Impaired Insulin Secretion and Enhanced Insulin Sensitivity in Cholecystokinin-Deficient Mice

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OBJECTIVE—Cholecystokinin (CCK) is released in response to lipid intake and stimulates insulin secretion. We hypothesized that CCK deficiency would alter the regulation of insulin secretion and glucose homeostasis.

RESEARCH DESIGN AND METHODS—We used quantitative magnetic resonance imaging to determine body composition and studied plasma glucose and insulin secretion of *CCK* gene knockout (CCK-KO) mice and their wild-type controls using intraperitoneal glucose and arginine infusions. The area of anti-insulin staining in pancreatic islets was measured by immunohistochemistry. Insulin sensitivity was assessed with euglycemic-hyperinsulemic clamps.

RESULTS—CCK-KO mice fed a low-fat diet had a reduced acute insulin response to glucose but a normal response to arginine and normal glucose tolerance, associated with a trend toward greater insulin sensitivity. However, when fed a high-fat diet (HFD) for 10 weeks, CCK-KO mice developed glucose intolerance despite increased insulin sensitivity that was associated with low insulin secretion in response to both glucose and arginine. The deficiency of insulin secretion in CCK-KO mice was not associated with changes in β-cell or islet size.

CONCLUSIONS—CCK is involved in regulating insulin secretion and glucose tolerance in mice eating an HFD. The impaired insulin response to intraperitoneal stimuli that do not typically elicit CCK release suggests that this hormone has chronic effects on β-cell adaptation to diet in addition to acute incretin actions.
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Obesity and type 2 diabetes are epidemic in both developing and developed countries, and consumption of high-fat diets (HFDs) is thought to be a contributing factor. In particular, consumption of an HFD promotes excess energy intake and contributes to the development of obesity and insulin resistance (1) and to abnormalities of fat metabolism (2). An HFD also stimulates the release of gastrointestinal hormones including cholecystokinin (CCK), glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (3–5), all of which are secreted from the intestine as ingested food is being processed for absorption. Each of these gut hormones enhances insulin secretion and facilitates glucose tolerance (6).

CCK is secreted by intestinal I cells in response to consumption of lipid and is involved in modulating intestinal motility, stimulating pancreatic enzyme secretion, enhancing gall bladder contraction, and regulating meal size (2,7,8). Pure preparations of natural porcine CCK, the synthetic octapeptide of CCK (CCK-8), or synthetic caerulein, which contains a COOH-terminal pentapeptide identical to CCK, have all been found to stimulate insulin secretion in vivo and in vitro (9,10). CCK-8 and CCK-33 are potent stimuli for insulin release, an effect mediated by the CCK1-receptor (CCK1R) both in vitro and in animal models (11–13). Although the role of CCK action on insulin secretion is controversial in healthy humans (14,15), there is evidence that CCK decreases postprandial glucose levels and potentiates increased insulin levels in humans with type 2 diabetes (15,16). CCK also potentiates arginine- and amino acid–induced insulin in normal human subjects (17). To clarify the role of CCK in insulin and glucose homeostasis, we used mice with a targeted global deletion of the *CCK* gene (CCK knockout [CCK-KO]) mice and their wild-type controls.

RESEARCH DESIGN AND METHODS

The CCK-KO mice were back-crossed for more than 10 generations onto a C57BL/6 J genetic background, and all mice were genotyped by PCR analysis of tail DNA (18). Male CCK-KO mice and wild-type controls (C57BL/6 J background) were generated in an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited facility under conditions of controlled illumination (12:12-h light-dark cycle; lights on from 0600 to 1800 h) and temperature (22°C).

CCK-KO and wild-type mice were housed individually beginning at 9 weeks of age. All animals received free access to either a low-fat diet (LFD) (5% fat content) or a matched HFD (20% butter fat by weight; Research Diets, New Brunswick, NJ) and water starting at 10 weeks of age for 10 weeks (19). Body weight and food intake were weighed by using a top-loading balance (±0.01 g, Adenturer SL; Ohaus, Pine Brook, NJ). All animal protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Intraperitoneal glucose and arginine administration. After a 5-h fast, wild-type and CCK-KO mice (n = 8/group) were injected i.p. with 2 mg/g body wt glucose (Sigma, St. Louis, MO) in saline. Tail blood was collected prior to glucose administration and at 15, 30, 60, and 120 min thereafter. On a separate day, wild-type and CCK-KO mice (n = 8/group) were injected i.p. with 4.8 mmol/kg body wt arginine (Sigma) in saline following 5-h fasting. Blood was collected before arginine was given, and 5, 10, 15, 20, 30, and 60 min afterward. Plasma glucose was determined using a Freestyle glucometer (Abbot Diabetes Care, Alameda, CA).

