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The Metabolic State of Diabetic Monkeys Is Regulated by Fibroblast Growth Factor-21

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Fibroblast growth factor (FGF)-21 has been recently characterized as a potent metabolic regulator. Systemic administration of FGF-21 reduced plasma glucose and triglycerides to near normal levels in genetically compromised diabetic rodents. Importantly, these effects were durable and did not come at the expense of weight gain, hypoglycemia, or mitogenicity. To explore the therapeutic properties of FGF-21 in a nongenetically modified primate species, and thus demonstrate the potential for efficacy in humans, we evaluated its bioactivity in diabetic nonhuman primates. When administered daily for 6 wk to diabetic rhesus monkeys, FGF-21 caused a dramatic decline in fasting plasma glucose, fructosamine, triglycerides, insulin, and glucagon. Of significant importance in regard to safety, hypoglycemia was not observed at any point during the study. FGF-21 administration also led to significant improvements in lipoprotein profiles, including lowering of low-density lipoprotein cholesterol and raising of high-density lipoprotein cholesterol, beneficial changes in the circulating levels of several cardiovascular risk markers/factors, and the induction of a small but significant weight loss. These data support the development of FGF-21 for the treatment of diabetes and other metabolic diseases. (*Endocrinology* 148: 774–781, 2007)

NUMEROUS REPORTS DOCUMENT the increasing incidence of type 2 diabetes and the potential ensuing pandemic (1). These daunting statistics, coupled with the only moderately effective nature of current antidiabetic drugs, place a high demand on the global biomedical research community to provide safer and more effective therapies for the prevention and treatment of type 2 diabetes (2, 3).

Recently, select members of the fibroblast growth factor (FGF) family (4) have been described as potential new drug candidates to combat metabolic diseases (5, 6). Specifically, FGF-19 (7, 8) and FGF-21 (9, 10) have demonstrated an ability to beneficially regulate metabolism. Transgenic mice expressing either human FGF-19 or FGF-21 exhibit reduced adiposity and resistance to diet-induced metabolic disturbances. Moreover, both FGF-19 and FGF-21 significantly improve the overall metabolic state of diet-induced or genetically modified diabetic mice when the proteins are administered systemically over time. However, although FGF-19 and FGF-21 are similar in their metabolic activity, FGF-21 is free of the proliferative and tumorigenic effects (9–11) that were documented for some members of FGF family, including FGF-19 (4, 12, 13). Thus, we have focused our attention on further defining the therapeutic potential of FGF-21.

Whereas the use of FGF-21 has been demonstrated in numerous studies spanning a wide variety of rodent models (9, 10), its therapeutic effects in higher species remained to be determined. The natural spontaneous development and progression of diabetes in *Macaca mulatta* (rhesus monkey), as well as its overall similarity to the human disease, provided the ideal advanced nonclinical model for assessing the effects of FGF-21 (14–18). The current report details the bioactivity of FGF-21 in a well-defined group of diabetic rhesus monkeys after daily administration for 6 wk in a dose-escalating fashion. The results demonstrate the impressive metabolic control achieved through therapeutic administration of FGF-21.

Materials and Methods

Animal care, pharmacokinetics in mice and cynomolgus primates, and the protocol of the FGF-21 efficacy study in diabetic rhesus monkeys

The protocols used in studies in mice and cynomolgus monkeys were approved by the Eli Lilly Research Laboratories Institutional Animal Care and Use Committee. FGF-21 was administered to male CD-1 (20–25 g) as a single iv or sc administration of 1.0 mg/kg. Blood samples were obtained at 0.08 (iv only), 0.25 (sc only), 0.5, 1, 4, 8, 12, and 24 h after administration from three animals per time point and processed to plasma. FGF-21 was administered to male cynomolgus monkeys (2.5–3.0 kg) as a single iv or sc administration of 0.5 mg/kg. Blood samples were obtained by cardiac puncture at 0.08, 0.25, 0.5, 1, 2, 4, 6 (sc only), 8, 12, 24, 36, 48, 72, and 96 h after administration from two animals per treatment group and processed to plasma. Plasma concentrations of FGF-21 were determined by ELISA.

The study protocol to evaluate FGF-21 effects in male diabetic rhesus monkeys was approved by the University of Maryland, Baltimore, Institutional Animal Care and Use Committee. For this purpose, a total of

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Abbreviations: FGF, Fibroblast growth factor; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; NMR, nuclear magnetic resonance; PPAR, peroxisome proliferator-activated receptors; TG, triglyceride; VLDL, very-low-density lipoprotein.

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six diabetic monkeys were used for a period of 117 d. The study included a baseline phase of vehicle injections followed by FGF-21 administration in a dose-escalating fashion and a 54-d washout period (Fig. 1A). The baseline phase started on d 0 and ended on d 21. Dosing of monkeys with 30, 100, and 300 μg/kg of FGF-21 was initiated on d 22, 36, and 50, respectively, and carried out for 2 wk in each dosing phase. The washout period began on d 64 and concluded on d 117. Vehicle and FGF-21 were delivered daily by sc administration, and animals were untreated during the washout period. Blood draws were performed on days according to Fig. 1A on animals after an approximately 16-h fasting interval. During baseline and FGF-21 phase, blood samples were taken 1 h before injection (approximately 24 h after the last dose). Animal body weights were measured weekly and food intake daily throughout the course of the study.

