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Pancreatic beta cell failure in obese mice with human-like CMP-Neu5Ac hydroxylase deficiency

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science

in

Biology

by

Sarah Kavaler

Committee in charge:

Professor Jane J. Kim, Chair
Professor Stephen M. Hedrick, Co-Chair
Professor Gen-Sheng Feng

2011

The Thesis of Sarah Kavaler is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California, San Diego

2011

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ABSTRACT OF THE THESIS

Pancreatic beta cell failure in obese mice with human-like CMP-Neu5Ac hydroxylase deficiency

by

Sarah Kavaler

Master of Science in Biology

University of California, San Diego, 2011

Professor Jane J. Kim, Chair

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Type 2 diabetes is highly prevalent in human populations, particularly in obese individuals, and is characterized by progressive pancreatic beta cell dysfunction and insulin resistance. Most mammals, including old world primates, express two major kinds of sialic acids, *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc), typically found at the distal ends of glycoconjugate chains at the cell surface. Humans are uniquely unable to produce endogenous Neu5Gc due to an inactivating mutation in the *CMAH* (CMP-Neu5Ac hydroxylase) gene. The CMAH enzyme catalyzes the generation of CMP-Neu5Gc by the transfer of a single oxygen atom to the acyl group of CMP-Neu5Ac. Here, we show that mice bearing a human-like deletion of the *Cmah* gene exhibit fasting hyperglycemia and glucose intolerance following a high fat diet. This

phenotype is caused not by worsened insulin resistance but by compromised pancreatic beta cell function associated with a 65% decrease in islet size and area and 50% decrease in islet number. Obese *Cmah* null mice also show an ~40% reduction in response to insulin secretagogues *in vivo*. These findings show that human evolution-like changes in sialic acid composition impair pancreatic beta cell function and exacerbate glucose intolerance in mice. This may lend insight into the pathogenesis of type 2 diabetes in obese humans.

INTRODUCTION

Two hallmarks in the pathogenesis of type 2 diabetes are insulin resistance and failure of pancreatic β cell compensation. Obesity in humans causes a state of insulin resistance, requiring increased insulin secretion to maintain relatively normal glucose levels. This increase in insulin secretion is usually accompanied by an increase in β cell mass. In individuals that develop type 2 diabetes, β cell compensation declines and relative insulin insufficiency develops, leading to glucose intolerance and eventually frank diabetes (1).

Sialic acids represent a class of 9-carbon monosaccharides that are abundantly expressed at the outermost region of the mammalian cellular glycocalyx. They are dominated by two major types: N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc). Cellular Neu5Gc is generated by the transfer of a single oxygen atom to the acyl group of Neu5Ac, catalyzed by the CMAH (CMP-Neu5Ac hydroxylase) enzyme in the cytosol (2). Both Neu5Ac and Neu5Gc are transported into the Golgi, where they may be used as donors to newly synthesized glycoproteins and glycolipids destined for the cell surface.

Although humans and chimpanzees share nearly 99% genetic identity, one rare difference is a human-specific inactivating mutation in the CMAH gene. The human mutation in CMAH consists of a 92 bp deletion in exon 6 that results in a frame-shift and premature translation termination, producing a small 72 amino acid fragment. This deletion is universal in all human populations, but absent in all other mammals, including the African great apes (3). The mutation in the CMAH gene is thought to have occurred

approximately 2-3 million years ago, just prior to the emergence of the genus Homo (3), and resulted in the complete absence of endogenous Neu5Gc expression in all human tissues (4).

Humans demonstrate a marked propensity to develop type 2 diabetes. Although strong environmental factors such as diet certainly contribute to this phenotype, there is some evidence to suggest that intrinsic differences in pancreatic beta cell function exist between humans and other species. For example, although features of type 2 diabetes have been observed in obese rhesus monkeys and other old world primates (5, 6), pancreatic β cell function appears to be more severely impaired in humans. Adult rhesus monkeys have significantly lower fasting glucose levels, markedly higher fasting insulin levels, and a higher acute insulin response to glucose when compared to humans in both lean and obese states (7). Islet size and area are also significantly larger in monkeys (8). Insulin secretion is universally biphasic, with the first phase representing the first spike of insulin release occurring within 10 minutes after glucose exposure. While loss of first-phase insulin secretion is described as one of the earliest detectable defects in humans during the prodrome to overt diabetes (9), the loss of first-phase insulin release is a very late event in monkeys (10).

