

# Regulation of insulin secretion, glucokinase gene transcription and beta cell proliferation by adipocyte-derived Wnt signalling molecules

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## Abstract

**Aims/hypothesis** Adipocytes secrete signalling molecules that elicit responses from target cells, including pancreatic beta cells. Wnt signalling molecules have recently been identified as novel adipocyte-derived factors. They also regulate insulin secretion in pancreatic beta cells and the cell cycle. The aim of this study was to investigate the effect of adipocyte-derived Wnt signalling molecules on insulin secretion and beta cell proliferation.

**Methods** Human adipocytes were isolated to generate fat cell-conditioned medium (FCCM). Ins-1 cells were stimulated with FCCM and transiently transfected with reporter genes. Proliferation assays using [<sup>3</sup>H]thymidine incorporation were carried out in Ins-1 cells and primary islet cells. Insulin secretion from primary islets was assessed by radioimmunoassay. Gene expression in primary islets was assessed by Taqman PCR.

**Results** Treatment with human FCCM increased the transcription of a T cell-specific transcription factor reporter gene (TOPFLASH) in Ins-1 cells (241%,  $p < 0.05$ ). FCCM induced the proliferation of Ins-1 cells (1.8 fold,  $p < 0.05$ ) and primary mouse islet cells (1.6 fold,  $p < 0.05$ ). Antagonizing Wnt signalling with secreted Frizzled-related protein 1

(FRP-1) inhibited the proliferative effect induced by Wnt3a and FCCM on Ins-1 cells by 49 and 41%, respectively. In addition, FCCM led to a twofold ( $p < 0.05$ ) induction of cyclin D1 promoter activity in Ins-1 cells. Furthermore, FCCM stimulated insulin secretion (204% of controls,  $p > 0.05$ ) in primary mouse islets, and this stimulation was inhibited by sFRP-1. At a molecular level, canonical Wnt signalling induced glucokinase gene transcription in a peroxisome proliferator-activated receptor  $\gamma$ -dependent fashion, thereby defining the glucokinase gene as a novel Wnt target gene. **Conclusions/interpretation** Taken together, these data show that adipocyte-derived Wnt signalling molecules induce beta cell proliferation and insulin secretion in vitro, suggesting a novel mechanism linking obesity to hyperinsulinaemia.

**Keywords** Adipocyte · Beta cells · Islet · Obesity · Wnt signalling

## Abbreviations

FCCM	fat cell-conditioned medium
HNF	hepatocyte nuclear factor
LEF	lymphoid enhancer-binding factor
LRP	LDL receptor-related protein
sFRP-1	secreted Frizzled-related protein 1
PPAR $\gamma$	peroxisome proliferator-activated receptor $\gamma$
TCF	T cell-specific transcription factor
TCF7L2	transcription factor 7-like 2

## Introduction

Obesity is associated with insulin resistance, hyperinsulinaemia and beta cell hyperplasia [1–5]. Hyperinsulinaemia can result from either islet hyperplasia or hypersecretion of

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insulin from the individual beta cell. Hyperinsulinaemia compensates for insulin resistance to maintain normoglycaemia. However, hyperinsulinaemia per se deteriorates insulin resistance [6] and is thought to trigger the progression of the metabolic syndrome [7, 8]. The mechanisms linking obesity and insulin resistance to hyperinsulinaemia and beta cell hyperplasia are incompletely understood [3]. Adipocytes are hormonally active and adipocytokines are able to elicit a response from beta cells [5]. Studies have shown that adipocytes secrete Wnt signalling molecules to regulate adipocyte differentiation [9–11]. In a previous study we demonstrated that Wnt3a and Wnt10b are secreted by mature human adipocytes and regulate hormone secretion from adrenocortical cells in vitro [12].

Wnts are ligands for serpentine Frizzled receptors and LDL receptor-related protein (LRP) coreceptors. Upon ligand binding to both receptors, canonical Wnt signalling is activated, resulting in a stabilisation of  $\beta$ -catenin. Subsequently,  $\beta$ -catenin coactivates the transcription factors of the T cell-specific transcription factor (TCF) and lymphoid enhancer-binding factor (LEF) families on canonical Wnt target genes [13]. Common genetic variation within the gene encoding transcription factor 7-like 2 (*TCF7L2*) has recently been shown to be associated with an increased risk of type 2 diabetes in humans. However, the mechanisms through which *TCF7L2* affects glucose metabolism are not clear [14, 15]. Frizzled receptors and LRP coreceptors are expressed in pancreatic beta cells [16–18].

