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Lipid transport in cholecystokinin knockout mice

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HIGHLIGHTS

- Energy homeostasis in cholecystokinin knockout (CCK-KO) mice was determined.
- Fat absorption in CCK-KO mice was normal in response to high-fat feeding.
- CCK-KO mice had increased energy expenditure during high-fat feeding.
- CCK-KO mice had impaired lipid transport from the small intestine to tissues.
- CCK-KO mice were resistant to high fat diet-induced obesity.

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ABSTRACT

Cholecystokinin (CCK) is released in response to lipid feeding and regulates pancreatic digestive enzymes vital to the absorption of nutrients. Our previous reports demonstrated that cholecystokinin knockout (CCK-KO) mice fed for 10 weeks of HFD had reduced body fat mass, but comparable glucose uptake by white adipose tissues and skeletal muscles. We hypothesized that CCK is involved in energy homeostasis and lipid transport from the small intestine to tissues in response to acute treatment with dietary lipids. CCK-KO mice with comparable fat absorption had increased energy expenditure and were resistant to HFD-induced obesity. Using intraduodenal infusion of butter fat and intravenous infusion using Liposyn III, we determined the mechanism of lipid transport from the small intestine to deposition in lymph and adipocytes in CCK-KO mice. CCK-KO mice had delayed secretion of Apo B48-chylomicrons, lipid transport to the lymphatic system, and triglyceride (TG)-derived fatty acid uptake by epididymal fat in response to acute treatment of intraduodenal lipids. In contrast, CCK-KO mice had comparable TG clearance and lipid uptake by white adipocytes in response to TGs in chylomicron-like emulsion. Thus, we concluded that CCK is important for lipid transport and energy expenditure to control body weight in response to dietary lipid feeding.

1. Introduction

Cholecystokinin (CCK) is secreted by intestinal endocrine cells in response to lipid and protein [1–3]. This fat-induced stimulation of CCK is dependent on the formation and secretion of chylomicrons [4]. CCK is involved in stimulating gallbladder contraction, intestinal motility and pancreatic secretion of insulin and pancreatic enzymes [5–11]. In addition, CCK reduces meal size and energy expenditure and delays gastric emptying [4,12–16]. Peripheral CCK acts on CCK receptor 1 (CCK-1R) on vagal afferent nerves projecting to the brain and consequently reduces meal size [12,16–19]. Conversely, the application of either CCK-1R antagonists or vagal deafferentation abolishes the satiating effect of peripheral CCK [20–22]. Duodenal administration of dietary lipids stimulates brown adipose tissue (BAT) thermogenesis via intestinal CCK-1R [23]. Peripheral CCK has been shown to attenuate whole-body energy expenditure via CCK-1R, but not via CCK receptor 2 (CCK-2R) [14,15,24–28]. Central CCK also regulates energy expenditure [13,26,28–31]. Thus, fat-induced CCK plays important roles in lipid transport and metabolism, glucose homeostasis and energy homeostasis between food intake and energy expenditure.

Consumption of a high-fat diet (HFD) increases peripheral CCK secretion in both humans and rodents [32–34]. Controversially, HFD does not increase the plasma level of CCK in humans [35]. It also reportedly reduces sensitivity to the satiating effect of peripheral CCK in HFD-induced obese animals [36–38]. CCK knockout (CCK-KO) mice have comparable food intake and slightly enhanced energy expenditure and malabsorption of saturated fatty acids after chronic consumption of HFD [39]. Although CCK-KO animals on HFD have enhanced insulin sensitivity, CCK has no effect on glucose uptake by white adipose tissue and skeletal muscle [39]. Thus, lipid metabolism in CCK-KO mice might be

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an important regulatory mechanism to improve insulin sensitivity. The objective of the present experiments was to determine the involvement of endogenous CCK in energy homeostasis and lipid transport from the small intestine to tissues using the CCK-KO mouse model.

2. Materials and methods

2.1. Animals

Male CCK-KO mice and their wild-type (WT) littermates were generated onto a C57BL/6J genetic background [39–41] and housed in an AAALAC-accredited facility under conditions of controlled illumination (12:12-h light–dark cycle, lights on from 0600 to 1800 h). All animal protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

2.2. Body weight, food intake and metabolic rate

Based on our published protocols [11,39], CCK-KO and WT mice (n = 10 per group) were individually housed at 10 weeks of age and received a semi-purified high-fat pelleted diet (HFD, 20% butter fat by weight; Research Diets, Inc., New Brunswick, NJ) or pelleted chow (LFD, 5% fat diet, Harlan Teklad, Madison, WI) for 10 weeks. Body weights and food intake were recorded twice a week using a top-loading balance (±0.01 g, Adventurer SL, Ohaus Corp., Pine Brook, NJ). Cohorts of CCK-KO and WT mice were acclimatized to individual metabolic cages in an Oxymax system (Columbus Instruments, Columbus, OH) for 3 days. Metabolic rate and food intake were recorded at 15-min intervals for 2 days using the manufacturer's software.

