Inhibition of secreted frizzled-related protein 5 improves glucose metabolism

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Rulifson IC, Majeti JZ, Xiong Y, Hamburger A, Lee KJ, Miao L, Lu M, Gardner J, Gong Y, Wu H, Case R, Yeh W, Richards WG, Baribault H, Li Y. Inhibition of secreted frizzled-related protein 5 improves glucose metabolism. Am J Physiol Endocrinol Metab 307: E1144–E1152, 2014. First published November 4, 2014; doi:10.1152/ajpendo.00283.2014.—Elucidating the role of secreted frizzled-related protein 5 (SFRP5) in metabolism and obesity has been complicated by contradictory findings when knockout mice were used to determine metabolic phenotypes. By overexpressing SFRP5 in obese, prediabetic mice we consistently observed elevated hyperglycemia and glucose intolerance, supporting SFRP5 as a negative regulator of glucose metabolism. Accordingly, Sfrp5 mRNA expression analysis of both epididymal and subcutaneous adipose depots of mice indicated a correlation with obesity. Thus, we generated a monoclonal antibody (mAb) against SFRP5 to ascertain the effect of SFRP5 inhibition in vivo. Congruent with SFRP5 overexpression worsening blood glucose levels and glucose intolerance, anti-SFRP5 mAb therapy improved these phenotypes in vivo. The results from both the overexpression and mAb inhibition studies suggest a role for SFRP5 in glucose metabolism and pancreatic β-cell function and thus establish the use of an anti-SFRP5 mAb as a potential approach to treat type 2 diabetes.

secreted frizzled-related protein 5; diabetes; obesity; pancreatic β-cell; Wnt

Genome-wide association studies (GWAS) have identified multiple genomic loci associated with susceptibility to type 2 diabetes (T2D). One such locus reported by several groups is highly associated with T2D risk, transcription factor 7-like 2 (TCF7L2), which is located in the noncoding region of a gene in the Wnt-signaling pathway (10, 12, 35, 36, 38, 39, 46). Although expressed in many tissues, the variant in TCF7L2 associates with impaired pancreatic β-cell insulin secretion and β-cell survival (8, 9, 17, 18, 20, 21, 33, 37, 40, 41, 47). The commonality of the TCF7L2 variants among the population and across ethnicities, combined with the reported associated risk, places TCF7L2 as the strongest identified genetic risk factor for T2D (12, 44). Findings such as this fuel the incentive to better understand how modulation of the Wnt signaling pathway drives T2D pathogenesis and determine whether a Wnt pathway-associated protein could be a suitable therapeutic target for treating this disease.

Secreted frizzled-related proteins (SFRPs) comprise a family of secreted proteins that contain an NH2 terminus cysteine-rich domain (CRD) homologous to that found in the Wnt-binding seven-transmembrane receptor, Frizzled (24, 31). Via their CRD, SFRPs bind to Wnt proteins, thereby blocking their interactions with Frizzled and inhibiting canonical Wnt signaling (7). Of the family members, Sfrp5 mRNA expression in mice has been shown to be sensitive to metabolic queues and changes with fat mass expansion (16, 25, 27). The role of SFRP5 in metabolism, however, remains unclear due to contradictory phenotypes observed with different lines of Sfrp5-deficient mice. In the first published study, Sfrp5-deficient mice were reported to exhibit severe glucose intolerance and hepatic steatosis upon consumption of a high-fat/high-sugar diet; both phenotypes were reversed upon adenovirus-mediated SFRP5 overexpression (27). The authors proposed that, in the context of metabolic stress, SFRP5-secreting adipocytes exert anti-inflammatory effects via noncanonical regulation of the JNK signaling pathway to ameliorate disease. However, in a different study, Sfrp5-deficient mice were reported to exhibit mild improvement in glucose tolerance upon consumption of a high-fat diet (25). In this case the authors proposed that the presence of SFRP5 negatively regulates mitochondrial biogenesis, leading to an increase in adipocyte size and number, thus worsening disease. Further confirming the story is that Ouchi et al. (27) reported SFRP5 mRNA in human visceral adipose tissue increases with obesity, whereas Ehrlund et al. (11) reported a paucity of detectable SFRP5 mRNA and SFRP5 protein expression in white adipose depots from obese humans. Additionally, there are contradictory findings on the effect of SFRP5 on primary adipocytes and 3T3-L1 adipocytes in cell culture (3, 23) and whether circulating SFRP5 directly or inversely correlates with obesity, insulin resistance, and T2D (2, 13, 14, 22, 42). However, recent GWAS of obese vs. lean individuals indicate that polymorphisms in the SFRP5 locus associate with decreased abdominal and subcutaneous fat in obese men (43). These seemingly inconsistent findings highlight the need to further define the potential involvement of SFRP5 in diabetes and obesity. Herein we show that overexpression of SFRP5 in vivo consistently exacerbated both hyperglycemia and glucose intolerance and that chronic treatment of diabetic mice with an anti-SFRP5 monoclonal antibody (mAb) improved glucose metabolism. In all scenarios we observed no significant impact of SFRP5 modulation on body weight but instead found evidence suggesting that SFRP5 may play a role in regulating pancreatic β-cell function.