Although glycosylation of the Glut2 transporter has been shown to affect its expression in pancreatic β cells and impair insulin secretion (11), few other studies to date have addressed how changes in glycan structure at the cell surface modulate glucose homeostasis. To investigate the role of CMAH in glucose metabolism we studied Cmah null mice that were generated using Cre-mediated excision of exon 6, yielding a genotype

essentially identical to the human mutation (4). We find that CMAH null mice fed a high fat diet manifest worsened glucose tolerance secondary to pancreatic β cell dysfunction. These data show that sialylation with N-glycolylneuraminic acid plays an important role in β cell function and that its loss contributes to β cell dysfunction in mice. We speculate that this human evolutionary loss of CMAH function may contribute to the development of type 2 diabetes in human obesity.

MATERIALS AND METHODS

Mutant Mice

Cmah knockout mice were created via Cre-LoxP-mediated deletion of 92 bp in exon 6 (4). This frame shift mutation produces a 72 amino acid nonfunctional peptide fragment that mimics the human mutation. Mice with the null mutation were backcrossed onto the C57/BL6 background for at least 10 generations prior to study. Wild-type C57/BL6 mice purchased from Harlan Laboratories were used as controls. Only male mice were used for study. All animal procedures adhered to University of California, San Diego institutional guidelines for the ethical treatment of animals.

In vivo metabolic testing

The study was initiated when mice were 3 months of age. Mice were placed on either a normal chow diet (NCD) containing 16% kcal from fat, or a high fat diet (HFD) containing 60% kcal from fat (Research Diets, Inc). Body weight measurements were obtained weekly.

For glucose tolerance (GTT) or insulin tolerance (ITT) testing, mice were placed into weight-matched groups. Animals were allowed to feed overnight and then fasted for 6 hours (GTT) or 4 hours (ITT). After collection of basal blood work, either dextrose (1g/kg) or recombinant human insulin (0.50 units/kg, Novolog) was injected intraperitoneally. Blood samples were drawn by tail vein sampling at 0, 15, 30, 60, 90, and 120 min after injection for whole blood glucose and/or plasma insulin measurement. Blood glucose was measured using a OneTouch Ultra 2 glucometer. Plasma insulin was quantified using the Ultra Sensitive Mouse Insulin ELISA kit (ALPCO).

To evaluate first-phase insulin secretion, mice were fasted for 6 hours, then subjected to dextrose (2 g/kg) or arginine (1 g/kg) challenge via intraperitoneal injection. Plasma insulin was measured at 0, 2, 5, 15, and 30 minutes after injection.

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using glucose and insulin concentrations obtained after 6 hours of food withdrawal, using the following formula: [fasting blood glucose (mg/dl) × fasting insulin (μU/ml)] ÷ 405 (12).

Glucose-stimulated insulin secretion studies in isolated islets

Primary mouse islets were isolated via intraductal collagenase (Roche) digestion and density centrifugation as described previously (60). Following isolation, islets were handpicked and maintained in DMEM without phenol red, 5.5 mM glucose plus 10% (v/v) FBS (Sigma-Aldrich), and penicillin and streptomycin. Following overnight incubation, glucose stimulated insulin secretion (GSIS) studies were performed. Briefly, islets were incubated first in Krebs Ringer HEPES buffer for a 1 hour starvation period. Islets were then incubated in Krebs Ringer HEPES buffer containing either 2.8 or 16.7mM glucose for 1 hour. Insulin secretion was measured using the Ultra Sensitive Insulin ELISA kit (ALPCO). All samples were run in triplicate. Values were adjusted to reflect insulin secretion per islet per hour in each sample.

Immunohistochemistry

To identify Neu5Gc expression in pancreatic tissues, deparaffinized tissue sections were blocked for endogenous peroxidase and endogenous biotin and overlaid with 0.5% fish gelatin in phosphate-buffered saline Tween 20. Sections were then incubated with chicken immunoglobulin Y or chicken anti-Neu5Gc antibody, followed by detection of specific binding

using biotinylated anti-chicken and horse radish peroxidase-labeled streptavidin. Nuclei were counterstained with Mayers and the slides were aqueous mounted for viewing and analysis.

For islet morphometric study, paraffin-embedded pancreatic tissues were first stained with rabbit anti-insulin (DAKO N1542) and anti-glucagon (DAKO A0565) antibodies. Secondary antibodies for immunofluorescence detection were Cy3-conjugated anti-rabbit (Jackson 111-165-144) and Alexa-488 nm conjugated anti-guinea-pig (Molecular Probe S-40174A). Specimens were viewed on a Zeiss AxioObserver Z1 microscope and 24-bit TIFF images were acquired with a Zeiss AxioCam digital camera driven by Zeiss AxioVision v.3.1 software. Images were processed with Adobe Photoshop C32 9.0. Morphometry was performed on a minimum of five mutants and five wild-type controls using Image-Pro Plus v.5.0.1 (Media Cybernetics). Relative pancreatic areas of β-cells were calculated as described previously (13). Mean islet size and number were determined using NIH Image J software (Bethesda, Md., USA).

