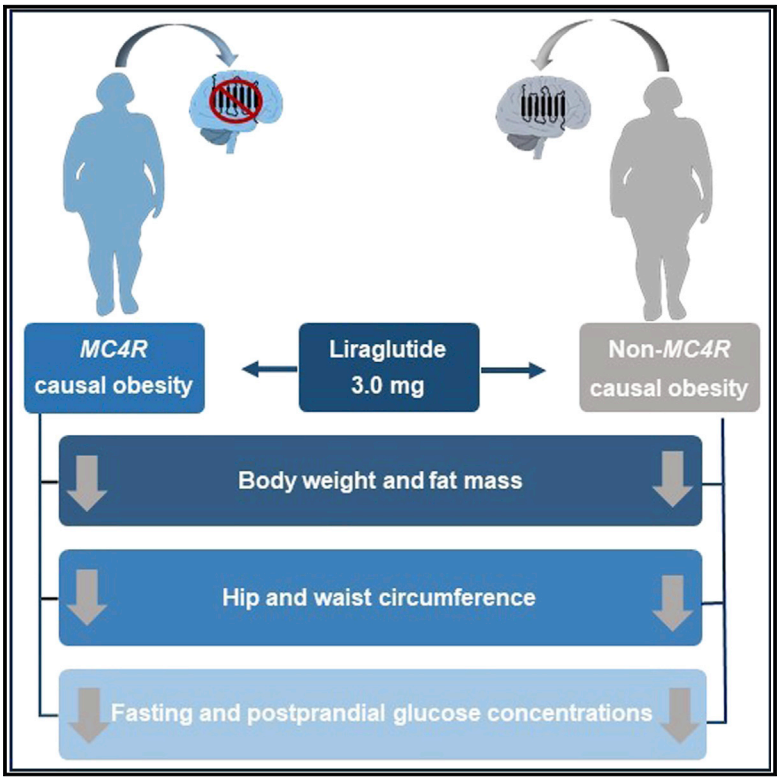
Clinical and Translational Report

Cell Metabolism

Patients with Obesity Caused by Melanocortin-4 Receptor Mutations Can Be Treated with a Glucagon-like Peptide-1 Receptor Agonist

Graphical Abstract



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In Brief

Iepsen et al. show that the diabetes and obesity drug liraglutide, which has appetite-suppressing effects, caused weight loss in obese patients with mutations in the appetite-regulating *melanocortin-4 receptor* (*MC4R*). These results show that the appetite effects of liraglutide are independent of the MC4R pathway and offer therapeutic opportunities for patients with *MC4R* causal obesity.

Highlights

- Fully functional MC4Rs are not required for GLP-1 RA-mediated weight loss
- Liraglutide caused a 6% weight loss in patients with *MC4R* mutations and controls
- Fat mass, waist circumference, and glucose concentrations improved with treatment
- Liraglutide is an effective treatment of the most common form of monogenic obesity

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Patients with Obesity Caused by Melanocortin-4 Receptor Mutations Can Be Treated with a Glucagon-like Peptide-1 Receptor Agonist

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SUMMARY

Pathogenic mutations in the appetite-regulating *melanocortin-4 receptor* (*MC4R*) represent the most common cause of monogenic obesity with limited treatment options. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) cause weight loss by reducing appetite. We assessed the effect of the GLP-1 RA liraglutide 3.0 mg for 16 weeks in 14 obese individuals with pathogenic *MC4R* mutations (BMI 37.5 ± 6.8) and 28 matched control participants without *MC4R* mutation (BMI 36.8 ± 4.8). Liraglutide decreased body weight by 6.8 kg ± 1.8 kg in individuals with pathogenic *MC4R* mutations and by 6.1 kg ± 1.2 kg in control participants. Total body fat, waist circumference, and fasting and postprandial glucose concentrations similarly decreased in both groups. Thus, liraglutide induced an equal, clinically significant weight loss of 6% in both groups, indicating that the appetite-reducing effect of liraglutide is preserved in *MC4R* causal obesity and that liraglutide acts independently of the MC4R pathway. Thus, liraglutide could be an effective treatment of the most common form of monogenic obesity.

INTRODUCTION

Pathogenic mutations in the hypothalamic appetite-regulating *melanocortin-4 receptor* (*MC4R*) are the most common cause of monogenic juvenile-onset obesity with a global prevalence of up to 6% (Vaisse et al., 2000; Farooqi et al., 2000; Hainerová et al., 2007) and even higher in developing countries where consanguinity exists (Saeed et al., 2015). Individuals with pathogenic *MC4R* mutations are characterized by early-onset obesity, hyperphagia, increased linear growth (Farooqi et al., 2003; Vollbach et al., 2017; Branson et al., 2003), and a lower tendency for developing hypertension despite their obesity (Greenfield et al., 2009). As obesity is strongly associated with an increase in morbidity and mortality (Berrington de Gonzalez et al., 2010; Whitlock et al., 2009), prevention and treatment of obesity is much needed. Only few studies have been reported regarding the ability of patients with monogenic *MC4R* mutations to lose weight with standard treatment modalities. Thus, in pediatric patients with pathogenic mutations, it has previously been shown that weight loss may be achieved by dieting (Hainerová et al., 2007), but the ability to maintain the weight loss is impaired (Reinehr et al., 2009). Results from bariatric surgery have shown that patients with monogenic *MC4R* mutations may benefit from operations such as the Roux-en-Y gastric bypass (RYGB) (Aslan et al., 2011) but do not seem to achieve the same degree of postoperative weight loss as individuals with common obesity, perhaps due to a higher degree of eating disorders in this particular patient group (Bonnefond et al., 2016). Previous pharmacological interventions, such as the monoamine re-uptake inhibitor sibutramine (now withdrawn due to the risk of adverse cardiovascular events) in patients with hypothalamic obesity, have shown less efficacy compared to patients with non-hypothalamic related obesity (Danielsson et al., 2007). A recent study has shown that 28 days of treatment with the MC4R-agonist setmelanotide resulted in a modest weight loss of 3.5 kg in six heterozygous carriers with *MC4R* mutations compared to 0.85 kg in the placebo group (Collet et al., 2017). Notably, however, weight losses of 51 kg and 20.5 kg with setmelanotide treatment have been shown in two individuals with pro-opiomelanocortin (POMC) deficiency (Kühnen et al., 2016), probably reflecting a more limited effect of the MC4R agonist in heterozygous *MC4R* mutation carriers. Thus, even with the results of setmelanotide, safe and effective treatment options in patients with *MC4R* causal obesity remain scarce.

