Endocrinization of FGF1 produces a neomorphic and potent insulin sensitizer

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Fibroblast growth factor 1 (FGF1) is an autocrine/paracrine regulator whose binding to heparan sulphate proteoglycans effectively precludes its circulation1–4. Although FGF1 is known as a mitogenic factor, FGF1 knockout mice develop insulin resistance when stressed by a high-fat diet, suggesting a potential role in nutrient homeostasis3,4. Here we show that parenteral delivery of a single dose of recombinant FGF1 (rFGF1) results in potent, insulin-dependent lowering of glucose levels in diabetic mice that is dose-dependent but does not lead to hypoglycaemia. Chronic pharmacological treatment with rFGF1 increases insulin-dependent glucose uptake in skeletal muscle and suppresses the hepatic production of glucose to achieve whole-body insulin sensitization. The sustained glucose lowering and insulin sensitization attributed to rFGF1 are not accompanied by the side effects of weight gain, liver steatosis and bone loss associated with current insulin-sensitizing therapies. We also show that the glucose-lowering activity of FGF1 can be dissociated from its mitogenic activity and is mediated predominantly via FGF receptor 1 signalling. Thus we have uncovered an unexpected, neomorphic insulin-sensitizing action for exogenous non-mitogenic human FGF1 with therapeutic potential for the treatment of insulin resistance and type 2 diabetes.

Increases in the prevalence of obesity and insulin resistance and the associated incidence of difficult-to-manage type 2 diabetes have become world-wide public health problems as well as a financial burden for the health care system, emphasizing the urgent need for improved insulin-sensitizing therapies. Thiazolidinediones are highly effective oral medications for type 2 diabetes that act through the nuclear receptor peroxisome proliferator activated receptor γ (PPARγ) to control networks of genes involved in adipogenesis, lipid metabolism and insulin sensitization. However, the unique sensitization benefits of thiazolidinediones are compromised by detrimental side effects, including weight gain, bone loss and congestive heart failure, suggesting that targeting downstream effectors of PPARγ may evoke fewer side effects yet retain insulin-sensitizing potential5. In this regard, FGF1, a member of the endocrine FGF subfamily whose expression is regulated by PPARγ in adipose tissue, has been identified as an effective glucose-lowering agent in rodents6–7. Recently, FGF1, the prototype of the FGF family of proteins, was also found to be transcriptionally regulated by PPARγ in adipose tissue, and Fgf1 knockout mice exhibit an aggressive insulin-resistant phenotype when stressed by a high-fat diet8,9. The affinity of FGF1 for heparan sulphate proteoglycans results in autocrine/paracrine signalling and limited serum exposure, distinguishing it from the endocrine FGFs, which include FGF21 (refs 1, 2, 8, 9). These studies, combined with those demonstrating that FGF receptor agonist-antibodies modulate glucose homeostasis10 raised the question as to whether endocrinization of the non-endocrine FGF1 could elicit glucose-lowering effects.

To explore its therapeutic potential, recombinant murine FGF1 (rFGF1) was injected subcutaneously (subQ) into genetically induced (ob/ob and db/db) as well as diet-induced obese (DIO) insulin-resistant mice. We found that a single injection of 0.5 mg kg⁻¹ rFGF1 was sufficient to attain normoglycaemia in the severely hyperglycaemic ob/ob mice (Fig. 1a). Maximal glucose lowering was achieved within 18–24 h, and sustained effects observed for more than 48 h. Moreover, this effect was dose dependent, but even at the maximal dose (2.0 mg kg⁻¹) it did not result in hypoglycaemia (Fig. 1b and data not shown). Potent glucose lowering was

**Figure 1 | Acute rFGF1 injection lowers glucose in diabetic mice.** a, Blood glucose levels in ob/ob mice after rFGF1 injection (filled bars, n = 6) or control vehicle (open squares, n = 4) injection. b, Dose response of rFGF1 on blood glucose levels in ob/ob mice 24 h after injection (n = 3). c–f, Blood glucose levels after rFGF1 injection in db/db (n = 3) (c), DIO (n = 6) (d), normal-chow-fed (n = 8) (e) and fasted ob/ob (n = 6 for control; n = 5 for rFGF1) (f) mice. g, Blood glucose levels in ob/ob mice before (open bars) and 24 h after injection of murine FGF1 peptides (control, FGF1, FGF2: n = 4; FGF9, FGF10: n = 2). h, Blood glucose levels in ob/ob mice after human rFGF1 (filled bars, n = 4) or control vehicle (open bars, n = 4). C, control. Recombinant FGF9 peptides (0.5 mg kg⁻¹) or control vehicle (PBS) were injected subcutaneously to ad libitum fed mice unless otherwise noted. Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; **P < 0.01.

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observed in both db/db and DIO mouse models, and rFGF1 was effective when delivered either intraperitoneally or intravenously in ob/ob mice, independently of exogenous heparin (Fig. 1c, d and Extended Data Fig. 1a–c). rFGF1 had no effect on blood glucose or insulin levels in normoglycaemic Chow-fed mice (Fig. 1e and Extended Data Fig. 1d, e). Consistent with the known effects of FGF1 on feeding11–13, we observed a transient suppression of food intake that correlated with a temporary decrease in body weight (Extended Data Fig. 1f–i and data not shown). However, injection of rFGF1 similarly reduced glucose levels under fasting conditions, dissociating the glucose-lowering effects of rFGF1 from its effect on food intake (Fig. 1f). FGF1 is considered the universal ligand for FGF receptors (FGFRs) in its ability to bind and activate each of the alternatively spliced forms of the four tyrosine kinase FGF receptors (FGFR1–FGFR4), whereas other members of the FGF superfamily demonstrate receptor specificity1. To determine whether other autocrine/paracrine FGFs have similar blood glucose lowering activity when given pharmacologically, we tested a selection of FGFs with specificities covering all seven FGFR receptors. FGF1 seems unique in its ability to lower blood glucose; FGF2, FGF9 and FGF10 failed to do so (Fig. 1g). Furthermore, recombinant human FGF1 (hFGF1) was similarly able to normalize blood glucose in diabetic mice, suggesting an evolutionarily conserved pathway (Fig. 1h).

