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Article

The Effects of LY2405319, an FGF21 Analog, in Obese Human Subjects with Type 2 Diabetes

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SUMMARY

Fibroblast growth factor 21 (FGF21) is a recently discovered metabolic regulator. Exogenous FGF21 produces beneficial metabolic effects in animal models; however, the translation of these observations to humans has not been tested. Here, we studied the effects of LY2405319 (LY), a variant of FGF21, in a randomized, placebo-controlled, double-blind proof-of-concept trial in patients with obesity and type 2 diabetes. Patients received placebo or 3, 10, or 20 mg of LY daily for 28 days. LY treatment produced significant improvements in dyslipidemia, including decreases in low-density lipoprotein cholesterol and triglycerides and increases in high-density lipoprotein cholesterol and a shift to a potentially less atherogenic apolipoprotein concentration profile. Favorable effects on body weight, fasting insulin, and adiponectin were also detected. However, only a trend toward glucose lowering was observed. These results indicate that FGF21 is bioactive in humans and suggest that FGF21-based therapies may be effective for the treatment of selected metabolic disorders.

INTRODUCTION

Recent projections indicate that more than 550 million people will be afflicted with diabetes by 2030 (Whiting et al., 2011). Most patients with diabetes have other features of the metabolic syndrome—defined by the presence of three or more of the following signs and symptoms: glucose intolerance, abdominal obesity, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C) levels, and hypertension (Moller and Kaufman, 2005). A limitation of currently available drug classes for the treatment of risk factors associated with the metabolic syndrome is that no single agent (e.g., statin, fibrate, or metformin) is able to address more than one comorbid condition. Thus, many individual therapies are usually prescribed in combination, leading to tolerability issues, poor patient compliance, and suboptimal outcomes, all of which provide an incentive for continuing the search for new therapeutic approaches.

Fibroblast growth factor 21 (FGF21) is a recently identified circulating protein that regulates insulin sensitivity along with lipid and energy metabolism. We initially discovered FGF21 in a glucose uptake screen in 3T3-L1 adipocytes (Kharitonenkov et al., 2005) and subsequently demonstrated that the administration of FGF21 to obese diabetic rodents markedly improved hyperglycemia, lowered elevated triglycerides (TGs), and reduced body weight (Coskun et al., 2008; Kharitonenkov et al., 2005; Wente et al., 2006). Importantly, the administration of exogenous FGF21 to obese diabetic rhesus monkeys promoted improvements in glucose, body weight, and circulating lipids (Kharitonenkov et al., 2007). The metabolic effects of native FGF21 or its derivatives and mimetics have also been replicated by other investigators in rodents and nonhuman primates (Foltz et al., 2012; Hecht et al., 2012; Mu et al., 2012; Smith et al., 2013; Véniant et al., 2012; Xu et al., 2009). As shown in rodents, the mechanisms underlying the pleiotropic actions of FGF21 include enhanced total body insulin sensitivity (Berglund et al., 2009) and improved pancreatic islet β cell function (Wente et al., 2006) along with the suppression of islet glucagon secretion (Kharitonenkov et al., 2005), reduced hepatic lipogenesis, and the induction of energy expenditure via brown fat activation (Coskun et al., 2008; Fisher et al., 2012; Kharitonenkov et al., 2005). At a molecular level, FGF21 interacts with the FGF receptor 1c only in tissues expressing the cofactor Klotho β (Adams et al., 2012a). Although most of the effects of FGF21 were discovered empirically in the previously described laboratory experiments, the Klotho β-dependent tissue specificity is consistent with the predominant effects of FGF21 occurring in liver and adipose tissue.

There are important potential differences between the physiologic role(s) of FGF21 and the pharmacologic effects of exogenous FGF21 (Kliewer and Mangelsdorf, 2010; Angelin et al., 2012). However, some aspects of FGF21 pharmacology appear related to its potential physiology. For example, the phenotype of increased adipose mass in FGF21 knockout mice is generally consistent with the reported effects of FGF21 overexpression in transgenic mice or dosing at pharmacological levels (Adams et al., 2012b; Badman et al., 2009; Hotta et al., 2009; Kharitonenkov et al., 2005). In addition, treatment of mice with low doses of FGF21, resulting in plasma concentrations only 30% to 50% above endogenous levels, results in effects that are similar to (albeit attenuated) higher doses. Thus, the effect of increasing adiponectin levels, a marker of FGF21 action on adipose tissue, occurs with a clear dose response ranging from pronounced with higher pharmacological doses to more modest at doses that achieve levels found in the physiological range (Holland et al., 2013). This relationship is also consistent with data