Wnts induce cell proliferation in a variety of tissues, including embryonic pancreatic endocrine cells [18]. In addition, genetic ablation of *Lrp5* causes impaired insulin secretion in mice, and this occurs in concert with decreased expression of the genes for glucokinase and HNF transcription factors, which regulate glucose sensing in beta cells. Wnt ligands stimulate insulin secretion in vitro [17]. Furthermore, a recent paper by Rulifson et al. [19] demonstrated that Wnt signalling regulates the proliferation of pancreatic beta cells in vitro and in vivo.

The aim of this study was to investigate whether Wnt signalling molecules secreted by adipocytes act on beta cells to regulate insulin secretion and beta cell proliferation.

## Methods

**Plasmid constructs** The TOPFLASH plasmid is a TCF luciferase reporter plasmid. It contains two sets of three copies of the TCF binding site upstream of the thymidine kinase minimal promoter. The plasmid FOPFLASH contains mutated TCF binding-sites and serves as a negative control. Both plasmids are commercially available (Upstate, Cell signalling solutions, Charlottesville, VA, USA). The constitutively active mutant of  $\beta$ -catenin, S45A, was

generated by T. Hagen (Wolfson Digestive Diseases Centre, University of Nottingham, UK) as described previously [20]. The cDNA bearing the Ser45→Ala mutation has been inserted into the pcDNA3 expression vector (Invitrogen, Karlsruhe, Germany). The glucokinase reporter gene contains a luciferase reporter under the control of the rat glucokinase promoter, spanning –1003/+196 of the beta cell-specific gene [21]. The expression vector for PPAR $\gamma$  contains the full-length cDNA transcript of the gene, as described previously [22]. The cyclin D1 reporter gene contains a luciferase reporter under the control of the promoter (spanning –1745/+134) of the human cyclin D1 gene [23].

The plasmid pRL-TK (Promega, Mannheim, Germany) serves as an internal control for transfection efficiency. The vector contains a cDNA encoding *Renilla* luciferase under the control of the herpes simplex virus thymidine kinase promoter.

**Cell culture and transfection of DNA** The rat insulinoma cell line Ins-1 has been described previously [24]. Ins-1 cells were cultured in RPMI 1640 medium containing 11 mmol/l glucose supplemented with 10% FCS, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin [24]. Cells were trypsinised and transiently transfected with Fugene 6 reagent (Roche, Grenzach-Wyhlen, Germany) according to the manufacturer's protocol.

Co-transfections were carried out with a constant amount of DNA, which was maintained by adding the vector pcDNA3 (Invitrogen, Karlsruhe, Germany). For each transfection experiment 0.5  $\mu$ g of the *Renilla* luciferase reporter gene (plasmid pRL-TK) and 0.5  $\mu$ g of the firefly reporter gene were added to each well to check for transfection efficiency (the relative luciferase activities presented in the figures are derived from firefly/*Renilla* ratios. Where indicated, cells were incubated with fat cell-conditioned medium (FCCM), conditioned-medium from Wnt3a secreting L cells or the respective control media 24 h before harvest. The luciferase assay was performed as described previously [25, 26].

**Proliferation assays** The proliferation assay has been described previously [27, 28]. In brief, Ins-1 cells were resuspended in RPMI 1640 medium supplemented with 10% FCS, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin and  $5 \times 10^4$  cells were cultured in round-bottomed, 96 well culture plates. Where indicated sFRP-1 (R&D Systems, Minneapolis, MN, USA) was added at a concentration of 10 ng/ml. The proliferation activity was determined by [ $^3$ H]thymidine uptake. Cells were pulsed with 37 MBq [ $^3$ H]thymidine per well (Amersham Pharmacia Biotech, Braunschweig, Germany) 18 h before harvesting. After harvesting, thymidine incorporation was assessed using 25  $\mu$ l of a  $\beta$ -scintillation cocktail (Perkin Elmer, Rodgau-

Jugesheim, Germany) and a micro scintillation counter (Trillux, Wallach, Germany). Cellular proliferation was expressed relative to the respective control medium.