The pronounced glucose-lowering efficacy of a single injection of rFGF1 led us to investigate the effects of serial injections. ob/ob mice injected with 0.5 mg kg−1 every other day for 35 days (chronic treatment) did not develop any apparent resistance to rFGF1, exhibiting sustained glucose lowering with minimal changes in body weight or composition (Fig. 2a, b and Extended Data Fig. 2a, b). A similar glucose-lowering effect was also seen in pair-fed ob/ob mice, indicating that the transient reduction in food intake after chronic treatment with rFGF1 does not account for the beneficial glucose-lowering effects (Extended Data Fig. 2c, d). The fasting blood glucose levels of chronically rFGF1-treated ob/ob mice were 50% lower than in PBS-treated control mice, and remained lower throughout the glucose tolerance test (GTT) with a coincident decrease in insulin levels. Furthermore, rFGF1-treated mice showed a marked improvement in insulin sensitivity as measured by an insulin tolerance test (ITT) (Fig. 2e–g). Although there was no significant effect on free fatty acids, cholesterol and triglyceride levels in serum (Extended Data Table 1 and Extended Data Fig. 2e, f), chronic treatment with rFGF1 decreased hepatic steatosis and increased liver glycogen content, as shown histologically and by quantitative analyses (Fig. 2h–n). No significant changes were detected in serum metabolic hormone levels (Extended Data Fig. 2g).

Furthermore, chronic treatment with rFGF1 of DIO mice, a strain that more closely models the majority of human type 2 diabetes, also resulted in pronounced and sustained lowering of blood glucose levels (Fig. 2i) as well as increased insulin sensitization as measured by GTT and ITT (Fig. 2j, k). These beneficial effects in DIO mice were observed without significant changes in body weight, organ weights and feeding trends (Extended Data Fig. 2h–j).

The potent and rapid normalization of blood glucose by parenteral rFGF1 led us to investigate potential insulin secretagogue or insulin mimetic activities of rFGF1. rFGF1 administered by injection had no significant effect on glucose-stimulated insulin secretion in isolated pancreatic islets and did not increase serum insulin levels under basal conditions or during a GTT, indicating that exogenous FGF1 does not stimulate pancreatic β-cell insulin release, either ex vivo or in vivo (Fig. 2d and Extended Data Fig. 3a–c). Next, we tested rFGF1 efficacy in mice rendered diabetic by streptozotocin–induced destruction of insulin-producing β-cells. In this diabetic mouse model (STZ mice), rFGF1 alone failed to lower blood glucose levels, indicating that rFGF1 is not an insulin mimic (Fig. 3a). However, pretreatment of STZ mice with rFGF1 markedly enhanced the glucose-lowering effects of exogenously supplied insulin (Fig. 3b, c). Conversely, the rFGF1-dependent increase in metabolic clearance rate was severely blunted when insulin secretion was inhibited by somatostatin (Fig. 3d–g). Improved sensitivity to insulin often correlates with reduced systemic inflammation; indeed, chronic treatment of ob/ob mice with rFGF1 decreased serum levels of several inflammatory cytokines (eotaxin, keratinocyte chemoattractant (KC), Mip-1β and interleukin-3) (Extended Data Fig. 3d). These results demonstrate that rFGF1 operates in an insulin-dependent manner to lower blood glucose, and suggest that parental rFGF1 may act as an insulin sensitizer.

To investigate the physiological mechanisms of rFGF1 action further, we performed hyperinsulinaemic–euglycaemic clamp studies in DIO mice chronically treated with vehicle or rFGF1. The steady-state glucose infusion rate during the clamp was ~75% higher in rFGF1-injected mice, indicating increased responsiveness to insulin (Extended Data Fig. 4a). The ability of insulin to suppress hepatic glucose production was improved in rFGF1-injected mice, revealing increased hepatic insulin sensitivity as a long-term consequence of rFGF1 injection (Fig. 4a). Liver gene expression analyses from chronically rFGF1-treated DIO mice revealed significant reductions in macrophage markers, for example F4/80 and Cd11c, and in inflammatory cytokines, for example Il-1α, Il-1β and Tnf-α (Extended Data Fig. 4b). Furthermore, whole-body and insulin-stimulated glucose disposal rates were ~47% and ~80% higher, respectively, in ob/ob mice with rFGF1 decreased serum levels of several inflammatory cytokines (eotaxin, keratinocyte chemoattractant (KC), Mip-1β and interleukin-3) (Extended Data Fig. 3d). These results demonstrate that rFGF1 operates in an insulin-dependent manner to lower blood glucose, and suggest that parental rFGF1 may act as an insulin sensitizer.