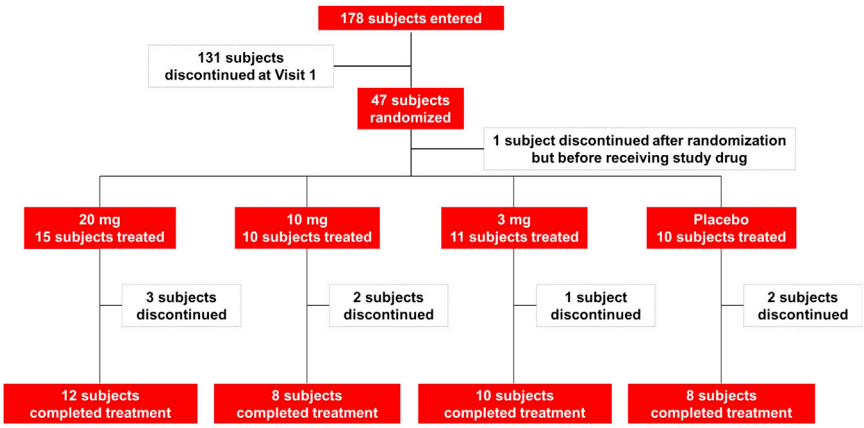

Figure 1. Trial Profile
Numbers of subjects initially screened and subsequently randomized to each of four treatment groups are depicted. Subject disposition (number of subjects who discontinued treatment or completed the study) are also shown.

demonstrating reduced adiponectin levels in chow-fed FGF21 knockout mice (Holland et al., 2013; Lin et al., 2013).

The beneficial pharmacology observed in preclinical models indicated that FGF21 and its analogs or mimetics hold promise as innovative therapeutics and may represent a future class of "glucose plus" medicines for treating metabolic disorders (Moller, 2012). Toward that end, drug discovery efforts yielded LY2405319 (LY), an investigational FGF21 variant suitable for early-phase clinical development. LY is indistinguishable from native human FGF21 in cell-based, rodent, and nonhuman primate assays with respect to potency, selectivity, and multiple measures of biological activity and efficacy (Adams et al., 2013a; Kharitonenkov et al., 2013).

The goals of early clinical studies were to characterize LY for safety and pharmacokinetics in healthy volunteers and to assess its potential effects for ameliorating abnormalities commonly associated with type 2 diabetes (T2DM). Initial clinical experience with LY included a single ascending dose study in 19 healthy men who received doses ranging from 0.6 to 30 mg and a multiple ascending dose study in 32 men and women who received doses of LY ranging from 1 to 20 mg daily for 7 days. There was a statistically significant dose-dependent increase in HDL-C and a decrease in TGs in the 7-day study (data not shown). There were no serious adverse events or clinically meaningful safety concerns in either study. These investigations provided safety, tolerability, pharmacokinetic, and limited pharmacodynamic data to support a 28-day phase 1b proof-of-concept trial in obese subjects with T2DM-exploring doses of LY up to 20 mg per day. The translational medicine study described here reports pharmacodynamic metabolic effects of an FGF21-derived molecule in humans.

RESULTS

Patient Characteristics

Of the 178 subjects who signed informed consent and underwent screening procedures, 47 met the inclusion criteria and were randomly assigned to treatment, 46 received at least one dose of the study drug, and 38 completed the study (Figure 1). Clinical characteristics of the 46 individuals receiving the study drug are shown in Table 1. Patients had been diagnosed with T2DM for an average of 7.4 years prior to enrollment, 43 of the 46 were receiving metformin, and 11 were treated with a statin. Baseline characteristics were similar across treatment groups, the exception being fasting glucose, which was included as a covariate variable in the statistical analysis.

LY2405319 Plasma Exposure

LY plasma exposure was measured at several time points on day 28 of the study, 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, and 48 hr after dose administration. Average steady-state circulating plasma concentrations of LY on day 28 were 17.5 ± 4 ng/ml in subjects who received the 3 mg daily dose, and levels were 67.3 ± 25 ng/ml and 150 ± 49 ng/ml in subjects treated with 10 and 20 mg, respectively.