Materials and Methods

Animal studies. All animal experiments were approved by the Institutional Animal Care and Use Committee of Amgen and carried for in accordance with the Guide for the Care and Use of Laboratory Animals (8th ed.) (26). Mice were housed in an air-conditioned room at 22 ± 2°C with a 12:12-h light-dark cycle (0600–1800).

B6.Cg-Lepob/J mice, B6D2F1/J mice, and C57BL/6J mice were purchased from The Jackson Laboratory. All animals had access to ad libitum feed; B6.Cg-Lepob/J mice C57BL/6J lean mice and B6D2F1/J–lean mice were fed a regular chow diet (2020x; Harlan-Teklad), and...
B6D2F1/J-DIO and C57BL/6J-DIO mice were fed 60 kcal% fat diet (D12492; Research Diet) for 7 and 12 wk, respectively, prior to initial dosing. All animals had access to water (reverse osmosis-purified) via an automatic watering system. B6.Cg-Lepob/J, B6D2F1/J-lean, B6D2F1/J-DIO, and C57BL/6J-DIO mice were 6, 12, 12, and 20 wk old, respectively, at the initiation of in vivo studies.

Adeno-associated virus (AAV) containing vector alone [empty vector (EV)] or SFRP5, driven by the EF1α promoter, was administered via the tail vein using a U-100 insulin syringe. B6D2F1/J-DIO mice received 8 \times 10^{12} virus particles per mouse in PBS; B6D2F1/J-lean mice received 1.6 \times 10^{12} virus particles per mouse in PBS; and B6.Cg-Lepob/J mice received 4 \times 10^{12} virus particles per mouse in PBS.

Generation of the mouse anti-SFRP5 mAb. A soluble mouse SFRP5-huFc protein containing full-length mouse SFRP5 cDNA (GenBank accession no. NM_018780.3) with human Fc fused to the C-terminus was expressed in Chinese hamster ovary cells and purified to homogeneity. This protein was emulsified in TiterMax Gold adjuvant (Sigma-Aldrich) and used to immunize C57BL/6J mice (The Jackson Laboratory). Mice with the highest specific titers after eight rounds of immunization were selected for final immunization with protein in PBS, and hybridomas were generated by electrofusion of their spleens and lymph nodes (bilateral popliteal, superficial inguinal, and lumbar) with Sp2/0-Ag14 mouse myeloma cells (ATCC) at a 1:1 ratio, similar to previous reports (45).

Hybridoma clones that bound to soluble SFRP5-human Fc were identified by two rounds of ELISA screening, with purified protein as the capturing agent. From these clones, several were selected on the basis of their unique selective binding activity to mouse recombinant SFRP5 protein over mouse recombinant SFRP3 protein in a counter ELISA screen. Hybridomas found to secrete anti-SFRP5 IgG antibodies were then cloned by limiting dilution. One antibody was chosen for in vivo testing on the basis of binding and activity in the TCF luciferase assay.