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Figure 3 | rFGF1 induces insulin-dependent glucose uptake. a, Blood glucose levels in STZ-induced diabetic mice 8 h after subcutaneous injection of control PBS (open bars) or rFGF1 (0.5 mg kg\(^{-1}\), filled bars). b, c, ITT (b) and area under the ITT curve (AUC; c) for control and rFGF1-treated STZ mice (open and filled squares and bars, respectively; n = 4). d–g, Blood glucose level (d, e) and metabolic clearance rate (MCR; f, g) in ob/ob mice after control PBS (open bars) or rFGF1 injection (0.2 mg kg\(^{-1}\) intravenously, filled bars) before (d, f) or 1 h after (e, g) infusion of somatostatin (SOM) (n = 5). Values are means and s.d. Statistics by two-tailed t-test: *P < 0.05; **P < 0.01.

rFGF1-injected mice, reflecting enhanced peripheral sensitivity to insulin (Fig. 4b, c and Extended Data Fig. 4c–f). Consistent with the hepatic and peripheral insulin-sensitizing effects of rFGF1, insulin-stimulated AKT signalling was enhanced in both the liver and muscle of chronically rFGF1-treated DIO mice (Extended Data Fig. 4g, h). Taken together, these findings demonstrate that chronic administration of rFGF1 leads to sustained glucose lowering and whole-body insulin sensitization.

The above studies demonstrating robust and sustained glucose lowering in multiple diabetic mouse models raised the possibility of paren- teral administration of rFGF1 as a diabetic therapy. In support of this notion, diabetic mice chronically treated with rFGF1 were phenotypically normal in terms of locomotor activity, oxygen consumption and respiratory exchange ratio (Extended Data Fig. 4i–n), suggesting that chronic treatment with rFGF1 does not evoke adverse pleiotropic effects. Although endogenous FGF1 has been shown to have a role in adipose remodelling, histological examination of this tissue found no abnormalities after chronic treatment with rFGF1 (Extended Data Fig. 4o). Furthermore, serum creatine kinase levels did not change in chronically rFGF1-treated ob/ob mice, indicating the absence of muscle tissue damage (Extended Data Fig. 4p).

Although thiazolidinediones are the only therapeutic insulin sensitizers, their application is limited by adverse side effects, including weight gain, increased liver steatosis and bone fractures\(^7\). Notably, chronic treatment with rFGF1 did not lead to weight gain, and there was a decrease in hepatic steatosis (Fig. 3f, Extended Data Fig. 2i, j and Extended Data Table 1). Similar to thiazolidinediones, endocrine FGF21, which has recently been shown to have potential therapeutic blood glucose-lowering effects, has also been associated with a loss of bone density\(^4\). In contrast, bone mineral density, trabecular bone architecture and cortical bone thickness were not affected by chronic treatment with rFGF1 in DIO mice, as determined by micro-computed tomography analyses (Extended Data Fig. 4q, r). Furthermore, total and high-molecular-weight serum adiponectin levels were not altered by chronic treatment with rFGF1, differentiating its mechanism of action from the adiponectin-dependent glucose-lowering effects of FGF21 (ref. 15, 16) (Extended Data Fig. 4s). As the prototype of a growth factor family, rFGF1 has the potential to induce unwanted cell proliferation, and concern resides in whether the mitogen properties of rFGF1 could be dissociated from its glucose-lowering activities. To address this question, we generated an FGF1 ligand, rFGF1\(^{\text{ANT}}\), lacking the first 24 residues from the amino terminus. On the basis of the crystal structures of FGF1–FGFR complexes, the truncation was predicted to have significant effects on the binding affinity of FGF1 for all FGFRs, and similar truncations have been shown to decrease FGF1 mitogenicity. Consistent with this prediction, rFGF1\(^{\text{ANT}}\) showed a marked decrease in binding affinity for FGFRs compared with the native ligand, yet was still able to bind FGFR1c and FGFR2c, although with lower affinity (Extended Data Fig. 5a, b). In vitro, rFGF1\(^{\text{ANT}}\) showed a somewhat attenuated ability to activate intracellular signals downstream of FGFRs but showed a severe decrease in mitogenic activity (Fig. 4d and Extended Data Fig. 6a). Parenteral delivery of rFGF1\(^{\text{ANT}}\) dose-dependently lowered blood glucose levels in both genetically induced and diet-induced mouse models of diabetes (Fig. 4e and Extended Data Fig. 6b). rFGF1\(^{\text{ANT}}\) also retained the feeding suppression effects observed with rFGF1 (Extended Data Fig. 6c). The synthetic effects of exogenous rFGF1 on physiology, such as glucose homeostasis and feeding behaviour, therefore differ from, and are independent of, its classical role as a growth factor and mitogen.

In exploring the receptor dependence of the observed glucose-lowering effects, we speculated that the effects were mediated through FGFR1, on the basis of its known role in insulin sensitivitiy\(^7\) and the observation that rFGF1\(^{\text{ANT}}\) retained FGFR1c-binding affinity in our surface plasmon resonance studies. Furthermore, our previous studies on Fgf1 knockout mice implicated adipose tissue as a major site of FGF1 action. Accordingly, we generated mice lacking Fgfr1 predominantly in adipose tissue (aP2-Cre:Fgfr1\(^{\text{fl/fl}}\), R1 knockout mice). Indeed, although rFGF1 potently lowered blood glucose levels in control diabetic mice, it failed to lower glucose levels in the R1 knockout mice (Fig. 4g), indicating a requirement for FGFR1. Consistent with this, rFGF1\(^{\text{ANT}}\) similarly failed to affect blood glucose levels in R1 knockout mice (Extended Data Fig. 6d). An FGF1 analogue with severely attenuated FGFR-mediated signalling in vitro (FGF1 L29–D155; rFGF1\(^{\text{ANT2}}\)) did not significantly affect blood glucose levels in diabetic mice (Extended Data Fig. 6e, f), further supporting