**Human tissues** Tissue samples of human white adipose tissue were obtained from women (20–35 years of age) undergoing surgical mammary reduction ( $n=10$ ). The patients were otherwise healthy and free of metabolic or endocrine diseases. The BMI range of the donors was between 21.4 and 29.2 kg/m<sup>2</sup> ( $25.4\pm 2.8$ , mean $\pm$ SD). Consent was obtained from the patients after the nature of the procedure was explained, and the study was approved by the ethics committee of the Heinrich-Heine-University Düsseldorf, Germany (study number 2292).

**FCCM** The isolation of adipocytes and preparation of FCCM have been described previously [29]. In brief, adipose tissue samples of 20–60 g wet weight were obtained from surgical mammary reductions and immediately transported to the laboratory in DMEM/Nutrient Mix F12 (DMEM/F12, Life Technologies, Karlsruhe, Germany) supplemented with 2% BSA, 100 U/ml penicillin and 100 µg/ml streptomycin. After removal of fibrous material and blood vessels, the adipose tissue was minced and digested in Krebs Ringer bicarbonate buffer (KRB) containing 2% BSA and 120 U/ml collagenase type I from *Clostridium histolyticum* (Sigma) in a shaking water bath for 45–60 min at 37°C. The digested tissue was then filtered through nylon gauze (250 µm mesh) and washed with KRB containing 0.1% BSA. For culturing, 2 ml of isolated floating adipocytes was transferred into culture flasks (Becton Dickinson, Heidelberg, Germany) containing 5 ml of cell culture medium (DMEM/F12 containing 15 mmol/l HEPES and 2.5 mmol/l L-glutamine, supplemented with 1.125 g/l NaHCO<sub>3</sub>, 100 units/ml penicillin and 100 µg/ml streptomycin). Cells were kept at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>/95% air and cultured for 24 h. The conditioned medium was subsequently collected, carefully avoiding the lipid floating on the top, and kept frozen at –20°C until used. DMEM/F12 medium incubated without adipocytes was used as control medium for the treatment of Ins-1 cells. The glucose concentration in the control medium and the FCCM was adjusted to 11 mmol/l prior to the treatment of Ins-1 cells or primary beta cells.

**Wnt3a-conditioned medium from L cells** The preparation of conditioned medium from Wnt3a-secreting L cells has been described previously [30]. L cells expressing Wnt3a were obtained from the American Type Culture Collection (CRL-2647; contact through LGC Promochem, Wesel, Germany). Conditioned medium from wild-type L cells served as a negative control.

**Preparation of mouse islets** Wild-type mice (C57BL/6, 6 months old) were used as donors. The mice were obtained from the animal facility of the University Hospital Düsseldorf and the study was conducted in accordance with the Principles of Laboratory Care. Mice were killed and islets were isolated using the intraductal collagenase digestion technique as described previously [31]. Islets were purified, handpicked and thereafter incubated overnight with FCCM or the respective control medium (see above). Glucose concentrations in the FCCM and control medium were adjusted to 11 mmol/l before treatment of the islets. The next day, insulin in the supernatant was measured using a radioimmunoassay kit.

The generation of a single cell culture has been described previously [31]. In brief, islets were dissociated into single cells by trypsinising in Hanks' balanced salt solution and thereafter cultured in RPMI 1640 medium (GIBCO BRL, Karlsruhe, Germany) containing 4.5 mmol/l glucose supplemented with 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin.