Insulin, glucagon, leptin, and adiponectin measurements. Plasma insulin, leptin, and adiponectin were determined using commercial enzyme-linked immunosorbent assay kits, and glucagon was determined by mouse endocrine LINCO*plex* kits (Millipore, St. Charles, MO). All samples were processed according to manufacturers' protocols. Briefly, 10-μL plasma samples with 1% dipeptidyl peptidase IV (DPPIV) inhibitor were added to each well of a microtiter plate precoated with antipeptide monoclonal antibodies or mixed beads, and the detection antibody was added to the captured molecules. After incubation, absorbance was measured with either a microplate reader (Synergy

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HT; BioTek Instruments, Richmond, VA) or a Luminex instrument (LX200; Millipore, Austin, TX). The final concentrations were calculated using standards provided with the enzyme-linked immunosorbent assay kit and Luminex kit.

Fat and lean body masses. Fat and lean masses were determined using an EchoMRI whole-body composition analyzer (Houston, TX) (20). The EchoMRI is a quantitative nuclear magnetic resonance instrument that provides precise and noninvasive measurements of whole-body composition parameters that include total body fat and lean mass in living rodents.

Immunofluorescent microscopy and morphometric analysis. The pancreata of wild-type and CCK-KO mice (n = 4/group) were collected from 5-h fasted mice and fixed in 4% paraformaldehyde for 30 min, followed by incubation in 30% sucrose overnight at 4°C (21). Cryosections (8 μm) were cut and immunostained with rabbit anti-insulin (1:200, sc-9168; Santa Cruz Biotechnology). After being washed with PBS, sections were incubated with Cy5-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch Laboratory, West Grove, PA). Sections were viewed under a Nikon confocal microscope (Nikon DECLIPSE C1; Nikon, Tokyo, Japan). Images of islets were captured, and islet area (n = 34 for wild-type mice and n = 31 for CCK-KO mice) was subjected to morphometric analysis using MetaMorph Image Analysis software (Molecular Devices, Downingtown, PA).

Euglycemic-hyperinsulinemic clamp. CCK-KO and wild-type mice were anesthetized with ketamine and xylazine (90 mg/kg and 8 mg/kg). A chronic indwelling catheter was inserted into the right external jugular vein and externalized behind the head (22), and mice were allowed to recover for at least 4 days before study. Following a 5-h fast, a primed continuous infusion of insulin (bolus of 62 mU/kg, followed by 3.5 mU/min/kg; Novolin Regular, Novo Nordisk, Clayton, NC) was given to raise plasma insulin levels as previously described (22). A bolus of 10 μCi of [U-^{14}C]2-deoxy-*d*-glucose (American Radiolabeled Chemicals, St. Louis, MO) was infused at t = 75 min to allow the determination of glucose uptake (Rg) in specific tissues. For tail vein sampling, mice were restrained in a Tailveiner (Braintree Scientific, Braintree, MA). Blood samples (~30 μL) were taken to determine plasma glucose levels and tracer activity at time t = 0, 30, 60, 80, 90, 100, 110, and 120 min. Additional blood (~40 μL) was taken at t = 0, 100, and 120 min to determine basal and clamped insulin levels. All erythrocytes from the collected blood samples were washed in saline and infused back into the mice to minimize anemia. At the conclusion of the studies, mice were killed and liver, fat pads, and skeletal muscle were quickly dissected. Another cohort of animals (n = 3–4/group) was used to determine basal glucose turnover rate. Briefly, 5-h fasted animals were infused with a bolus of tracer glucose (6.24 μCi per animal), followed by a continuous infusion of 0.1 μCi/min of [^{3}H]glucose, and tail blood was collected at 30, 50, 60, 70, 80, and 90 min for determining glucose and insulin levels. Mean glucose turnover rate was determined by the average rate of appearance of [^{3}H]glucose at 60–90 min.

Calculations and statistical analysis. Glucose responses to the intraperitoneal glucose tolerance test (IPGTT), and insulin secretion after intraperitoneal glucose or arginine, were computed as the average increment above basal for the values after injection. All values are expressed as means ± SE. Parametric statistical analyses, one-way ANOVA and two-way ANOVA, followed by Bonferroni posttest for multiple comparisons, were analyzed by GraphPad Prism (version 5.0; San Diego, CA). Differences were considered significant relative to the wild-type mice at the same time point if P values were < 0.05.

RESULTS

For mice fed the LFD, initial/final body weight and body lean/fat mass in CCK-KO mice (n = 9/group) were comparable with those of wild-type mice (Supplementary Table 1). For mice fed the HFD, initial body weight and lean mass in CCK-KO mice were significantly less than those in wild-type mice. Although mice fed the HFD had increased body weight and fat mass, the CCK-KO mice remained significantly leaner and had less fat than wild-type mice ($P <$ 0.05 for both). Fasting plasma glucose, insulin, glucagon, leptin, and adiponectin were comparable in CCK-KO and wild-type mice fed the LFD or the HFD (Table 1).

Insulin secretion and glucose metabolism on an LFD. Prior to the IPGTT, CCK-KO mice had comparable basal plasma glucose and insulin as wild-type mice after a 5-h fast (Fig. 1*A* and *C*). During the intraperitoneal glucose infusion, the CCK-KO mice had comparable plasma glucose curves and mean area under the curve (AUC) for plasma glucose but significantly reduced insulin at 15 min compared with the wild-type mice (Fig. 1*A–C*). The insulin and glucose responses to arginine infusion were similar in the CCK-KO and wild-type animals (Fig. 1*D–F*).