Statistical Analysis

All values are expressed as means ± SEM unless otherwise noted. We used analysis of variance (ANOVA) to determine differences between groups, and repeated measures ANOVA testing for comparisons over time. *P* values of <0.05 were considered significant.

RESULTS

Cmah null mice demonstrate fasting hyperglycemia and impaired glucose tolerance

The study was initiated when *Cmah* null and wild-type (WT) control mice were 3 months of age. Mice were placed on either a normal chow diet (NCD) containing 16% kcal from fat, or a high fat diet (HFD) containing 60% kcal from fat. As shown in Figure 1, HFD-fed *Cmah*$^{-/-}$ mice gained less weight than WT controls, with a significant difference in body weight by 5 weeks of HFD feeding (*Cmah-/-:* 33.5 ± 1.4 vs. WT: 37.4 ± 0.85 g, p = 0.04) (Figure 1A and B). Food intake measured over a 4 week period in HFD-fed mice showed that *Cmah*$^{-/-}$ mice ate a smaller quantity than WT mice (*Cmah-/-:* 2.33 ± 0.03 vs. WT: 2.50 ± 0.08 g/mouse/day, p = 0.03), although this difference was not significant when normalized to body weight.

We conducted *in vivo* metabolic studies after 5 weeks of either HFD or NCD, when *Cmah*$^{-/-}$ and WT mice were approximately 4 months of age. Although mice on NCD had similar body weights, *Cmah*$^{-/-}$ mice demonstrated significantly higher blood glucose after a 4 hour fast (*Cmah*$^{-/-}$*:* 174 ± 4 vs. WT: 150 ± 4 mg/dL, p = 0.01) (Figure 1C). HFD mice were placed in weight-matched groups in order to determine the effect of genotype independent of weight. HFD significantly increased fasting blood glucose values in both genotypes, consistent with obesity-induced insulin resistance. However, HFD-fed *Cmah*$^{-/-}$ mice exhibited further worsening in fasting hyperglycemia when compared to diet-matched controls (*Cmah*$^{-/-}$*:* 217 ± 9 vs. WT: 194 ± 9 mg/dL, p = 0.04) (Figure 1C).

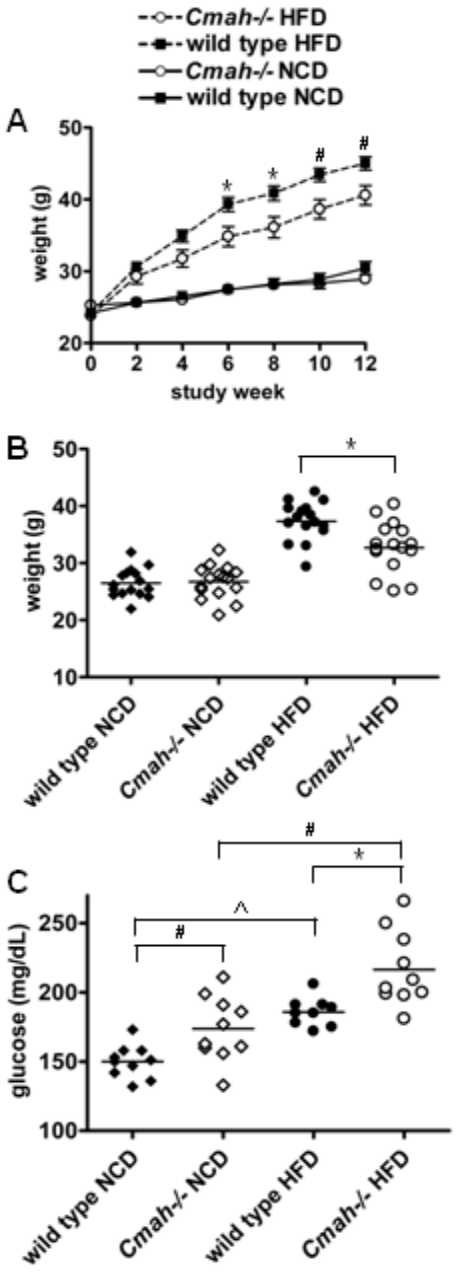

Figure 1. Effect of *Cmah* inactivation on body weight and fasting glucose

(A) Weights of *Cmah* null and wild-type mice on normal chow (NCD) and 60% high fat diet (HFD) (n=16 per group) .

(B) Body weight distribution of *Cmah* null and wild-type mice after 5 weeks of HFD feeding.

(C) Fasting blood glucose values in *Cmah* null and wild-type mice on both NCD and HFD following a 4 hour fast after 5 weeks of HFD feeding (n=10 per group).

Data are represented as the mean ± SEM. *$p<0.05$, $^{\#}p<0.01$ ^$p<0.001$.