**Semiquantitative TaqMan PCR** Pancreatic islets from 6-month-old wild-type mice (C57BL/6) were isolated and treated with recombinant Wnt3a protein (10 ng/ml) (R&D Systems) for 24 h. Total RNA was extracted using the RNeasy MiniKit (Qiagen, Hilden, Germany) including a DNase I digestion step (New England Biolabs, Ipswich, UK) and reversely transcribed with the random-primed first-strand cDNA Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. For negative control reactions, the reverse transcription step was omitted. β-Actin was used as an internal control. The glucokinase-specific primers were selected using the software PrimerExpress (PE Applied Biosystems, Foster City, CA, USA) (glucokinase: forward: 5'-CAC-AAT-GAT-CTC-CTG-CTA-CT-3', reverse: 5'-TTC-TGC-ATC-TCC-TCC-ATG-TA-3'; β-actin: forward: 5'-CCT-GAA-CCC-TAA-GGC-CAA-CCG-3', reverse: 5'-GCT-CAT-AGC-TCT-TCT-CCA-GGG-3'). Semiquantitative TaqMan PCR was carried out using an ABI PRISM 7700 Sequence Detector (PE Applied Biosystems) as follows: 40 cycles of denaturation at 95°C for 15 s and annealing/elongation at 58°C for 1 min. All experiments were carried out in triplicate and average cycling threshold ( $C_t$ ) units were obtained as the average of the results. Relative quantification of the glucokinase expression was performed using the comparative  $C_t$  method in separate tubes. All data are expressed as the means $\pm$ SD of three independent experiments as described previously [32].

**Statistical analysis** All data are presented as means $\pm$ SEM. Statistical analysis was performed using Student's *t* test. Significance was assumed at a *p* value of less than 0.05.

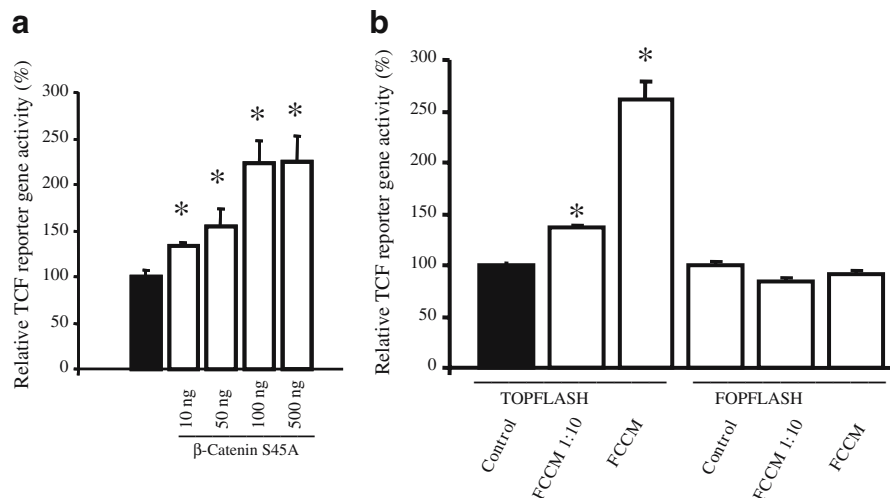
## Results

*Adipocyte-derived factors induce Wnt signalling in pancreatic beta cells* Wnts have been discovered as novel autocrine and paracrine factors secreted by adipocytes [9, 11, 12]. In addition, Wnt signalling molecules and Frizzled receptors are expressed in the endocrine pancreas of both mice and humans [12, 16, 33] and regulate insulin secretion [17] and beta cell proliferation [19]. In contrast, the canonical Wnt signalling pathway cannot be activated in glucagon-producing alpha cells [34, 35]. We investigated canonical (i.e.  $\beta$ -catenin) Wnt signal transduction in insulin-producing Ins-1 cells. As shown in Fig. 1a, co-transfection of a constitutively active mutant of  $\beta$ -catenin (S45A) [20] activates the transcription of a canonical TCF reporter gene (TOPFLASH). The activation is dose-dependent with a maximum effect using 500 ng of the  $\beta$ -catenin mutant ( $225 \pm 27\%$ ,  $p < 0.05$ ).

To investigate the functional interaction between adipocytes and beta cells through the Wnt signalling pathway we used FCCM to treat Ins-1 cells. We found a concentration-dependent induction of a TCF reporter gene (TOPFLASH) after incubation with FCCM for 24 h (Fig. 1b). TOPFLASH transcription was increased to  $136 \pm 3\%$  of the control level ( $p < 0.05$ ) by FCCM diluted 1:10 and to  $262 \pm 16\%$  ( $p < 0.05$ ) by undiluted FCCM. This effect was comparable to the maximum activation seen by co-transfecting the  $\beta$ -catenin mutant S45A. FCCM had no significant effect on a mutated TCF reporter plasmid (FOPFLASH;