The body weight and fat mass of mice in the two genotypes fed the LFD and used in the euglycemic clamp (n = 6 per group) were not different (Table 2). Basal insulin in the CCK-KO mice was lower than that in the wild-type mice, but the difference was not significant (Table 2). During the clamp, plasma insulin and glucose reached steady state by 80 min and were similar for both groups (Fig. 2*A*; Table 2). The mean overall glucose infusion rate (GIR) at steady state in the clamp (90–120 min) tended to be increased (P = 0.06) compared with that of the wild-type mice (Fig. 2*B* and *C*). The tracer estimates of Rg in the epididymal (visceral) and inguinal white adipose tissue, as well as for soleus, tibialis anterior, and extensor digitorum longus muscles, were comparable for the two groups (Fig. 2*D*). These findings suggest that whole-body insulin sensitivity may be increased in the CCK-KO compared with wild-type animals, and may account for the normal intraperitoneal glucose tolerance in the presence of reduced glucose-stimulated insulin secretion. However, glucose uptake by skeletal muscle and adipose tissue does not explain the trend toward increased insulin sensitivity in CCK-KO mice.

Insulin secretion and glucose metabolism on an HFD. Prior to the IPGTT, basal plasma glucose and insulin were similar in the CCK-KO and wild-type mice (Fig. 3*A* and *C*). During the IPGTT, CCK-KO mice were glucose intolerant (Fig. 3*A* and *B*) with significantly reduced insulin secretion relative to the control animals maintained on HFD ($P <$ 0.0001) (Fig. 3*C*). Relative to mice fed the LFD, wild-type mice fed the HFD had significantly increased insulin and reduced plasma glucose in response to the arginine challenge (Figs. 1 and 3). However, the CCK-KO mice on HFD had less arginine-induced insulin release compared with the wild-type controls (Fig. 3*D–F*).

TABLE 1
Plasma parameters in CCK-KO mice after a 10-week period of LFD or HFD

	LFD		HFD	
	Wild-type	CCK-KO	Wild-type	CCK-KO
Glucose (mg/dL)	144.65 ± 3.64	150.33 ± 6.15	163.17 ± 8.00	156.00 ± 8.10
Insulin (ng/mL)	0.61 ± 0.14	0.57 ± 0.08	1.09 ± 0.21	0.88 ± 0.14
Glucagon (pg/mL)	58.10 ± 9.36	69.54 ± 10.21	36.43 ± 2.54	36.59 ± 3.64
Leptin (ng/mL)	1.53 ± 0.43	2.29 ± 0.63	5.83 ± 0.89*	3.49 ± 0.37
Adiponectin (μg/mL)	31.56 ± 8.19	28.64 ± 7.11	45.24 ± 1.78	43.95 ± 1.87

Data are means ± SEM. Plasma in CCK-KO and wild-type mice (n = 7–10/ group) was collected after a 5-h fast following either a 10-week LFD or an HFD at 10 weeks of age. *Significant difference ($P < 0.05$) compared with LFD-treated wild-type controls.