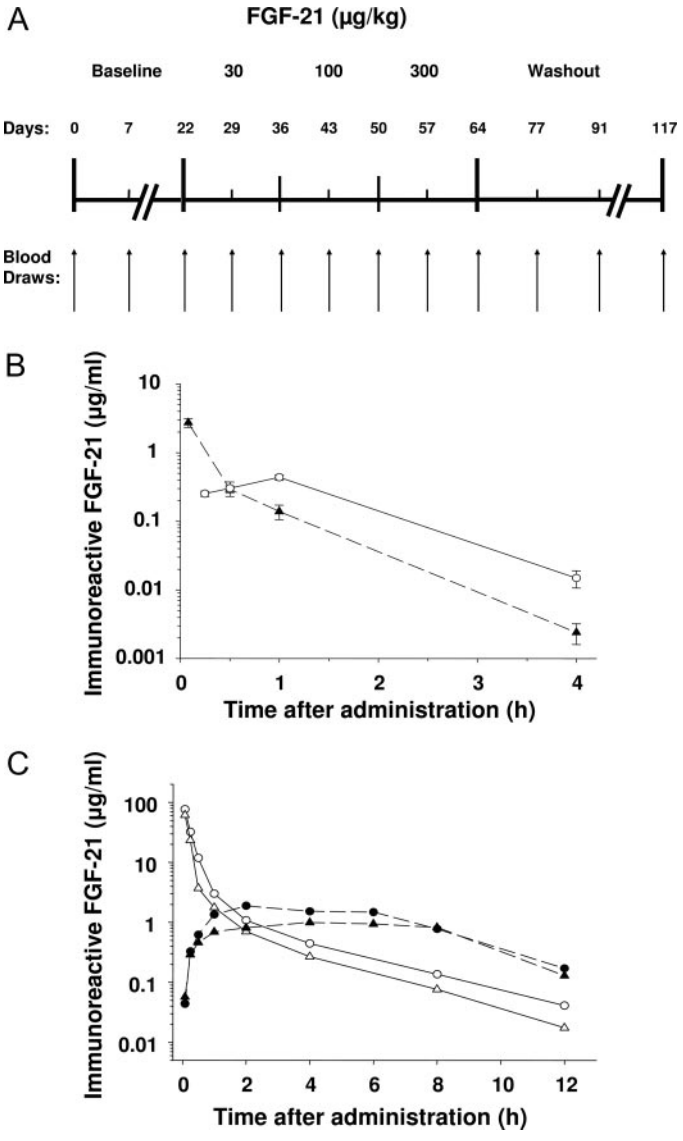

FIG. 1. FGF-21 study design in diabetic rhesus monkeys, and pharmacokinetic profile of FGF-21 in mice and monkeys. A, Baseline, FGF-21 injection, and washout phases are shown. Blood draws were carried out on days as indicated by the *arrows*. B, Plasma profile after administration of FGF-21 to CD-1 mice as a single iv (*closed symbols*) or sc administration (*open symbols*) of 1 mg/kg. Data represent the mean (±SD) (three animals/time point/group). C, Plasma profile after administration of FGF-21 to cynomolgus monkeys as a single iv (*open symbols*) or sc administration (*closed symbols*) of 0.5 mg/kg. Data represent profiles from individual animals.

FGF-21 expression and purification

Human recombinant FGF-21 was expressed, purified from *Escherichia coli* as described (9), and formulated in bacteriostatic NaCl (0.9%) (vehicle).

FGF-21 ELISA

Plasma samples were analyzed for concentrations of immunoreactive FGF-21 using anti-FGF-21 antibodies (raised in rabbits against full-length FGF-21 protein) by the sandwich ELISA method. The wells of a 96-well microtiter plate were coated overnight at 4 C with an affinity purified rabbit polyclonal anti-FGF-21 antibody at a concentration of 1 μg/ml (0.1 ml/well). All assay steps were carried out in 0.1-ml/well additions with 1-h incubations at room temperature. Samples and standards were diluted in cynomolgus monkey, rhesus monkey, or CD-1 mouse plasma. After washing, biotinylated affinity purified anti-FGF-21 (1:2500 dilution) was added and detected with a 1:4000 dilution of streptavidin-horseradish peroxidase. Samples were analyzed in duplicate and, when appropriate, at multiple dilutions. The standard curve range for the assays was 0.39–50 ng/ml.

Blood plasma analyses

Blood glucose, fructosamine, and triglycerides (TGs) were determined by Antech Diagnostics (New York, NY). Fasting plasma insulin and glucagon were measured using ELISA kits from Linco Diagnostic Services, Inc. (St. Charles, MO). Total cholesterol, low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) were measured using a Hitachi 912 clinical chemistry analyzer. Cytokine analysis was done by Rules-Based Medicine, Inc. (Austin, TX) using human antigen Multi-Analyte Profiles (MAP) kits. All clinical chemistry, lipid composition, cytokine, and other analyses presented in this report were performed on the blood samples collected 24 h after the last dose unless stated otherwise.