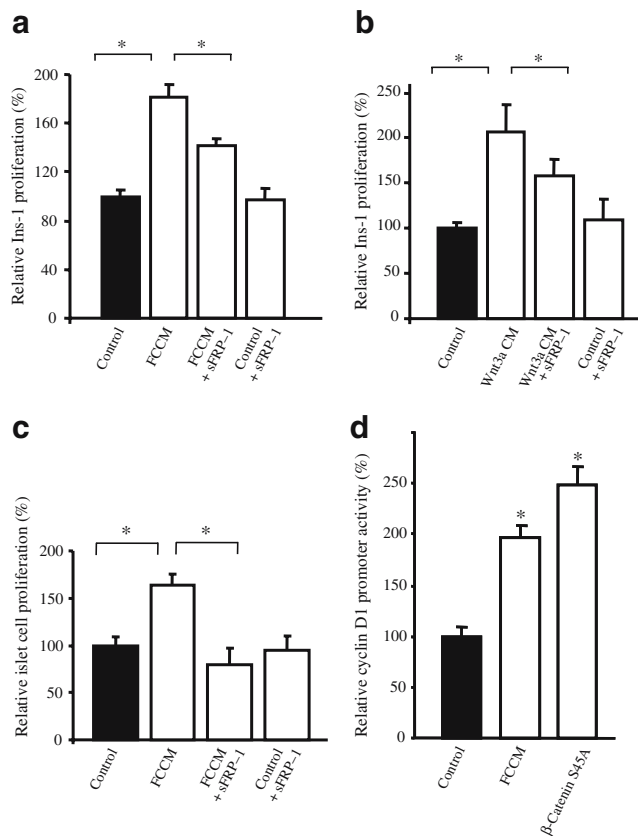
Fig. 1b). Thus, our data show an interaction between adipocytes and insulin-producing cells through the Wnt signalling pathway in vitro.

*Adipocyte-derived factors induce beta cell proliferation through Wnt signalling* We assessed the effect of Wnts and FCCM on Ins-1 proliferation by [ $^3$ H]thymidine incorporation. Figure 2b shows that conditioned medium from L cells containing Wnt3a, an activator of canonical Wnt signalling, increases the proliferation of Ins-1 cells to  $207 \pm 27\%$  of the control level. Similarly, treatment of Ins-1 cells with FCCM leads to an increase in proliferation to  $181 \pm 11\%$  of the control level ( $p < 0.05$ ; Fig. 2a). Similarly, treatment of Ins-1 cells with FCCM leads to an increase to  $181 \pm 11\%$  in cell proliferation ( $p < 0.05$ ; Fig. 2a). To test whether the proliferation seen in the presence of FCCM is mediated through the Wnt signalling pathway we used the soluble Wnt antagonist sFRP-1 to treat Ins-1 cells, together with FCCM or Wnt3a-conditioned medium from L cells. The proliferative effects of Wnt3a-conditioned medium from L cells and FCCM were both inhibited by the Wnt-antagonist sFRP-1. After adding 10 ng/ml of recombinant sFRP-1 to the respective conditioned medium, the FCCM-mediated proliferation was inhibited by 42% (Fig. 2a) and the Wnt3a-mediated proliferation was inhibited by 49% (Fig. 2b). Treatment with sFRP-1 in the absence of FCCM had no effect on basal Ins-1 proliferation (Fig. 2a,b). Similar results were obtained in primary murine islet cells. FCCM increased islet cell proliferation to 164% of the level with



**Fig. 1** Adipocyte-derived factors induce Wnt signalling in Ins-1 beta cells. **a** Intact canonical Wnt signalling in Ins-1 beta cells. Activation of a luciferase reporter gene under the control of TCF-binding-sites (TOPFLASH) in response to increasing amounts of a constitutively active mutant of  $\beta$ -catenin (S45A) in Ins-1 cells. **b** Activation of a TCF reporter gene in Ins-1 beta cells by FCCM. Ins-1 cells were transfected with the TOPFLASH reporter gene and treated with FCCM

or the respective control medium. The FOPFLASH reporter gene bearing mutated TCF binding sites showed no response to FCCM and served as a negative control. The luciferase activity is expressed as percentage of the mean value of the activity measured in the untreated controls. Values are means  $\pm$  SE of three independent experiments, each performed in triplicate. \* $p < 0.05$  vs untreated control (Student's *t* test)



