Pancreatic Function in *Carboxyl-Ester Lipase* Knockout Mice

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Key Words

Carboxyl-ester lipase • MODY • Exocrine dysfunction

Abstract

Background/Aims: *CEL*-MODY is a monogenic form of diabetes and exocrine pancreatic insufficiency due to mutations in the carboxyl-ester lipase (*CEL*) gene. We aimed to investigate endocrine and exocrine pancreatic function in *CEL* knockout mice (*CELKO*). **Methods:** A knockout mouse model with global targeted deletion of *CEL* was investigated physiologically and histopathologically, and compared to littermate control *CEL*^{+/+} mice at 7 and 12 months on normal chow and high-fat diets (HFD), i.e. 42 and 60% fat by calories. **Results:** *CELKO*^{+/+} and *CELKO*^{-/-} mice showed normal growth and development and normal glucose metabolism on a chow diet. Female *CELKO*^{-/-} mice on 60% HFD, on the other hand, had increased random blood glucose compared to littermate controls ($p = 0.02$), and this was accompanied by a reduction in glucose tolerance that did not reach statistical significance. In these mice there was also islet hyperplasia, however, α - and β -islet cells appeared morphologically normal and pancreatic exocrine function was also normal. **Conclu-**

sion: Although we observed mild glucose intolerance in female mice with whole-body knockout of *CEL*, the full phenotype of human *CEL*-MODY was not reproduced, suggesting that the pathogenic mechanisms involved are more complex than a simple loss of *CEL* function.

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Over the past decade, the study of monogenic forms of diabetes has revealed important new insights into mechanisms of normal β -cell function and β -cell dysfunction, and has facilitated new treatment strategies as in certain cases of neonatal diabetes [1, 2]. Some monogenic mutations in which the disrupted gene seems to affect the progenitor cells for both the endocrine and exocrine cells cause diabetes associated with pancreatic exocrine dysfunction [3–6]. Recently, we have identified a new form of monogenic diabetes (*CEL*-MODY; MODY: maturity-onset diabetes of the young), characterized by β -cell dysfunction, exocrine pancreatic insufficiency and muta-

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tions in the carboxyl-ester lipase (*CEL*) gene, which appear to affect the functional enzyme by decreased stability and secretion in vitro [7]. *CEL* expression in acinar cells but not in islets of healthy C57BL mice has been shown by immunohistochemistry [8]. *CEL* has broad substrate specificity and hydrolyzes mono-, di-, and triacylglycerols, but also cholesteryl and retinyl esters and lysophosphatidylglycerols, and has been referred to by a number of names such as bile-salt-dependent lipase, carboxyl ester hydrolase, cholesterol ester hydrolase, cholesterol esterase, sterol ester hydrolase, phospholipase A1. Human pancreatic juice has been demonstrated to sometimes contain isoforms of pancreatic *CEL* of two different molecular weights, proposed to correspond to heterozygosity for VNTR length. Incomplete glycosylation of the PEST sequences encoded by the VNTR is a less likely explanation, as glycosylation has been shown to be important to *CEL* secretion [9]. Previous studies of the *CEL* knockout mouse model (*CELKO*) have revealed impaired intestinal absorption of esterified cholesterol [10, 11] as well as a significant reduction in the number of chylomicrons and size of the intestinal lipoproteins [12]. In the present study, we have further characterized the endocrine and exocrine pancreatic phenotype of this model.