Effects on Plasma Lipids and Lipoproteins

Table 2 provides a summary of metabolic and glycemic parameters for each of the four treatment groups at baseline and after 28 days of daily subcutaneous injections of LY or placebo. Final low-density lipoprotein cholesterol (LDL-C) concentrations were unchanged in the 3 mg treatment group, whereas reductions of 29.5% and 20.2% were observed for the 10 and 20 mg groups, respectively (Table 2). Significant reductions in LDL-C occurred as early as day 7, the maximal lowering effect of LY occurring between study days 14 and 21 (Figure 2). Relative to baseline, significant mean decreases in fasting TG levels were observed as early as day 2 for all three dosing groups, and maximal TG lowering was observed between days 2 and 7 (Figure 2). These reductions were maintained for the duration of the treatment period and were significantly different from baseline and placebo at the 10 and 20 mg dose levels (Table 2; Figure 2). In comparison to baseline and placebo values, total cholesterol concentrations were also lowered in patients treated with 10 (−19.2%) or 20 mg (−15.4%) of LY (Table 2). Additionally, an increase of 15% to 20% in HDL-C occurred across all three dose groups (Table 2). These improvements in dyslipidemia were accompanied by a shift to a less atherogenic apolipoprotein (apo) profile. In comparison to baseline and placebo, both 10 and 20 mg dose levels of LY reduced apoC-III by approximately 35%, whereas apoB was reduced by 25.1% and 21.6%, respectively (Table 2). ApoAII was also decreased (−18.3%) from baseline and placebo values in the 20 mg treatment group (Table 2).

Effects on Body Weight, Glucose, and Other Parameters

Reductions in mean body weight relative to baseline were significant for the 10 (−1.75 kg) and 20 mg (−1.49 kg) dose groups, the maximal effect being observed at the 10 mg dose level (Table 2). Notably, these changes were smaller in comparison to placebo and did not reach statistical significance for either dose group (10 mg [p = 0.1011] and 20 mg [p = 0.1211]). Regardless of dose level, the least squares mean difference in the change

Table 1. Subject Baseline Demographics

Treatment Group	Placebo	3 mg	10 mg	20 mg	Overall
Subjects Randomized	10	11	10	15	46
Female	6	4	3	7	20
Male	4	7	7	8	26
Origin - White	10	11	10	13	44
Origin - African American	0	0	0	1	1
Origin - Asian	0	0	0	1	1
Metformin use	10	11	9	13	43
Duration T2DM (years) (SD)	7.7 (5.9)	9.8 (6.8)	5.0 (2.7)	7.2 (4.6)	7.4 (5.3)
Age (years) (SD)	58.6 (6.3)	56.5 (7.3)	59.6 (9.2)	56.9 (10.2)	57.7 (8.4)
BMI (kg/m^2) (SD)	32.7 (3.5)	31.3 (2.8)	33.4 (3.2)	31.5 (4.4)	32.1 (3.6)
Baseline FG (mg/dl)[a] (SD)	161.5 (15.9)	179.2 (36.5)	152.8 (30.6)	186.1 (32.4)	171.9 (32.3)
Screening HbA1c (%) (SD)	7.55 (0.60)	8.30 (1.08)	7.80 (0.89)	8.10 (0.79)	7.96 (0.87)

The table summarizes clinical characteristics of study subjects at baseline (randomization) by treatment group.
[a]Significant difference ($p < 0.05$) in baseline fasting glucose among the four treatment arms.

from baseline of fasting glucose was not statistically different versus baseline or placebo over the 28-day treatment period (Table 2). A glucose-lowering trend ($p = 0.12$) for a dose response across was observed across LY dose groups (Figure 3). Fasting insulin levels were significantly reduced in the 20 mg dose group in comparison to baseline values (Table 2).

Plasma concentrations of adiponectin increased in comparison to baseline over the 28-day treatment period for all three LY dose levels, and the mean change in the 20 mg dose group was significant in comparison to placebo on day 28 (Table 2; Figure 3). Adiponectin circulates in one of three forms: as a trimer, in a low-molecular-weight complex, or in a high-molecular-weight complex. LY treatment for 28 days at the 20 mg dose level was sufficient to substantially change the adiponectin complex distribution. The proportion of high-molecular-weight adiponectin, a potential contributor to the efficacy of thiazlodinedione insulin sensitizers (Pajvani et al., 2004), increased from 25.0% ± 3.3% at baseline to 39.6% ± 3.9% ($p < 0.02$). A corresponding decrease was observed for the circulating adiponectin trimer (45.2% ± 4.3% to 29.0% ± 2.7% of the protein's population [$p < 0.02$]), whereas an abundance of low-molecular-weight adiponectin remained unchanged. Significant effects ($p < 0.03$) in complex formation were also detectable after 14 days of LY treatment, albeit with slightly less magnitude.

