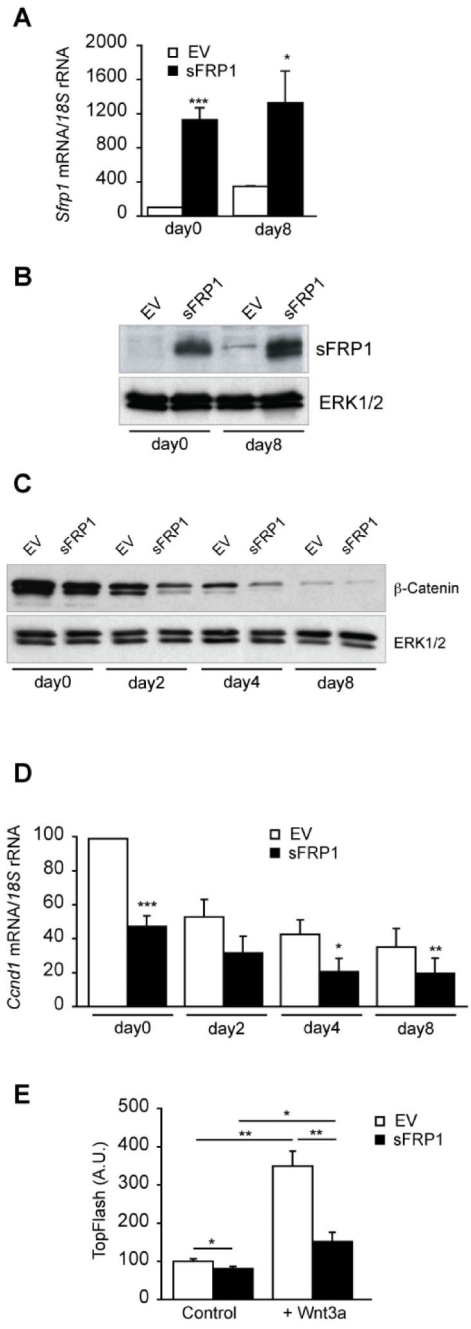

Figure 2. SFRP1 is an antagonist of the Wnt/β-catenin signalling in 3T3-L1 adipocytes

Preadipocytes were infected with a retrovirus carrying Human SFRP1 or vector alone (EV). **(A)** SFRP1 mRNA levels, normalized to 18S rRNA levels, were measured using real time RT PCR at day 0 and day 8 of differentiation in SFRP1 and EV expressing cells. *P<0.05, ***P<0.001 versus EV. **(B)** Whole-cell lysates were extracted at day 0 and day 8 of differentiation in SFRP1 and EV expressing cells. Representative immunoblots of SFRP1 and ERK1/2 (loading control). **(C)** Whole-cell lysates were extracted at indicated time points of SFRP1 and EV differentiated with MDI and analysed by immunoblotting.

Representative immunoblots of β-catenin and ERK1/2 (loading control). **(D)** CyclinD1 (*Ccnd1*) mRNA levels, normalized to 18S rRNA levels, was measured at the indicated hours of differentiation in SFRP1 and EV cells differentiated with DI. *P<0.05, **P<0.01,***P<0.001 versus EV. **(E)** Topflash reporter activity in 3T3-L1 cells expressing SFRP1 or an empty vector (EV) treated with MDI ± Wnt3a (10ng/mL) for 48hrs. Results are expressed as fold difference relative to EV. Results are presented as mean ± SEM of at least 3 independent experiments, performed in triplicates. *P<0.05, **P<0.01.