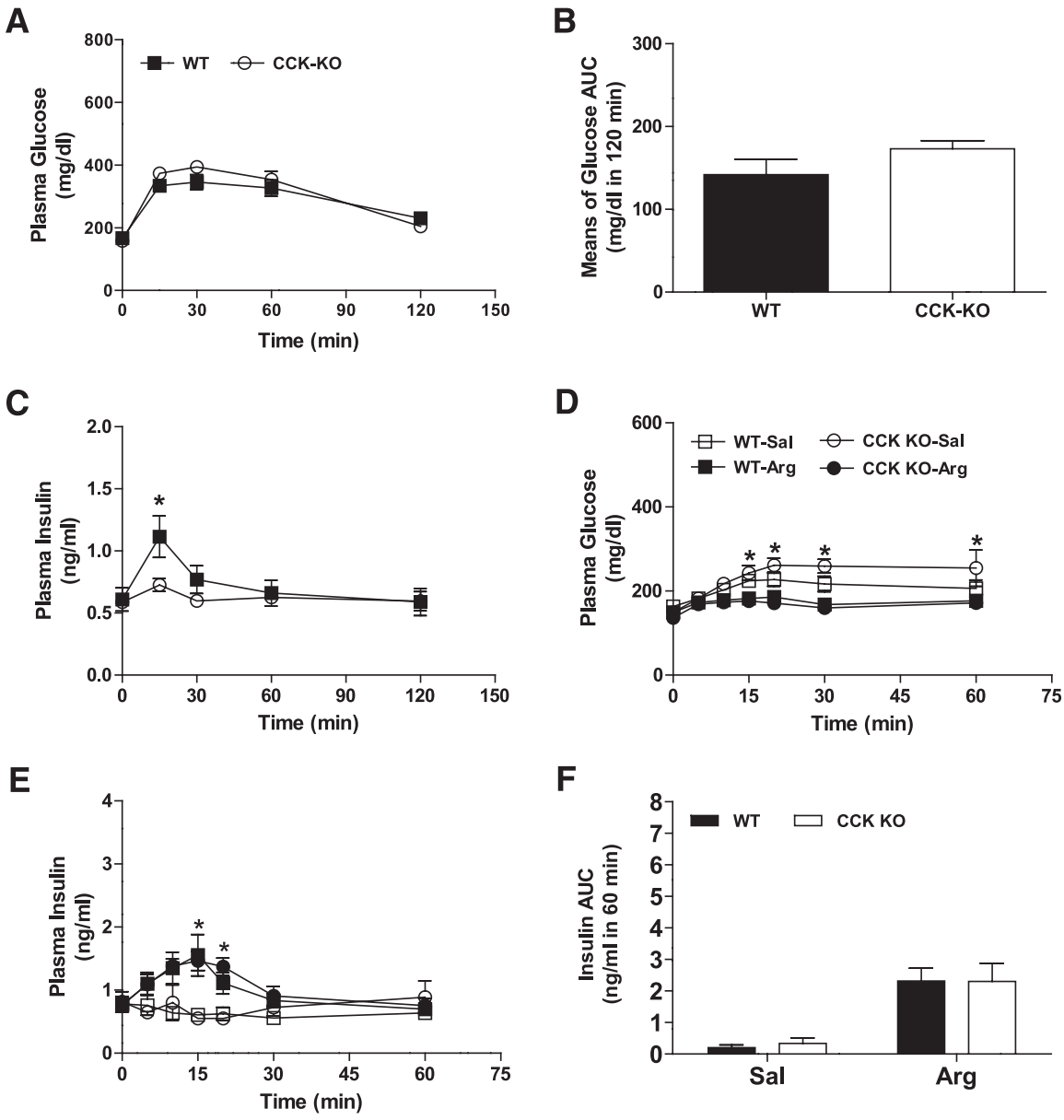

FIG. 1. IPGTT and arginine stimulation test (AST) in mice fed the LFD. IPGTT (2 mg/g body wt) in 5-h–fasted wild-type and CCK-KO mice maintained on an LFD. *A*: Plasma glucose. *B*: Means of glucose AUC. *C*: Plasma insulin over 120 min. Arginine stimulation test (4.8 mmol/kg body wt i.p.) in 5-h–fasted mice maintained on an LFD. *D*: Plasma glucose. *E*: Plasma insulin. *F*: Mean insulin AUC over 60 min. Data are expressed as means ± SEM. *Significant differences relative to the wild-type groups at the same time point ($P < 0.05$).

The cohorts of CCK-KO and wild-type mice fed the HFD and used for the euglycemic clamp had comparable body weight and body composition, thus minimizing the possibility that there would be an effect of body weight or fat mass on insulin sensitivity (Table 2). During the clamp studies, CCK-KO mice required more exogenous glucose to maintain euglycemia relative to wild-type mice (Fig. 4*B*), and the mean GIR at 90–120 min in the CCK-KO mice was significantly higher than in the wild-type mice ($P < 0.05$) (Fig. 4*C*). Interestingly, insulin sensitivity in the CCK-KO mice was similar whether they were receiving an LFD or HFD (Supplementary Fig. 1*E*). In contrast, the mean GIR was less in the wild-type mice on an HFD than in the wild-type mice on an LFD. Despite the changes in insulin sensitivity, basal glucose turnover on LFD and HFD was similar in wild-type and CCK-KO mice (Supplementary Fig. 1*C* and *D*), indicating that CCK is involved in the modulation of insulin sensitivity. These findings suggest that unlike wild-type mice, CCK-KO mice did not develop diet-induced insulin resistance. The Rg in the white adipocytes and skeletal muscles of the CCK-KO and wild-type mice was reduced comparably following 10 weeks on the HFD (Fig. 4*D*). These findings imply that although CCK-KO mice are protected against diet-induced insulin resistance, the HFD causes a sufficient impairment of insulin secretion in these animals to cause glucose intolerance.

The islets of CCK-KO mice maintained on an HFD were of a size comparable to the size in islets of wild-type mice as determined by the area of anti-insulin staining in pancreatic islets by immunohistochemistry (Fig. 5). These

TABLE 2
Body weight, body composition, and insulin level during the euglycemic-hyperinsulinemic clamp

	LFD		LFD	
	Wild-type	CCK-KO	Wild-type	CCK-KO
Initial body weight (g)	26.50 ± 1.04	24.38 ± 0.52	25.13 ± 0.94	24.37 ± 1.03
Final body weight (g)	28.25 ± 1.11	26.63 ± 0.44	30.92 ± 1.67	28.60 ± 0.52
Final fat mass (g)	1.79 ± 0.31	1.40 ± 0.13	5.02 ± 0.78*	4.64 ± 0.53*
Final lean mass (g)	24.31 ± 1.09	23.25 ± 0.45	24.96 ± 1.41	24.74 ± 0.82
Basal insulin (ng/mL)	0.57 ± 0.08	0.34 ± 0.05	0.62 ± 0.10	0.77 ± 0.16
Clamp insulin (ng/mL)	1.84 ± 0.15	1.56 ± 0.37	2.00 ± 0.25	2.00 ± 0.20

Data are means ± SEM. Body weight and body composition in CCK-KO and wild-type mice (n = 6/group) were measured before and after either a 10-week LFD or a 10-week HFD at 10 weeks of age. Plasma insulin and glucose was determined at baseline and during a euglycemic-hyperinsulinemic clamp study. *Significant difference ($P < 0.05$) relative to LFD-treated wild-type mice in one-way ANOVA with Bonferroni posttest.

results suggest that reduced β-cell number is not a factor in the reduced level of glucose- or arginine-induced insulin secretion in the CCK-KO mice.