Lipoprotein subclass analysis

Subclass analysis and very-low-density lipoprotein (VLDL)-triglyceride (TG) determination were performed on plasma samples by proton nuclear magnetic resonance (NMR) spectroscopy using the LipoProfile test (LipoMed, Raleigh, NC). The lipoprotein subclasses were defined by particle size as follows: small HDL, 7.3–8.8 nm; large HDL, 8.8–13 nm; small LDL, 18.8–19.7 nm; medium LDL, 19.8–21.2 nm; large LDL, 21.3–23.0 nm; small VLDL, 27–35 nm; medium VLDL, 35–60 nm; and large VLDL, 60–200 nm.

Statistics and data presentation

Repeated-measures analysis was applied for multidose designs in which each animal received the same treatment within each time period and used to analyze blood glucose, TGs, fructosamine, insulin, glucagon, total cholesterol, HDL-c, LDL-c, and animal body weights. For the baseline and FGF-21 dosing phases, the data presented on the graphs represent the average of the readouts for the following days: baseline: d 0, 7, and 22; 30-μg/kg dose: d 29 and 36; 100-μg/kg dose: d 43 and 50; and 300-μg/kg dose: d 57 and 64. During the washout period, individual data for d 77, 91, and 117 are plotted. For lipoprotein NMR spectroscopy and cytokine analysis, a paired *t* test was applied and the measurements taken at d 0 and d 64 (end of 300-μg/kg dose phase) were compared. Statistical significance was determined using SAS (version 8.2) statistical analysis program (SAS Institute, Cary, NC). Results are typically shown as means ± SE.

Results

FGF-21 pharmacokinetic dose-selection studies in mice and normal cynomolgus monkeys

FGF-21 exerted glucose-lowering activity in diabetic rodents (9) and was further evaluated for efficacy in a dose-response fashion. For that purpose, we used a daily dosing paradigm for 7 d in *ob/ob* mice and measured blood glucose

24 h after the last injection. The ED_{50} for glucose lowering under these conditions was determined to be 65 μg/kg (data not shown). Subsequently, the pharmacokinetic profile of FGF-21 on single dose administration was characterized and used to establish a relationship of daily exposure (area under the curve) to pharmacodynamic activity (glucose lowering). The exposure determined for the ED_{50} dose in the mouse was used as the target for the starting exposure point at which to dose FGF-21 in the diabetic monkeys.

The pharmacokinetics of FGF-21 was determined in male CD-1 and *ob/ob* mice and lean and obese male cynomolgus monkeys. FGF-21 was administered as a single sc dose or daily dose for 7 d of 1 mg/kg to mice or 0.5 mg/kg to primates. The clearance of FGF-21 was approximately 2- to 3-fold slower in the primate compared with mouse (Table 1 and Fig. 1, B and C). Subcutaneous bioavailability was similar between the two species. No differences were noted in the clearance between CD-1 and *ob/ob* mice or lean and obese primates when normalized for body weight (data not shown). Although not determined directly, the assumption was made that the pharmacokinetic profile of FGF-21 is similar in rhesus and cynomolgus macaques. Accounting for the slower clearance in primate, the dose of FGF-21 to provide equivalent daily exposure based on area under the curve to the ED_{50} dose determined in the mouse was 30 μg/kg. Assuming that the sensitivity of diabetic rhesus monkeys to the biological activity of FGF-21 was similar to that of the *ob/ob* mouse, it might be anticipated that this dose could yield a glucose-lowering response. However, because we did not have an indication of the relative interspecies sensitivity to FGF-21, 30 μg/kg was chosen as the lowest tested dose with subsequent escalation to 100 and then 300 μg/kg.

FGF-21 pharmacodynamic effects in diabetic monkeys

Glycemic control. Before FGF-21 administration, mean baseline fasting plasma glucose in these animals was 119 ± 8.1 mg/dl (Fig. 2A), which is approximately two times normal and well above the diagnostic limit for type 2 diabetes in this species (14). Notably, FGF-21 lowered mean fasting plasma glucose from the overtly diabetic level to near normal when administered for 6 wk (Fig. 2A). Consistent with the effect on plasma glucose and indicating a durable impact on glycemic control, systemic delivery of FGF-21 also led to a reduction in fructosamine levels for each dose phase (Fig. 2B). Fasting plasma glucose and fructosamine were on average trending toward to preadministration levels during the washout period.

TABLE 1. Summary of pharmacokinetic parameters for FGF-21 in mouse (CD-1 mice) and primate (cynomolgus monkeys)

	Intravenous		Subcutaneous	
	Mouse	Primate	Mouse	Primate
Dose (mg/kg)	1	0.5	1	0.5
AUC (μg/h/ml)	1.2	2.4	0.98	1.2
CL (ml/h/kg)	803	217	1024	435
$T_{1/2}$ (h)	0.5	2.0	0.6	4.3
Cmax (μg/ml)	4.3	6.0	0.44	0.14
Tmax (h)	NA	NA	1	4
%F	NA	NA	78	50

AUC, Area under the plasma concentration curve; CL, clearance; $T_{1/2}$, half-life; Cmax, maximal plasma concentration; Tmax, time to Cmax; %F, absolute bioavailability; NA, not available.