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**Figure 4** Chronic administration of rFGF1 is insulin sensitizing.

a–c, Insulin-stimulated suppression of hepatic glucose production (HGP) (a), steady-state glucose disposal rate (b) and insulin-stimulated glucose disposal rate (c) measured during hyperinsulinaemic-euglycaemic clamps on DIO mice after 3 weeks of control PBS (Veh., open bars, n = 11) or rFGF1 (0.5 mg kg\(^{-1}\) subcutaneously every other day; filled bars, n = 9) treatment.

d, Proliﬁcative activity (measured by the absorbance of formazan at 600 nm) of NIH3T3 cells treated with rFGF1 (red) or rFGF1\(^{\text{ANT}}\) (blue) at the indicated concentrations (experiment repeated three times). e, Blood glucose levels of ob/ob mice treated with control PBS (open symbols, n = 12), rFGF1 (0.5 mg kg\(^{-1}\) subcutaneously; ﬁlled symbols, n = 8) or rFGF1\(^{\text{ANT}}\) (0.5 mg kg\(^{-1}\) subcutaneously; red dashed line, n = 6). f, Blood glucose levels of DIO mice treated with control PBS (open bars), rFGF1 (0.5 mg kg\(^{-1}\) subcutaneously, ﬁlled bars), or rFGF1\(^{\text{ANT}}\) (0.5 mg kg\(^{-1}\) subcutaneously, stripped bars) at the indicated times (n = 10). g, Blood glucose levels in 8-month-old mice fed on a high-fat diet: Fgfr1\(^{\text{fl/fl}}\) (WT, n = 5) and aP2-Cre:Fgfr1\(^{\text{fl/fl}}\) (R1 knockout, KO), n = 4 mice at 0 h (open bars) and 24 h (ﬁlled bars) after treatment with rFGF1 (0.5 mg kg\(^{-1}\) subcutaneously). Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; ***P < 0.001; ****P < 0.0005; n.s., not significant.
the notion that FGFR1-mediated signalling is required for the glucose-lowering effects of parenteral rFGF1. Taken together, these studies identify exogenous or endocrinized rFGF1 as a potent and long-lasting insulin sensitizer that seems to circumvent the adverse side effects associated with other diabetic therapies.

Previously we identified a role for endogenous FGF1 in the adaptive remodelling of visceral adipose tissue in response to nutrient fluctuations. The profound metabolic dysregulation observed in FGF1 knock-out mice when stressed by a high-fat diet was associated with decreased vascularity in visceral adipose depots, consistent with the known role of FGF1 in angiogenesis. In contrast, our present findings identify a neo-morphic insulin-sensitizing action for FGF1 in which systemic delivery of the normally autocrine/paracrine FGF1 through parenteral routes results in potent and sustained correction of hyperglycaemia accompanied by whole-body insulin sensitization.

The apparent divergent activities in vivo now ascribed to FGF1 seem to closely parallel those of FGF21, which has been assigned locally restricted roles in adipose tissue as well as systemic glucose-lowering activities. However, FGF21 circulates as a true endocrine hormone, whereas the high-affinity heparan sulphate proteoglycan-binding activity and serum lability of FGF1 restrict its endogenous actions to local tissues, resulting in the rapid clearance of exogenous FGF1 from the circulation. In addition, these two FGFs have disparate FGFR specificities: whereas FGF1 can bind and signal through each of the alternatively spliced forms of the FGFRs, FGF21 signalling requires a heterodimeric β-klotho–FGFR complex. Our findings indicate that the metabolic effects of exogenous FGF1 are mediated through FGFR1 in adipose tissue; however, additional studies will be necessary to exclude the involvement of additional receptors and/or tissues in the body-wide effects attributed to parenterally delivered rFGF1. In conclusion, given that the glucose-lowering effects of rFGF1 were not accompanied by side effects associated with current insulin sensitizers, along with the discovery that non-mitogenic rFGF1 retains glucose-normalizing capability, rFGF1 and its derivatives may hold therapeutic promise.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 7 August 2013; accepted 29 May 2014.

Published online 16 July 2014.

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Acknowledgements We thank L. Chong, J. Alvarez, S. Kaufman, B. Collins, X. Zhao, S. Liu, A. Jurzdzinski, A. Bleeker, K. Bijsterbosch, D. Gh and G. Bandyopadhyay for technical assistance, and L. Ong and C. Brondos for administrative assistance. Computed tomography was performed at the Veterans Medical Research Foundation. R.M.E. is a Howard Hughes Medical Institute Investigator at the Salk Institute and March of Dimes Chair. M.D. is supported by National Institutes of Health (NIH) grants (DK075778, DK090962, HL088093, HL105278 and ES010337), the Glenn Foundation for Medical Research, the Leona M. and Harry B. Helmsley Charitable Trust, Ipsen/Biomeasure, the California Institute for Regenerative Medicine and The Ellison Medical Foundation, C.L. and J.M.O. are funded by the Netherlands Organisation for Scientific Research (NWO) and the Dutch Diabetes Foundation (grant 1153154, 632886 and 1043199); J.W.J. by the European Research Council (grant IRG-277169), the Human Frontier Science Program (CA000113-2011-C), the Netherlands Organisation for Scientific Research (Vidi grant 016.126.338), the Dutch Digestive Remodelling grant W08-01-01 and the Dutch Diabetes Foundation (grant 2012.00.1937); J.M.O. by NIH grants (DK-033651, DK-074868, T32-DK-007494, DK-063491 and PO1-DK054441-14A1) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development/NIH through cooperative agreement of U54-HD-012303-25 as part of the specialized Cooperative Centers Program in Reproduction and Infertility Research; M.M. by the National Institute of Dental and Craniofacial Research grant (DE13686); and M.A. by an F32 Ruth L. Kirstchich National Research Service Award (National Institute of Diabetes and Digestive and Kidney Diseases).