Finally, concentrations of β-hydroxybutyrate were also compared to baseline and placebo and were shown to be elevated in all three LY dose groups on day 28 (Table 2).

Adverse Events

There were three serious adverse events during this study. On study day 27, one subject in the 20 mg dose group had a severe reaction, including a drop in blood pressure, urticaria, and pruritis, requiring treatment with intravenous antihistamines and corticosteroids. This subject had a drug antibody titer of 1:320 at day 28 and a higher peak titer of 1:1,280 by day 35. The other two serious adverse events (cholecystitis [day 26] and optic neuropathy [day 38 after the last dose of study drug]) were assessed by the investigator as related to pre-existing diseases in the patients.

Three subjects discontinued because of adverse events that were considered related to treatment: one individual with hypersensitivity, one with elevated liver enzymes, and a third with injection site reactions, headache, and urticaria that had no detected antibodies to LY. The majority (94.5%) of the remaining treatment-emergent adverse events were mild in severity. Injection-site-related adverse events were the most frequently reported across all doses but were more prevalent at the 10 and 20 mg dose levels.

Drug antibodies were measured in each patient and were observed in 20% of placebo-treated patients and in 55%, 80%, and 87% of patients treated with 3, 10, and 20 mg, respectively. Titers of $\geq$1:32 occurred in no placebo-treated patients and 9%, 60%, and 47% of patients in the 3, 10, and 20 mg groups, respectively. Drug antibodies were present in a greater proportion of patients with injection site reactions but did not affect pharmacokinetics or pharmacodynamics of LY.

DISCUSSION

This study reports the pharmacology and efficacy of an FGF21-derived compound administered to human subjects—in this case, obese patients with T2DM. At the end of the 28-day treatment period, there were statistically and clinically meaningful effects on lipids and body weight in comparison to baseline. A dose-dependent trend in the lowering of fasting glucose was also observed. Although LY is indistinguishable from native FGF21 in both in vitro and in vivo preclinical laboratory testing (Adams et al., 2013a; Kharitonenkov et al., 2013), it is important to emphasize that the effects described in the current report were pharmacologic and may not reflect the underlying physiologic role(s) of FGF21 in humans (Angelin et al., 2012). The mean concentrations of LY observed in this study increased in a dose-proportionate manner and ranged from 10- to over 100-fold greater than previously reported for endogenous FGF21 in diet-induced obese and leptin-deficient (*ob/ob*) mice (Hale et al., 2012) or in humans, including obese patients with T2DM (Chavez et al., 2009; Angelin et al., 2012). Importantly, plasma LY levels were also within the range of efficacious exposures of LY (or human recombinant FGF21) achieved in animal models (Coskun et al., 2008; Kharitonenkov et al., 2005; Wente et al., 2006; Kharitonenkov et al., 2013).

The impact of LY treatment on all four lipid parameters (total cholesterol, LDL, HDL, and TGs) was rapid, occurring as early as 2 days after the initiation of dosing in the case of fasting TGs, and appeared to reach a maximum effect within 7 to 21 days. The top end of the explored dose range appeared to achieve maximum efficacy for lipids, the 10 mg dose providing effects indistinguishable from those at the 20 mg dose. Prominent reductions in apoCIII may have contributed to TG lowering, given the known role of apoCIII in the regulation of TG clearance