Methods

CELKO Mice

Previously described *CELKO* mice [10] were rederived by embryo transfer and kindly provided by Dr. Laurence Panicot, Beth Israel Deaconess Medical Center, Boston, Mass., USA. All mice were on a 129Sv-C57BL/6-FVB mixed genetic background, so for all experiments littermates were used as controls. The animals were kept on a 12 h light, 12 h dark cycle and fed a standard rodent chow (Mouse Diet 9F; Taconic Farms, Germantown, N.Y., USA) ad libitum unless otherwise indicated. In the high-fat diet (HFD) experiments, the mice were fed either a 42% fat by calories diet (TD 88137; Harlan Teklad, Madison, Wisc., USA) or a 60% fat by calories diet (D12492; Research Diets Inc., New Brunswick, N.J., USA). Heterozygous mice (*CEL*+/-) were used for breeding. The experimental groups consisted of homozygous knockout mice (*CEL*-/-) compared to littermate controls (*CEL*+/+) referred to as WT (wild-type). Genotyping was performed by PCR using genomic DNA isolated from tail snips. One downstream and two upstream primers for *CEL* were synthesized to differentiate WT, heterozygous, and homozygous knockout mice. The upstream primer UP-WT (5' TCG TGG AGG GCG TCA ACA 3'), and the downstream primer DOWN (5' CCC AAG GGT CCA ACA CGG TA 3'), amplify a 744-bp fragment of the WT *CEL* gene. The upstream primer UP-KO (in the neo gene; 5' CTG CAC GAC GCG AGC TG 3') and primer DOWN amplify a 608-bp fragment of the disrupted *CEL*. Thus, a single band of 608 bp was scored as disruption of both *CEL* alleles, i.e. a *CELKO* mouse. All protocols for animal use and euthanasia were approved by the Institutional

Animal Care Committee of the Joslin Diabetes Center and Harvard Medical School in accordance with National Institutes of Health guidelines or, for studies conducted in Norway, by the Norwegian Research Animal Care Committee in accordance with the Norwegian Animal Welfare Act.

Study Groups and High-Fat Diets

The investigations of chow-fed mice at 7 months included 4 mice in each group, and investigations at 12 months included males [*n* = 3 *CEL*-/-, 4 WT] and females [*n* = 8 *CEL*-/-, 7 WT]. For the high-fat challenges, we used HFDs consisting of either 42.0% fat, 42.7% carbohydrate and 15.3% protein (% of kcal) ('42% HFD'; males [*n* = 5 *CEL*-/-, 5 WT] and females [*n* = 5 *CEL*-/-, 5 WT], 9 months old at baseline) or 60.0% fat, 20% carbohydrate and 20% protein (% of kcal) ('60% HFD'; males [*n* = 8 (*CEL*-/-), 10 WT] and females [*n* = 7 *CEL*-/-, 7 WT], 14 weeks old at baseline). The mice were fed the diets for 12 weeks prior to physiological testing and for 16 weeks prior to morphological studies.

Physiological Parameters

We measured fed and fasting blood glucose levels by a Glucometer Elite (Bayer Health Care, Tarrytown, N.Y., USA) using blood from tail snips. For other analyses, blood was collected in chilled heparinized tubes and centrifuged (5–10 min at 5,000 rpm) and the supernatants were collected and stored at -20°C. Plasma insulin and glucagon levels were measured by ELISA using mouse insulin and glucagon standards, respectively (Crystal Chem Inc., Chicago, Ill., USA). We measured blood glucose and plasma insulin levels in the random-fed state between 8:30 and 11:00 a.m. or in the morning after a 14- to 16-hour overnight fast. Serum triglyceride levels were measured by colorimetric enzyme assay (GPO-Trinder Assay; Sigma, St. Louis, Mo., USA). Free fatty acid levels were measured using the NEFA-Kit-U (Amano Enzyme, Osaka, Japan). We measured serum amylase by an enzyme assay (Raichem, San Diego, Calif., USA).

For glucose tolerance tests (GTTs), mice were fasted overnight (14 h) then injected intraperitoneally (i.p.) with glucose (2 g/kg body weight [b.w.]). Tail vein blood glucose was measured as described above at 0, 15, 30, 60, and 120 min after injection. For insulin tolerance tests (ITTs), fed mice were injected i.p. with insulin (1 U/kg b.w. for females, 1.5 U/kg b.w. for males; Humulin, Lilly, Indianapolis, Ind., USA), and tail vein blood glucose was measured 0, 15, 30, and 60 min after injection. We performed stimulated acute-phase insulin secretion tests after an overnight fast (14 h). Tail vein blood glucose was measured and blood for insulin analyses collected and treated as described above at 0, 15, 30, and 60 min after an i.p. injection of either glucose (3 g/kg b.w.; GSIS) or a combination of glucose in the same dose as above and arginine (0.3 g/kg b.w., Arg-GSIS).