DISCUSSION

Patients with type 2 diabetes have relative β-cell insufficiency and impaired insulin-dependent stimulation of glucose disposal in skeletal muscle and adipocytes as well as impaired suppression of hepatic glucose production (23). We hypothesized that the gut hormone CCK might be important in some of these defects. The absence of CCK increased insulin sensitivity both in the setting of LFD and HFD independent of body weight or adiposity. Our findings also implicate CCK in the stimulation of insulin secretion, although this effect was dependent on diet. The normal arginine response in CCK-KO mice, coupled with normal intraperitoneal glucose tolerance, suggests that islet secretion in these animals is appropriate. However, despite increased insulin sensitivity, CCK-KO animals were glucose intolerant when maintained on a chronic HFD, and this was associated with deficient insulin secretion in response to both glucose and arginine. These findings support a role for CCK, a gut hormone strongly influenced by lipid ingestion, in the β-cell adaptation to high-fat feeding and increased body adiposity. The fact that insulin secretion in CCK-KO mice was impaired in response to intraperitoneal stimuli that do not stimulate CCK release suggests a role for CCK on β-cell function beyond that of an acute secretagogue.

We used a global CCK-KO mouse model to study the effect of endogenous CCK in glucose homeostasis in the current study. As previously reported, these animals appeared phenotypically normal (24) and had body weight; adiposity; and plasma glucose, insulin, glucagon, leptin,

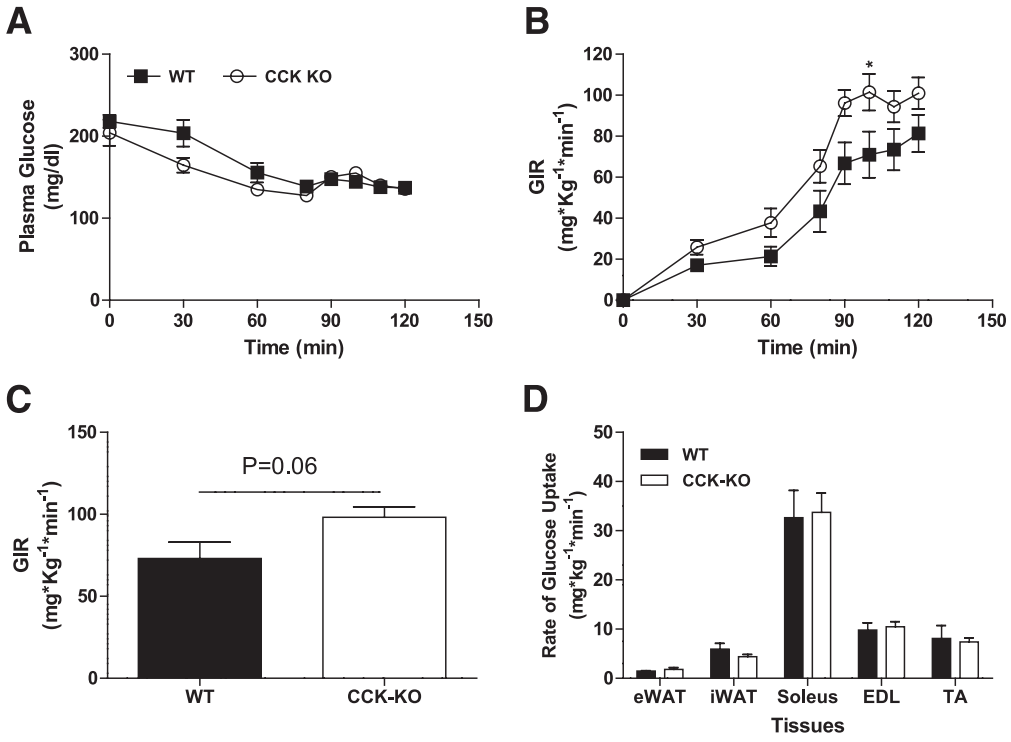

FIG. 2. Euglycemic-hyperinsulinemic clamps in mice fed an LFD. *A*: Blood glucose levels. *B*: GIR. *C*: Means of GIR at 90–120 min during the euglycemic clamp. *D*: Rg into selected tissues. Data are expressed as means ± SEM for six animals per group. *Significant differences relative to the wild-type mice ($P < 0.05$).