Table 2. Summary of Change in Metabolic and Glycemic Parameters

	Placebo	3 mg	10 mg	20 mg
LDL-C (mmol/l)				
Baseline	2.78	2.72	2.35	3.11
Week 4	2.76	2.71	1.66	2.48
LS mean change from baseline to week 4 (%, SE)	−0.751% (6.9) n = 7	−0.099% (6.0) n = 10	−29.5% (7.8)[a,b] n = 8	−20.2% (4.56)[a,b] n = 13
HDL-C (mmol/l)				
Baseline	1.02	1.16	1.12	1.13
Week 4	1.00	1.34	1.29	1.35
LS mean change from baseline to week 4 (%, SE)	−2.29% (4.4) n = 8	15.6% (3.4)[a,b] n = 10	15.2% (3.9)[a,b] n = 8	19.5% (3.07)[a,b] n = 13
Apolipoprotein A2 (g/l)				
Baseline	0.347	0.378	0.352	0.351
Week 4	0.326	0.374	0.314	0.286
LS mean change from baseline to week 4 (%, SE)	−6.02% (3.4) n = 8	−1.09% (2.9) n = 9	−10.7% (3.3)[b] n = 8	−18.3% (2.60)[a,b] n = 13
Apolipoprotein B (g/l)				
Baseline	1.01	0.855	0.832	1.00
Week 4	1.07	0.873	0.623	0.784
LS mean change from baseline to week 4 (%, SE)	5.76% (5.0) n = 8	2.12% (5.3) n = 10	−25.1% (6.5)[a,b] n = 7	−21.6% (3.99)[a,b] n = 13
Apolipoprotein C-III (g/l)				
Baseline	0.180	0.123	0.130	0.142
Week 4	0.176	0.104	0.086	0.091
LS mean change from baseline to week 4 (%, SE)	−2.30% (4.5) n = 7	−15.2% (5.2)[b] n = 9	−34.0% (5.1)[a,b] n = 8	−35.4% (3.63)[a,b] n = 13
Triglycerides (mmol/l)				
Baseline	3.13	1.68	1.98	2.14
Week 4	3.20	1.25	1.07	1.18
LS mean change from baseline to week 4 (%, SE)	2.48% (6.1) n = 8	−25.9% (9.9)[b] n = 10	−46.2% (9.0)[a,b] n = 8	−44.6% (6.57)[a,b] n = 13
Total cholesterol (mmol/l)				
Baseline	5.14	4.72	4.40	5.28
Week 4	5.11	4.82	3.56	4.47
LS mean change from baseline to week 4 (%, SE)	−0.632% (3.6) n = 8	2.13% (3.5) n = 10	−19.2% (4.2)[a,b] n = 8	−15.4% (2.76)[a,b] n = 13
Glucose, Fasting (mmol/l)				
Baseline	8.97	9.95	8.49	10.3
Week 4	9.14	9.75	8.11	9.75
LS mean change from baseline to week 4 (mmol/l, SE)	0.176 (0.58) n = 8	−0.195 (0.52) n = 10	−0.372 (0.59) n = 8	−0.581 (0.463) n = 13
Insulin (pmol/l)				
Baseline	65.7	67.4	88.4	88.4
Week 4	62.8	58.6	55.1	52.3
LS mean change from baseline to week 4 (pmol/l, SE)	−2.85 (19.5) n = 8	−8.77 (17.4) n = 10	−33.2 (19.6) n = 8	−36.0 (15.3)[b] n = 13
Adiponectin (ng/ml)				
Baseline	4160	6230	5020	6830
Week 4	4610	8080	7930	12500
LS mean change from baseline to week 4 (ng/ml, SE)	458 (976) n = 8	1850 (869)[b] n = 10	2910 (972)[b] n = 8	5650 (784)[a,b] n = 12

(*Continued on next page*)

Table 2. *Continued*

	Placebo	3 mg	10 mg	20 mg
β-Hydroxybutyrate (mg/dl)				
Baseline	1.24	1.17	1.33	1.13
Week 4	0.868	2.08	2.18	2.32
LS mean change from baseline to week 4 (mg/dl, SE)	−0.372 (0.39) n = 8	0.906 (0.35)[a,b] n = 10	0.852 (0.39)[a,b] n = 8	1.19 (0.306)[a,b] n = 13
Weight (kg)				
Baseline	88.7	85.4	95.6	86.3
Week 4	88.5	84.7	93.8	84.8
LS mean change from baseline to week 4 (kg, SE)	−0.229 (0.62) n = 8	−0.679 (0.56) n = 10	−1.75 (0.65)[b] n = 8	−1.49 (0.492)[b] n = 13

The table summarizes mean (±SEM) values for several measured metabolic parameters by treatment group at baseline versus study endpoint (week 4). In addition, the percent change from baseline for each measured parameter is also noted. LS, least squares.
[a]$p < 0.05$ in comparison to placebo.
[b]$p < 0.05$ in comparison to baseline.

(Zheng et al., 2010). ApoB, a component of LDL particles, was also reduced as expected on the basis of the observed LDL-lowering effect. Notably, changes in lipid parameters were very similar to effects we have previously reported after the administration of FGF21 or LY to obese rhesus monkeys with dyslipidemia (Adams et al., 2013a; Kharitonenkov et al., 2007).