To further explore these differences in fasting glucose, we subjected all cohorts to glucose tolerance testing. NCD-fed *Cmah*$^{-/-}$ were mildly glucose intolerant, with an increase in area-under-the-curve glucose excursion when compared to WT controls following intraperitoneal dextrose injection (Figure 2A and B). HFD-fed *Cmah*$^{-/-}$ and WT mice demonstrated higher glucose values compared to NCD-fed lean mice, consistent with obesity-induced insulin resistance. However, HFD-fed *Cmah*$^{-/-}$ mice exhibited more severely impaired glucose tolerance when compared to HFD-fed WT controls (Figure 2A and B). Plasma insulin measured concurrently during glucose tolerance testing showed no differences in area-under-the-curve insulin secretion between NCD-fed groups. Insulin values were significantly raised in HFD-fed groups, reflecting hyperinsulinemia in response to obesity-induced insulin resistance. Interestingly, insulin secretion was reduced in HFD-fed *Cmah*$^{-/-}$ mice when compared to diet-matched controls (Figures 2C and D), suggesting that impaired insulin secretion contributed to glucose intolerance in these mice.

We then performed insulin tolerance testing in NCD and HFD mice. NCD-fed *Cmah* null mice showed higher blood glucose values when compared to WT following intraperitoneal insulin injection (Figure 2E). However, HFD fed mice showed no differences between genotypes following intraperitoneal insulin injection when measuring either absolute glucose values (Figure 2F) or percent glucose decrease (data not shown), confirming that differences in glucose tolerance in the obese state were not secondary to differences in insulin sensitivity. When we performed homeostatic model assessment (HOMA) analysis as a measure of insulin resistance (12), HOMA-IR values

were higher in fasted NCD-fed *Cmah*$^{-/-}$ mice, indicating the presence of moderate insulin resistance (*Cmah*$^{-/-}$*:* 16.70 ± 2.16 vs. WT: 7.49 ± 1.30, p = 0.04). HFD significantly increased HOMA-IR values in both genotypes, consistent with severe obesity-induced insulin resistance. However, HOMA-IR values on HFD were similar between genotypes, indicating that insulin resistance was not further worsened in obese *Cmah-/-* mice (Figure 2G).

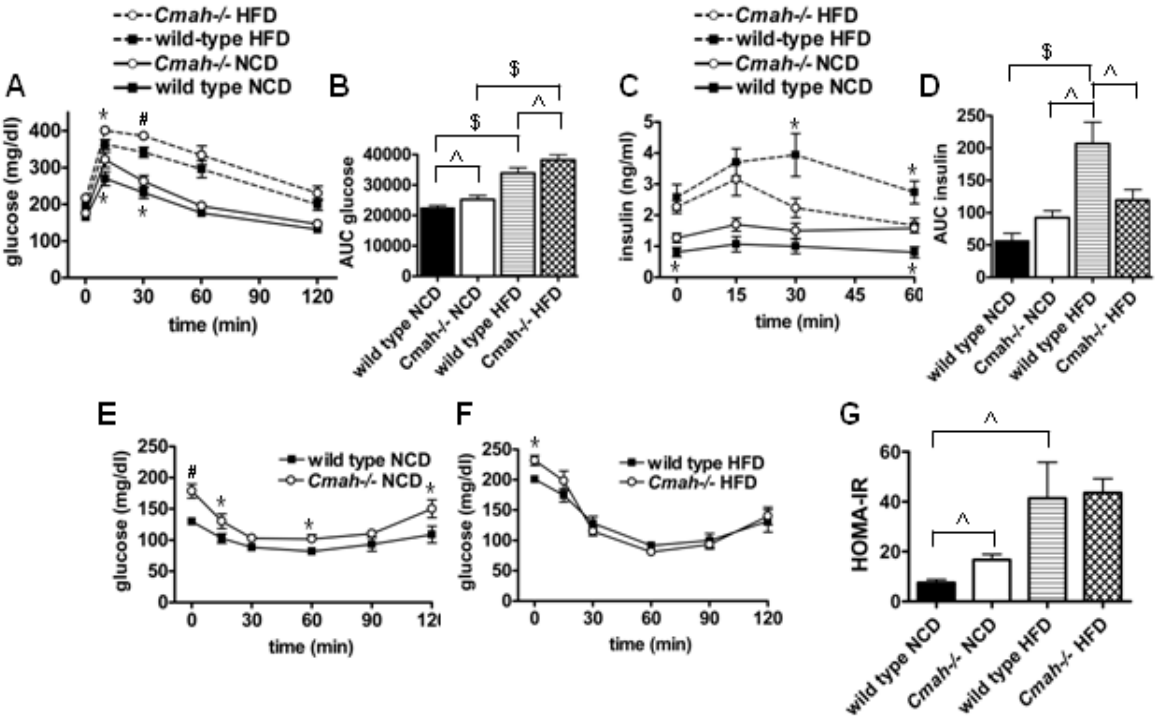

Figure 2. Impaired glucose tolerance in *Cmah* null mice

(A) Intraperitoneal (IP) glucose tolerance testing in both NCD and HFD mice. Whole-blood glucose was measured following IP dextrose 1 g/kg. *$p<0.05$ vs. diet-matched WT, $^{\#}p<0.01$ vs. diet-matched WT (n=7 per group).