The MC4R is primarily located in the central nervous system (CNS) with particular abundancy in the paraventricular nucleus (PVN) and the arcuate nucleus (ARC) of the appetite-regulating center in the hypothalamus (Mountjoy et al., 1994). In the ARC,

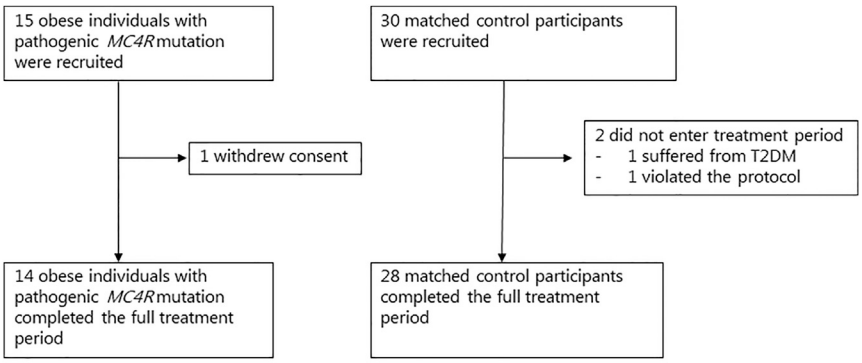

Figure 1. Participant Flow Diagram

15 obese individuals with pathogenic *MC4R* mutation and 30 matched control participants were recruited. One individual with pathogenic *MC4R* mutation withdrew consent, one control participant suffered from undiagnosed T2DM, and one control participant violated the protocol restrictions. Thus, 14 individuals with pathogenic *MC4R* mutations and 28 control participants completed the study and where subject for data analyses. See also Figure S1.

two distinct neuron populations are critical for the regulation of food intake; anorexigenic proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons produce CART and the MC4R agonist α-melanocyte-stimulating hormone (α-MSH). Orexigenic neuropeptide Y (NPY) and agouti-related peptide (AgRP) neurons produce NPY and the MC4R antagonist AgRP (Ellacott and Cone, 2004). In the non-fed state, POMC neurons are inhibited while AgRP neurons are activated, resulting in inhibition of MC4R signaling and subsequently increased appetite and food intake. Conversely, in the fed state, POMC neurons are activated and AgRP neurons inhibited, resulting in increased MC4R activation and consequently decreased appetite and food intake (Elmquist et al., 1999). Pathogenic mutations in the *MC4R* cause low MC4R functionality, leading to increased appetite sensation and thereby obesity (Huszar et al., 1997).

Glucagon-like peptide-1 (GLP-1) is a peptide hormone primarily secreted from the intestinal L cells upon meal intake (Drucker et al., 2017). GLP-1 is well known for its appetite-inhibiting and glucose-lowering effects (Flint et al., 1998; Nauck et al., 1993), and agonists of the GLP-1 receptor (GLP-1 RAs) are used for the treatment of obesity and type 2 diabetes mellitus (T2DM) (Madsbad, 2016).

The mechanisms behind GLP-1 RA-induced appetite inhibition, -reduced food intake, and thus weight loss seem to be the result of combined GLP-1 receptor (GLP-1R) activation in the periphery (via vagal afferents innervating the gut and hepatoportal bed; Kanoski et al., 2011) and the CNS (Tang-Christensen et al., 1996), where it enters the brain via leaks in the blood-brain barrier (BBB) (Secher et al., 2014) and targets GLP-1Rs in the hypothalamus (Göke et al., 1995).

However, it is still unclear which neuron populations in the hypothalamus are involved in the action of the GLP-1 RA liraglutide. It has been reported that peripherally administered liraglutide is taken up and internalized in the cytoplasm of POMC/CART neurons in mice (Secher et al., 2014). In rats, peripherally administered, fluorescently labeled liraglutide is taken up by the circumventricular organs (area postrema, the median eminence, and the subfornical organ) but also reaches the PVN (Secher et al., 2014). However, direct injection of the GLP-1R antagonist exendin (9–39) into the ARC, but not into PVN, abolished the effect of liraglutide on food intake and body weight, suggesting that the ARC, and not the PVN, is involved in the weight-regulating effect of liraglutide (Secher et al., 2014). In contrast to this finding, another study has shown that

Table 1. Participant Characteristics

	MC4R Group (n = 14)	Control Group (n = 28)	Estimated Difference	p Value
Male ratio (%)	53.6	57.1	3.5% (−3.01 −3.72)	0.8
Age (y)	32.8 ± 13.5	42.8 ± 10.2	10.0 (2.4 −17.7)	0.01
Weight (kg)	122.4 ± 24.9	112.6 ± 18.9	9.7 (−4 −23)	0.3
BMI (kg/m^2)	37.5 ± 6.8	36.8 ± 4.8	0.7 (−2 −4.4)	0.7
Lean body mass (kg)[a]	67.5 ± 13.6	63.6 ± 11.2	3.9 (−4.0 −11.9)	0.4
Total body fat (kg)	52.1 ± 15.2	47.5 ± 11.0	4.6 (−3.7 −12.9)	0.3
Total body fat (%)	44.5 ± 6.9	43.8 ± 5.7	0.7 (−3.3 −4.7)	0.6
Liver fat (%)	6.4 ± 1.8	9.5 ± 1.7	3.1 (−2.4 −8.5)	0.3
Muscle fat (%)	3.0 ± 0.4	2.4 ± 0.5	0.6 (−1.1 −2.2)	0.5
Fasting plasma glucose (mmol/L)	5.3 ± 0.5	5.7 ± 0.6	0.2 (−0.3 −0.8)	0.9
Fasting serum insulin (pmol/L)	108.3 ± 66.5	124.4 ± 169.4	16.0 (−61.3 −29.3)	0.7
Fasting C-peptide (nmol/L)	0.9 ± 0.3	1.2 ± 0.41	0.2 (−0.5 −0.05)	0.6
HOMA-IR (F_{PG}xF_{INS}/22.5)	3.6 ± 2.3	4.7 ± 3.0	1.08 (−2.9 −0.8)	0.3
Matsuda (10.000/(√(F_{INS} × F_{PG} × 2H_{INS} × 2H_{PG})))	4.3 ± 2.8	2.8 ± 1.8	1.6 (−0.18 −3.3)	0.08

Data are shown as mean ± standard deviation (SD). Estimated differences between groups are presented as mean with 95% CI. F_{PG}, fasting plasma glucose (mg/dL); F_{INS}, fasting insulin (μL/mL); 2H_{PG}, 2 hr plasma glucose (mg/dL); 2H_{INS}, 2 hr insulin (μL/mL). See also Tables S1–S3.

[a]Lean body mass is without bone mineral content.