Body weight was reduced over the 28-day treatment period (Figure 3). As with the lipids, 10 mg seemed to be the maximum effective dose in this study. Given that body weight typically takes at least 6 months to reach a maximum reduction, whether due to pharmacology or lifestyle intervention, it is possible that the 10 and 20 mg doses would diverge with longer treatment. In animal models, FGF21 administration lowers body weight via increased metabolic rate and without suppressing food intake (Coskun et al., 2008). Given that neither caloric intake nor energy expenditure were measured in the current study, it is not yet possible to assess the mechanisms underlying weight loss in humans. However, significant increases in β-hydroxybutyrate are suggestive of LY-enhanced fatty acid oxidation, thus implying that reduced body weight may be related to an increase in total body energy expenditure. Future clinical studies with FGF21-based therapies should assess mechanisms that may underlie the weight loss effect; these could include appetite (visual analog scale), food intake, resting metabolic rate, body composition, and fat distribution.

The LY effects on glucose were not as robust as expected on the basis of studies in both rodent and nonhuman primate models of T2DM (Adams et al., 2013a; Kharitonenkov et al., 2005; Kharitonenkov et al., 2007). Although decreases from baseline in fasting glucose were larger for all doses of LY in comparison to placebo, none of these changes were statistically significant. The time course of glucose response differed from that of the lipids, showing no evidence of being different from placebo or dose-dependent until the 21- and 28-day assessments. Although baseline glucose was included as a covariate in the final statistical analysis, given the small sample size, baseline differences may have confounded the interpretation of the glucose data. Further studies that incorporate longer periods of treatment and alternative patient populations (e.g., with impaired fasting glucose or T2DM with varying degrees of hyperglycemia at baseline) may be needed in order to elucidate the true potential glycemic effects of exogenous FGF21 treatment.

Interestingly, a prominent reduction in mean fasting insulin levels was observed—this replicates the effects of FGF21 demonstrated earlier in rodents and nonhuman primates (Kharitonenkov et al., 2005; Kharitonenkov et al., 2007). As with lipids and body weight, fasting insulin showed a dose-dependent lowering at 28 days with a maximum effect observed with the 20 mg dose. This finding is consistent with a potential improvement in insulin sensitivity, although direct measures of insulin action were not evaluated in this study.

Plasma adiponectin levels were also increased in a dose-dependent manner, which was consistent with the ability of FGF21 to robustly induce adiponectin levels in animal models

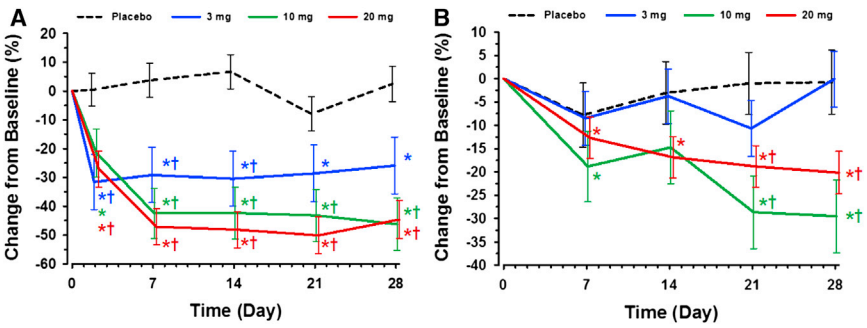

Figure 2. Effect of LY2405319 on Triglycerides and LDL-Cholesterol
Time course of mean (±SEM) change from baseline in fasting plasma TGs (A) and LDL-C (B). *$p < 0.05$ from baseline, †$p < 0.05$ from placebo.