(B) Area-under-the-curve (AUC) glucose during IPGTT. $^{\wedge}p<0.05$, $^{\$}p<0.001$

(C) Plasma insulin values measured during IPGTT. *$p<0.05$ vs. diet-matched WT (n=7 per group).

(D) Area-under-the-curve (AUC) insulin during IPGTT. $^{\wedge}p<0.05$, $^{\$}p<0.001$.

(E-F) Insulin tolerance testing in (A) NCD-fed or (B) HFD-fed mice. Whole-blood glucose was measured following IP insulin 0.50 units/kg. *$p<0.05$ vs. diet-matched WT (n=7 NCD-fed males mice per group and n=6 HFD-fed male mice per group).
Data are represented as the mean ± SEM.

(G) Homeostatic model assessment as a measure of insulin resistance (HOMA-IR) (n=7 per group). $^{\wedge}p<0.05$.

Cmah null mice show impaired insulin secretion in response to glucose and arginine stimulation _in vivo_.

Since we did not observe significant differences in insulin sensitivity between genotypes on HFD, we next conducted *in vivo* measurements of acute insulin secretion in response to intraperitoneal glucose or arginine to assess pancreatic β cell function. There were no genotype-specific differences in NCD-fed mice in insulin secretory responses to either secretagogue. HFD enhanced the response of WT mice to glucose stimulation, indicating pancreatic β cell compensation in the face of increased peripheral insulin resistance. By contrast, the insulin secretory response to glucose was significantly diminished in HFD-fed *Cmah*$^{-/-}$ mice compared to diet-matched WT controls (Figure 3A and B).

To investigate potential defects in distal steps of the stimulus-secretion pathway, we administered arginine *in vivo*. Arginine is known to trigger insulin secretion by inducing membrane depolarization, subsequent calcium influx, and insulin exocytosis (14), while bypassing glucose transport and glycolysis. Again, acute insulin secretion was markedly higher in response to arginine in HFD-fed wild-type mice when compared to NCD-fed groups, consistent with compensatory hyperinsulinemia. Moreover, we again observed a significant decrease in insulin secretion in HFD-fed *Cmah*$^{-/-}$ mice when compared to HFD-fed WT controls (Figure 3C and D).

Glucose-stimulated insulin secretion in isolated islets in _Cmah_ null mice

In an effort to further characterize the nature of pancreatic β cell dysfunction, pancreatic islets of mice were isolated and subjected to either low (2.8 mM) or high (16.7 mM) concentrations of glucose. Insulin secretion was then measured and corrected for the total number of islets in each sample. Glucose-stimulated insulin secretion was higher in islets isolated from NCD-fed *Cmah*$^{-/-}$mice when compared to diet-matched controls following exposure to high glucose concentrations (*Cmah*$^{-/-}$*:* 1.72 ± 0.28 vs. WT: 0.65 ± 0.13 ng/hour/islet, p=0.02) (Figure 3E), perhaps in response to their moderately insulin resistant state. Insulin secretion was considerably higher in HFD-fed wild-type mice when compared to their NCD-fed counterparts, consistent with severe obesity-associated insulin resistance and compensatory hyperinsulinemia. In contrast, insulin secretion in response to low glucose dosage was significantly lower in islets of HFD-fed *Cmah*$^{-/-}$ mice compared to their diet-matched controls in response to low glucose concentrations (*Cmah*$^{-/-}$*:* 1.03 ± 0.32 vs. WT: 2.79 ± 0.50 ng/hour/islet, p=0.03) but was not significantly changed in response to high glucose concentrations (Figure 3E).