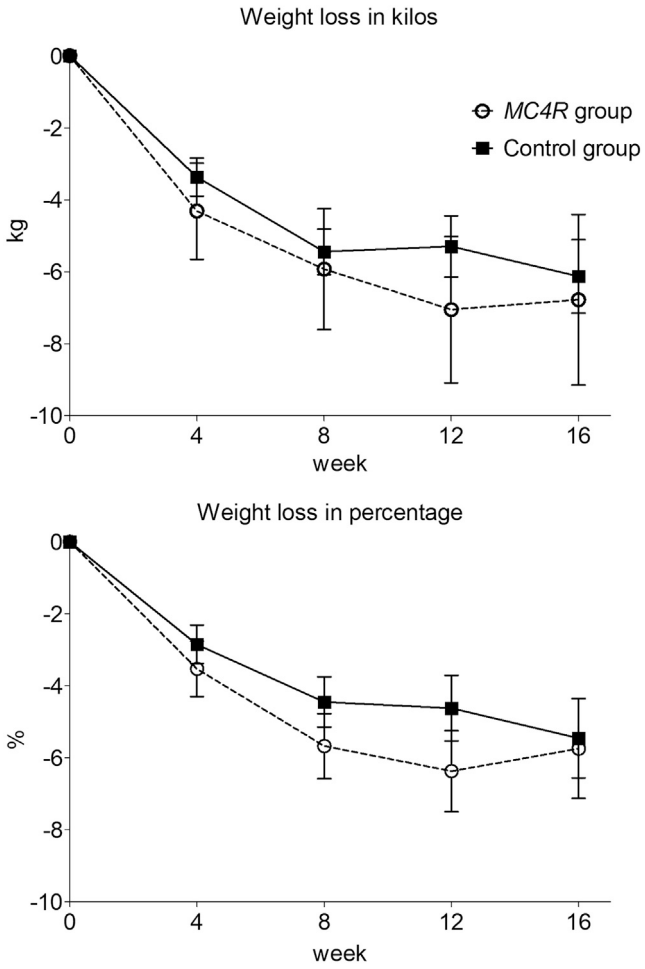

Figure 2. Liraglutide Induces Weight Loss in Individuals with Pathogenic *MC4R* Mutations and Control Participants

Weight loss in kilograms (upper graph) and percentage (lower graph) from 0–16 weeks of liraglutide treatment in 14 individuals with pathogenic *MC4R* mutations and 28 control participants. Data, including error bars, are shown as mean ± SEM. Open circles and dashed line: *MC4R* group. Closed squares and solid line: control group.

endogenous GLP-1 injections into the PVN, but not the ARC, decreased food intake in rats (Sandoval et al., 2008). Moreover, a recent study showed that direct infusion of liraglutide in the hypothalamus increased MC4R expression in contrast to peripherally administered liraglutide (Kaineder et al., 2017). Interestingly, mice with heterozygous *MC4R* mutations displayed a full anorectic response to intraperitoneal liraglutide treatment (Nonogaki et al., 2011), suggesting that MC4R activation may not be necessary for anorexia mediated by peripherally injected liraglutide.

Combined with the limited treatment options for the most common form of monogenic obesity, we therefore hypothesized that the GLP-1 RA liraglutide could circumvent MC4R-induced appetite regulation and thereby constitute a treatment option for individuals with obesity causal *MC4R* mutations. Thus, in this study, we assessed the effects of the GLP-1 RA liraglutide on weight loss in humans with disrupted MC4R signaling. We gave liraglutide 3.0 mg daily to individuals with pathogenic *MC4R* mutations and matched non-mutation carriers for 16 weeks. In this study design, we are able to elucidate whether a fully functional MC4R receptor is required for liraglutide-mediated GLP-1R effects in humans. Furthermore, the study results point to a new understanding of whether liraglutide could be a treatment option in this otherwise relatively treatment-resistant patient group.

RESULTS AND DISCUSSION

Clinical Trial Design and Patient Characteristics

Participants were recruited either as parents to a child diagnosed with pathogenic *MC4R* mutation or as young adults with pathogenic *MC4R* mutation (minimum age of 18 years) and were previously enrolled at the Children's Obesity Clinic, Department of Pediatrics, Copenhagen University Hospital Holbæk, Denmark, in the period from August 2014 to April 2016. Out of 1,044 children and adolescents, 25 (2.4%) carried a pathogenic *MC4R* mutation. As obesity resulting from pathogenic *MC4R* mutations has dominant inheritance (Farooqi et al., 2003), one of the biological parents will carry the mutation. Thus, parents to a child or an adolescent diagnosed with pathogenic *MC4R* mutation were invited to the Children's Obesity Clinic for blood sampling and subsequently mutation screening. If the parents met the inclusion criteria (see below), they were invited to participate in the full study either as case (if mutation carrier) or as control (if non-carrier). As genotyping was performed after the intervention, *MC4R* mutation carrier status was unknown during the intervention in these individuals.

Two matched control individuals per case were included in the study; therefore, additional control participants were recruited from family, relatives, and friends to minimize social and behavioral differences between individuals with pathogenic *MC4R* mutation and control participants.

Inclusion criteria were body mass index (BMI) above 28 and age above 18 and under 65 years.

Exclusion criteria were any acute or chronic diseases, including known diabetes, or patients taking any pharmaceuticals with known effects on glucose and lipid metabolism, appetite, or food intake. Pregnant or breast feeding women were excluded.

In total, 15 obese individuals with pathogenic *MC4R* mutation and 30 matched control participants were recruited for the study. One individual with pathogenic *MC4R* mutation withdrew consent, one control participant suffered from undiagnosed T2DM, and one control participant violated the protocol instructions. Thus, 14 individuals with pathogenic *MC4R* mutations and 28 control participants completed the study and were subject to data analyses. See Figure 1 for participant flow diagram.

Study participation comprised one full test day before and one full test day after 16 weeks of treatment with liraglutide 3.0 mg daily, including three control visits during the treatment period (weeks 5, 9, and 13). At the two full test days, the participants underwent a 3 hr 75 g oral glucose tolerance test (OGTT) as well as dual-energy X-ray absorptiometry (DEXA) assessed body composition and magnetic resonance spectroscopy (MRS) assessed ectopic fat accumulation in liver and muscle. The two groups were similar with regards to BMI, body weight, body composition, and glucose regulation. See Table 1 for participant characteristics.

Table 2. Treatment Effect of Liraglutide on Body Composition and Glucose Regulation