Expression analysis. An RNeasy Mini kit (Qiagen) was used for RNA extraction and purification from mouse tissues. All extracted RNA was treated with RNase-free DNase (Qiagen) for removing genomic DNA. RNA was subjected to quantitative real-time PCR using a ViiA real-time PCR System (Life Technologies) according to the manufacturer’s instructions. All of the PCR reagents were from Life Technologies. For Fig. 2A, gene expression values were normalized to Gapdh as an internal control. For Fig. 3, three different endogenous control genes, Gapdh, Tbp, and Rn18s, were used to validate changes in Sfrp5. Gene sequences for probes and primers are as follows: Sfrp5 (Life Technologies): Mm01194236_m1, probe spans...
exons 2–3, RefSeq NM-018780.3; Sfrp2 (Life Technologies): Mm01213947_m1, probe spans exons 2–3, RefSeq NM-009144.2.

**T cell factor luciferase assays.** A β-catenin/T cell factor (TCF)-luciferase reporter system was used to measure Wnt signaling activity under various conditions in vitro. For TCF luciferase experiments, 293 Super Top Flash cells (1, 30) were grown in DMEM (Cellgro) and seeded in 96-well plates (Costar) at 3.5 × 10⁴ cells/well. The following day, reagents were mixed accordingly, incubated at room temperature for 30 min, and then added to cells: recombinant mouse WNT3a and recombinant mouse SFRP5 (R & D Systems) and anti-SFRP5 mAb. After overnight incubation at 37°C, plates were removed from the incubator and equilibrated to room temperature for 30 min, and then Steady-Glo (Promega) was added to each well according to the manufacturer’s instructions. Luciferase activity was measured using an Envision plate reader (Perkin-Elmer).

**Pharmacokinetic study and antibody dosing.** Six-week-old B6.Cg-Lepob/J mice were dosed via intraperitoneal injection with 30 ml/kg body wt anti-SFRP5 mAb in PBS. Terminal samples were collected at 1, 3, 6, 24, 48, 72, 96, and 168 h postinjection. Three mice were collected per time point. The concentration of serum anti-SFRP5 mAb was determined by ELISA using recombinant mouse SFRP5-coated plates (R & D Systems), followed by serum samples, anti-mouse IgG-HRP (Jackson ImmunoResearch), and a tetramethylbenzidine substrate solution (BD Biosciences). Samples were run in triplicate. For chronic studies, mice were dosed via intraperitoneal injections twice/wk with the indicated amount of anti-SFRP5 mAb in PBS. The IgG isotype control antibody was made in-house (Amgen).

**In vivo measurements.** Before in vivo studies commenced, mice were randomized into treatment groups based on body weight, fasting blood glucose levels, and fasting serum insulin levels. For some studies, mice were also randomized based on percent fat and lean muscle mass using a Minispec LF90 TD-NMR analyzer (Bruker Optics). Blood glucose levels were measured using an Accu-check Aviva blood glucose meter (Roche), and serum insulin levels were measured using an ultrasensitive mouse insulin ELISA kit (Alpco).

For oral glucose tolerance tests, mice were fasted for 4 h beginning at 0700 on the day of the experiment. Blood samples to determine blood glucose levels were obtained from the tail vein immediately before glucose injection (0 min) and 15, 30, and 60 min post-glucose challenge. The concentration of glucose used for the study, 10 vs. 20 mg/kg body wt, was determined based on fasting blood glucose levels

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**Fig. 2. Effects of SFRP5 overexpression in B6.Cg-Lepob mice.** Six-week-old B6.Cg-Lepob male mice were injected intravenously with AAV-EV (●; n = 15) or AAV-SFRP5 (○; n = 17). A: expression of Sfrp5 in liver, pancreas, brown adipose tissue (BAT), white adipose tissue (WAT), and duodenum 2 wk after AAV-EV (open bars) vs. AAV-SFRP5 (black bars) injection. Data represent the average relative fold expression of Sfrp5, normalized to Gapdh, from 6 animals/cohorts. B and C: 2 wk postinjection, fasted mice were tested for response to glucose challenge by oral gavage (10 mg/kg body wt glucose; B), and AUC was calculated (C). D–F: baseline and 2-wk postinjection fasting blood glucose levels (D), fasting insulin levels (E), and body weights (F). G: %body composition was determined by NMR analysis. H: ratio of fasting terminal serum proinsulin/C-peptide (pmol) 2 wk postinjection. Values are means ± SE, 2-way ANOVA. AUC: means ± SD, unpaired 2-tailed t-test. Proinsulin to C-peptide: means ± SE, unpaired 2-tailed r-test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

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and the degree of glucose intolerance observed in the mice at the time of the study. Serum C-peptide, insulin, and proinsulin levels were measured by ELISA (Alpco) from 4-h-fasted terminal samples. Pancreas proinsulin and C-peptide content levels were determined by ELISA (ALPCO) using snap-frozen pancreas samples from treated mice; results were normalized to total protein content and presented as pM/mg protein.