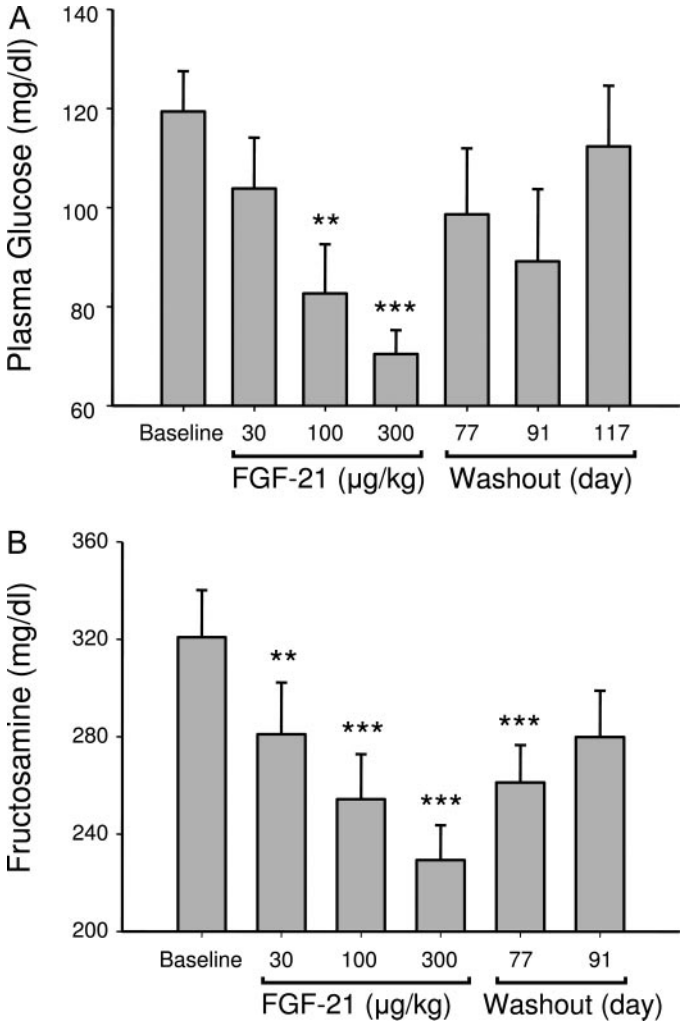

FIG. 2. FGF-21 effects on glycemia. The values (±SE) shown are the average of the measurements in all six animals taken on days during each phase of the study according to *Materials and Methods*. *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$, compared with baseline. Fasted blood glucose (A) and fructosamine (B) levels in diabetic rhesus monkeys at the different phases during the study.

Lipid metabolism. Although FGF-21 lowers total plasma triglycerides (TG) in diabetic rodents (9), it has not been known to date whether the molecule regulates lipid parameters in nonhuman primates. The effects of FGF-21 on lipids in obese rhesus monkeys were evaluated by both clinical chemistry analysis (Fig. 3, A–D) and NMR spectroscopy (Table 2).

The mean baseline TG level, as assessed by clinical chemistry analyzer, was 626.1 ± 215.2 mg/dl. Relative to baseline, FGF-21 reduced the mean plasma TGs by approximately 34%, 52%, and 69% at the doses of 30, 100, and 300 μg/kg, respectively. During the washout period, TGs trended upward in a time-dependent manner and reached preadministration levels at the end of the washout phase (Fig. 3A).

In a similar manner, FGF-21 significantly reduced total cholesterol and LDL-c by approximately 17%, 25%, 35%, and