	MC4R Group (n = 14)				Control Group (n = 28)				Estimated between-Group Difference	
	Baseline	Week 16	Mean Difference	p Value	Baseline	Week 16	Mean Difference	p Value	Mean Difference	p Value
Anthropometric Measurements										
Weight (kg)	122.4 ± 6.7	115.6 ± 6.7	−6.8 ± 1.8	0.003	112.7 ± 3.6	106.5 ± 3.7	−6.1 ± 1.2	<0.0001	−0.7 (−5.1 −3.8)	0.9
BMI (kg/m^2)	37.5 ± 1.8	35.4 ± 1.9	−2.0 ± 0.5	0.001	36.8 ± 0.9	34.8 ± 0.9	−2.0 ± 0.4	<0.0001	0.006 (−1.4 −1.4)	0.8
Waist circumference (cm)	110.6 ± 3.5	105.3 ± 3.9	−5.4 ± 1.5	0.003	114.5 ± 2.7	108.1 ± 3.0	−6.5 ± 1.7	<0.0001	1.1 (−4.0 −6.2)	0.4
Hip circumference (cm)	127.0 ± 3.8	120.8 ± 4.1	−6.3 ± 1.3	0.001	120.3 ± 1.9	114.4 ± 1.7	−5.8 ± 1.1	<0.0001	−0.4 (−4.1 −3.2)	0.7
Body Composition										
Lean body mass (kg)[a]	67.5 ± 3.6	66.6 ± 3.7	−0.9 ± 0.4	0.1	63.6 ± 2.1	62.8 ± 2.2	−0.8 ± 0.4	0.1	−0.1 (−1.4 −1.2)	0.9
Total fat mass (kg)	52.1 ± 4.1	47.1 ± 4.6	−5.0 ± 1.5	0.007	47.5 ± 2.1	42.3 ± 2.1	−5.2 ± 1.0	<0.0001	0.2 (−3.4 −3.8)	0.9
Total fat percentage (%)	44.5 ± 1.9	42.0 ± 2.3	−2.5 ± 0.8	0.009	43.8 ± 1.1	41.2 ± 1.2	−2.6 ± 1.1	<0.0001	0.12 (−1.8 −2.0)	0.7
Liver fat percentage (%)	6.4 ± 1.8	5.4 ± 2.5	−1.05 ± 1.7	0.6	9.5 ± 1.7	6.5 ± 1.4	−3.0 ± 1.7	0.07	1.9 (−3.1 −7.1)	0.4
Muscle fat percentage (%)	3.0 ± 0.4	2.9 ± 0.8	−0.1 ± 0.8	0.9	2.4 ± 0.5	3.0 ± 0.6	0.35 ± 1.6	0.7	0.5 (−2.9 −1.9)	0.7
Glucose Regulation										
Fasting p-glucose (mmol/L)	5.3 ± 0.5	5.1 ± 0.4	−0.25 ± 0.1	0.047	5.7 ± 0.1	5.3 ± 0.09	−0.4 ± 0.1	0.002	0.18 (−0.18 −0.5)	0.3
Incremental $_{AUCglucose}$ (mmol/L × min)	305 ± 40	160 ± 51	−145 ± 35	0.02	310 ± 29	235 ± 29	−75 ± 36	0.01	−70 (−185 −45)	0.08
Fasting s-insulin (pmol/L)	108.3 ± 17.8	112.2 ± 23.3	3.9 ± 14.8	0.8	124.3 ± 13.1	103.9 ± 10.6	−20.4 ± 10.8	0.07	24.3 (−13.2 −61.8)	0.2
Incremental$_{AUCinsulin}$ (pmol/L × min)	66,958 ± 10,640	61,932 ± 12,384	−5,026 ± 4,134	0.2	91,359 ± 11,743	84,161 ± 13,117	−7,198 ± 14,413	0.2	2,172 (−28,402 −32,746)	0.8
Fasting C-peptide (nmol/L)	0.9 ± 0.08	1.03 ± 0.12	0.045 ± 0.06	0.5	1.18 ± 0.08	1.06 ± 0.06	−0.13 ± 0.07	0.06	0.17(−0.04 −0.39)	0.2
Incremental$_{AUCc\text{-}peptide}$ (nmol/L × min)	295 ± 25	264 ± 29	−29 ± 24	0.2	369 ± 27	355 ± 29	−14 ± 30	0.5	−14 (−110 −81)	0.5
HOMA-IR ((F_{PG} × F_{INS})/22.5)	3.6 ± 0.6	3.6 ± 0.8	−0.02 ± 0.5	0.8	4.7 ± 0.6	3.6 ± 0.4	−1.1 ± 0.5	0.04	1.07 (−0.4 −2.6)	0.2
Matsuda (10.000/($\sqrt{}$(F_{INS} × F_{PG} × $2H_{INS}$ × $2H_{PG}$))[b]	4.4 ± 0.8	6.6 ± 1.3	2.2 ± 0.8	0.02	2.7 ± 0.4	4.8 ± 0.7	2.03 ± 0.6	0.001	0.18 (−1.8 −2.1)	0.9

Within- and between-group differences from baseline to week 16. Within-group differences are shown as mean ± SEM, and estimated between-group differences are shown with mean and 95% CI. F_{PG}, fasting plasma glucose (mg/dL); F_{INS}, fasting insulin (μL/mL); $2H_{PG}$, 2 hr plasma glucose (mg/dL); $2H_{INS}$, 2 hr insulin (μL/mL). As data were not normally distributed for glucose, C-peptide, and insulin concentrations, data are shown as raw mean ± SEM and the p values are given for log-transformed data.

[a]Lean body mass is without bone mineral content.

[b]Matsuda index analyses are performed on n = 13 in the *MC4R* group and n = 26 in the control group.

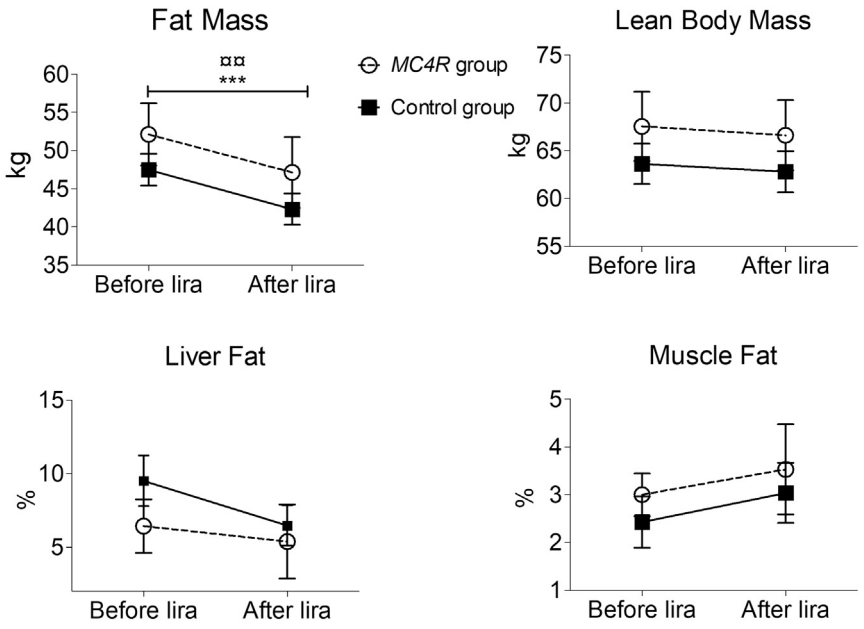

Figure 3. Liraglutide Reduces Fat Mass, but not Lean Body Mass, in Individuals with Pathogenic *MC4R* Mutations and Control Participants

Lean body mass (kg), total fat mass (kg), muscle fat percentage, and liver fat percentage before and after liraglutide treatment in 14 individuals with pathogenic *MC4R* mutations and 28 control participants. Data, including error bars, are shown as mean ± SEM. Open circles and dashed line: *MC4R* group. Closed squares and solid line: control group. *MC4R* group: ¤¤p < 0.01, control group: ***p < 0.0001. See also Figure S2.

The project and associated biobank were approved by the ethical committee in Copenhagen (reference number: H-1-2013-093), and the study was performed in accordance with the Helsinki Declaration II. Participation was voluntary, and the individuals could at any time retract their consent to participate (ClinicalTrials.gov; identifier: NCT02082496).