**Fig. 2** Adipocyte-derived Wnts induce Ins-1 and beta cell proliferation. **a** FCCM induces the proliferation of Ins-1 beta cells. Ins-1 cells were incubated in the presence of FCCM or the respective controls. FCCM-induced proliferation of Ins-1 cells can be inhibited by antagonising Wnt signalling, as assessed by treatment with recombinant sFRP-1 (10 ng/ml) where indicated. sFRP-1 had no effect on unstimulated Ins-1 proliferation. Proliferation is expressed relative to cells treated with control medium. **b** Wnt3a-conditioned medium induces the proliferation of Ins-1 cells. Cells were treated with Wnt3a-conditioned medium (Wnt3a CM) from L cells or the respective controls. Wnt3a-induced proliferation can be inhibited by adding sFRP-1 (10 ng/ml). sFRP-1 had no effect on unstimulated Ins-1 proliferation. **c** FCCM induces the proliferation of primary murine islet cells. Islet cells were incubated in the presence of FCCM or the respective controls. sFRP-1 (10 ng/ml) was added where indicated. FCCM induced the proliferation of primary mouse islet-cells. sFRP-1 had no effect on unstimulated islet cell proliferation. The proliferation was expressed relative to cells treated with control medium. Values are means $\pm$ SE of three independent experiments, each performed in triplicate. \* $p$ <0.05 (Student's  $t$  test). **d** Adipocyte-derived factors induce the transcription of a cyclin D1 reporter gene in Ins-1 beta cells. Co-transfection with  $\beta$ -catenin S45A and FCCM both induced the transcription of a luciferase reporter gene under the control of the cyclin D1 promoter. The luciferase activity is expressed as a percentage of the mean value of the activity measured in the untreated controls. Values are means $\pm$ SE of three independent experiments, each performed in triplicate. \* $p$ <0.05 vs control (Student's  $t$  test)

control medium and this effect was abolished by adding sFRP-1 to FCCM (Fig. 2c). These data demonstrate that activation of Wnt signalling is one mechanism through which FCCM induces Ins-1 proliferation.

We explored the molecular mechanisms involved in the proliferative effect of FCCM on Ins-1 cells through Wnts by investigating the transcriptional regulation of the cyclin D1 reporter gene by fat cell products. The promoter of the gene encoding cyclin D1 contains binding sites for TCF/LEF transcription factors, which are known to be coactivated by  $\beta$ -catenin upon activation of canonical Wnt signalling [23]. Cyclin D1 promotes the transition from G1 to S phase by inhibiting the retinoblastoma protein (Rb) and thereby induces proliferation in multiple cell types, including pancreatic endocrine cells [36]. Consequently, overexpression of cyclin D1 has been shown to induce beta cell proliferation in vitro and in vivo [36, 37]. We questioned whether activation of Wnt signalling in Ins-1 beta cells by fat cell products could induce cyclin D1 reporter gene transcription. Transient transfection studies using a cyclin D1 luciferase fusion gene and an expression vector for  $\beta$ -catenin S45A into Ins-1 cells confirmed the regulation of the cyclin D1 promoter by  $\beta$ -catenin. Co-transfection of  $\beta$ -catenin S45A led to an increase in cyclin D1 transcription to  $249\pm 18\%$  of the control level (Fig. 2d). In addition, we found that FCCM induced cyclin D1 promoter activity ( $197\pm 11\%$ ,  $p$ <0.05; Fig. 2d). These results demonstrate the activation of the cyclin D1 promoter in response to fat cell products and suggest that this is one mechanism through which adipocytes induce the proliferation of beta cells.

*FCCM stimulates insulin secretion in primary murine islets* Islets were isolated from adult (6 months old) wild-type (C57BL/6) mice and transferred into primary culture. After incubation with FCCM for 24 h, insulin secretion from primary islets was stimulated to 204% of the control level and this effect was abolished after adding 10 ng/ml sFRP-1 to the FCCM (Fig. 3a). These data demonstrate that adipocyte-derived Wnts make a major contribution to the induction of insulin secretion in pancreatic cells by FCCM.