2.3. Fat mass and parameters in the plasma and liver

Fat and lean body masses were determined using an EchoMRI whole body composition analyzer (Houston, TX) [39,41]. After 10 weeks on LFD or HFD, fat pads, the liver and plasma were carefully collected and weighed from 5-h fasted mice. The glucose level was determined in tail blood using a Freestyle glucometer (Abbot Diabetes Care, Alameda, CA). Hepatic lipids were extracted using the Folch method [42]. Triacylglycerides (TGs) and cholesterol (CHOL) in the plasma and the liver were determined using Randox triglyceride kits (Antrim, UK) and Infinity cholesterol kits (Thermo Electron, Noble Park, Victoria, Australia), respectively. According to the manufacturer's protocol, diluted plasma was mixed with 200 μl enzyme reagent and then incubated at 37 °C for 30 min [11]. The absorbance was read at 500 nm using a microplate reader (Synergy HT; BioTek Instruments, Richmond, VA). Plasma insulin and leptin were determined using commercial enzyme-linked immunosorbent assay kits (Millipore, St. Charles, MO) [11]. Briefly, 10-μl samples were added to each well of a precoated microtiter plate, and the detection antibody was added to the captured molecules. After incubation, absorbance was measured with a microplate reader and the final concentrations were calculated using standards provided with the enzyme-linked immunosorbent assay kits.

2.4. Fat absorption

Using our published protocol [39,43], animals (n = 7–10 per group) consumed the HFD mixed with sucrose polybehenate (Research Diets, Inc.) for 4 days and their fecal pellets were collected for analysis on the final day. Fatty acids in fecal pellets were extracted, methylated and analyzed by a gas chromatography system (Shimadzu GC 2010) equipped with a DB-23 Column (J & W Scientific, Folsom, CA) and Schimadzu Class EZStart 7.4 software [39,43]. The percentage of fat absorption was determined based on the ratio of total fatty acids to behenic acid in the diet and in the feces.

2.5. Lymphatic TG output

Male CCK-KO and WT mice (n = 4–5 per group) fed LFD received a surgery for duodenal cannulation and lymphatic cannulation in the main mesenteric lymph duct based on our published methods [44,45]. After surgery, the animals were infused via the duodenal catheter with a saline solution containing 5% glucose at a rate of 0.3 ml/h to compensate for fluid and electrolyte loss due to lymphatic drainage. The animals were allowed to recover for 24 h in a warm chamber (approximately 30 °C). Fasting state lymph was collected after a 1-h infusion of saline before a lipid emulsion consisting of 4 μmol/h butter fat (The Kroger Corp., Cincinnati, OH) in a 19 mM sodium taurocholate solution was infused. To remove gastric emptying as a potential confounding factor for the transport of lipids to the lymph, the lipid emulsion was infused intraduodenally at a constant rate of 0.3 ml/h for 6 h. Lymphatic levels of TGs and CHOL were determined using Randox triglycerides and Infinity cholesterol kits.

2.6. Lipid uptake by adipocytes and lipid clearance assay

Two experiments (either intraduodenal or intravenous infusion of TGs) were performed to determine lipid uptake by adipose tissue. For intraduodenal TG infusions, CCK-KO and WT mice (n = 5 per group) maintained on LFD received a duodenal infusion of a lipid emulsion containing 4 μmol/h butter fat and labeled with [9,10-^{3}H]oleic acid, 1 μCi/0.3 ml (Perkin Elmer, Boston, MA) 24 h after recovery from the duodenal cannulation. After the 2-h infusion, inguinal and epididymal adipose tissue was collected on dry-ice. For intravenous infusions, we used Liposyn III (intravenous fat emulsion), which acts like a chylomicron and can transport TGs directly to all tissues including the adipose tissues, liver and heart [46]. The 5-h fasted mice (n = 6–7 per group) received 100 μl of Liposyn III (20% fat) mixed with 0.25 μCi ^{3}H-TGs per mouse over 30 min and tail blood (20 μl) was collected at 2, 4, 6, 10, 20 and 30 min post-injection. Based on previous reports [47,48], chylomicrons in the plasma are cleared in the rodents within 30 min. After 30 min, adipose tissue was collected on dry-ice. According to our published protocols [45,48], tissue lipids were extracted using the Folch method [42] and radioactivity in the plasma and tissues was measured by liquid scintillation counting.

2.7. Lymph apolipoprotein determination

Based on our published protocol [45], lymph samples were loaded into a 4–20% Mini-Protean TGX gel (Bio-Rad Laboratories, Hercules, CA) and gels were run at a constant voltage (60 V) until the protein standards were well separated. Proteins were then transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories) for 2 h at 300 mA. After blocking nonspecific binding sites on the membranes, membranes were then incubated with the polyclonal rabbit anti-rat Apo B antibody (1:6000 dilution) and then incubated with horseradish peroxidase-conjugated goat anti-rabbit antibodies (Dako, Cytomation). Detection of Apo B48 was achieved by using the enhanced chemiluminescence system (ECL Western Blotting Detection Reagents, Amersham Biosciences, Buckinghamshire, UK), and HyBlot CL films (Denville Scientific Inc.) were used for development and visualization of the membranes. Apo B secretion during each subsequent hour of infusion was quantified by subtracting the 0 h (fasting) lymph Apo B content from relative Apo B levels at each hourly time point.

2.8. Statistical analysis

All values are expressed as mean ± SE. Parametric statistical analyses and one-way and two-way ANOVAs, followed by the Bonferroni posttest for multiple comparisons, were analyzed by GraphPad™ Prism (version 6.0, San Diego, CA). Differences were considered