16 Weeks of Liraglutide Treatment Decreases Body Weight, Body Fat Percentage, and Waist and Hip Circumference in Obese Individuals with Pathogenic MC4R Mutations and Control Participants

After 16 weeks of liraglutide treatment, the *MC4R* group had lost −6.8 ± 1.8 kg (p = 0.003) and the control group −6.1 ± 1.0 kg (p < 0.0001), with no difference between groups (p = 0.9). In percentage, the weight loss was −5.7% ± 1.4% and −5.5% ± 1.1%, respectively, with no group difference (p = 0.9). See Figure 2. In addition, the weight loss was accompanied by decreases in total fat mass (−2.5% ± 0.8%, p = 0.009, and −2.6% ± 1.1%, p < 0.0001, in the *MC4R* group and in the control group, respectively) as well as decreases in waist circumference (−5.4 ± 1.5 cm, p = 0.003, and −6.5 ± 1.7 cm, p < 0.0001) and hip circumference (−6.3 ± 1.3 cm, p = 0.001, and −5.8 ± 1.1 cm, p < 0.0001) in the *MC4R* group and control group, respectively, with no difference between groups. Lean body mass did not decrease in either group. As for ectopic fat accumulation, liver fat percentage tended to decrease in the control group (−3.0% ± 1.7%, p = 0.07), but not in the *MC4R* group (−1.05% ± 1.7%, p = 0.6). However, there was no difference between groups (p = 0.4). Muscle fat percentage did not change in the two groups. See Table 2 and Figures 3 and S2.

16 Weeks of Liraglutide Treatment Improves Glucose Regulation in Obese Individuals with Pathogenic MC4R Mutations and Control Participants

Both groups achieved significant decreases in fasting plasma glucose: −0.25 ± 0.1 mmol/L, p = 0.047, in the *MC4R* group and −0.4 ± 0.1 mmol/L, p = 0.002, in the control group, with no difference between groups, p = 0.3. Postprandial plasma glucose (evaluated as the incremental area under the curve [$iAUC_{0–180}$] for glucose) decreased significantly by 47.5% in the *MC4R* group and by 24% in the control group, with no difference between groups, p = 0.08. Fasting serum insulin and fasting C-peptide concentrations, as well as postprandial concentrations ($iAUC_{0–180}$ for insulin and $iAUC_{0–180}$ for C-peptide), did not change within or between groups after treatment. Hepatic insulin resistance evaluated by homeostasis model assessment for insulin resistance (HOMA-IR) improved in the control group, but not in the *MC4R* group. However, both groups improved their peripheral insulin sensitivity estimated by the Matsuda index by 2.2 ± 0.8, p = 0.02, and by 2.03 ± 0.6, p = 0.001, for the *MC4R* group and the control group, respectively. See Table 2 and Figure 4.

16 Weeks of Liraglutide Treatment Improves Lipid Concentrations in Obese Individuals with Pathogenic MC4R Mutations and Control Participants

Finally, both groups decreased their concentrations of total plasma cholesterol (−0.3 mmol/L) and plasma triglycerides, although it was only significant in the control group. There was no difference between groups. Plasma HDL and plasma LDL cholesterol did not change within or between groups. Systolic and diastolic blood pressure did not change significantly; however, the *MC4R* group had significantly decreased diastolic blood pressure after treatment compared to the control group. Pulse increased in both groups (8.6 ± 2.4 bpm, p = 0.003, and 5.7 ± 1.9 bpm, p = 0.006, in the *MC4R* group and control group, respectively), with no difference between groups (p = 0.4). See Table 3.

Finally, gastrointestinal side effects were generally reported as mild and transient. Thus, after 4 weeks of treatment, 57.1% of *MC4R* mutation carriers and 64.4% of control participants reported nausea (primarily of mild intensity), and after 16 weeks of treatment nausea was prevalent in 14.3% of *MC4R* mutation carriers and 17.9% of control participants. See Table S1 for reported gastrointestinal side effects. No other adverse events were reported.

Liraglutide as Treatment for MC4R Causal Obesity

This is the first study to show that monogenic obesity caused by pathogenic *MC4R* mutations can be treated with a GLP-1 RA

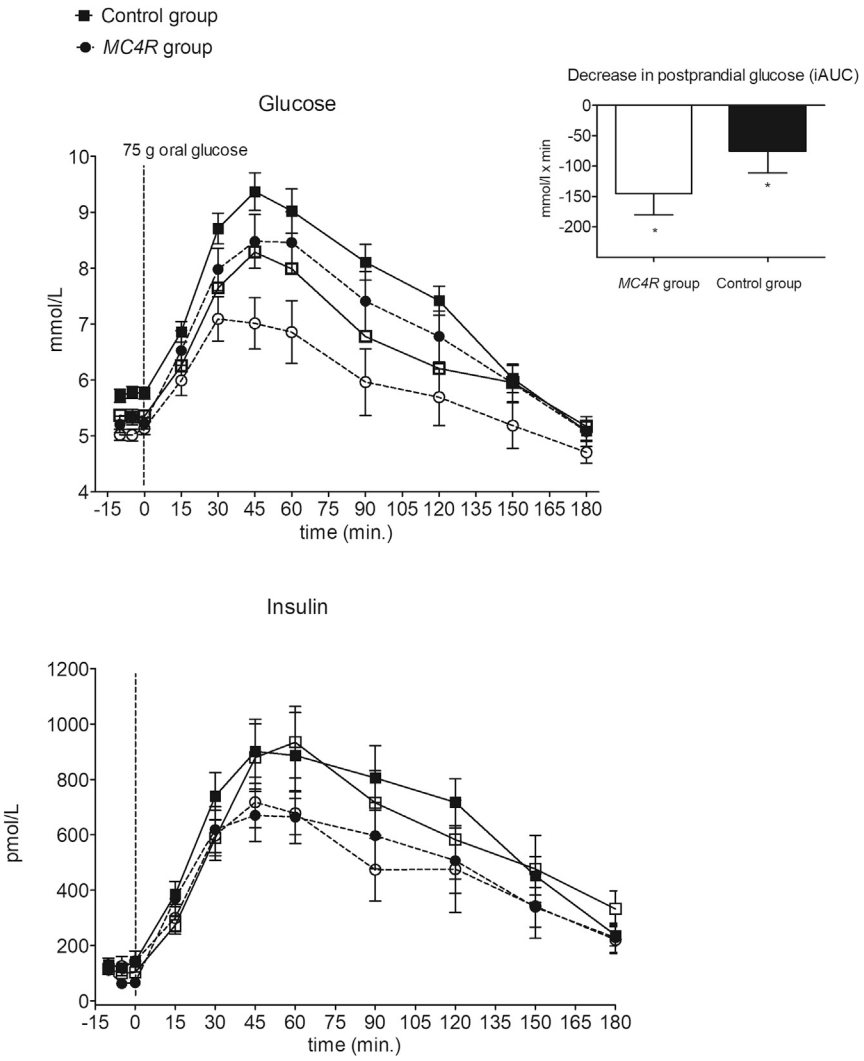

Figure 4. Liraglutide Decreases Fasting and Postprandial Glucose Concentrations in Individuals with Pathogenic *MC4R* Mutations and Control Participants

Glucose and insulin concentrations during an OGTT before and after liraglutide treatment in 14 individuals with pathogenic *MC4R* mutations and 28 control participants. Data, including error bars, are shown as mean ± SEM. Closed circles and dashed line: *MC4R* group before liraglutide. Open circles and dashed line: *MC4R* group after liraglutide. Closed squares and solid line: control group before liraglutide. Open squares and solid line: control group after liraglutide. Column bar plot upper right corner: decrease in postprandial glucose concentrations ($iAUC_{glucose}$) after liraglutide treatment. Black bar, control group; white bar, *MC4R* group. *$p < 0.05$.