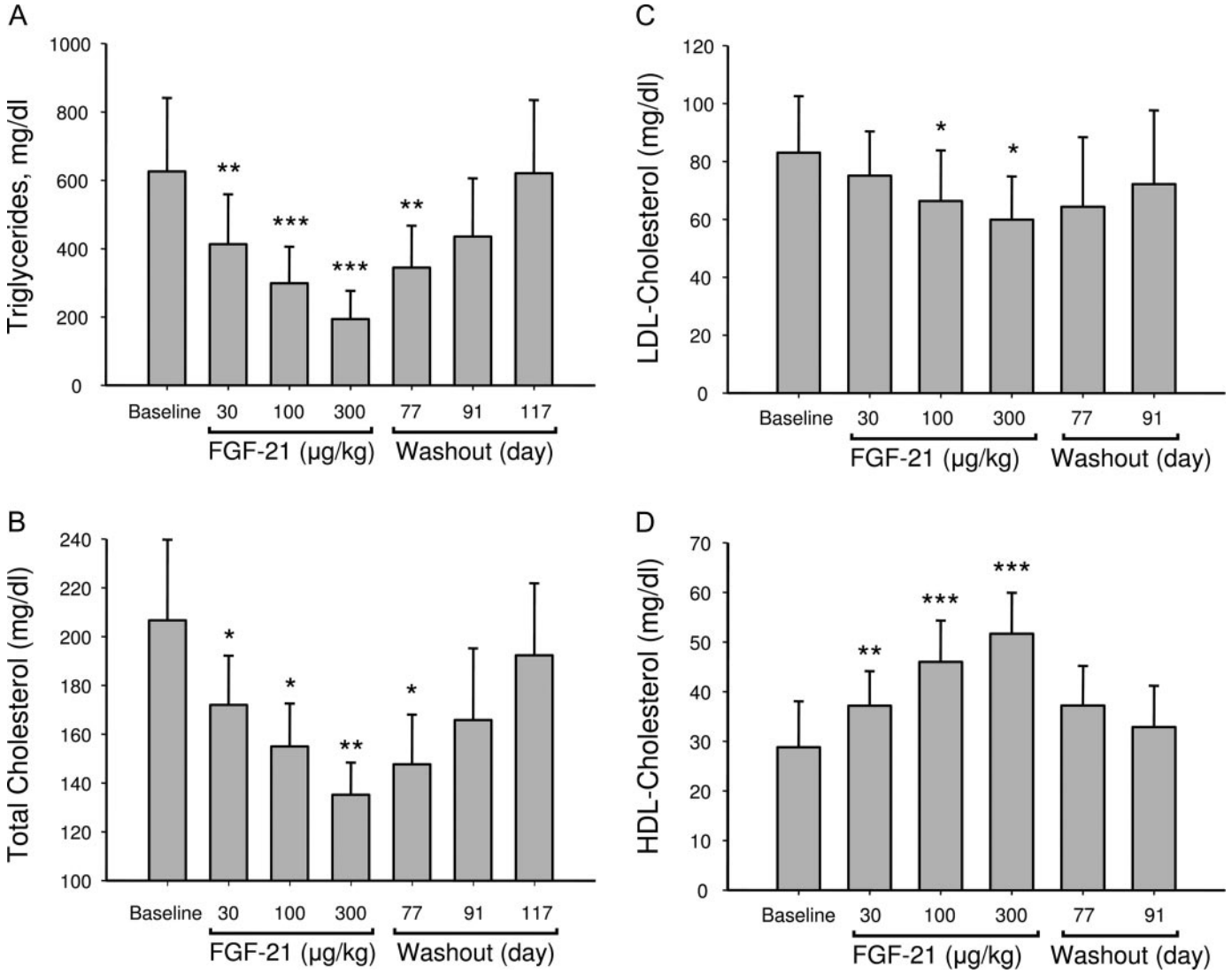

FIG. 3. FGF-21 effects on lipids. The values (±SE) shown are the average of the measurements in all six animals taken on days during each phase of the study according to *Materials and Methods*. *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$, compared with baseline. Fasted triglycerides (A), total cholesterol (B), LDL-c (C), and HDL-c (D) levels in diabetic rhesus monkeys at the different phases during the study.

10%, 21%, and 28%, respectively, at 30-, 100-, and 300-μg/kg doses of FGF-21 (Fig. 3, B and C). In contrast, HDL-c was elevated by approximately 29%, 60%, and 79% in response to FGF-21 (Fig. 3D). During the washout period, total cholesterol and LDL-c trended upward, and HDL-c trended downward (Fig. 4, B–D).

TABLE 2. Administration of FGF-21 affects lipoprotein profiles in diabetic rhesus monkeys

	d 0	d 64	*P* value
VLDL total (nmol/liter)	196 ± 164	27 ± 36	0.046
Large VLDL (nmol/liter)	25 ± 18	5 ± 6	0.021
Medium VLDL (nmol/liter)	59 ± 45	10 ± 13	0.033
Small VLDL (nmol/liter)	111 ± 109	13 ± 20	0.076
LDL total (nmol/liter)	2888 ± 944	1346 ± 728	0.024
Small LDL (nmol/liter)	2685 ± 973	1138 ± 863	0.046
Medium small LDL (nmol/liter)	522 ± 234	215 ± 167	0.008
Large HDL (μmol/liter)	6 ± 5	12 ± 2	0.102
VLDL-TGs (mg/dl)	371 ± 279	64 ± 51	0.03

The values (±SE) shown are the average of the measurements in all six animals on the blood samples collected at d 0 and 64. *P* values represent statistical significance as compared to d 0.

NMR spectroscopy was used to assess various lipid parameters on samples from the baseline and the 300-μg/kg dose phase. No statistically significant change was observed in the size of VLDL, LDL, and HDL particles indicating that the FGF-21-induced differences in lipoprotein levels may be due to alterations in particle numbers. Indeed, relative to baseline, the 300-μg/kg dose of FGF-21 resulted in 52% and 86% reduction in concentrations of total LDL and VLDL, respectively (Table 2). More specifically, the change in LDL was mainly accounted for by lowering of small LDL particles. As for VLDL, the changes were due to a significant decrease in numbers of large and medium VLDL particles (Table 2). Although the concentration of small VLDL was decreased as well (89%), it did not reach statistical significance. A trend for elevation of large HDL was also observed (Table 2).