Fat malabsorption was measured by a previously described method by comparing the fecal excretion of fat and the non-absorbable dietary marker sucrose polybehenate to the ratio of benzoic acid to other dietary fatty acids, using gas chromatography analysis as previously described [13]. We analyzed the enzymatic activity of elastase in mouse feces by fluorometry as described by Zenker et al. [14].

Islet Isolation and Assessment of Gene Expression

Islets were obtained by collagenase digestion as previously described [15] and were maintained in RPMI-1640 media supple-

mented with 7 mM glucose and 10% v/v FCS. After overnight incubation, gene expression studies were performed in healthy handpicked islets. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Valencia, Calif., USA) and cDNA was generated by reverse transcriptional DNA synthesis (Applied Biosystems, Foster City, Calif., USA). Gene expression for mouse *CEL*, *glucagon*, *insulin*, *glut-2*, *PDX-1*, *MafA*, *Neuro-D1* and *amylase* was determined by qRT-PCR [16] using appropriate primers (sequences available on request), and normalized for β -actin.

Morphology and Tissue Preparation

Weight and blood glucose levels were decided in fed mice before they were anesthetized and exsanguinated. Blood was collected and prepared as described above. The pancreas was rapidly dissected and divided into two or three parts. Pancreatic samples to be studied by light microscopy were weighed, fixed in pre-chilled zinc-formalin (Z-Fix), then paraffin-embedded, sectioned, hematoxylin and eosin (HE)-stained, and examined by direct microscopy. We measured islet area in all islets in one section from each pancreatic sample using the ColorView Soft Imaging System. Other sections were prepared by immunohistochemical staining for amylase or triple immunostaining for insulin, glucagon and somatostatin. Liver and epididymal fat samples were fixed in 10% formalin, paraffin-embedded, sectioned and HE-stained. Other liver sections were treated with periodic acid-Schiff reagent for immunohistochemical staining of glycogen using standard protocols. For lipid staining, liver and pancreas samples were snap-frozen in liquid nitrogen or embedded in OCT 4853 (Tissue-Tek, Sakura, Japan) and frozen on dry ice, cross-sectioned at -30°C and stained for lipids with Oil-red-O (Polyscientific, Gaithersburg, Md., USA). Tissue samples for protein extraction were snap-frozen in liquid nitrogen and stored at -80°C , while samples for RNA extraction were either directly homogenized in tissue lysis buffer (pancreas), put in RNeasy lysis buffer (Ambion, Austin, Tex., USA) (liver) or snap-frozen (other tissues) and then stored briefly at -80°C .

Immunohistochemistry

Immunohistochemical analyses of pancreas sections were performed as previously described [17]. Antibodies to the following proteins were used: amylase (# sc-12821; Santa Cruz Biotechnology, Santa Cruz, Calif., USA, and donkey anti-goat cy2-conjugated secondary antibody; Jackson Labs, Bar Harbor, Me., USA), insulin (# 4011-01; Linco Research, St. Charles, Mo., USA, and donkey anti-guinea pig-Texas red secondary antibody, Jackson Labs), glucagon (# G2654; Sigma, and donkey anti-mouse-Texas red secondary antibody; Jackson Labs), and somatostatin (# n1551; Dako, and donkey anti-rabbit cy2-conjugated secondary antibody; Jackson Labs).

Statistical Analysis

We employed two-tailed Student's *t* tests of independent groups with assumption of unequal variances and a significance level of 5%. For the GTTs, we also performed analyses of variance (ANOVA) for repeated measures, using baseline measurements as a covariate. Due to non-normal distribution, we used the Mann-Whitney U test for comparisons of the islet area. Statistical analyses were carried out using SPSS.