RESULTS

Overexpression of SFRP5 worsens glucose metabolism. To assess the function of SFRP5 on glucose metabolism, we used an AAV-mediated gene delivery approach to overexpress SFRP5 in various disease models. First, we examined the impact of overexpressed SFRP5 in the B6D2F1 diet-induced obese (DIO) model (15). Upon being fed a high-fat diet, B6D2F1 mice gain significant body weight and exhibit hyperglycemia and insulin resistance compared with littermates on a normal chow diet (15). Additionally, pancreatic β-cells from B6D2F1-DIO mice exhibit defective insulin secretion, leading to a more diabetic-like model than the C57Bl/6-DIO mouse model. B6D2F1-DIO mice injected with AAV-SFRP5 demonstrated further worsened glucose intolerance at 2, 4, and 6 wk compared with AAV-EV control mice (Fig. 1, A, B, and C, respectively). Increased glucose intolerance was paralleled by significantly higher fasting blood glucose levels (Fig. 1D) and higher fasting serum insulin levels (Fig. 1E) over time, suggesting that overexpression of SFRP5 exacerbated insulin resistance in this model. However, despite indications of impaired metabolism, B6D2F1-DIO mice injected with SFRP5 exhibited only minor, insignificant increases in body weight compared with control mice over 6 wk of evaluation (Fig. 1F).