Europe PMC Funders Author Manuscripts

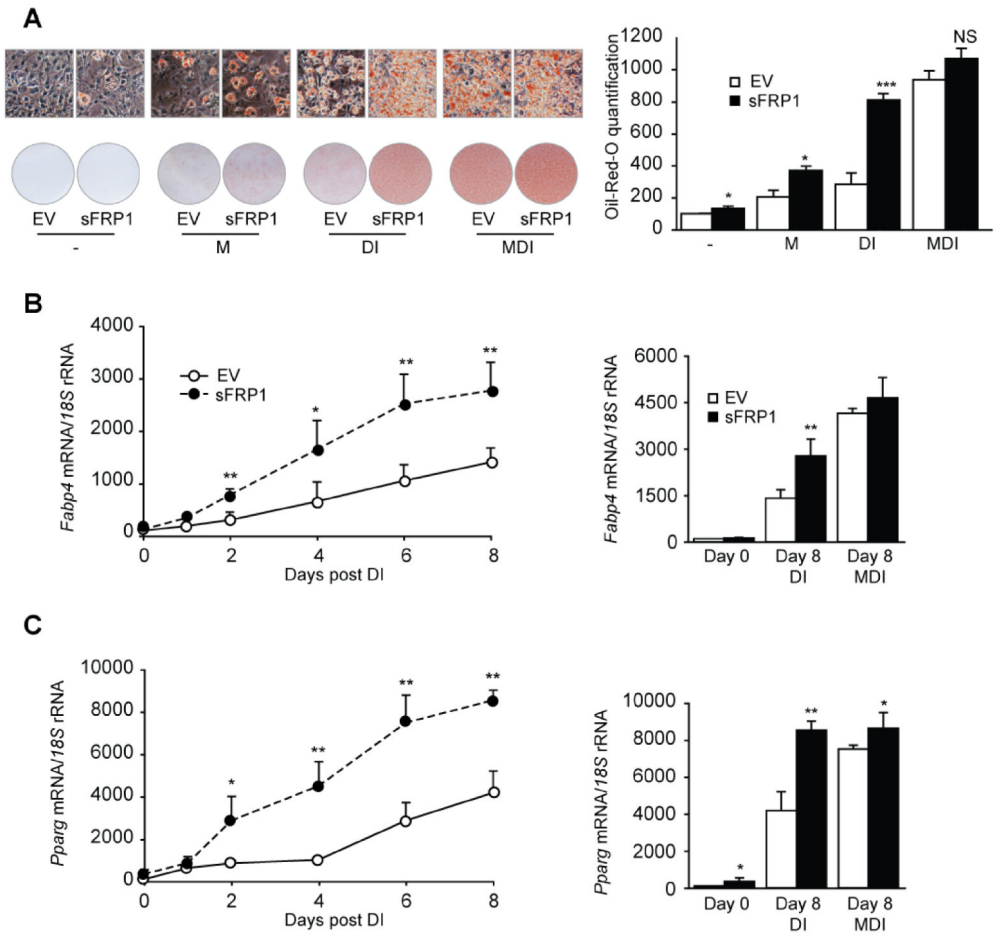

Figure 3. SFRP1 promotes adipogenesis in 3T3-L1 preadipocytes

(A) SFRP1 and EV 3T3-L1 cells were differentiated or not (-) with submaximal conditions: IBMX alone (M) or dexametasone and insulin (DI) or with full differentiation cocktail (MDI). Cells were stained with Oil Red-O to visualize lipid droplets 8 days post induction. Staining was quantified at 540nm and expressed as the percentage of EV (-). *P<0.05, ***P<0.001 versus EV. **(B)** *Fabp4* and **(C)** *Pparg* and mRNA levels, normalized to *18S* rRNA levels, were measured using real time RT PCR at the indicated hours of differentiation in SFRP1 (Black circles and bars) and EV (White circles and bars) cells differentiated with DI. *P<0.05, **P<0.01 versus EV.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