*Wnt/ $\beta$ -catenin signalling activates glucokinase gene transcription in the presence of PPAR $\gamma$*  To explore the molecular mechanisms underlying the stimulatory effect of FCCM (Fig. 3a) and Wnt signalling [17] on insulin secretion we tested whether glucokinase is a direct target gene for canonical Wnt signalling. Figure 3b shows that the transcription of a glucokinase reporter gene [21] is induced by an active  $\beta$ -catenin mutant (S45A) after co-transfection of a plasmid encoding PPAR $\gamma$ . In contrast,  $\beta$ -catenin has no effect on glucokinase gene transcription in the absence of exogenous PPAR $\gamma$  (Fig. 3b). Accordingly, we found that incubation with recombinant Wnt3a protein (10 ng/ml) increased glucokinase mRNA expression in primary mouse islets (Fig. 3c), as assessed by semiquantitative PCR. These results define the glucokinase gene as a novel target gene

for canonical Wnt signalling involving the transcription factor PPAR $\gamma$ .

## Discussion

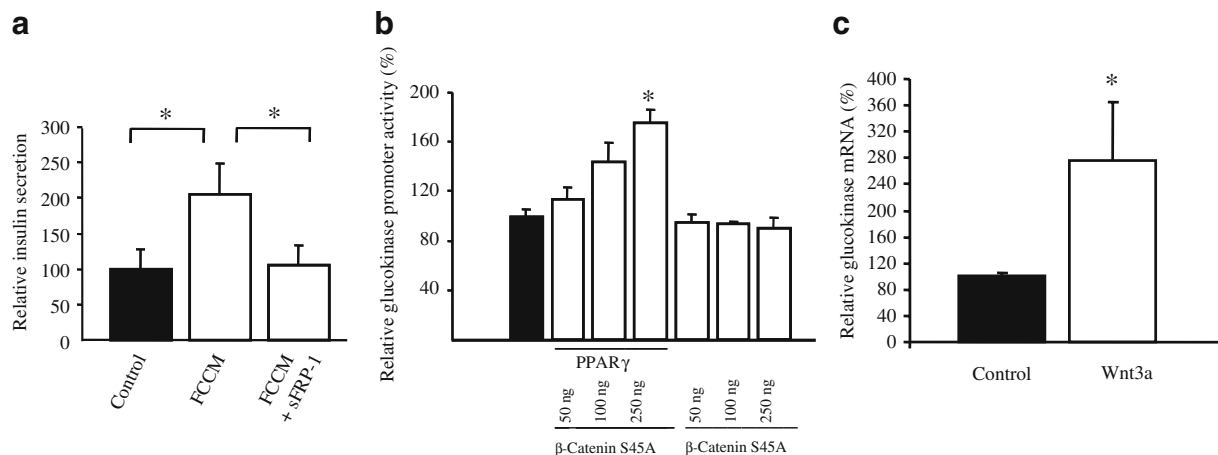
Our data presented here indicate that adipocyte-derived Wnts are, at least in part, responsible for mediating beta cell proliferation in vitro. This is suggested by the finding that the proliferative effect of FCCM on beta cells is inhibited when the Wnt signalling pathway is blocked (Fig. 2a,c). In line with this, we here demonstrate the induction of the cyclin D1 promoter, containing TCF/LEF consensus-sites [23], by fat cell secretory products (Fig. 2d). We used human FCCM as an established in vitro model to mimic the effect of fat cells on target cells [12, 29]. FCCM contains a variety of metabolites and signalling molecules, including known adipocytokines such as leptin, adiponectin, IL-6, TNF- $\alpha$  and resistin. However, these adipocytokines are not thought to mediate beta cell proliferation [5, 38, 39]. In addition to these known adipocytokines, Wnt signalling molecules are secreted by adipocytes [9, 12]. In line with our current study, there is evidence that the Wnt signalling pathway regulates glucose sensing in pancreatic beta cells [17] and prenatal and postnatal beta cell development in mice [18, 19].