(liraglutide) as efficiently as in "common" obesity. Thus, 4 months of treatment, including 5 weeks of dose escalation with liraglutide 3.0 mg daily, resulted in a similar, clinically relevant weight loss of 6% body weight in obese individuals with and without pathogenic *MC4R* mutations. The weight losses of 6.8 kg in the *MC4R* group and 6.1 kg in the control group were comparable to those reported by other weight management studies with 3.0 mg liraglutide after 4 months (Pi-Sunyer et al., 2015; Wadden et al., 2013; Astrup et al., 2009); notably, the weight loss was obtained without any lifestyle counseling, diet, or exercise intervention. Currently available weight loss interventions have shown less efficient results in obese patients with *MC4R* mutations (Reinehr et al., 2009; Danielsson et al., 2007; Bonnefond et al., 2016). Furthermore, treatment with the MC4R agonist setmelanotide resulted in a modest weight loss of 2% body weight in individuals with heterozygous *MC4R* mutations (Collet et al., 2017), whereas liraglutide 3.0 mg induced a superior weight loss of 6%. Thus, liraglutide is a safe and effective treatment in this particular group of patients with *MC4R* causal obesity with regards to both weight and fat reduction and lowering of fasting and postprandial glucose concentrations.

Additional Health Benefits from Treatment

For both groups, the weight loss was accompanied by decreases in fat mass, but not lean mass, reductions in waist and hip circumference, and fasting and postprandial plasma glucose concentrations as well as improvements in insulin sensitivity measured by the Matsuda index. It is well known that, besides weight loss, liraglutide treatment is associated with reductions in fasting and postprandial glucose concentrations, as well as reductions in cardio-metabolic risk factors such as waist circumference and blood pressure, and is thereby protective against prediabetes and T2DM (Pi-Sunyer et al., 2015). Furthermore, in patients with T2DM, liraglutide has been shown to lower the risk of cardiovascular events as well as to reduce the cardiovascular mortality rate (Marso et al., 2016). Consistent with this, our results show that treatment with liraglutide in *MC4R* causal obesity, besides weight loss, includes improvements in the metabolic profile and thereby a diminished risk of future co-morbidities. Furthermore, treatment of obesity in itself is a highly valued outcome with regards to health-related quality of life (Kroes et al., 2016). Of note, the *MC4R* group had lower diastolic blood pressure after treatment compared to the control group, which may be partly reflected by a general lower tendency for developing hypertension despite obesity (Greenfield et al., 2009), likely due to central melanocortinergic effects on the sympathetic nervous system (SNS) (do Carmo et al., 2017). However, we did not observe any significant difference in blood pressure between the groups before treatment.

Liraglutide has recently been shown to reduce the prevalence of histologically verified non-alcoholic fatty liver disease (NAFLD) (Armstrong et al., 2016) as well as MRS-assessed liver fat content in obese women with polycystic ovary syndrome (PCOS) (Frossing et al., 2018). In our study, both groups met the criteria for NAFLD with 6.4% and 9.5% liver fat in *MC4R*

Table 3. Treatment Effect of Liraglutide on Lipids and Blood Pressure

	MC4R Group (n = 14)				Control Group (n = 28)				Estimated between-Group Difference	
	Baseline	Week 16	Mean Difference	p Value	Baseline	Week 16	Mean Difference	p Value	Mean Difference	p Value
Lipid Assessment										
Total cholesterol (mmol/L)	4.74 ± 0.2	4.45 ± 0.2	−0.28 ± 0.14	0.06	4.84 ± 0.16	4.55 ± 0.16	−0.29 ± 0.12	0.02	0.008 (−0.39 −0.41)	0.8
HDL cholesterol (mmol/L)	1.17 ± 0.08	1.12 ± 0.08	−0.05 ± 0.03	0.09	1.22 ± 0.08	1.20 ± 0.06	−0.02 ± 0.03	0.4	0.03 (−0.12 −0.06)	0.7
LDL cholesterol (mmol/L)	2.9 ± 0.2	2.8 ± 0.2	−0.15 ± 0.14	0.3	2.78 ± 0.16	2.71 ± 0.16	−0.07 ± 0.08	0.4	0.08 (−0.34 −0.23)	0.6
Triglycerides (mmol/L)	1.47 ± 0.16	1.31 ± 0.14	−0.16 ± 0.09	0.1	1.85 ± 0.16	1.43 ± 0.13	−0.42 ± 0.13	0.003	0.26 (−0.15 −0.66)	0.5
Cardiovascular Assessment										
Systolic blood pressure (mmHg)	127.6 ± 4.5	127.6 ± 4.6	0.07 ± 4.1	0.9	135 ± 4.0	132.5 ± 3.3	−2.1 ± 2.1	0.3	2.18 (−6.3 −10.6)	0.6
Diastolic blood pressure (mmHg)	76.9 ± 2.4	75.4 ± 2.5	−1.5 ± 2.0	0.5	81.2 ± 2.3	83.4 ± 1.9	2.3 ± 1.4	0.1	3.8 (−8.8 −1.14)	0.03
Pulse (bpm)	64.3 ± 2.0	72.9 ± 1.7	8.6 ± 2.4	0.003	67.0 ± 1.7	73.0 ± 2.0	5.7 ± 1.9	0.006	2.9 (−3.4 −9.2)	0.9

Within- and between-group differences from baseline to week 16. Within-group differences are shown as mean ± SEM, and estimated between-group differences are shown with mean and 95% CI.

group and control group, respectively (above 5% is generally considered diagnostic; Hardy et al., 2016). After liraglutide treatment, liver fat content was non-significantly reduced by 16% and by 32% in the *MC4R* group and the control group, respectively. To our knowledge, liver fat accumulation in *MC4R* mutation carriers has not been assessed previously. However, MC4 receptors are expressed in rat liver cells (Malik et al., 2012), and *MC4R*-deficient mice had a 3-fold higher content of lipids in the liver compared to wild-type mice on a standard chow diet, suggesting that these mice are more prone to develop NAFLD independent of obesity status (Lede et al., 2017). This may explain the somewhat reduced ability of our *MC4R* mutation carriers to reduce their hepatic fat content compared to the control group (16% reduction in the *MC4R* group versus 32% reduction in the control group, although non-significant), which perhaps is also reflected in the unchanged HOMA-IR.