Fig. 1. Expression of mouse *CEL* mRNA in CELKO and WT mice. *CEL* expression at the mRNA level in the pancreas of WT mice (white bar) and *CEL* $^{-/-}$ mice (CELKO; black bar) was assessed by quantitative real-time PCR.

Results

General Characteristics

CELKO mice were genotyped by PCR as described in Methods, and qRT-PCR of pancreas confirmed the absence of *CEL* expression in the CELKO mice (fig. 1). *CEL* $^{+/+}$, $^{+/-}$ and $^{-/-}$ mice showed normal weight development, lifespan and behavior. When compared to gender-matched controls, CELKO mice showed no weight difference on both normal chow (21.6% fat by calories) and 42 and 60% HFDs (fig. 2a).

Glucose Homeostasis

When placed on a 60% HFD, *CEL* $^{-/-}$ mice gained weight in a similar fashion to controls. Over a 12-week period, males increased their body weights by 69.4 (*CEL* $^{-/-}$) and 70.3% (WT), respectively, while females had 78.0 and 55.0% weight gain, respectively (fig. 2b). Random fed blood glucose levels were mildly, but statistically significantly higher in female *CEL* $^{-/-}$ mice compared to WT controls (146 ± 3 mg/dl [*CEL* $^{-/-}$] and 126 ± 3 mg/dl [WT], $p = 0.002$; fig. 2c), but not in males. Male mice fed the 60% HFD for 12 weeks had increased random fed glucose values compared to age-matched mice on chow diet (189 ± 12 vs. 130 ± 1 mg/dl, $p = 0.0004$ [*CEL* $^{-/-}$], and 214 ± 36 vs. 131 ± 17 mg/dl, $p = 0.02$ [WT]; fig. 2c). WT females fed the 60% HFD for 12 weeks also had increased random fed glucose values compared to age-matched mice on chow diet (151 ± 8 vs. 110 ± 1 mg/dl, $p = 0.004$). Compared to controls, female *CEL* $^{-/-}$ mice appeared to have decreased glucose tolerance on both normal chow and on the 42 and 60% HFDs (fig. 3); however, these differences did not reach statistical sig-

Fig. 2. Weight and glucose homeostasis in CELKO and WT mice. Tail or hind leg vein blood was drawn in the random fed state at 9–11 a.m. and glucose was measured by glucometer. Plasma insulin was measured by ELISA. Results are given as mean $\pm$ SEM. WT, white bars and circles; CEL $^{-/-}$, black bars and circles. **a** Male mice fed the 60% HFD had increased body weight compared to age-matched mice on chow (* $p = 0.002$ [CEL $^{-/-}$], $p = 0.02$ [WT]), but there were no significant differences between CEL $^{-/-}$ and WT mice in either test group. **b** CEL $^{-/-}$ and WT mice were given a 60% HFD challenge for 12 weeks. There were no differences in rate of weight gain for either males or females, although CEL $^{-/-}$ males weighed borderline significantly less than controls at 3 weeks ($p = 0.05$). **c** Random fed glucose values were increased in the 60% HFD group compared to age-matched mice on chow for males (* $p = 0.0004$ [CEL $^{-/-}$], $p = 0.02$ [WT]) and WT females (** $p = 0.004$). Random fed blood glucose was weakly, but statistically, increased in female CEL $^{-/-}$ mice compared to WT controls following a 60% HFD (** $p = 0.02$).

nificance. Surprisingly, male CEL $^{-/-}$ mice on the 42% HFD had somewhat better glucose tolerance than controls, with a 32% reduction in the area under the curve ($p = 0.03$). This was confirmed by ANOVA for repeated measures ($F = 13.456$; $p = 0.008$). There were no significant differences in fasting blood glucose for any group (fig. 3), and fasting glucose levels increased after 12 weeks on the 60% HFD compared to baseline in both CEL $^{-/-}$ and control mice (males: +82% [CEL $^{-/-}$] and +90% [WT]; females: +36% [CEL $^{-/-}$] and +54% [WT]).