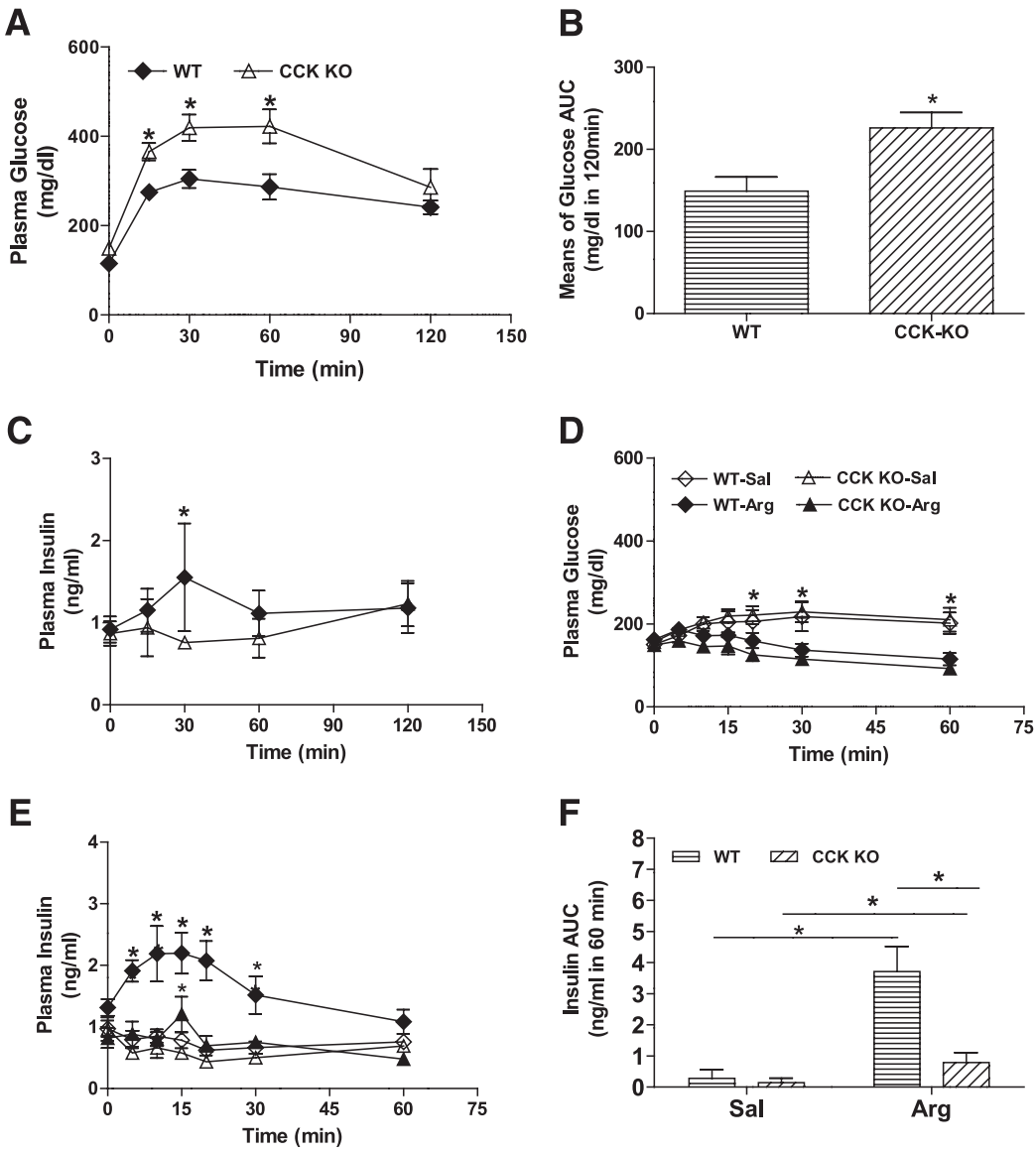

FIG. 3. IPGTT and arginine stimulation (AST) tests in mice fed the HFD. IPGTT (2 mg/g body wt) in 5-h–fasted wild-type and CCK-KO mice maintained on an HFD. *A*: Plasma glucose. *B*: Means of glucose AUC. *C*: Plasma insulin over 120 min. Arginine stimulation test (4.8 mmol/kg body wt i.p.) in 5-h–fasted mice maintained on an HFD. *D*: Plasma glucose. *E*: Plasma insulin. *F*: Insulin AUC over 60 min. Data are expressed as means ± SEM. *Significant differences relative to the wild-type groups at the same time point ($P < 0.05$).

and adiponectin levels similar to levels in wild-type mice. We used two distinct means of stimulating the β-cells because CCK has been reported to potentiate both glucose- and arginine-induced insulin (11,17,25,26). The CCK1R is present in pancreatic β-cells (27), and the action of CCK on insulin secretion is mediated via the CCK1R pathway (28). Although the CCK-KO mice on an LFD had lower glucose-stimulated insulin secretion than the wild-type mice, they had comparable glucose tolerance. The fact that these animals were more insulin sensitive during a glucose clamp suggests that the insulin response to intraperitoneal glucose is appropriately reduced to accommodate greater whole-body insulin action (29). The normal insulin response to intraperitoneal arginine in LFD-fed CCK-KO animals is consistent with this interpretation and suggests that with this diet, CCK is not necessary for normal β-cell function.