Liraglutide Is Fully Effective with Respect to Reducing Body Weight and Lowering Glucose in Spite of a Defective MC4R Pathway

As MC4Rs are predominantly expressed in hypothalamic nuclei (Mountjoy et al., 1994), our finding of a preserved appetite inhibiting effect of liraglutide in obese patients with pathogenic *MC4R* mutations suggests that the hypothalamic melanocortinergic system is not a key pathway for GLP-1 RA-mediated weight loss. Alternative routes for liraglutide-mediated anorexia may be through GLP-1R activation of a local GABA neuron inhibiting the orexigenic NPY/CART neurons, as suggested by Secher et al. (2014), or by direct GLP-1R activation on PVN neurons (Sandoval et al., 2008), thereby circumventing the MC4R. Interestingly, this is also in agreement with a recent study that demonstrated that mice lacking GLP-1Rs in the hypothalamus, as well as mice specifically lacking GLP-1Rs in the PVN and POMC neurons, all responded fully to peripherally administered GLP-1 RAs (liraglutide and exendin-4) with weight loss and lowering of glucose concentrations (Burmeister et al., 2017). Thus, liraglutide seems to be fully effective with respect to reducing body weight and lowering glucose in spite of a defective MC4R pathway.

Limitations of Study

We have a relatively small number of participants with pathogenic *MC4R* mutations (n = 14), but we have compensated for this by including cases and controls in a 1:2 ratio, respectively. We have not included a placebo group, since the weight-lowering effect of liraglutide is well established and the present weight reduction of approximately 6 kg after 4 months is similar to those reported in previous studies (Pi-Sunyer et al., 2015; Astrup et al., 2009; Wadden et al., 2013).

Also, cases and controls were recruited within the same family (as parents to a child heterozygous for a pathogenic *MC4R* mutation) or as friends and relatives to avoid any socioeconomic and environmental differences otherwise often existing between cases and controls. Thus, three out of nine *MC4R* mutation families comprised a mix of cases and controls. However, there was no weight response difference between the families.

As homozygous *MC4R* mutation carriers are extremely rare in the obese population (Farooqi et al., 2003), the participants recruited for this study were all heterozygous carriers. This means that in spite of reduced MC4R activity, we cannot exclude the possibility that liraglutide may have improved the impaired MC4R signaling. However, all variants in this study have been shown in functional studies to result in complete loss of function (see Table S2 for included variants), which most likely explains the obese phenotype of the carriers. Thus, unless liraglutide is capable of completely restoring the activity of all the variants, it would seem unlikely that liraglutide would be able to generate a completely normal weight loss if the response was exclusively transmitted via the MC4R.

Thus, with our 14 *MC4R* mutation carriers and 28 matched control subjects and a weight response difference of 0.7 ± 2.2 kg between groups (−6.8 kg in the *MC4R* group and −6.1 kg in the control group), we can conclude that there is no weight response difference between the groups and, accordingly, that liraglutide is effective in individuals with *MC4R* causal obesity. However, larger-scale studies confirming the results of preserved weight loss effects of GLP-1 RAs in individuals with obesity causal *MC4R* mutations are warranted, as well as studies of the ability of liraglutide to maintain weight loss after initial weight loss by dieting, as shown in individuals with common obesity (Wadden et al., 2013; Astrup et al., 2009). However, given that our patients responded normally to the treatment, it seems reasonable to hypothesize that liraglutide will also facilitate weight maintenance in patients with obesity caused by *MC4R* mutations.

Conclusions

We demonstrate that the weight-reducing and glucose-lowering effects of the GLP-1 RA liraglutide are preserved in spite of defective MC4R activity in patients with obesity caused by *MC4R* mutations. Therefore, we propose liraglutide 3.0 mg as a relevant treatment in the most common form of monogenic obesity.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and three tables and can be found with this article online at https://doi.org/10.1016/j.cmet.2018.05.008.

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AUTHOR CONTRIBUTIONS

Conceptualization and methodology, S.S.T., J.J.H., S.M., T.H., E.W.I., and J.-C.H.; Formal Analysis, E.W.I. and J.Z.; Investigation, E.W.I.; Resources, M.H., T.H., H.S.T., E.L.H., and J.-.C.H.; Writing – Original Draft, E.W.I. with help from S.S.T.; Writing – Review & Editing, J.J.H., S.M., S.S.T., T.H., J.-C.H., E.L.H., H.S.T., M.H., and J.Z.; Visualization, E.W.I., J.Z., and S.S.T.; Supervision, S.S.T. and J.J.H.; Project Administration, E.W.I. The corresponding authors E.W.I. and S.S.T. confirm full access to data and final responsibility for the decision to submit for publication.

DECLARATION OF INTERESTS

S.S.T. and T.H. hold stocks in Novo Nordisk A/S. J.J.H. has acted as consultant and received speaker honoraria for Novo Nordisk. S.M. served as a consultant or adviser to Novartis Pharmaceuticals, Novo Nordisk, Merck, Sharp and Dome, Pfizer A/S, Abbott Laboratories, Sanofi Aventis, Astra Zeneca, Johnson & Johnson, Rosche, Mankind, BMS, Antarcia, Boehringer Ingelheim, Eli Lilly, and Amgen. S.M. received a fee for speaking from Novo Nordisk, Merck, Sharp and Dome, Astra Zeneca, Johnson & Johnson, Abbott Laboratories, Pfizer A/S, Roche, Schering-Plough, Sanofi-Aventis, Eli Lilly, Novartis Pharmaceuticals, BMS, and Boehringer Ingelheim. The remaining authors have nothing to disclose.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Human plasma and serum	Clinical Trial	H-1-2013-093/ NCT02082496
Human DNA	Clinical Trial	H-1-2013-093/ NCT02082496
Deposited Data		
Raw Data File	Mendeley Data	https://dx.doi.org/10.17632/dryd5vjvz4.1
Software and Algorithms		
Prism 5.0	GraphPad	https://www.graphpad.com/
SPSS 23.0	IBM	https://www.ibm.com/products/spss-statistics
Other		
Liraglutide, Victoza	Novo Nordisk A/S	https://www.victoza.com/
Measuring station (weight and height)	SECA	https://www.seca.com/
Omron 705IT blood pressure device	Omron	https://omron.eu/en/home
GE Lunar iDXA Scanner	GE Healthcare	http://www3.gehealthcare.com/en
3T Achieva MR-imaging system and 1T Panorama HFO MR-imaging system	Philips Medical Systems	https://www.usa.philips.com/healthcare
Dimension Vista (glucose and cholesterol analyses)	Siemens Healthcare	https://www.healthcare.siemens.com/
COBAS 6000 (insulin and C-peptide analyses)	Roche Diagnostics	http://www.roche.dk

CONTACT FOR REAGENT AND RESOURCE SHARING

Requests for further information or data should be directed to and will be fulfilled by the Lead Contact, Signe S. Torekov (torekov@sund.ku.dk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Genotyping

Children and adolescents attending the Children's Obesity Clinic were screened for pathogenic *MC4R* mutations using a novel target region capture sequencing platform, using genomic DNA extracted from peripheral blood lymphocytes, as previously described in detail (Gao et al., 2014). Five different heterozygous pathogenic mutations were identified in the cohort based on previous *in vitro* pathogenic studies (see Table S2 for *MC4R* variants).