Author Contributions J.M.S., J.W.J., M.D. and R.M.E. designed and supervised the research. J.M.S., J.W.J., M.A., R.T.Y., C.L., A.R.A., J.M.O., M.M., M.D. and R.M.E. analysed data. J.M.S., J.W.J., M.A., R.T.Y., C.L., A.R.A., J.M.O., M.M., M.D. and R.M.E. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to R.M.E. (evans@salk.edu) or M.D. (downes@salk.edu).

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STZ mice. Mice were housed in a temperature-controlled environment with a 12-h light/12-h dark cycle and handled in accordance with Salk IACUC guidelines complying with US legislation. Male ob/ob mice (B6.V-Lepob129S7/J; Jackson Laboratories) and male C57BL/6J mice received a standard diet (MI laboratory rodent diet 5001; Harlan Teklad) or high-fat diet (high-fat diet (60%) diet F3282; Bio-Serv) and acidified water ad libitum. STZ-induced diabetic mice in the C57BL/6J background were purchased from Jackson Laboratories. aP2-Cre mice (B6.Cg-Tg(Fabp4-cre)1Rev/J; Jackson Laboratories) were crossed to FGFFR1 floxed mice (B6.129S4-Fgfr1tm1Sor/J; Jackson Laboratories) to generate aP2-Cre;Fgfr1fl/fl mice. Solutions (0.1 mg ml\(^{-1}\) in PBS) of mouse FGF1 (Prospective), human FGF1 (Prospect), mouse FGF2 (Prospect), mouse FGF3 (Prospect), and mouse FGF10 (BD Systems) were injected as described. Heparin sodium salt (Sigma) was premixed with mFGF1 peptide before injection.

Serum analysis. Blood was collected by tail bleeding either in the ad libitum fed state or after overnight fasting. Free fatty acids (Wako), triglycerides (Thermo) and cholesterol (Thermo) were measured using enzymatic colorimetric methods, following the manufacturer’s instructions. Serum insulin levels were measured using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Millipore) and Bio-Plex Pro (Bio-Rad) kits.

Histological analysis. Tissues were fixed in 4% phosphate-buffered formalin, embedded in paraffin, and sectioned at 4 μm. Sections were stained with hematoxylin and eosin or periodic acid–Schiff reagent in accordance with standard procedures.

Metabolite analysis. Hepatic concentrations of triglycerides, free cholesterol and total cholesterol were measured in homogenized livers using commercial kits (Roche Diagnostics, Wako Chemicals and DiaSys) after lipid extraction according to Bligh and Dyer; lipids were redissolved in 2% Triton X-100 in water. Glycogen in liver was determined after extraction of 100 mg of liver with KOH.

Metabolic studies. GTTs were conducted after fasting overnight. Mice were injected intraperitoneally with 1 g of glucose per kilogram body weight, and blood glucose was measured at 0, 15, 30, 60, and 120 min using a OneTouch Ultra glucometer (Lifecare Inc.). ITTs were conducted after 3 h of fasting. Mice were injected intraperitoneally with 2 U of insulin per kilogram body weight (Humulin R; Eli Lilly) and blood glucose was measured at 0, 15, 30, 60, and 90 min using a OneTouch Ultra glucometer. Real-time metabolic analyses were conducted in a Comprehensive Lab Animal Monitoring System (Columbus Instruments). CO\(_2\) production, O\(_2\) consumption, RQ (relative rates of carbohydrate versus fat oxidation) and ambulatory counts were determined for six consecutive days and nights, with at least 24 h for adaptation before data recording. Analysis of total body composition was performed with an EchoMRI-100 (Echo Medical Systems, LLC).

STZ mice. STZ mice (Jackson Laboratory) injected subcutaneously with control PBS or 0.5 mg kg\(^{-1}\) rFGF1 were subsequently fasted for 8 h before blood glucose measurements (OneTouch Ultra glucometer). STZ mice injected subcutaneously with control PBS or 0.5 mg kg\(^{-1}\) rFGF1 were subsequently fasted for 8 h before injection of 0.25 U kg\(^{-1}\) insulin (Humulin R), and blood glucose was monitored at 0, 30, 60 and 120 min (OneTouch Ultra glucometer).

Hyperinsulinemic–euglycemic clamp in DIO mice. Mouse clamps were performed as described previously\(^{13}\). Male C57BL/6J mice received a high-fat diet (60%, diet F3282; Bio-Serv) for 16 weeks and treated with vehicle (PBS) or FGF1 (0.5 mg kg\(^{-1}\)) by subcutaneous injection every 48 h for 3 weeks before clamp studies. In brief, mice implanted with dual jugular catheters 3 days previously were fasted for 6 h, then equilibrated for 90 min with tracer (5.0 μCi h\(^{-1}\), 0.12 ml h\(^{-1}\) d-3-[\(^{13}\)C]glucose; NEN Life Science Products). A basal blood sample was then drawn via tail vein to calculate basal glucose uptake. The insulin (8 μU kg\(^{-1}\) min\(^{-1}\) at 2 μl min\(^{-1}\); Novo Nordisk) plus tracer (5.0 μCi h\(^{-1}\)) and glucose (50% dextrose at variable rate; Abbott) infusions were initiated simultaneously, with the glucose flow rate adjusted to reach a steady-state blood glucose concentration (~120 min). Steady state was confirmed by stable plasma tracer counts during the final 30 min of clamp. Blood was taken at 110 and 120 min for the determination of tracer-specific activity. At steady state, the rate of glucose disappearance or the total glucose disposal rate is equal to the sum of the rate of endogenous or hepatic glucose production and the rate of exogenous glucose infusion. The insulin-stimulated glucose disposal rate is equal to the sum of glucose fluxes minus the basal glucose turnover rate.