Consistent with clinical chemistry plasma triglyceride re-

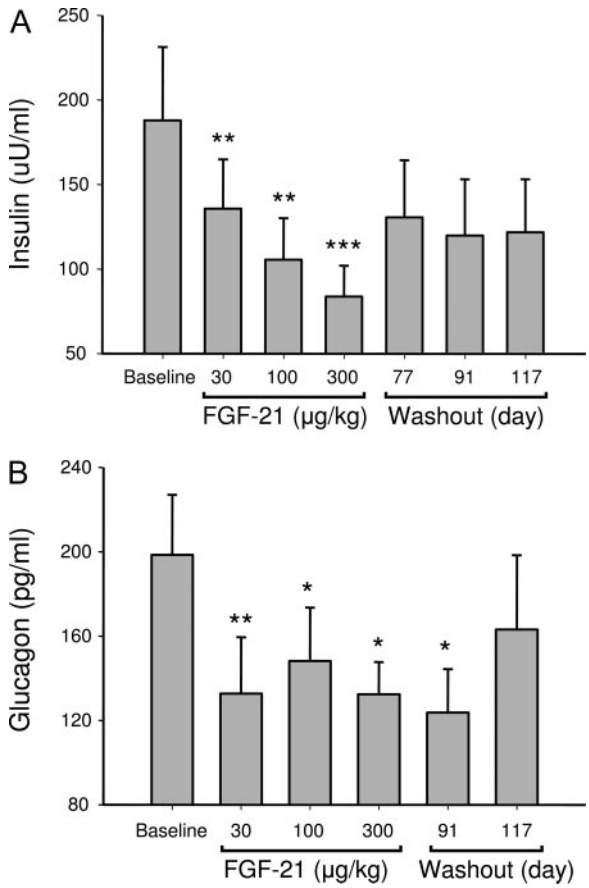

FIG. 4. FGF-21 administration lowers insulin and glucagon. The values (±SE) shown are the average of the measurements in all six animals taken on days during each phase of the study according to *Materials and Methods*. *, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.001$ compared with baseline. Fasted insulin (A) and glucagon (B) levels in diabetic rhesus monkeys at the different phases during the study.

sults, VLDL-TG content was significantly and dramatically reduced relative to baseline level on 6 wk of FGF-21 administration (Table 2).

Plasma circulating factors. Either forced FGF-21 overexpression in transgenic mice or systemic administration of the protein to diabetic rodents affected circulating levels of insulin and glucagon (9). At baseline, the mean fasting insulin level was 188 ± 43.4 μU/ml reflecting the hyperinsulinemic condition in these animals (14). The administration of FGF-21, however, led to a statistically significant and dramatic reduction in plasma insulin levels during all dose phases (Fig. 4A). Although during the washout period, insulin was on average 34% lower than preadministration baseline, these differences did not reach statistical significance.

In contrast to insulin, fasting plasma glucagon levels were more variable over the course of the study (Fig. 4B). At the 30-μg/kg dose, glucagon was significantly lower than baseline (~33%) but then climbed slightly during dosing at the 100 μg/kg. At the 300-μg/kg dose, the concentration of glucagon in circulation dropped down to approximately 33% compared with mean baseline. During the washout phase, glucagon levels trended upward in a time-dependent manner.

To monitor for additional FGF-21 effects, we measured the expression levels of 78 other polypeptides in the circulation at d 0 and at the end of the 300-μg/kg dose phase (d 64) using human Multi-Analyte Profiles (MAP) kit (Rules-Based Medicine, Inc.). In addition to insulin and glucagon, we observed FGF-21-induced changes in the levels of several other circulating factors (Table 3).

Body weights and food consumption. The administration of FGF-21 led to a slight but statistically significant weight loss during each dosing phase compared with preadministration. On average, the 30-μg/kg dose resulted in approximately 1% reduction, the 100-μg/kg dose led to 2.5% lowering, and the 300-μg/kg dose yielded in 4% percent body weight reduction from baseline. During the washout period, all animals began to gain weight, and the average body weight level trended upward over time (Table 4).

Food consumption was assessed daily and converted into an estimated weekly caloric intake. Although the estimated caloric intake was variable over the course of the study, there was a trend for its reduction that did not reach statistical significance at any time point during the study relative to mean baseline measurements (data not shown). During the washout phase, the estimated caloric intake returned to the baseline level.

Discussion

Protein therapeutics have played a prominent role in the treatment of diabetes mellitus since the discovery of insulin in 1921 by Banting and Best. This legacy has been refined and extended over the years as exemplified by the introduction of human recombinant insulin, Humulin, the first engineered human insulin, insulin lispro (Humalog), and the recent introduction of the glucagon-like peptide-1 receptor agonist, exenatide IV (Byetta). Several other secreted proteins have also demonstrated favorable metabolic effects in animal models of diabetes including adiponectin (19–21), FGF-19 (7, 8), and most recently FGF-21 (9, 10).

The bioactivity of FGF-21 was first identified in a mouse

TABLE 3. Administration of FGF-21 affects the levels of circulating polypeptides in diabetic rhesus monkeys

	d 0	d 64	*P* value
Adiponectin (μg/ml)	2.7 ± 0.4	4.6 ± 0.3	0.034
apoC-III (μg/ml)	39.8 ± 9.1	20.1 ± 5.8	0.026
IL-8 (pg/ml)	2159 ± 608	632 ± 204	0.016
Factor VII (ng/ml)	36.85 ± 4.34	23.62 ± 2.42	0.015
RANTES (ng/ml)	17.5 ± 1.4	13.1 ± 1.0	0.048
SAP (μg/ml)	2.05 ± 0.18	1.04 ± 0.15	0.003
TIMP-1 (ng/ml)	39.0 ± 3.8	23.1 ± 1.7	0.049
vWF (μg/ml)	57.27 ± 5.65	41.72 ± 3.12	0.021
apoA-I (mg/ml)	0.22 ± 0.04	0.27 ± 0.02	0.085
CRP (μg/ml)	14.6 ± 7.9	1.9 ± 0.4	0.069
PAI-1 (ng/ml)	17.5 ± 1.4	13.1 ± 1.0	0.077

The values (±SE) shown are the average of the measurements in all six animals on the blood samples collected at d 0 and 64. *P* values represent statistical significance as compared to d 0. apo, Apolipoprotein; RANTES, regulated upon activation, normal T-cell expressed, and presumably secreted; SAP, serum amyloid P-component; TIMP-1, tissue inhibitor of metalloproteinase 1; vWF, von Willebrand factor; CRP, C-reactive protein; PAI-1, plasminogen activator inhibitor type 1.