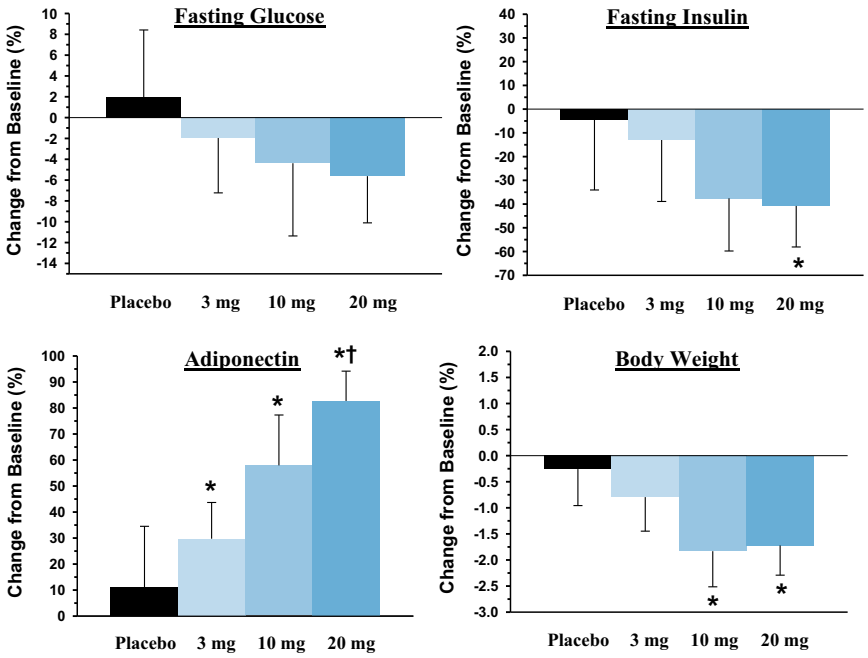

Figure 3. Effect of LY2405319 on Body Weight and Selected Metabolic Parameters

Mean change from baseline (±SEM) in fasting glucose, insulin, adiponectin, and body weight. *p < 0.05 from baseline, and †p < 0.05 from placebo.

(Adams et al., 2012a; Adams et al., 2012b; Adams et al., 2013b; Kharitonenkov et al., 2007). Recently, it has been demonstrated that adiponectin plays an important role in the propagation of the metabolic effects of FGF21 (Holland et al., 2013; Lin et al., 2013). Importantly, low adiponectin levels in humans are correlated with obesity and insulin resistance (Rasmussen-Torvik et al., 2012), and treatment of rodents with exogenous adiponectin has been shown to augment insulin action (Berg et al., 2001; Turer and Scherer, 2012). LY also substantially changed adiponectin complex distribution, increasing the proportion of high-molecular-weight adiponectin. Importantly, increasing levels of high molecular weight adiponectin per se have been identified as a correlate of human efficacy in the case of antidiabetic thiazolidinedione treatment (Pajvani et al., 2004).

Interestingly, relative to lean normal subjects, circulating levels of FGF21 are reportedly elevated (by 1.5- to 2.0-fold) in patients with obesity and T2DM (Chen et al., 2008; Dushay et al., 2010). It is also apparent that plasma FGF21 levels are more prominently increased in patients with nonalcoholic fatty liver disease (Dushay et al., 2010). These observations have led to the hypothesis that obesity, fatty liver disease, and T2DM may be states of relative FGF21 "resistance." Nevertheless, treatment of obese, hyperglycemic, *ob/ob* mice with FGF21 can still normalize hyperglycemia despite markedly elevated endogenous FGF21 levels in these animals (Hale et al., 2012; Kharitonenkov et al., 2005). The intrasubject variability of circulating FGF21 levels in humans has also been reported to be as great as 100-fold (Gälman et al., 2008; Angelin et al., 2012). Given this, as well as the fact that obesity and T2DM are clearly heterogeneous syndromes rather than discrete disorders (Drong et al., 2012), it will be important in larger future investigations to determine which patient characteristics are potentially associated with more favorable metabolic responses to FGF21-based therapy. Given that endogenous FGF21 levels were not measured in the present study, future human studies should also determine whether this key parameter is a predictor of response and the extent to which endogenous levels might be modulated by FGF21-based therapies.

Treatment with LY was generally well tolerated. However, skin rash and hypersensitivity in two 20 mg subjects, as well as injection site reactions in several other patients, were notable in this study. We speculate that these effects are unrelated to FGF21 action per se but might represent specific aspects of the LY molecule or the formulation used in this study. Recent reports have highlighted impaired growth hormone signaling and bone loss as two possible safety concerns that pertain to the future development and utility of FGF21-based therapies. Transgenic mice which overexpress FGF21 exhibit moderate growth retardation and evidence of growth hormone resistance (Inagaki et al., 2008). Another recent report also shows that FGF21 transgenic mice have reduced bone density, and FGF21 treatment of mice also produced a similar effect (Wei et al., 2012). Neither bone biomarkers nor bone mineral density were evaluated in the current study and would most likely be uninformative given the study size and duration. Nevertheless, these recently reported findings in rodents merit further investigation in any future human studies of FGF21 or related analogs.

In summary, daily administration of LY, a human FGF21 analog, provided clear evidence of clinically meaningful effects on several metabolic comorbidities associated with T2DM. Although maximum efficacy appeared to be rapidly achieved within the LY dose range studied for lipids, a maximally efficacious dose was not clearly established for other key endpoints, such as adiponectin and fasting glucose. Thus, the exploration of higher doses and longer treatment periods would be necessary in order to assess the full range of LY effects and the potential to achieve meaningful antidiabetic efficacy. Robust effects on fasting insulin were consistent with potential insulin sensitization. Prominent increases in adiponectin, high-molecular-weight adiponectin and β-hydroxybutyrate also indicate that specific pathways which are modulated by FGF21 in rodents are also operative in humans and are likely contributors to secondary metabolic benefits of FGF21.