Insulin sensitivity as measured by an ITT was normal in female mice on chow diet or after 12 weeks on a 42% HFD (fig. 4a), or 12 weeks on a 60% HFD (fig. 4b) and in

12-month-old males (data not shown). The 60% HFD induced insulin resistance in male mice as measured by the ITT (fig. 4b), but no differences were detected between the genotypes. No statistical differences were observed between CEL $^{-/-}$ and WT mice for fasting plasma insulin levels (data not shown) or random fed plasma insulin levels (fig. 5a). Likewise, the insulin secretory response was not different between CEL $^{-/-}$ females and controls following arginine-enhanced glucose stimulation (fig. 5b, c). Fasting serum leptin values were not significantly different between CEL $^{-/-}$ females and controls after 12 weeks on the 42% HFD (19.9 ± 7.7 and 14.1 ± 9.6 , respectively).

Fig. 3. GTTs in CELKO and WT mice on chow or HFD. Open circles, WT; filled circles, CEL^{-/-}. Results are given as mean $\pm$ SEM. A GTT was performed in (a) females and (b) males after a 12- to 14-hour fast by i.p. injection of glucose (2 g/kg b.w.). Although the

60% HFD induced decreased glucose tolerance compared to age-matched chow-fed mice, particularly in males, there were no differences between CEL^{-/-} mice and controls in any group.

Fig. 4. ITTs in CELKO and WT female mice on chow or HFD. Open circles, WT; filled circles, CEL^{-/-}. Results are given as mean $\pm$ SEM. An ITT was performed by i.p. injection of insulin (0.75 U/kg b.w.) in 12-month-old (a) and 7-month-old (b) mice. There were no differences between CEL^{-/-} females and controls at either (a) chow diet (n = 5; left panel) or 42% HFD (n = 5; right panel), or (b) 60% HFD (n = 7; left panel). The 60% HFD induced insulin resistance in male mice (n = 8 CEL^{-/-}, 10 WT; B, right panel), but no differences were detected between the genotypes.

Fig. 5. Insulin response and pancreatic morphology in CELKO and WT mice. An arginine-enhanced glucose-stimulated insulin secretion test was performed by the i.p. injection of a combination of 3 g/kg b.w. of glucose and 0.3 g/kg b.w. of arginine and collection of blood for insulin analysis at 0, 15, 30, and 60 min. Insulin was measured by ELISA. Results are given as mean $\pm$ SEM. Open circles, WT; filled circles, CEL^{-/-}. No statistically significant differences were observed between chow-fed CEL^{-/-} and in 12-month-old WT mice for random fed plasma insulin (**a**; graph shows results for females) or in an arginine-enhanced glucose-

stimulated insulin secretion test (**b, c**; females, $n = 5$). Islet size and number as well as the organization of the exocrine pancreatic tissue was normal in HE-stained sections from CEL^{-/-} male mice and not different from controls in either 12-month-old chow-fed mice (**d**) or in 7-month-old chow-fed mice after a 60% HFD (**e**) (10 $\times$ resolution). **f** Triple immunohistochemical staining for insulin (blue), glucagon (red) and somatostatin (green) showed normal distribution of the corresponding endocrine β -, α - and δ -cell types in 12-month-old chow-fed males (40 $\times$).

CEL^{-/-} females showed a trend towards higher random fed plasma glucagon levels compared to controls (69.1 ± 11.4 [CEL^{-/-}, $n = 3$] and 36.9 ± 11.2 [wt, $n = 2$]; $p = 0.07$), which was supported by a trend towards increased glucagon gene expression in isolated islets as

shown by qRT-PCR; however, neither reached statistical significance. By contrast, there were no differences for plasma glucagon between CEL^{-/-} and WT males (plasma glucagon: 40.4 ± 8.2 [CEL^{-/-}, $n = 3$] and 35.6 ± 5.7 [wt, $n = 4$]). No differences in expression levels were detected