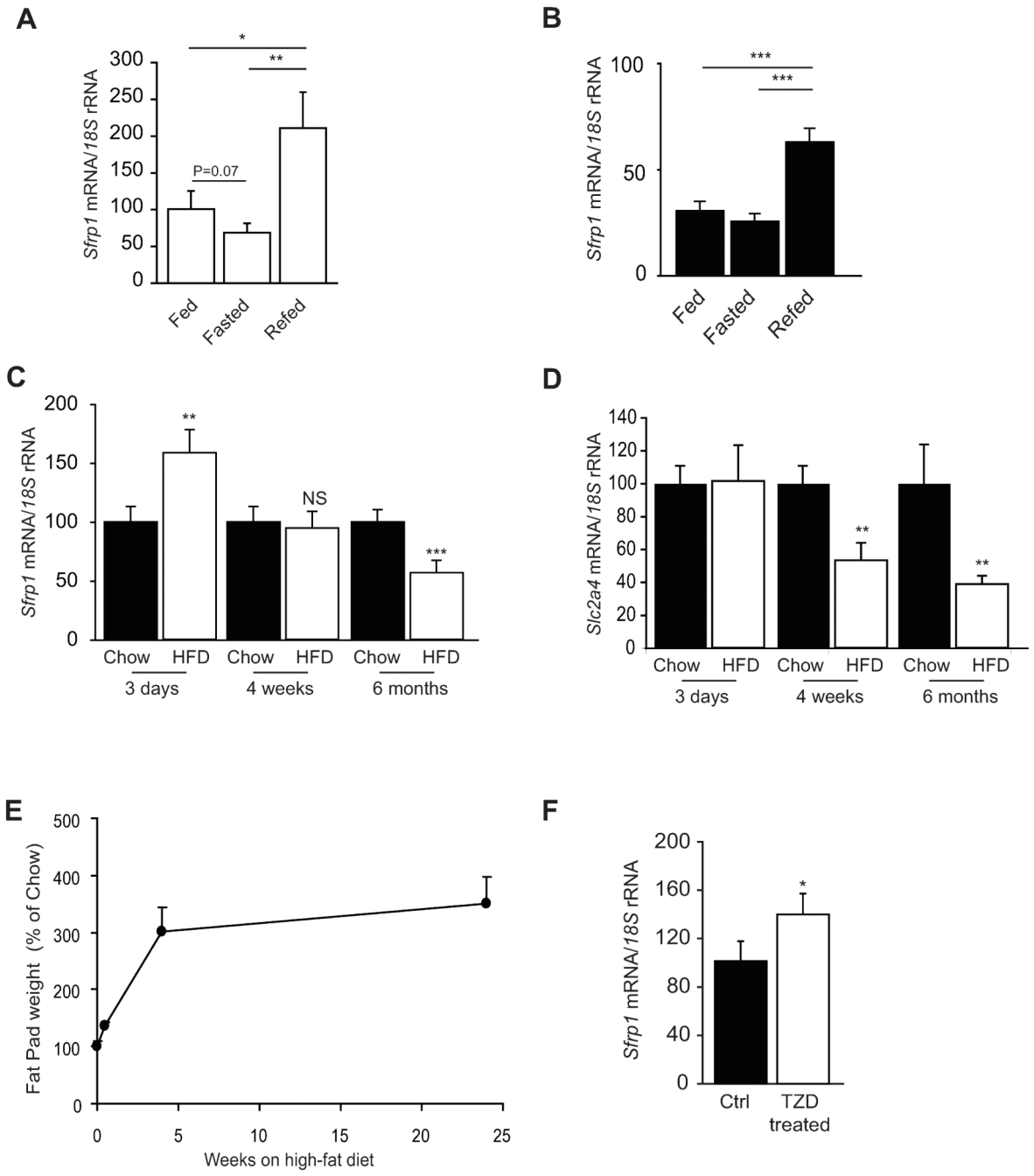

Figure 4. Sfrp1 is regulated by nutritional signals, genetic obesity and metabolic status in vivo

Sfrp1 mRNA levels, normalized to 18S rRNA, were measured in **(A)** whole epididymal adipose tissue and **(B)** mature adipocytes from 8-Week-old male C57/Bl6 mice challenged under the following conditions: fed (n = 8–9), fasted (24 h) (n = 7–8), and refed (24 h) (n =7–8). *P<0.05, **P<0.01, ***P<0.001. **(C)** *Sfrp1* mRNA and **(D)** Glut4 (*Slc2a4*) mRNA levels were measured in whole adipose tissue from C57/Bl6 mice fed either chow diet or high fat diet for 3 days (Acute), 4 weeks (Short term) or 6 months (long term) post weaning (n=7-8). **P<0.01, ***P<0.001 versus chow fed mice. **(E)** Changes in epididymal fat pad

weights during HFD feeding relative to chow feeding **(F)** Expression data from 4 months old C57/Bl6 mice fed rodent chow supplemented with or without rosiglitazone (TZD) for 3 weeks (n=9-10). *P<0.05 versus untreated mice.