EXPERIMENTAL PROCEDURES

Study Design and Patients

Eligible subjects were between the ages of 18 and 75 years old (inclusive) with a body mass index (BMI) of 25 to 40 kg/m^2 (inclusive) and had a diagnosis of T2DM prior to screening on the basis of the disease diagnostic criteria (World Health Organization) classification, and a fasting C peptide >1 ng/ml. Patients were ineligible if they used insulin, thiazolidinediones, dipeptidyl-peptidase IV

inhibitors, or exenatide during the previous 3 months or had recently experienced severe hypo- or hyper-glycemia. Patients who were found eligible for the study by initial screening procedures but were receiving metformin and one additional oral antidiabetic medication were removed from treatment of the second medication and rescreened after a minimum 14-day washout period to confirm that eligibility criteria were met prior to randomization. Patients were required to have HbA1c values of 7% to 10%, the exception being those who were receiving metformin and an additional oral antidiabetic medication, in which case HbA1c values were required to be 6.5% to 9.5% inclusive in order to account for the inevitable rise in glucose that accompanies the discontinuation of a second agent. Females were required to be surgically sterilized or postmenopausal. All individuals exhibited acceptable results on clinical laboratory tests, vital signs, and electrocardiogram at screening.

All patients gave written informed consent, and the protocol was approved by the local ethics committee and institutional review boards at each participating center. This study complied with the Declaration of Helsinki and its amendments and was performed in accordance with Good Clinical Practice guidelines. The trial was registered at http://clinicaltrials.gov (#NCT01869959).

Randomization and Masking

Patients were randomly assigned in an equal proportion to one of the four treatment groups. Individuals were stratified at the site level on the basis of treatment (assigned dose level), metformin treatment (yes or no), and fasting glucose level. Subjects and investigators were masked to drug versus volume-matched placebo at every LY dose level.

Procedures

After the initial screening, patients were randomized to the study drug (3, 10, or 20 mg of LY) or placebo and continued this treatment for 28 days. Subcutaneous injections were administered daily, and volumes of placebo were chosen to match the volumes of the three doses of LY. At baseline and treatment days 2, 7, 14, 21, and 28, body weight was measured, and fasting blood samples were collected and analyzed for lipid and glycemic parameters. Throughout the treatment and followup periods, patients were to continue on their current diet and exercise regimen in addition to their stable metformin and/or statin treatment (where applicable).

Analytical Methods

LY concentrations were analyzed with an LY antibody and a validated ELISA with a lower limit of quantification of 250 pg/ml. Insulin was measured by an immunoenzymatic assay (Beckman Coulter). Glucose was determined by a hexokinase method (Roche Diagnostics). Total cholesterol and TGs were measured with Center for Disease Control methods with the use of a Roche Modular Analyzer (performed by Covance Central Laboratory Services). Direct HDL-C and LDL-C were determined enzymatically (Roche). β-hydroxybutyrate was determined enzymatically (ARUP Laboratories). ApoAI, apoAII, and apoC-III were determined by immunonephelometry (Siemens Healthcare Diagnostics) and immunoturbidimetry (Kamiya Biomedical), respectively. ApoB was determined by nephelometry (Roche). Total adiponectin was determined by ELISA (R&D Systems). Adiponectin complex distribution was assessed with a gel filtration and a fast-protein-liquid chromatography-based assay followed by western blotting as previously reported (Schraw et al., 2008).

Statistical Methods

Sample size calculations were based on the interpatient variability of fasting glucose observed in similar internal studies (SD approximately 0.58 mmol/l). It was determined that eight patients per group would provide at least 90% power (one-sided alpha of 0.05) to detect an average fasting glucose decrease of 0.83 mmol/l (assuming the highest dose achieves this effect) in comparison to placebo by using a linear trend test. Pharmacodynamic analyses were conducted on all data from all evaluable patients. Safety analyses were conducted for all enrolled and randomized patients under the intent-to-treat principle.

For each measured parameter, a mixed effect linear model was used with change from baseline as the response variable, treatment, day, and treatment by day interaction as fixed effects, baseline value as covariate, and subject as random effect. Pairwise treatment comparisons between LY dose groups and placebo were performed for each day. The 90% confidence intervals and the We calculated SEM differences change from baseline in comparison placebo group using the within-subject error and degrees of freedom derived from the mixed effect model. Statistical significance was claimed when $p < 0.05$ from a two-sided test.

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