Maintenance on the HFD increased adiposity in wild-type and CCK-KO mice but did not affect fasting insulin or glucose. After 10 weeks on the HFD, CCK-KO mice gained less body weight and fat mass than wild-type mice, consistent with our previous observation in these animals at different ages (30). Further, glucagon, leptin, and adiponectin, factors that influence insulin sensitivity (31,32), were also not altered in CCK-KO mice fed the HFD relative to wild-type controls, although HFD plasma leptin was appropriately increased in the wild-type mice fed the HFD relative to those fed the LFD. Consistent with the lack of weight gain and the findings in mice on an LFD, the CCK-KO mice fed an HFD were more insulin sensitive than the wild-type controls. Despite this, they had impaired glucose tolerance associated with almost absent insulin responses to intraperitoneal glucose and arginine. These findings support a role for CCK in the β-cell adaptation to high-fat feeding and increased body adiposity. The importance of this role is underscored by the fact that in the absence of CCK, insulin secretion was insufficient to

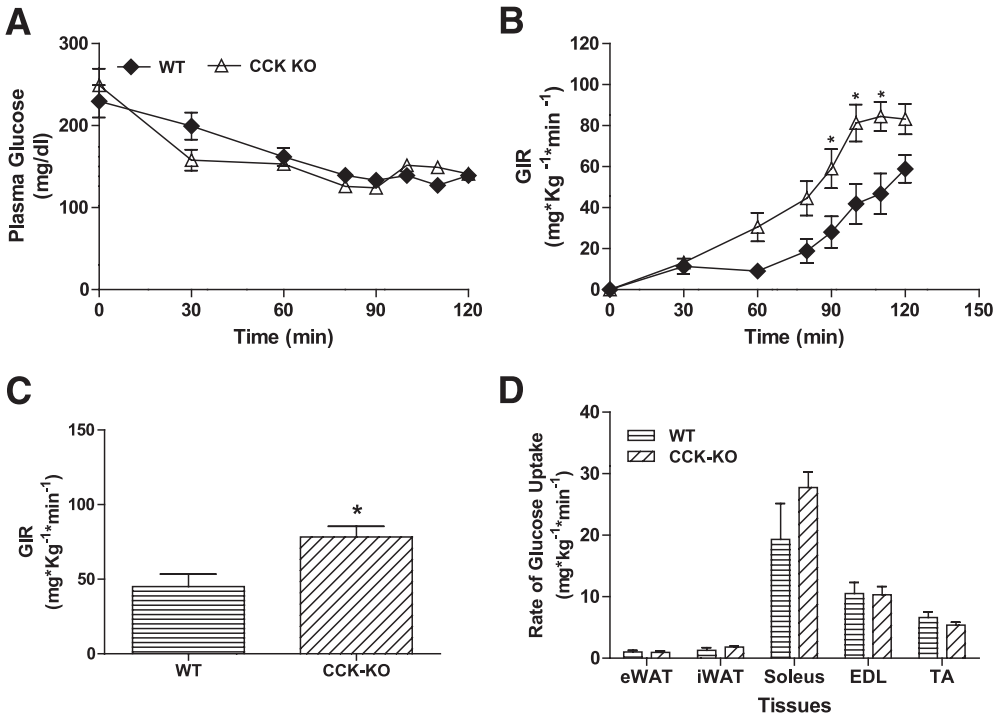

FIG. 4. Euglycemic-hyperinsulinemic clamps in mice fed an HFD. *A*: Blood glucose levels. *B*: GIR. *C*: Means of GIR at 90–120 min during the euglycemic clamp. *D*: Rg into selected tissues. Data are expressed as means ± SEM for six animals per group. *Significant differences relative to the wild-type mice ($P < 0.05$).

control blood glucose even with greater whole-body insulin action.

CCK is released in response to enteral stimuli, particularly protein and lipid-containing meals, but does not increase in the circulation in response to intravenous or intraperitoneal glucose or arginine (4,33,34). Previous work has demonstrated that HFD increases plasma insulin and CCK in rats and healthy men (35,36), making this perturbation a logical stressor of CCK-KO animals. We did not formally test the incretin action of CCK in this study, in that we did not measure insulin secretion stimuli that would acutely stimulate CCK release from I-cells. However, our findings suggest that CCK is necessary for β-cell adaptation to high-fat feeding and that this effect can be detected even in the absence of elevated circulating plasma CCK or increased CCK release. Because an examination of the islets in the CCK-KO animals did not demonstrate changes in islet number or size, the implication from our