Europe PMC Funders Author Manuscripts

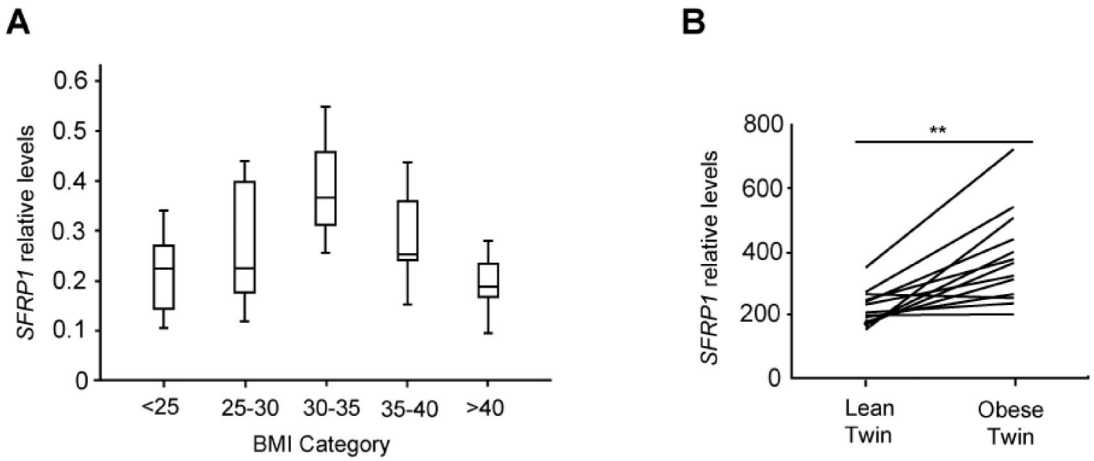

Figure 5. Regulation of SFRP1 in human adipose tissue

(A) SFRP1 mRNA levels, normalised to Cyclophilin A, in subcutaneous adipose tissue of 31 white European females (Group B). For all subjects, BMI cut-off follows WHO classification (BMI<25 n=6, BMI 25-30 n=5, BMI 30-35 n=3, BMI 35-40 n=5, BMI>40 n=12). Analysis of variance (ANOVA) did not reach statistical significance, p=0.059. **(B)** Microarray SFRP1 expression were measured in subcutaneous adipose tissue of 13 pairs of monozygotic twins discordant for weight (Group C). Paired t-test p=0.005.

Europe PMC Funders Author Manuscripts

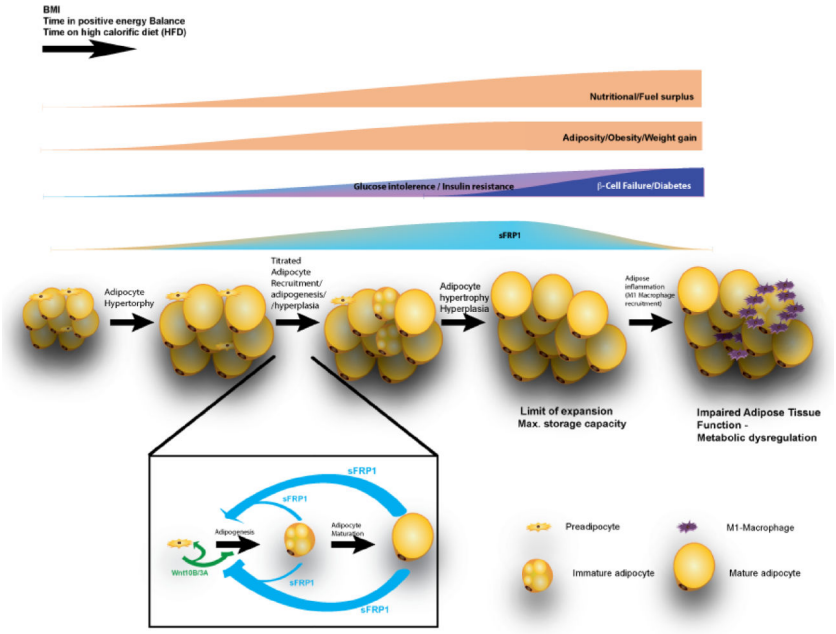

Figure 6. Postulated role of SFRP1 action in adipose tissue plasticity

Europe PMC Funders Author Manuscripts