Glucose flux measurements. Mice were equipped with a permanent catheter in the right atrium via the jugular vein\(^{16}\) and were allowed a recovery period of at least 3 days. After the recovery period, the mice were placed in experimental cages with access to drinking water. All infusion experiments were performed in conscious, unrestrained mice. Steady-state glucose fluxes were determined during three periods: first, basal hyperglycaemic; second, after administration of FGF1; and third, after somatostatin infusion. During the entire experiment, mice were infused with a solution containing a tracer of [U-\(^{13}\)C]glucose (10 mg ml\(^{-1}\)) (Cambridge Isotope Laboratories) at an infusion rate of 0.6 ml h\(^{-1}\). After the basal period (~3 h), mice were administered a bolus of recombinant FGF1 (0.5 mg kg\(^{-1}\)) and steady-state glucose fluxes were determined after blood glucose levels were stable (about 2 h). Finally, somatostatin (10 μg ml\(^{-1}\)) was added to the infusate and effects on glucose fluxes were monitored. During the experiment, blood glucose levels were determined every 15 min using a Lifescan EuroFlash glucometer. For GC-MS analysis of [U-\(^{13}\)C]glucose, blood spots on filter paper were collected from the tail vein every 30 min.

 Purification of FGF and FGF receptors. Human FGFR3 (M1–D155), N-terminally truncated human FGFR2 (FGFR2NT(K25–D155) and FGFR2NT(L29–D155)) and human FGFR homologous factor 1B (FHFB1; M1–T181) were expressed in Escherichia coli cells and purified from the soluble bacterial cell lysate fraction by heparin affinity, ion-exchange and size-exclusion chromatography. The minimal ligand-binding domain of human FGFR1c (D142–E365), FGFR2b (A140–E369), FGFR2c (N149–E368), FGFR3c (D147–E365) and FGFR4 (Q144–D355) was refolded in vitro from bacterial inclusion bodies and purified by published protocols.

FGF1–FGFR binding analysis by surface plasmon resonance spectroscopy. Real-time binding experiments were performed with a Biacore 2000 surface plasmon resonance (SPR) spectrometer (Biacore AB/GE Healthcare). FGF1–FGFR binding was studied at 25 °C in HBS-EP buffer (10 mM HEPES-NaOH pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) polysorbate 20). FGF1 and FGFR1NT were covalently coupled to flow channel of a research-grade CM5 chip (~60–99 fmol mm\(^{-2}\)). FHFB1, which shares structural similarity with FGFR1 but does not show any FGF binding, was coupled to the control flow channel of the chip (~63 fmol mm\(^{-2}\)). Increasing concentrations of the ligand-binding domain of FGFR1 (5–960 nM) were injected over the chip at a flow rate of 50 μl min\(^{-1}\), and at the end of each protein injection (180 s), HBS-EP buffer (50 μl min\(^{-1}\)) was flowed over the chip for 180 s to monitor dissociation. The chip surface was then regenerated by injecting 10–50 μl of 1.5 M NaCl in 10 mM HEPES-NaOH pH 7.5. The data were processed with BiaEvaluation software (Biacore AB/GE Healthcare). For each FGF1 injection over the chip, the non-specific responses from the FHFB1 control flow channel were subtracted from the responses recorded for the FGF1 flow channel. Where possible, equilibrium dissociation constants (K\(_d\) values) were calculated from fitted saturation binding curves. The fitted curves were judged to be accurate on the basis of on the distribution of the residuals (even and near 0) and \(\chi^2\) (~10% of \(R_{\text{max}}\)).

Mitogenicity assay. NIH3T3 cells from ATCC (not recently tested for mycoplasma contamination) were seeded in 96-well plates at a density of 5 × 10\(^{4}\) cells per well. After 24 h the cells were washed with PBS and then serum-starved for 24 h. Starved cells were stimulated with FGF1 or FGF1NT (0.097–25 ng ml\(^{-1}\)) for 24 h, and the number of viable cells was measured using the MTT assay.

Intracellular signalling assay. To analyse Akt signalling, mouse tissues were homogenized in Bio-Plex cell lysis kit solution (Bio-Rad) and lysates were analysed for total and phospho-Akt, following the manufacturer’s instructions for the Bio-Plex phosphoprotein detection system (Bio-Rad). For in vitro FGF1 signalling assays, HEK293 cells were seeded in six-well plates and grown to 70–80% confluence. After overnight serum starvation in DMEM medium, cells were treated with recombinant peptides for 15 min, washed in ice-cold PBS and frozen in liquid nitrogen. Cells were lysed in sample buffer, sonicated, resolved on SDS–PAGE, and blotted to poly (vinylidine difluoride) membranes for antibody detection. Antibodies against pFRS2α, ERK1/2, phospho-ERK1/2, Akt and phospho-Akt were purchased from Cell Signalling Technology.