TABLE 4. FGF-21 induces weight loss in diabetic rhesus monkeys

	Mean (kg)	*P* value
Baseline	19.0 ± 1.4	NA
30-μg/kg dose	18.7 ± 1.4	0.011
100-μg/kg dose	18.5 ± 1.5	0.001
300-μg/kg dose	18.2 ± 1.5	0.000
d 77	18.3 ± 1.5	0.000
d 91	18.4 ± 1.4	0.011
d 117	18.7 ± 1.4	0.211

The values (±SE) shown are the average of the measurements in all six animals taken on days during each phase of the study according to *Materials and Method*s. *P* values represent statistical significance as compared to the baseline level. NA, Not available.

3T3-L1 adipocyte glucose uptake assay. Subsequently, FGF-21 has shown its potential therapeutic use in ameliorating hyperglycemia in a range of diabetic rodent models. In addition, transgenic mice overexpressing FGF-21 were found to be resistant to diet-induced metabolic abnormalities, including decreased body weight and fat mass (9). The present investigation extends these initial findings in rodents in a clinically validated model, the diabetic rhesus monkey (14–18).

Type 2 diabetes in rhesus monkeys presents with the same characteristics as those found in humans. The animals used in the study had elevated (significantly above normal) levels of fasting glucose, triglycerides, total cholesterol, LDL-c, and reduced HDL-c at baseline (Figs. 2A and 3, A–D) (14). Similar to the previous studies in rodents, FGF-21 was efficacious in correcting hyperglycemia and dyslipidemia, and the effects of FGF-21 administration appear to be longlasting because the data presented represent samples taken 24 h after the last injection of the protein. Of importance, when blood samples were assessed within 1 h after FGF-21 administration on d 29, 43, and 57, there was no indication of hypoglycemia at any dose of FGF-21 (data not shown) consistent with a similar observation that has been previously made in normal and diabetic rodents (9) and strongly suggesting no risk of hypoglycemia in humans.

Further support for a durable impact of FGF-21 on glucose levels was provided by assessing a more integrated measure of ambient plasma sugar, fructosamine. Although fructosamine levels have not been evaluated in age-matched normal rhesus monkeys, it is clear that FGF-21 administration led to a statistically significant reduction of plasma fructosamine in the diabetic animals used in the study (Fig. 2B). Moreover, fructosamine levels remained significantly below preadministration levels even 2 wk after the end of the administration phase.

The dyslipidemia observed at baseline in the diabetic rhesus monkeys is consistent with lipid disorders in diabetic humans and is considered to be a preeminent cardiovascular risk factor associated with diabetes in humans (18, 22). Several observations have linked the relationship between elevated plasma triglycerides and cholesterol, low levels of HDL-c, and increased cardiovascular risk in diabetic patients (22). At the 300-μg/kg dose, FGF-21 lowered fasting plasma TGs and total cholesterol by approximately 70% and 50%, respectively, while simultaneously raising HDL-c by nearly 80% (Fig. 3, A, B, and D). Elevated levels of LDL-c is another well-established risk factor for cardiovascular disease, and FGF-21 appeared to lower them in a statistically significant manner (Fig. 3C). Through NMR spectroscopy, the ability of FGF-21 to reduce LDL-c was accounted for by a significant reduction in the highly atherogenic (23, 24) small, dense LDL particles (Table 2).

Although in magnitude the observed FGF-21 effects on triglycerides, total cholesterol, and LDL-c levels may on average be considered comparable to what has been clinically documented for lipid-lowering therapies such as the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) (25, 26), 80% elevation of "good" HDL-c is remarkable. Most notably, this effect was achieved over a relatively short period of FGF-21 administration for 6 wk. Overall, data from this study indicate that systemic administration of FGF-21 substantially and beneficially impacts multiple aspects of lipid metabolism in diabetic monkeys, thus potentially minimizing the risks of the vascular dysfunction.

In addition to providing tight glucose control without the need for continuing glucose monitoring and improving lipid profiles in diabetic monkeys, FGF-21 administration also significantly altered the levels of several circulating polypeptide factors in the plasma of dosed animals. In line with our earlier observations in rodents (9), insulin and glucagon were profoundly lowered throughout the administration phase (Fig. 4, A and B). The observed decrease in apoC-III was consistent with lowering of total TGs and VLDL-TGs (Fig. 3A and Table 2) (24). In contrast, plasma apoA-1 trended higher (Table 2), likely reflective of elevation in HDL-c (Fig. 3D). The levels of several proinflammatory cytokines and cardiovascular markers (27), regulated on activation, normal T-cell expressed, and presumably secreted, factor VII, IL-8, serum amyloid P-component, tissue inhibitor of metalloproteinase 1, von Willebrand factor, and plasminogen activator inhibitor type 1 were also reduced after 6 wk of FGF-21 treatment (Table 3). Interestingly, another important cardiovascular factor, C-reactive protein, was lowered approximately 10-fold relative the beginning of the study (Table 3). However, despite the amplitude, this effect did not reach statistical significance for the group, which was the result of the high variation in C-reactive protein levels at d 0 of the study.