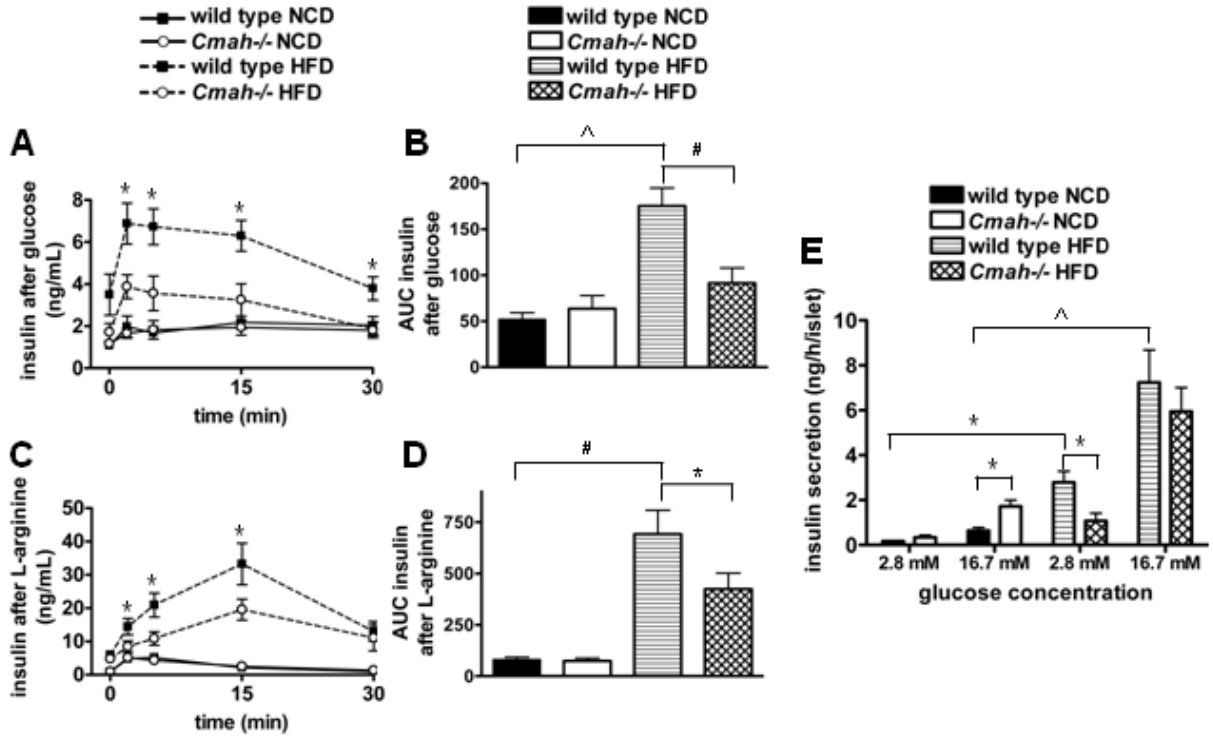

Figure 3. *Cmah* null mice show impaired insulin secretion in response to glucose and arginine stimulation

(A-B) *In vivo* insulin secretion following glucose injection. (A) Serum insulin was collected following IP dextrose 2 g/kg in NCD-fed and HFD-fed mice. (B) Data represent mean area-under-the-curve (AUC) insulin ± SEM (n = 7 male mice per group)

(C-D) *In vivo* insulin secretion following L-arginine injection. (C) Serum insulin was collected following IP arginine 1 g/kg in NCD-fed and HFD-fed mice.(D) Data represent mean area-under-the-curve (AUC) insulin ± SEM. (n=6 male mice per group).

(E) Glucose-stimulated insulin secretion (GSIS) studies in isolated islets from NCD-fed and HFD-fed mice. Insulin release was measured following stimulation with low (2.8mM) or high (16.7mM) glucose. Data represent mean ± SEM (n=3 per group).

*$p<0.05$, $^{\#}p<0.01$, ^$p<0.005$.

Islet size and area are significantly reduced in obese CMAH null mice.

To further investigate the divergence in insulin secretory responses between WT and "human-like" *Cmah*$^{-/-}$ mice, we examined pancreata by immunohistochemical analysis when the mice were 7 months of age and had been on HFD for 12 weeks. Prior studies of *Cmah*$^{-/-}$ mice have shown no evidence of Neu5Gc expression in null tissues (4). As predicted from the underlying genetic differences, we observed abundant expression of Neu5Gc in pancreatic islets of WT mice using an antibody specific for Neu5Gc (15) and no evidence of endogenous Neu5Gc expression in pancreatic tissues of *Cmah* null mice (Figure 4A).