Since previous studies suggested that SFRP5 directly affects adiposity (25, 27), we questioned whether the obese state of the B6D2F1-DIO mouse impaired the ability to detect a subtle change in body weight. Thus, we tested overexpression of SFRP5 in B6D2F1-lean mice fed a normal chow diet. Compared with control mice, within 2 wk, lean mice overexpressing SFRP5 exhibited a small but significant impairment in glucose tolerance (Fig. 1, G and H). In lean mice, fasting serum insulin levels are typically very low or undetectable. However, overexpression of SFRP5 in B6D2F1 lean mice led to a significant increase in fasting serum insulin levels within 2 wk (Fig. 1I). Nonetheless, we observed no significant difference in body weight between mice with or without SFRP5 overexpression (data not shown). Thus, in B6D2F1-DIO mice, SFRP5 overexpression worsens fasting blood glucose levels and glucose intolerance and leads to significantly increased serum insulin levels. The observation that serum insulin levels are elevated independent of obesity in this model suggests that SFRP5 may act at the level of the β-cell. Leptin-deficient B6.Cg-Lepob mice are characterized by a rapid increase in body weight by 4 wk of age, followed by hyperglycemia, glucose intolerance, and elevated plasma insulin due to severe insulin resistance (4). Despite their highly insulin-resistant state, B6.Cg-Lepob mice exhibit massive pancreatic β-cell hyperplasia; although the β-cells are themselves insulin resistant, they maintain responsiveness to glucose challenge and can secrete large amounts of insulin (19). Six-week-old B6-Cg-Lepob mice were injected with AAV-EV or AAV-SFRP5 at 8 wk of age, and glucose intolerance was assessed at 10 wk of age. As previously observed (4), we confirmed that B6-Cg-Lepob mice exhibited defective glucose tolerance. However, administration of AAV-SFRP5 significantly worsened glucose intolerance (Fig. 1J). Since SFRP5 inhibited WNT3a signaling in vitro (Figs. 4A and 4B), we hypothesized that SFRP5 may act at the level of the β-cell. Leptin-deficient B6.Cg-Lepob mice were characterized by a rapid increase in body weight by 4 wk of age, followed by hyperglycemia, glucose intolerance, and elevated plasma insulin due to severe insulin resistance (4). Despite their highly insulin-resistant state, B6.Cg-Lepob mice exhibit massive pancreatic β-cell hyperplasia; although the β-cells are themselves insulin resistant, they maintain responsiveness to glucose challenge and can secrete large amounts of insulin (19). Six-week-old B6-Cg-Lepob mice were injected with AAV-EV or AAV-SFRP5 at 8 wk of age, and glucose intolerance was assessed at 10 wk of age. As previously observed (4), we confirmed that B6-Cg-Lepob mice exhibited defective glucose tolerance. However, administration of AAV-SFRP5 significantly worsened glucose intolerance (Fig. 1J). Since SFRP5 inhibited WNT3a signaling in vitro (Figs. 4A and 4B), we hypothesized that SFRP5 may act at the level of the β-cell. Leptin-deficient B6.Cg-Lepob mice were characterized by a rapid increase in body weight by 4 wk of age, followed by hyperglycemia, glucose intolerance, and elevated plasma insulin due to severe insulin resistance (4). Despite their highly insulin-resistant state, B6.Cg-Lepob mice exhibit massive pancreatic β-cell hyperplasia; although the β-cells are themselves insulin resistant, they maintain responsiveness to glucose challenge and can secrete large amounts of insulin (19). Six-week-old B6-Cg-Lepob mice were injected with AAV-EV or AAV-SFRP5 at 8 wk of age, and glucose intolerance was assessed at 10 wk of age. As previously observed (4), we confirmed that B6-Cg-Lepob mice exhibited defective glucose tolerance. However, administration of AAV-SFRP5 significantly worsened glucose intolerance (Fig. 1J). Since SFRP5 inhibited WNT3a signaling in vitro (Figs. 4A and 4B), we hypothesized that SFRP5 may act at the level of the β-cell.
old B6.Cg-Lep^{ob} mice were injected with SFRP5 or EV and monitored for 2 wk (Fig. 2). To confirm overexpression of SFRP5, the relative fold increase in Sfrp5 was assessed in several tissues (Fig. 2A). As expected, SFRP5-treated mice displayed a significantly higher level of Sfrp5 mRNA in the liver compared with EV mice. Similar to the B6D2F1-DIO and lean models, by 2 wk postinjection we observed an elevation in glucose intolerance in SFRP5-overexpressing B6.Cg-Lep^{ob} mice compared with controls (Fig. 2, B and C). Although both cohorts of mice demonstrated an overall increase in fasting blood glucose levels over the course of the study, by 2 wk postinjection the SFRP5-overexpressing mice exhibited a significantly greater increase compared with controls (Fig. 2D). Distinct from fasting blood glucose levels, during the course of the study, fasting serum insulin levels did not change in control mice but did rise significantly in SFRP5-overexpressing control mice (Fig. 2E). Similar to the DIO and lean mice studies, no significant change in body weight (Fig. 2F) or percent body composition (Fig. 2G) between cohorts was observed in the B6.Cg-Lep^{ob} model. These observations led us to explore the potential effect of SFRP5 on pancreatic β-cell function. Analysis of serum collected at the end of the study revealed a significant disparity between cohorts in the ratio of serum proinsulin to C-peptide (Fig. 2H), an indicator of impaired insulin maturation and secretion. Thus, similar to the B6D2F1-DIO model, these data suggest that overexpression of SFRP5 in B6.Cg-Lep^{ob} mice may directly impair the normal insulin maturation process in pancreatic β-cells.

**Anti-SFRP5 mAb blocks the inhibitory action of SFRP5 in vitro.** Our overexpression studies implicated SFRP5 as a negative regulator of glucose metabolism. Additionally, we found Sfrp5 mRNA expression, when normalized to endogenous

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**Fig. 5.** Anti-SFRP5 mAb treatment improves glucose metabolism in B6.Cg-Lep^{ob} mice. A: pharmacokinetic analysis of anti-SFRP5 mAb in the serum of mice over time injected once with antibody (30 mg/kg body wt). Values are means ± SD, 3 mice/time point. B–H: 6-wk-old B6.Cg-Lep^{ob} mice were dosed twice/wk by intraperitoneal injection with 30 mg/kg body wt anti-SFRP5 mAb (n = 11) or an IgG isotype control antibody (n = 12) for 4 wk. B and C (B) and 4 wk (C) after the 1st dose, fasted mice were tested for response to glucose challenge by oral gavage (20 mg/kg body wt glucose). D: AUC for the oral glucose tolerance tests. E–G: fasting blood glucose levels (E), fasting serum insulin levels (F), and body weights (G) were recorded over time. H: ratio of fasting terminal serum proinsulin to C-peptide levels; n = 8/group, means ± SE, 2-tailed t-test. Other values are means ± SE, 2-way ANOVA.*p < 0.05; **p < 0.01; ***p < 0.001. Anti-SFRP5 indicates anti-SFRP5 mAb. NS, not significant.
controls, to increase in both epididymal and subcutaneous adipose depots from C57BL/6-DIO (B6-DIO) mice compared with C57BL/6-lean (B6-lean) mice (Fig. 3, A and B). Expression changes in Sfrp5 were also compared with changes in Sfrp2, the latter of which has been reported to not be affected by obesity in mouse adipose tissue.