Beta cell number is determined by their replication, neogenesis and apoptosis [5]. Animal studies in rodents revealed that the increased replication of beta cells is the major mechanism for the increased beta cell number seen in

non-diabetic obesity [40, 41]. Also, in humans, increased body weight is paralleled by an increase in beta cell mass [1, 2]. However, the mechanisms through which the increase in beta cell number is achieved appears to be different between species: In humans, neogenesis was found to be the major mechanism for the increase in beta cell mass in non-diabetic-obesity [4]. The data from our current study using rodent beta cells supports these previous findings as we find adipocyte-derived products to induce the proliferation of beta cells.

Our findings prompt the following question: how do adipocyte-derived Wnts reach pancreatic islets? Wnt signalling molecules are able to elicit paracrine as well as systemic effects on target tissues [42, 43]. Besides intra-abdominal and subcutaneous depots, adipocytes are also found within the pancreas [44]. Therefore, paracrine as well as endocrine stimulation of islets by adipocyte-derived Wnts appears to be possible and further studies are needed to distinguish between these mechanisms.

Hyperinsulinaemia can be caused by islet hyperplasia as well as by hypersecretion of insulin from the individual beta cell. Wnt signalling molecules have been found to regulate insulin secretion [17]. However, the underlying molecular mechanisms are not fully understood. Fujino et al. [17] found a decrease in genes that regulate glucose sensing in pancreatic beta cells in *Lrp5* knockout mice. In this study we demonstrate the induction of insulin secretion by fat cell products and show that this effect can be inhibited by blocking Wnt signalling (Fig. 3a), suggesting



**Fig. 3** Canonical Wnt signalling induces insulin secretion and glucokinase gene transcription. **a** FCCM stimulates insulin secretion through Wnts. sFRP-1 (10 ng/ml) inhibited the stimulation by FCCM. Values are means $\pm$ SE of three independent experiments, each performed in triplicate. \* $p$ <0.05 (Student's *t* test). **b** Canonical Wnt signalling activates glucokinase reporter gene transcription in the presence of PPAR $\gamma$ . Ins-1 cells were transiently transfected with a luciferase reporter gene under the control of the glucokinase promoter. Co-transfection of increasing amounts of  $\beta$ -catenin S45A activated glucokinase promoter activity only in the presence of PPAR $\gamma$ . The

luciferase activity is expressed as percentage of the mean value of the activity measured in the untreated controls. Values are means $\pm$ SE of three independent experiments, each performed in triplicate. \* $p$ <0.05 vs control (Student's *t* test). **c** Recombinant Wnt3a (10 ng/ml) increases glucokinase mRNA expression in primary mouse islets. Mouse islets were isolated and treated with recombinant Wnt3a. mRNA expression was assessed by semi-quantitative Taqman PCR. Values are means $\pm$ SE of three independent experiments, each performed in triplicate. \* $p$ <0.05 vs control (Student's *t* test)

that fat cells increase insulin secretion at least in part through the activation of Wnt signalling. On a molecular level, we identified the glucokinase gene promoter as a direct target for  $\beta$ -catenin in beta cells. Interestingly, the effect of  $\beta$ -catenin requires the presence of PPAR $\gamma$  (Fig. 3b). PPAR $\gamma$  is highly expressed in primary islets in alpha and beta cells [45]. This is in contrast to our previous findings in pancreatic endocrine cell lines, in which we demonstrated the loss of PPAR $\gamma$  expression [22]. Thus, Ins-1 cells are an excellent tool to investigate PPAR $\gamma$ -mediated effects without the need to knockdown the gene for this protein. PPAR $\gamma$  binds to its consensus motif within the glucokinase promoter [46, 47]. Here, we demonstrate a functional interaction between  $\beta$ -catenin and PPAR $\gamma$  in insulin-producing cells (Fig. 3b). We suggest that activated  $\beta$ -catenin coactivates PPAR $\gamma$ -mediated transcription at the glucokinase promoter, thereby defining the glucokinase gene as novel target gene for canonical Wnt signalling.

In conclusion, our data show that adipocyte-secreted products act on beta cells through the Wnt signalling pathway, resulting in an activation of cyclin D1 transcription and increased beta cell proliferation. In addition, fat cell products, through activation of canonical Wnt signalling, stimulate insulin secretion and glucokinase gene transcription in vitro. These data suggest a novel mechanism for obesity-induced beta cell hyperplasia and hyperinsulinaemia.

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**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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