Statistics and general methods. Age-matched and weight-matched mice (average cohort size six to ten, sufficient to enable statistical significance to be accurately determined) were randomly assigned to treatment or control groups. No animals were excluded from the statistical analysis, and the investigators were not blinded in the studies. Appropriate statistical analyses (t-test) were applied, assuming a normal sample distribution, as specified in the figure legends. No estimate of variance was made between each group.

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Extended Data Figure 1 | Acute rFGF1 injection lowers blood glucose levels and reduces food intake. a–c, Blood glucose levels in ob/ob mice after subcutaneous (sc, n = 3) or intraperitoneal (ip, n = 3) injection of rFGF1 (a), intravenous (iv) injection of rFGF1 (n = 6) or control vehicle (n = 5) (b), or subcutaneous injection of rFGF1 premixed with heparin (1.5 mg kg\(^{-1}\), n = 3) (c). d, Serum insulin levels 24 h after rFGF1 treatment under ad libitum fed or fasting conditions in chow-fed C57BL/6J mice (n = 10). e, Blood glucose levels in fasted, chow-fed mice after treatment with rFGF1 (n = 8). f, Food intake during 24 h after injection of rFGF1 in chow-fed C57BL/6J mice (n = 8). g–i, Food intake during indicated times after injection of rFGF1 in ob/ob (n = 6) (g), db/db (n = 3) (h) and DIO (n = 6) (i) mice. Control PBS (open bar) or murine rFGF1 (0.5 mg kg\(^{-1}\); filled bar) were injected subcutaneously to ad libitum fed mice unless otherwise noted. Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; **P < 0.01.
Extended Data Figure 2 | Chronic administration of rFGF1 lowers blood glucose independently of food intake and metabolic hormones. a–c, MRI analyses of fat (a) and lean mass content (b) at indicated days, and food intake (c) during chronic administration of control vehicle (n = 6) or rFGF1 (n = 8) in ob/ob mice. d, Random-fed blood glucose of a pair-fed cohort of ob/ob mice (red line, filled triangles, n = 12) plotted alongside blood glucose trends during chronic administration of control vehicle (black line, open squares, n = 6) or rFGF1 in ob/ob mice (black line, filled squares; n = 8). Pair-fed cohort food intake was restricted to equal the food intake of rFGF1-injected ob/ob mice throughout the 5-week trial. e–g, Serum cholesterol (e), free fatty acids (f) and metabolic hormone levels (g) after 5-week administration of control PBS or rFGF1 in ob/ob mice (n = 4). h–j, Body weight (h), tissue mass analyses (i) and food intake (j) of DIO mice after 4 weeks of rFGF1 treatment (n = 6). All injections were performed subcutaneously with control vehicle (PBS, open bars or symbols) or murine rFGF1 (0.5 mg kg\(^{-1}\); filled bars or symbols) to ad libitum fed mice every 48 h throughout chronic administration trials. Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; **P < 0.01.
Extended Data Figure 3 | rFGF1 does not stimulate insulin secretion and chronic administration reduces systemic inflammation. a, Glucose-stimulated insulin secretion (basal; 3 mM glucose; stimulated, 20 mM glucose) of ob/ob islets after 1 h pretreatment with control PBS (open bars) or rFGF1 (10 ng ml⁻¹, filled bars; n = 6). b, c, Time course of serum insulin (b) and blood glucose (c) levels in ob/ob mice after a single rFGF1 injection (0.2 mg kg⁻¹ intravenously; n = 8). d, Serum cytokines in ob/ob mice after 5 weeks of subcutaneous administration of control vehicle (PBS, open bars; n = 4) or rFGF1 (0.5 mg kg⁻¹ every other day, filled bars; n = 6) Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; **P < 0.01.
Extended Data Figure 4 | rFGF1 is an insulin sensitizer and does not affect bone morphology. a–d, Steady-state glucose infusion rate (a), hepatic inflammation-related gene expression (b), glucose tolerance tests (c) and body weights (d) of DIO mice after 3 weeks of administration of control PBS (n = 11) or rFGF1 (0.5 mg kg\(^{-1}\)) every other day; n = 9). e, f, Basal hepatic glucose production (e) and basal and clamped serum insulin concentrations (f) measured during hyperinsulinaemic–euglycaemic clamp studies of DIO mice after 3 weeks of administration of control PBS (n = 11) or rFGF1 (n = 6). g, h, Insulin induced phosphorylation of AKT in liver (g) and muscle tissues (h) of DIO mice after 3 weeks of administration of control PBS (n = 11) or rFGF1 (n = 12). i–n, Food intake (i), carbon dioxide production (j), heat production (k), total activity (l), respiratory exchange ratio (m) and oxygen consumption (n) of chronically rFGF1-treated DIO mice (3 weeks of treatment with control PBS (blue symbols, n = 4) or rFGF1 (red symbols, n = 4) measured in metabolic cages. o, Representative haematoxylin and eosin staining of inguinal white adipose tissue from DIO mice after 4 weeks of administration of control PBS (n = 6) or rFGF1 (n = 6). p, Serum creatine kinase levels in chronically rFGF1-treated ob/ob mice (n = 4). q, r, Bone mineral density (q) and trabecular bone thickness (Tb Th), trabecular bone space (Tb Sp) and cortical bone thickness (C Th) (r) in 4-week treated DIO mice analysed by micro-computed tomography (n = 6). s, Total and high-molecular-weight (HMW) serum adiponectin levels in ob/ob mice after 4 weeks of rFGF1 injections every 48 h (n = 4). Control vehicle (PBS, open bar), rFGF1 (0.5 mg kg\(^{-1}\) subcutaneously; filled bars). Scale bar, 100 µm. Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; **P < 0.01; ***P < 0.005; ****P < 0.001.
Extended Data Figure 5 | Binding affinities of rFGF1 and rFGF1\textsuperscript{INT} to FGFRs. Removal of the N terminus from FGF1 reduces the ligand’s binding affinity for FGFRs. a, Overlays of SPR sensorgrams of FGF1 binding to the ligand-binding domain of FGFRs and fitted saturation binding curves. Equilibrium dissociation constants (\(K_d\) values) were derived from the saturation binding curves. b, Overlays of SPR sensorgrams of FGF1\textsuperscript{INT} binding to the ligand-binding domain of FGFRs. Where possible, \(K_d\) values were calculated from fitted saturation binding curves.
Extended Data Figure 6 | rFGF1 and rFGF1\(^{\Delta NT}\) signal through FGFR1 in a dose-dependent manner. a, Western blot showing intracellular signalling in serum-starved HEK293 cells after a 15-min treatment with the indicated concentrations of PBS, rFGF1\(^{\Delta NT}\) or rFGF1. b, Dose response of glucose-lowering effects of subcutaneously delivered rFGF1\(^{\Delta NT}\) (striped bars) in comparison with rFGF1 (filled bars) in 12-week-old ob/ob mice (n = 8). c, Food intake in DIO mice during a 24 h period after injection of control PBS (open bar), rFGF1 (0.5 mg kg\(^{-1}\) subcutaneously; filled bars) or rFGF1\(^{\Delta NT}\) (0.5 mg kg\(^{-1}\) subcutaneously; striped bar, n = 10). d, Blood glucose levels in high-fat-diet-fed (8 months) Fgfr1\(^{+/+}\) (WT, n = 5) and aP2-Cre;Fgfr1\(^{+/+}\) (R1 knockout (KO), n = 4) mice at 0 h (open bars) and 24 h (filled bars) after treatment with rFGF1\(^{\Delta NT}\) (0.5 mg kg\(^{-1}\) subcutaneously). e, Western blot showing intracellular signalling in serum-starved HEK293 cells after a 15-min treatment with PBS or 10 ng ml\(^{-1}\) rFGF1, two independent preparations of rFGF1\(^{\Delta NT}\), and rFGF1\(^{\Delta NT2}\). f, Blood glucose levels in ob/ob mice at 0 h (open bars) and 24 h (filled bars) after treatment with rFGF1\(^{\Delta NT}\) and rFGF1\(^{\Delta NT2}\) (0.5 mg kg\(^{-1}\) subcutaneously; n = 2). Gel images are representative of at least three biological replicates. Values are means and s.e.m. Statistics by two-tailed t-test: *P < 0.05; ***P < 0.005.
Extended Data Table 1 | Metabolic parameters of ob/ob mice chronically treated with rFGF1