Because SFRP5 expression increased in obese mice and SFRP5 overexpression worsened diabetic phenotypes, we next questioned whether antagonizing SFRP5 function could lead to improvements in glucose metabolism. To test this hypothesis, we generated an anti-SFRP5 mAb directed against mouse SFRP5. The activity of this anti-SFRP5 mAb was assessed in vitro using a β-cat/Tcf luciferase reporter system (1). The addition of recombinant WNT3a to the reporter cells resulted in a dose-dependent increase in luciferase activity (Fig. 4A). In contrast, at a fixed concentration of WNT3a, the addition of recombinant SFRP5 suppressed the luciferase signal in a dose-dependent manner, whereas SFRP5 had no effect on the cells in the absence of WNT3a (Fig. 4B and data not shown). Anti-mouse SFRP5 mAb effectively impaired the inhibitory effect of recombinant mouse SFRP5 on WNT3a-mediated stimulation (Fig. 4C). Therefore, this anti-SFRP5 mAb antagonized the ability of SFRP5 to inhibit canonical Wnt signaling and was chosen to test whether SFRP5 inhibition could improve glucose metabolism in diseased mouse models.

Anti-SFRP5 mAb therapy improves diabetic phenotypes in vivo. To identify suitable dose levels and injection frequencies for subsequent efficacy studies, first the in vivo pharmacokinetic properties of the anti-SFRP5 mAb were determined. Six-week-old B6.Cg-Lepob mice were injected with 30 mg/kg body wt of antibody. At the indicated time intervals postinjection, terminal serum samples were collected, and the concentration of anti-SFRP5 mAb in serum was determined by ELISA (Fig. 5A). Based on this assessment, 6-wk-old B6.Cg-Lepob mice were dosed twice/wk with 30 mg/kg body wt of anti-SFRP5 or an isotype control IgG antibody for 4 wk. As shown, treatment with anti-SFRP5 mAb in B6.Cg-Lepob mice led to a significant improvement in glucose tolerance compared with control IgG-treated mice after 2 and 4 wk of injections (Figs. 5, B and C, respectively), the opposite effect of what was observed when SFRP5 was overexpressed in B6.Cg-Lepob mice (Fig. 2B). Calculating the area under the curve for the oral glucose tolerance tests shows a significant improvement in glucose tolerance after a prolonged anti-SFRP5 mAb treatment (Fig. 5D). Anti-SFRP5 mAb-treated mice also displayed a gradual and significant reduction in fasting blood glucose levels over time compared with control IgG-treated mice (Fig. 5E). The gradual decline in fasting blood glucose was accompanied by an increase in fasting serum insulin levels (Fig. 5F) and no change in body weight between cohorts (Fig. 5G). Since SFRP5 overexpression studies suggested a potential effect of SFRP5 on pancreatic β-cell function, we analyzed terminal serum from fasted mice and observed a reversal in the ratio of proinsulin to C-peptide in anti-SFRP5 mAb-treated mice vs. control IgG-treated mice (Fig. 5H). Thus, these data suggest that inhibition of SFRP5 by anti-SFRP5 mAb therapy improves insulin sensitivity and β-cell function, as demonstrated by reduced fasting blood glucose levels combined with improved glucose tolerance and increased levels of serum C-peptide.
The B6-DIO mouse is a pre-T2D and obesity model in that the animals exhibit mild hyperglycemia, impaired glucose tolerance, and insulin resistance but do not develop overt diabetes (5). Studies suggest that the β-cell dysfunction in these mice is due not to reduced β-cell mass, glucose metabolism, or steatosis but rather to a secretory defect (29). B6-DIO mice treated with anti-SFRP5 mAb therapy showed a mild but significant improvement in glucose tolerance compared with control antibody-treated mice (Fig. 6, A and B). Calculations for area under the curve in Fig. 6C parallel the values shown in Fig. 6, A and B. The improvement in glucose tolerance occurred without a significant change in body weight (data not shown) or percent fat or lean mass (Fig. 6D). Serum analysis of the anti-SFRP5-treated mice and controls at the time of harvest revealed no difference in the ratio of serum proinsulin to C-peptide (Fig. 6E). However, chronic treatment with anti-SFRP5 mAb resulted in a significantly improved ratio of proinsulin to C-peptide content in whole pancreas lysate (Fig. 6E), suggesting that inhibition of SFRP5 in this context enhanced insulin maturation in β-cells while reducing the secretory defect associated with this model.