Table 1. Serum lipid levels and exocrine pancreatic function

	Chow – 7 months		Chow – 12 months		42% HFD – 12 months	
	CEL+/+	CEL–/–	CEL+/+	CEL–/–	CEL+/+	CEL–/–
<i>Serum lipid levels</i>						
Total serum cholesterol, mg/dl	163 ± 11	188 ± 18	117 ± 11	132 ± 15	184 ± 18	218 ± 33
Serum triglycerides, mg/dl	111 ± 9	128 ± 15	57 ± 7	49 ± 10	98 ± 9	96 ± 9
Free fatty acids, mEq/l	1.42 ± 0.05	1.39 ± 0.06	0.77 ± 0.23	0.67 ± 0.18	1.15 ± 0.06	1.13 ± 0.07
<i>Exocrine pancreatic function</i>						
Serum amylase, U/l	1,028 ± 92	1,473 ± 171	748 ± 231	735 ± 186	2,833 ± 1,148	2,815 ± 228
Fat absorption, %	NI	NI	96 ± 2	95 ± 1	94 ± 4	84 ± 8
Fecal elastase, mU/g	NI	NI	144 ± 42	147 ± 56	178 ± 32	155 ± 35

The results represent mixed sex groups and are presented as mean ± SEM.

between CEL–/– and WT mice for insulin (Ins-1), the rate-limiting enzyme of glycolysis-glucokinase (GCK), the glucose transporter GLUT-2, or any of the transcription factors tested (PDX-1, Neuro-D1, MafA), as demonstrated by qRT-PCR of RNA extracted from isolated islets from 5 random fed 12-month-old female CEL–/– mice and 5 littermate controls.

Serum Lipid Levels and Exocrine Pancreatic Function

CEL participates in the absorption of cholesterol esters, and reduced lipid levels could indicate malabsorption. However, no differences were observed between CEL–/– mice and controls for serum cholesterol, triglycerides or free fatty acids (table 1). Exocrine pancreatic function as assessed by fecal fat absorption, measured as the ratio of excreted sucrose behenate (a dietary marker) to excreted lipids in the stool, was not decreased in CEL–/– mice fed chow or a 42% HFD (table 1). Serum amylase was within the normal range and did not differ between the genotypes on either chow diet or 42% HFD (table 1). Fecal elastase was statistically higher in CEL–/– mice (n = 4) compared to WT controls (n = 6) after the 60% HFD (p = 0.03), whereas no differences were seen in 12-month-old chow-fed mice or in mice fed the 42% HFD.

Morphological Studies

Morphological analyses of HE-stained pancreas sections revealed no pathology in the acinar tissue, and similar islet size and number between chow-fed CEL–/– mice and controls (fig. 5). For mice fed 60% HFD, islet area was statistically smaller in CEL–/– males compared to WT (22,233 ± 2,730 vs. 35,305 ± 4471 μm², respectively;

p = 0.03), whereas in females the islet area was statistically larger in CEL–/– than in controls (17,837 ± 2,535 vs. 7,659 ± 1,147 μm², respectively; p = 0.03). Immunostaining using antibodies against insulin, glucagon and somatostatin showed a relatively normal distribution of insulin-secreting β cells and glucagon-secreting α cells. There was no significant inflammation or fatty infiltration in the islets.

Of 3 chow-fed CEL–/– males, 2 had severe fatty infiltration of the liver on histological examination. This is compared to only 1 of 4 WT males, and in the latter, hepatic steatosis was classified as moderate. The steatosis was predominantly microvesicular and centrilobular. Following 16 weeks of a 42% HFD, 4 of 5 CEL–/– males had moderate or severe steatosis, compared to 3 of 5 controls. None of the chow-fed females had any evidence of liver steatosis, but 2 of 5 CEL–/– females showed moderate steatosis after the HFD. Some mice on HFD had a mild to moderate inflammation of the epididymal fat, but there was no difference between the genotypes. Fat cell size seemed similar between groups.

Discussion

Recently, we described a novel syndrome of pancreatic β-cell dysfunction and exocrine deficiency caused by mutations in the *CEL* gene [7]. These mutations resulted in reduction in functional enzyme, indicating that loss of function could be part of the molecular mechanism. In order to explore this further, we have studied the *CELKO* mouse model.