We detected no genotype differences in islet morphology of mice on NCD. Islet area was markedly increased by HFD in WT mice, consistent with β cell compensation and the observed increase in insulin secretion following glucose and arginine challenge *in vivo*. However, *Cmah-/-* mice failed to expand islet area when challenged with HFD. We noted that islet area was significantly smaller in HFD-fed *Cmah*$^{-/-}$ mice when compared to HFD-fed controls (*Cmah*$^{-/-}$*:* 1.54 ± 0.25% vs. WT: 4.39 ± 0.81%, p=0.01) (Figure 4B and C), consistent with diminished β cell compensation. Mean islet size in NCD-fed *Cmah*$^{-/-}$ mice was not significantly increased when compared to WT controls. However, HFD-fed *Cmah*$^{-/-}$ mice showed markedly reduced mean islet size when compared to HFD-fed controls (*Cmah*$^{-/-}$*:* 1.80 ± 0.19 x 10^4 vs. WT: 5.43 ± 0.34 x 10^4 um^2, p=0.0001) (Figure 4D). Furthermore, islet number normalized for pancreatic area was significantly lower in HFD-fed *Cmah*$^{-/-}$ mice when compared to diet matched controls (*Cmah*$^{-/-}$*:* 20.1

± 2.7 vs. WT: 41.0 ± 8.5 per mm^2 pancreatic area, p=0.02) (Figure 4E). We did not detect evidence of increased β cell proliferation or apoptosis by BrDU, Ki67, or TUNEL assay (data not shown), perhaps due to the extremely slow rates of β cell turnover observed in older mice (16). Immunohistochemical staining of islets with anti-CD45 antibodies detected no lymphocyte infiltrates, which would have implicated inflammation in the β cell failure of this model (data not shown).

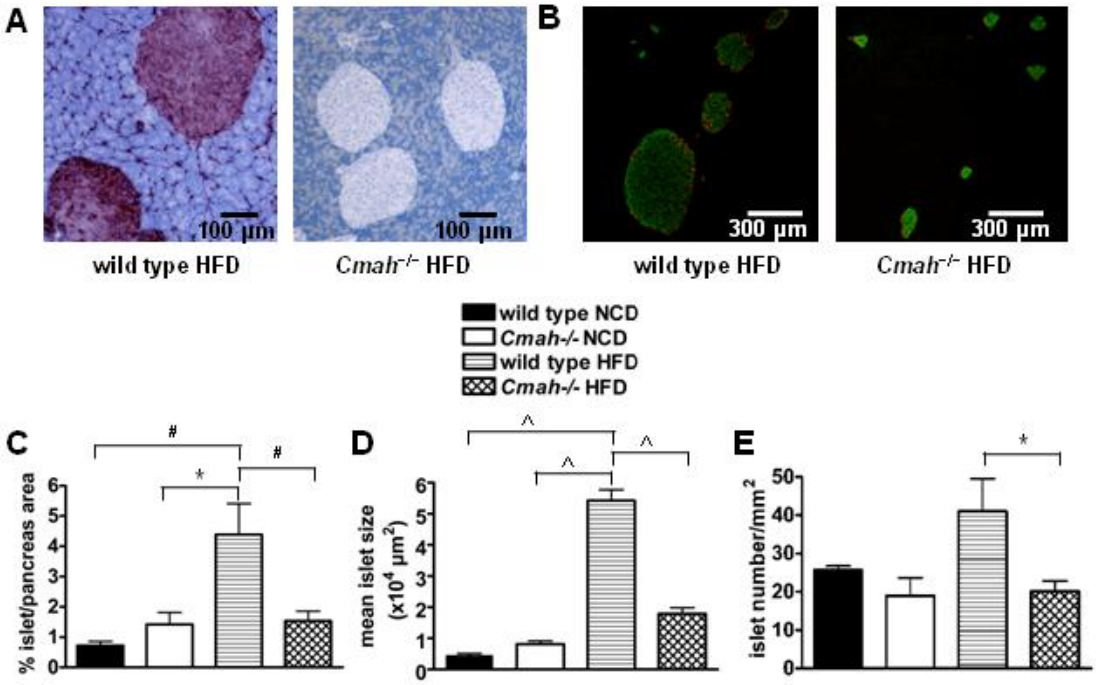

Figure 4. Islet size and area are significantly reduced in obese *Cmah* null mice
(A) Immunohistochemical analysis of pancreata using antibody specific for Neu5Gc in HFD-fed mice. Representative images are shown.
(B) Pancreatic islet sections from HFD-fed *Cmah* null and wild-type mice analyzed by fluorescence microscopy for insulin (green) and glucagon (red). Representative images are shown.
(C) Islet area expressed as a percentage of total pancreatic area in NCD-fed and HFD-fed mice (n=4-6 mice per group).
(D) Mean islet size in NCD-fed and HFD-fed *Cmah* null and wild-type mice (n=4-5 mice per group).
(E) Islet number normalized for pancreatic area in NCD-fed and HFD-fed mice (n=4-6 mice per group).
Data are represented as the mean ± SEM. *$p<0.05$, $^{\#}p<0.01$, ^$p<0.005$

DISCUSSION

Prospective studies of metabolic control in humans with type 2 diabetics indicate that, whereas insulin resistance is relatively constant, β cell failure is progressive in nature (17). The role of *CMAH* deletion in glucose metabolism and obesity has not been previously explored. In this study, we investigated the metabolic consequences of modifying sialic acid composition in the mammalian glycocalyx by using mice bearing a "human-like" mutation in the *Cmah* gene.