**DISCUSSION**

Numerous studies highlight Wnt ligands as regulators of mesenchymal cell fate and adipogenesis (6). SFRPs are evolutionarily conserved extracellular antagonists of Wnt signaling, and a study by Koza et al. (16) identified SFRP5 specifically as highly correlated with fat mass expansion. Since then, several independent research groups have sought to elucidate the role of SFRP5 in obesity and diabetes. Unfortunately, phenotypic characterization of Sfrp5-deficient mice generated by two different groups revealed contradictory outcomes when knockout mice were put on a high-fat diet. Therefore, in an effort to gain clarity and better understand the role of SFRP5 in different mouse models of diabetes, we employed two experimental strategies independent of possible developmental consequences: SFRP5 overexpression studies and SFRP5 inhibition studies using a mAb against SFRP5. Our results consistently indicate that SFRP5 overexpression worsened hyperglycemia and glucose intolerance and that inhibition via an anti-SFRP5 mAb improved phenotypes associated with glucose metabolism.

Although elevated Sfrp5 expression was detected in adipose tissue of obese mice, neither overexpression of SFRP5 nor inhibition of SFRP5 had any impact on body weight or percent body composition in the mouse models used for our studies. Immunohistochemical analysis of the white adipose tissue in mice overexpressing SFRP5 revealed some mild but inconsistent areas of inflammation and adipocyte degradation compared with control animals (data not shown). What we did observe, however, was regulation of C-peptide and proinsulin, suggesting that SFRP5 may act at the level of the β-cell. This hypothesis is consistent with previous studies where the effects of SFRP5 on pancreatic islets were reported (32). For example, IGF-binding protein-3 (IGFBP-3) was identified as an adipose-secreted factor controlling β-cell proliferation (28). Subsequently, it was demonstrated that Sfrp5 expression in islets is regulated by exogenous recombinant IGFBP-3 and that anti-IGFBP-3 antibody treatment increases β-cell proliferation in vitro by inhibiting Sfrp5 expression (32). These studies are also consistent with earlier studies demonstrating Wnt mediated signaling as a critical component of both embryonic and adult β-cell proliferation (34). Furthermore, Rebuffat et al. (32) showed that silencing Sfrp5 in β-cells increased both dephosphorylated β-catenin, an indicator of active Wnt signaling, and TCF-7L2 protein expression, thus linking SFRP5 as an important player in regulating β-cell function and survival under metabolic stress.

Despite the lack of effect of SFRP5 on body weight, our experimental strategy provided data showing that overexpression of SFRP5 elevated glucose intolerance and anti-SFRP5 mAb therapy improved glucose tolerance. These data align with those observed in the Sfrp5−/− mice described by Mori et al. (25). It is important to highlight that there could potentially be differences between rodents and humans, as evidenced already by Sfrp5 expression data (11, 16, 27). The contradictory findings in whether circulating SFRP5 is directly or inversely correlated with disease may shift attention more toward localized tissue-specific effects, such as effects on β-cell function. Using an anti-SFRP5 mAb approach to modulate SFRP5 activity allowed us to monitor inhibition of SFRP5 in different diabetic mouse models and eliminate possible misinterpretation that could arise from developmental abnormalities due to gene ablation. Our data support a role for SFRP5 in glucose regulation and β-cell function, providing an alternative perspective on how better to target SFRP5 as a therapy for diabetes.

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**DISCLOSURES**

All authors are/were employees of Amgen, Inc. and have stock options in the company.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