As gender differences in the penetrance of a diabetic phenotype are frequently encountered in mouse models [18, 19], we chose to study mice of both sexes. We found no evidence of altered pancreatic endocrine or exocrine function in the mice of either gender fed on a normal, low-fat chow diet through the age of 12 months. The human mutation carriers are not insulin-resistant, and neither were the mice.

To explore this further, we introduced two different HFDs, a common method of studying the interaction between genes and environment in the creation of diabetes and related syndromes. In this case, challenging the mice with a 42% HFD for 12 weeks was insufficient in inducing metabolic changes promoting excessive weight gain or a diabetic phenotype compared to chow diet, nor did it induce insulin resistance. Indeed, leptin values were only minimally elevated in the WT mice fed the 42% HFD compared to WT mice fed normal chow (data not shown). Consequently, it was not surprising that this diet did not provoke or unmask any differences in glucose homeostasis between *CEL*^{-/-} and control mice.

In contrast, male mice fed a 60% HFD for 12 weeks were significantly heavier than other mice of the same strain at 7 months of age, and were insulin-resistant, although there were no statistical differences between the genotypes. Female *CEL*^{-/-} mice fed the 60% HFD, on the other hand, developed random fed hyperglycemia compared to 60% HFD controls, despite normal insulin tolerance, suggesting a mild diabetic phenotype. The larger mean islet area of *CEL*^{-/-} females compared to controls likely indicates compensatory islet hyperplasia. However, analyses at the mRNA level showed no differences in the expression levels for insulin, the glucose transporter GLUT-2, or key transcription factors involved in β -cell growth. Furthermore, the insulin secretory response was similar between genotypes. This is in contrast to findings in humans with *CEL*-MODY, where mutations in *CEL* lead to reduced insulin secretion in mutation carriers and promote subsequent development of diabetes [7]. It is possible that the milder phenotype in the *CEL*KO mice is linked to the mixed 129Sv-C57BL/6-FVB genetic background, and that knockout of *CEL* in another strain might yield more severe impairment of metabolism. It is worth noting that mice with targeted disruption of the insulin receptor and insulin substrate-1 exhibit remarkably different phenotypes when created on a mixed genetic background or backcrossed onto individual inbred strains [20]. A similar scenario was recently observed in mice with a knockout of the uncoupling protein 2 gene [21]. It is also possible that the current study would not

have the power to detect a mild phenotype due to low penetrance of the genetic disorder. Thus, it is likely that creating the *CEL* KO on different inbred strains (e.g. C57BL/6, 129Sv, etc.) will unmask a phenotype that is influenced by one or more modifier genes close to the target locus.

In humans, the pancreatic exocrine disease as demonstrated by fecal elastase seems to precede diabetes, and all mutation carriers with diabetes exhibit steatorrhea. Mutations in *CEL* also lead to pancreatic lipomatosis in affected subjects [7, 22]. None of the mice, however, developed any exocrine dysfunction on either chow or HFD; indeed, fecal elastase was higher in *CEL*^{-/-} mice than in WT controls on the 60% HFD. The biological relevance of the fecal elastase test may be limited to a binary classification of 'low' or 'normal' fecal elastase, and may not indicate the degree of pancreatic exocrine dysfunction [23]. There are to our knowledge no generally acknowledged cutoff values for fecal elastase for mice. We suspect that all measured fecal elastase activity levels represent activities within the normal range, and that the observed statistical difference occurred by chance. The morphology of the exocrine pancreas was also normal in *CEL*KO mice at the age of 12 months, and there was no evidence of lipomatosis. A recent report based on studies of pancreatic triglyceride lipase (*PTL*) and *CEL* double knockout mice, revealed that disruption of both genes resulted in 40–50% reduction of TAG absorption [24]. *PTL* appeared to be the more important enzyme for retinyl ester hydrolysis, and *CEL* seemed likely to be the single enzyme responsible for cholesteryl ester hydrolysis. The study showed no difference in lipid absorption between WT and *CEL*^{-/-} mice even on a high-fat/high-cholesterol diet. These results indicate that knockout of only *CEL* in mice might not be expected to result in steatorrhea in the event of an intact lipase function, and that the type of dietary fat might influence whether steatorrhea would occur. We employed two different HFDs, based on milk fat ('western diet') and lard, respectively, thus covering a spectrum of fat types expected to coincide with the diets of the human patients with the syndrome, who do display clinically significant steatorrhea despite no indications of concomitant disruption of the lipase gene.