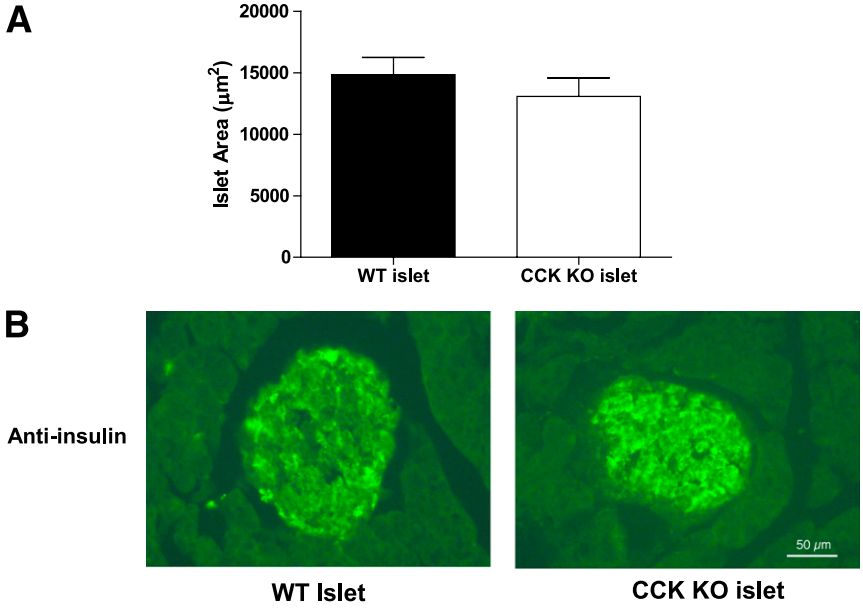

FIG. 5. Pancreatic morphology in fasted mice on an HFD. *A*: Anti-insulin islet area. *B*: Pancreatic islet mass stained with anti-insulin. Data are expressed as means ± SEM for four animals per group. (A high-quality digital representation of this figure is available in the online issue.)

data are for a functional impairment of insulin secretion in the chronic absence of CCK. This is a novel finding with clinical relevance because β-cell adaptation to obesity is now thought to be an essential compensation to maintain glucose homeostasis.

Surprisingly, insulin sensitivity was increased in the absence of CCK. The GIR in the CCK-KO mice on an LFD did not reach statistical significance, but the magnitude of the difference with wild-type controls, taken in the context of the findings with HFD, suggests an underpowered observation. After 10 weeks on an HFD, the insulin sensitivity in the CCK-KO mice was significantly higher than that of the wild-type animals. Wild-type mice maintained on the HFD had a 35.7% decrease in GIR relative to LFD-treated wild-type controls. In contrast, the GIR in the CCK-KO mice was reduced by only 16.1% relative to that in LFD-treated CCK-KO mice. Therefore, although maintenance on the HFD reduced insulin sensitivity in wild-type mice, the CCK-KO mice were less affected. Because we used mice from the two genotypes with comparable body weight and fat mass in the clamp study, adiposity per se is not likely to contribute to the greater insulin sensitivity in the absence of CCK. There is little support in the literature for direct effects of CCK on insulin sensitivity, making this a novel finding. In apparent contrast with our phenotype, Otsuka Long Evans Tokushima Fatty rats, which are genetically deficient in functioning CCK1R, are obese and diabetic (37). However, the nature of the genetic defect in these rats is not clear.

The increased whole-body insulin sensitivity in the CCK-KO mice was not explained by the results of Rg measurements in white adipocytes and skeletal muscle. Intraduodenal administration of CCK has been reported to decrease hepatic glucose production, ruling out the possibility that the improved insulin sensitivity of CCK-KO mice is secondary to the lack of intraduodenal CCK action (38). Recent evidence suggests that hepatic insulin sensitivity is regulated by the brain as well as by islet hormones and nutrient substrates (39). Our previous data have shown that CCK-KO mice have impaired fat absorption relative to wild-type mice following an HFD (30), and CCK-KO mice had a significant reduction of total hepatic fatty acids, especially palmitic acid and oleic acid (data not shown). It is possible that less hepatic lipid accumulation in CCK-KO mice results in improved hepatic insulin sensitivity. These differential effects on insulin action in these two genotypes cannot be attributed to either diet composition or hypercaloric challenge per se in the current study. Relevant to this, CCK1R is not detectable in the liver (18) even though CCK-KO mice had increased hepatic insulin sensitivity in the current study. The findings on hepatic insulin action in CCK-KO mice are different from observations in Otsuka Long Evans Tokushima Fatty rats during intraduodenal CCK infusion (38). Therefore, the effect of CCK on insulin action in the liver might not be through a direct CCK1R pathway; i.e., some other pathway related to hepatic lipid metabolism might be involved in insulin action. Further studies will be required to determine whether CCK has direct effects on insulin action or whether these are mediated through CNS regulation similar to CCK effects on food intake.

The role of CCK in the regulation of insulin secretion and glucose homeostasis has been debated for many years. In the present experiments, we demonstrate that CCK-KO mice have normal glucose tolerance and normal insulin secretion in response to arginine. Glucose-stimulated insulin secretion is reduced in these animals but seems to be appropriate for the degree of insulin sensitivity. Chronic intake of an HFD unmasks an important action of CCK to mediate a functional adaptation of insulin secretion to increased adiposity. The absence of CCK action in this setting causes glucose intolerance. Our findings also demonstrate a novel effect of CCK to regulate insulin sensitivity, an interesting and potentially important observation that compels further investigation.

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C.-M.L. researched data, contributed to discussion, and wrote and edited the manuscript. S.O. contributed to discussion and edited the manuscript. H.H.D. researched data and edited the manuscript. M.H. and D.L. researched data. D.H.K. researched data and edited the manuscript. M.L. edited the manuscript. D.D. contributed to discussion and edited the manuscript. S.C.W. researched data, contributed to discussion, and wrote and edited the manuscript. P.T. contributed to discussion and edited the manuscript.

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