Thus, four families carried the *Asp37ValTyr35X* variant (6 patients in total), two families carried the *Ala219Val* variant (4 patients in total), one family carried the *Arg165Gln* variant (1 patient), one family carried the *Gly181Asp* variant (1 patient), and one family carried both the *Thr112Met* variant (1 patient) and the *Asp37ValTyr35X variant* (1 patient) Thus, in total 14 carriers of pathogenic *MC4R* mutations from nine families were included in the study (see Table S3 for overview). The 28 control participants were screened for pathogenic *MC4R* mutations after the trial with no positive findings.

Self-Reported Weight Biography

BMI at age 20 was 29.8 kg/m^2 ± 7 and 27.6 kg/m^2 ± 4 in the *MC4R* group and control group, respectively. Highest BMI outside pregnancy was 39.6 kg/m^2 ± 8.8 and 38.2 kg/m^2 ± 4.5 in the *MC4R* group and control group, respectively. See Table 1 for further Participant Characteristics.

Ethical Issues

The project and associated biobank were approved by the ethical committee in Copenhagen (reference number: H-1-2013-093) and the study was performed in accordance with the Helsinki Declaration II. Participation was voluntary and the individuals could at any time retract their consent to participate. ClinicalTrials.gov Identifier: NCT02082496.

METHOD DETAILS

Study Drug

Liraglutide was administered as FlexPen devices (Victoza, Novo Nordisk A/S, Bagsvaerd, Denmark) by subcutaneous injection in the abdomen or thigh. Dosing was initiated at 0.6 mg daily, increasing to 3.0 mg daily over a 5 week long period (0.6 mg, 1.2 mg, 1.8 mg, 2.4 mg and 3.0 mg per week) continuing until 16 weeks of treatment. The liraglutide injection period was not accompanied by any lifestyle counselling, diet or exercise program. See Figure S2 for study design.

Control Visits

During the treatment period the participants attended monthly control visits at the Children's Obesity Clinic (three times in total). Weight, waist and hip circumference, blood pressure and, pulse were measured and fasting blood samples were taken at these visits.

Measurements

Morning weight was measured in light indoor clothes (weight model: SECA, Birmingham, UK), height, waist-and hip circumference (measured with non-elastic tape), blood pressure and pulse (Omron 705IT, Omron Healthcare, Kyoto, Japan) was measured and BMI calculated as the weight in kilos over the height in meters squared (kg/m^2).

Oral Glucose Tolerance Test

Participants met in the morning after an overnight fast at the Children's Obesity Clinic. A cannula was inserted in an antecubital vein and fasting blood samples were drawn at time point −10, −5, and 0 (for measurement of plasma glucose, serum insulin, serum C-peptide, plasma total cholesterol, plasma HDL cholesterol, plasma LDL cholesterol and plasma triglycerides) prior to ingestion of 75 g glucose dissolved in 250 mL water. Subsequently, blood samples were drawn 15, 30, 45, 60, 90, 120, 150, and 180 min after glucose ingestion for measurements of plasma glucose, serum insulin and serum C-peptide.

Dual Energy X-Ray Absorptiometry

Total fat mass and total lean body mass (fat free mass) were assessed by DEXA (GE Lunar iDXA Scanner, ME + 200179, GE Healthcare, Little Chalfont, UK), and performed by trained personnel.

Magnetic Resonance Spectroscopy

MR measurements were performed in 3T Achieva MR-imaging system (Philips Medical Systems, Best, the Netherlands) or in an open 1T Panorama HFO MR-imaging system (Philips Medical Systems, Best, the Netherlands) if body weight exceeded 120 kg. Patients were examined in the supine position. No respiratory triggering was used. Spectroscopy was positioned in the right lobe of the liver avoiding major blood vessels and intrahepatic bile ducts; and in the right psoas muscle. Details concerning MRS obtained by the 3T Achieva and the open IT Panorama, are described in detail in reference (Chabanova et al., 2013) and show good correlation between the two systems. All MR measurements and analyses were performed by trained personnel.

Blood Analyses

Plasma glucose was measured with the hexokinase/glucose-6-phosphate dehydrogenase method (Dimension Vista, Siemens Healthcare, Germany), serum insulin and serum C-peptide were measured with a sandwich ELISA technique (COBAS 6000, Roche Diagnostics, USA). Total plasma cholesterol was measured with a polychromatic endpoint technique (enzymatic determination), plasma HDL cholesterol was measured with an accelerator selective detergent methodology and serum triglyceride was measured by enzymatic determination (Dimension Vista, Siemens Healthcare, Germany). Plasma LDL cholesterol was calculated using the Friedewald formula: LDL cholesterol = total cholesterol - (triglyceride x 0.45+ HDL cholesterol).

QUANTIFICATION AND STATISTICAL ANALYSIS

Calculations

Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as follows (Wallace et al., 2004):

$$HOMA-IR = \frac{\left(fasting\ glucose\ \left(\frac{mmol}{L}\right)\ x\ fasting\ insulin\ \left(\frac{\mu IU}{mL}\right)\right)}{22.5}$$

The Matsuda index of insulin sensitivity was calculated with insulin in μU/mL and glucose in mg/dL (Matsuda and DeFronzo, 1999):

$$Matsuda\ Index = \frac{10.000}{\sqrt{(fasting\ insulin\ x\ fasting\ glucose\ x\ 2\ hr\ insulin\ x\ 2\ hr\ glucose)}}$$

Power Calculation

Sample size was calculated based on prior data indicating a standard deviation (SD) of 5.6 kg for weight change at the end of trial with 3 mg liraglutide (Astrup et al., 2009; Pi-Sunyer et al., 2015). Thus, to be able to detect a clinically relevant weight change difference of 5 kg between matched groups with a probability (power) of 0.8 and $p < 0.05$ we will need 12 experimental subjects and 24 matched control subjects.

Statistics

In order to asses any potential inter-family effects, a general linear model with family number (1-9) as an additional co-variate was performed; however, there were no differences between the nine families. Accordingly, data were analyzed contrasting the *MC4R* group with the matched control group. Treatment effect within the groups were calculated with paired t tests. Differences of the treatment effect between the groups were calculated using a general linear model with age and sex as covariates. Descriptive data are shown as mean ± standard deviation (SD). Insulin, C-peptide and glucose data were not normally distributed why these data are presented as raw mean ± standard error of the mean (SEM) and p values are shown for log-transformed data. For group comparisons, data are shown as mean ± SEM and estimated differences between groups are presented as mean with 95% confidence intervals (95% CI). Total and incremental areas under the curve (AUC) were calculated in GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, using the trapezoidal method. The remaining data were calculated with IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM. A two-tailed p value less than 0.05 was considered significant.

DATA AND SOFTWARE AVAILABILITY

Raw data file have been deposited at Mendeley Data (https://dx.doi.org/10.17632/dryd5vjvz4.1).