|                  | vehicle     | FGF1         | unit |
|------------------|-------------|--------------|------|
| Body weight (BW) | 70.0 ± 1.8  | 65.1 ± 1.9   | gram |
| Liver            | 4.26 ± 0.15 | 3.77 ± 0.22  | gram |
| jWAT             | 3.71 ± 0.09 | 3.22 ± 0.12  | gram |
| gWAT             | 1.46 ± 0.05 | 1.27 ± 0.05  | gram |
| Heart            | 160 ± 4     | 174 ± 10     | mg   |
| Liver % BW       | 6.1 ± 0.2   | 5.8 ± 0.2    | %    |
| iWAT % BW        | 10.6 ± 0.3  | 9.8 ± 0.3    | %    |
| gWAT % BW        | 4.2 ± 0.2   | 3.9 ± 0.2    | %    |
| Heart % BW       | 0.23 ± .004 | 0.26 ± .004  | %    |
| Triglycerides    | 247 ± 30    | 309 ± 22     | mg/dl|
| Free fatty acids | 0.85 ± 0.10 | 0.91 ± 0.09  | mEq/L|
| Cholesterol      | 269 ± 14    | 301 ± 8      | mg/dl|
| Glycogen         | 81 ± 8      | 167.22 ± 21  | μmol/g|

Male ob/ob mice injected subcutaneously with vehicle (PBS) or rFGF1 (0.5 mg kg⁻¹ every 48 h) for 36 days. Results are expressed as means ± s.e.m. (control, n = 6; rFGF1, n = 8). *P < 0.05; **P < 0.01.
Corrigendum: Endocrinization of FGF1 produces a neomorphic and potent insulin sensitizer

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Nature 513, 436–439 (2014); doi:10.1038/nature13540

This Letter should have declared the following competing financial interests: “The fibroblast growth factor (FGF) molecules and related methods of use reported in this study are covered in the following published patent applications and counterparts that derive priority: (1) PCT/US2011/032848, held by R.M.E., M.D., J.W.J., and J.M.S. (handled by Salk OTD); (2) PCT/US2013/044589, held by M.M., R.G., R.M.E., M.D. and J.M.S. (handled by NYU Office of Industrial Liaison/Technology Transfer); (3) PCT/US2013/044594, held by M.M., R.G., R.M.E., M.D. and J.M.S. (handled by NYU Office of Industrial Liaison/Technology Transfer); and (4) PCT/US2013/044592, held by M.M. and R.G. (handled by NYU Office of Industrial Liaison/Technology Transfer).”

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