CEL is expressed at both the mRNA and protein level in the liver [25]. The secreted enzyme has been suggested to be involved in the metabolism of neutral lipid esters of chylomicrons or chylomicron remnants, but studies have later shown that *CEL* deficiency in *CEL*^{-/-} mice had no effect on hepatic uptake of chylomicron-derived retinol esters and did not influence serum levels of retinol or ret-

inol-binding protein [26]. In accordance with previous reports, we observed no difference in serum cholesterol values. There are reports of altered liver function and morphology when the *HNF4A* gene causing *HNF4A-MODY* is disrupted [27, 28]. Our results demonstrate that male *CEL*^{-/-} mice on chow as well as on a 42% HFD had a higher incidence of moderate and severe liver steatosis compared to controls, however, the numbers were low and the result should be judged with caution. Otherwise, liver morphology was normal.

It should be noted that whole-gene deletion models such as the *CELKO* mouse are different from single-base deletions reported in the corresponding human disease. It is therefore possible that the mutation in *CEL-MODY* gives rise to an altered effect of the mutated gene product, such as retention in the endoplasmic reticulum or Golgi apparatus during posttranslational modification giving rise to endoplasmic reticulum stress or inflammation. This is supported by the fact that in humans the mutation is inherited dominantly, affected patients being heterozygotes, with one normal and one abnormal copy of *CEL*. Moreover, many late-onset human phenotypes are not apparent in mice [29, 30] and as noted above, the genetic background of the *CELKO* mouse might obscure the effect of the mutation. Indeed, previous studies have demonstrated a striking difference in phenotype when mouse knockouts or transgenics are created on different genetic backgrounds [19, 20, 31, 32].

Furthermore, there are important differences in the *CEL* gene structure between mouse and man. Mouse *Cel* is a single-copy gene spanning approximately 7.2 kb. In contrast, the 9,850-bp long human *CEL* is located in tandem with a pseudogene called *CELL* (or *CELP*), and *CEL* is suggested to have evolved from *CELL* through gene duplication, following which the original gene has become inactivated [reviewed in 9]. This gene duplication has not occurred in the mouse genome, and thus the human pseudogene (and not *CEL*) seems to be the equivalent of mouse *Cel* at the genetic level. In line with this, the most important elements in the promoters for the mouse and the human gene, respectively, are different [33]. The last *CEL* exon contains a VNTR encoding a highly variable number of repeats; in man, most frequently 16 repeats of 11 amino acids, but repeat numbers from 7 to 21 have been observed. The variability of the VNTR between species is high, with three repeats in mice. It has been clearly demonstrated that truncation of the C-terminal domain does not affect the enzymatic activity of *CEL*, although the interaction with bile salt may be compromised [34]. In spite of these differences, *CEL* is expressed at

comparable levels in the exocrine pancreas in both mouse and human [33]. However, the differences at the genetic level may indicate that *CEL* has obtained a somewhat different role in mouse and man throughout evolution, and that the functional overlap with other enzymes may differ. Thus, it is conceivable that the mouse may not be an optimal model for the *CEL-MODY* syndrome.

In conclusion, whole-body knockout of *CEL* in mice seems to promote a mild diabetic phenotype in female mice particularly after a 60% high-fat challenge, but did not recapitulate the full phenotype of human *CEL-MODY*. Most likely, the mechanism of *CEL-MODY* does not involve a simple loss-of-function effect. It is also possible that the role of *CEL* varies between species. Further studies are warranted to reveal the pathogenesis. We are currently investigating a transgenic mouse model where we seek to address these questions.

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