The CMAH enzyme catalyzes the conversion of Neu5Ac to Neu5Gc. Both Neu5Ac and Neu5Gc sialic acids are endogenously produced in all non-human vertebrates, including primates. In humans, an inactivating mutation of the *CMAH* gene has resulted in the evolutionary loss of Neu5Gc synthesis. Humans therefore express only Neu5Ac sialic acids at the cell surface. Here, we find that *Cmah* null mice with high fat diet-induced obesity demonstrate fasting hyperglycemia and impaired glucose tolerance. Although normal chow fed mice exhibit moderate insulin resistance, the interaction of *CMAH* deletion and high fat diet produced significant defects in insulin secretion with markedly reduced pancreatic islet size and area.

Prior studies in using *Cmah* null mice with the exon 6 deletion have shown that these mice have impaired hearing and delayed wound healing (4), as well as worsened neuromuscular disease when crossed with the mdx null model for Duchenne muscular dystrophy (20). Another knockout model of *Cmah*, using *neo*-cassette insertion into exon 5 (21), has been studied in the context of xeno-transplantation, producing robust anti-Neu5Gc antibody levels in response to immunization with Neu5Gc-expressing

thymocytes (22). In this case, Neu5Gc containing islets from syngeneic wild-type mice were transplanted into immunized *Cmah* null mice with streptozotocin-induced diabetes. These null mice demonstrated hyperglycemia due to the

rejection of transplanted islets and poor β cell engraftment. However, glucose metabolism was not studied in non-transplanted mice.

In the present study, our primary finding is that pancreatic islet mass appears to be significantly smaller in HFD-fed *Cmah*-/- mice, indicating failure of β cell compensation in response to severe obesity-induced insulin resistance. It is yet unclear how changes in sialic acid composition contribute to the expansion of β cell mass. Ongoing studies will determine whether this reduction in islet size and area in HFD-fed $Cmah^{-/-}$ mice accounts entirely for the decreased phenotype *in vivo*, or whether cellular defects in β cell function also exist. Prior work has shown that impaired glycosylation of Glut2 reduces its expression at the β cell surface, resulting in impaired glucose-stimulated insulin secretion but preserved arginine-stimulated insulin secretion (11). In our model, the insulin secretory response was reduced to both secretagogues. This could merely be explained by reduced islet mass, although it could also reflect possible defects in β cell function at more distal steps in the stimulus-secretion pathway.

Interestingly, activity of both the insulin receptor and IGF-1 receptor can be modified via desialylation by endogenous neuraminidase-1, which alters their ability to transduce net proliferative responses to insulin in skeletal myoblasts (23). β-cell specific mutations of the insulin receptor and IGF-1 receptor are also associated with reduced first-phase insulin secretion and impaired glucose tolerance (24-26). Defective insulin signaling has also been shown to diminish β cell replication and survival (27-29). Further work will elucidate whether *Cmah* inactivation and loss of Neu5Gc expression alter insulin receptor expression or action in β cells of obese mice.

An emerging literature shows that changes in sialic acid composition may affect cell function through several mechanisms. The evolutionary loss of Neu5Gc in humans has been studied in immunity, potentially determining human resistance or susceptibility to certain Neu5Gc- or Neu5Ac-preferring pathogens (18). Sialic acid modifications also affect their recognition and binding by Siglecs (sialic acid binding Ig-like lectins) at the cell surface. For example, Siglec-1, which is expressed specifically by resident and inflammatory macrophages, strongly prefers to bind Neu5Ac over Neu5Gc structures (19). Extracellular matrix proteins such as laminins and agrins also preferentially bind Neu5Ac rather than Neu5Gc (20).

In this study, we have expanded our understanding of *CMAH in vivo* by showing that it plays a role in systemic glucose metabolism in response to HFD. HFD-fed *Cmah* null mice manifest glucose intolerance secondary to pancreatic β cell dysfunction. These data suggest that human-like changes in sialic acid composition contribute to impaired glucose tolerance and β cell dysfunction in obese mice, which may lend insight into the development of type 2 diabetes in obese humans. Given the high prevalence of type 2 diabetes and its co-morbidities, it is clear that further study is necessary to understand how the evolutionary loss of *CMAH* and changes in sialic acid composition affect the pathogenesis and prevention of metabolic disease in humans.

This thesis, in full, is a reprint of the material as it appears in The Journal of the Federation of American Societies for Experimental Biology (FASEB) 2011. Kavaler, S.; Morinaga, H.; Jih, A.; Fan, W.; Hedlund, M.; Varki, A.; Kim J. *FASEB J*, 2011. Sarah Kavaler was the primary investigator and author of this paper.

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