Treatment of diabetic monkeys with FGF-21 led to a small but statistically significant weight loss (Table 4). The importance of this observation is further supported by the fact that weight reduction was to varying degrees observed in each and every animal (data not shown). Although we have previously reported that FGF-21 transgenic mice were protected from weight gain and fat accumulation (9), no effect of FGF-21 administration on body weight had been demonstrated in rodents.

The difference between our previous results in rodents and the current findings in monkeys on the ability FGF-21 to regulate animal weights can be related to several factors. Most of the earlier studies in mice examined dosing over 7-d period or less. It is possible that the longer duration of FGF-21 delivery or higher doses administered in this study may be important in eliciting the weight loss in primates. Alternatively or even in concert, human FGF-21 could be more biologically active in the primate resulting in a more pronounced pharmacological response than observed in ro-

dents. Finally, our previous work in rodents was carried out in genetically compromised leptin-deficient models of type 2 diabetes, which have different disease pathology than the animals used in this study with naturally occurring type 2 diabetes. The relevance of this distinction in disease etiology remains to be tested in human diabetics.

Although the beneficial effects of FGF-21 in the diabetic rhesus monkeys are unmistakable, the mechanism through which this naturally occurring protein exerts all these positive effects remains unclear. It is possible that an observed minor weight reduction could be a potential contributor. However, given the magnitude of the body weight-lowering effect in monkeys, and the absence of an appreciable impact on body weight in rodents, although robust glucose and lipid-lowering was demonstrated in both species (9, 10), it is unlikely that weight loss could be a predominant mechanism for the observed metabolic changes in monkeys. Moreover, given the fact of multiple changes in the animals' metabolic state (sustained glucose control, amelioration of dyslipidemia, improvements in lipoprotein and cardiovascular risk factor profiles, body weight reduction), it is conceivable that FGF-21 may be acting in a pleiotropic manner using diverse downstream mechanisms in different targeted tissues/organs that in turn determine the particular functional outcome.

Changes in fasting insulin levels on FGF-21 treatment (Fig. 4A) suggest enhancements in insulin sensitivity. FGF-21 also lowered glucagon, a contributor to the pathophysiology of diabetes (28). Lowering of both insulin and glucagon is consistent with our previous results in rodents (9). Adiponectin, associated with glucose-lowering, insulin-sensitizing, and anti-atherosclerotic actions (19–21), is another candidate to mediate FGF-21 activity as it was up-regulated on FGF-21 treatment (Table 2). Alternatively, the FGF-21-induced elevation in adiponectin may reflect improvements in glycemic and lipid states because this adipokine is also considered as a reliable biomarker for various metabolic dysfunctions (29). The same is true for alterations in the levels of other circulating polypeptides (Table 3) that may be secondary to changes in glucose and lipid profiles. Finally, the FGF-21-induced body weight lowering in diabetic monkeys could be partially mediated through trends of caloric intake modulation (data not shown), but further studies are ultimately needed to better understand the nature of this effect.

Whereas the pharmacological effects of FGF-21 on hyperglycemia and other metabolic parameters in this model are clear, the relationship between pharmacokinetic and pharmacodynamic activity is not. Due to the nature of the study design, it is not certain whether the progressive improvement in metabolic indices is dose dependent and/or related to the duration of FGF-21 administration. Studies in mice and primates indicate that the $T_{1/2}$ of FGF-21 was relatively short, leading to the hypothesis that FGF-21 may trigger a variety of cellular responses thus enabling pharmacodynamic action to extend long beyond the presence of the compound in circulation. Plasma exposure analysis in this study was complicated by the formation of antibodies to FGF-21 in dosed animals. Analysis of samples for anti-FGF-21 antibodies suggested that the immune response to FGF-21 occurred sometime after d 35 of the study (during the 100-μg/kg dosing period). Titers were present in all animals, although end point titers were low (generally $<$1:1000). The plasma exposure after 30 μg/kg dose was as expected based on cynomolgus monkey kinetics [maximal plasma concentration (maximal plasma concentration) of 3.7 ng/ml]; however, the remaining exposure data were compromised. The anti-FGF-21 response most likely interferes in the bioanalytic assessment of FGF-21 concentrations and could have acted as a carrier for the protein, confounding interpretation of the pharmacokinetic-pharmacodynamic relationship. Although we cannot clearly distinguish between these effects, it appears that the biological activity of FGF-21 was not neutralized by the presence of anti-FGF-21 antibodies, allowing interpretation of the pharmacological effect of this molecule on the studied biological end points.

Although current antidiabetic therapies generally provide a degree of glycemic improvement in patients, they rarely modify additional features of the disease (2). Of the marketed compounds, the PPARγ activators, or thiazolidinediones, are probably the most attractive with respect to their impact on multiple parameters associated with diabetes. However, this class of drugs possesses a variety of unwanted side effects such as weight gain and edema, which limits their therapeutic utility (30). In the present study, we demonstrated that FGF-21 provided sustainable glucose control without incidence of hypoglycemia and a substantial improvement in lipid abnormalities and several cardiovascular risk factors/biomarkers. In addition, FGF-21 stimulated mild weight loss with no sign of fluid retention or any other adverse clinical observations.

Because type 2 diabetes continues to be a growing global health concern, the need for more effective, safer therapies is increasingly important. Whereas the beneficial effects of FGF-21 have previously been demonstrated in rodent models, the present investigation extends these finding to a clinically relevant disease model in a higher species. Future studies in man will be required to fully explore therapeutic potential of FGF-21.

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