RESEARCH ARTICLE | Obesity, Diabetes and Energy Homeostasis

Energy homeostasis in apolipoprotein AIV and cholecystokinin-deficient mice

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Weng J, Lou D, Benoit SC, Coschigano N, Woods SC, Tso P, Lo CC. Energy homeostasis in apolipoprotein AIV and cholecystokinin-deficient mice. Am J Physiol Regul Integr Comp Physiol 313: R535–R548, 2017. First published July 28, 2017; doi:10.1152/ajpregu.00034.2017.—Apolipoprotein AIV (ApoAIV) and cholecystokinin (CCK) are well-known satiating signals that are stimulated by fat consumption. Peripheral ApoAIV and CCK interact to prolong satiating signals. In the present study, we hypothesized that ApoAIV and CCK control energy homeostasis in response to high-fat diet feeding. To test this hypothesis, energy homeostasis in ApoAIV and CCK double knockout (ApoAIV/CCK-KO), ApoAIV knockout (ApoAIV-KO), and CCK knockout (CCK-KO) mice were monitored. When animals were maintained on a low-fat diet, ApoAIV/CCK-KO, ApoAIV-KO, and CCK-KO mice had comparable energy intake and expenditure, body weight, fat mass, fat absorption, and plasma parameters relative to the controls. In contrast, these KO mice exhibited impaired lipid transport to epididymal fat pads in response to intraduodenal infusion of dietary lipids. Furthermore, ApoAIV-KO mice had upregulated levels of CCK receptor 2 (CCK2R) in the small intestine while ApoAIV/CCK-KO mice had upregulated levels of CCK2R in the brown adipose tissue. After 20 wk of a high-fat diet, ApoAIV-KO and CCK-KO mice had comparable body weight and fat mass, as well as lower energy expenditure at some time points. However, ApoAIV/CCK-KO mice exhibited reduced body weight and adiposity relative to wild-type mice, despite having normal food intake. Furthermore, ApoAIV/CCK-KO mice displayed normal fat absorption and locomotor activity, as well as enhanced energy expenditure. These observations suggest that mice lacking ApoAIV and CCK have reduced body weight and adiposity, possibly due to impaired lipid transport and elevated energy expenditure.

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CCK, ApoAIV-KO, CCK-KO, ApoAIV/CCK-KO, and wild-type (WT) mice (C57BL/6J background) were generated in an AAALAC-accredited facility under conditions of controlled illumination (12:12-h light-dark cycle, lights from 0600 to 1800). All KO mice were backcrossed for >10 generations onto a C57BL/6J genetic background, and all mice were genotyped by PCR analysis of tail DNA (37, 39). WT mice in the experiment of lipid uptake by adipocytes were obtained from Jackson Laboratory (Bar Harbor, ME). All mice were housed individually beginning at 10 wk of age. Starting at 10 wk of age, all animals received free access to either a low-fat diet (LFD; 5% butter fat content) or a matched high-fat diet (HFD; 20% butter fat by weight; Research Diets, New Brunswick, NJ), in addition to water, for 20 wk. All animal protocols were approved by the Institutional Animal Care and Use Committee at Ohio University and the University of Cincinnati.

qPCR for relative mRNA measurement. ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice (n = 6–8 mice per group) maintained on chow diets at 15 wk of age were fasted for 5 h with water access. The small intestine and epididymal fat tissue of fasted mice were collected on dry ice. Total RNA was isolated, and first-strand cDNA was synthesized from 1 μg total RNA (91). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed in a 27-μl final reaction volume with an Applied Biosystems RT-PCR instrument using SYBR green RT-PCR master mixes (Life Technologies, Warrington, UK). RT-PCR conditions were conducted as follows: 95°C for 3 min for one cycle, followed by 40 cycles of 95°C for 30 s and 60°C for 30 s. Threshold cycle readings for each of the unknown samples were used, and the results were analyzed in Excel using the ΔΔCt method (46). Cyclophilin mRNA levels from each sample were used as internal controls to normalize the mRNA levels. The sequences of the primers (Integrated DNA Technologies, Coralville, IA) were as follows: mouse CCK1R, 5′-AAGAGGATGGCGGACTGT-3′ (forward) and 5′-CATGACGAGTGTTAGG-3′ (reverse); mouse CCK 2 receptor (CCK2R), 5′-GTCAACAAATGTTGGTTCC-3′ (forward) and 5′-TTATCACCATCAGAGCGAGCCTC-3′ (reverse); mouse CCK, 5′-TACGCGGATACATCCAGGCAGG-3′ (forward) and 5′-ACTTAAATAGTAGACTCAAGCC-3′ (reverse); mouse ApoAIV, 5′-ACCAGGACTAAGCAGCAGA-3′ (forward) and 5′-TGTCTGAAAGAGGGTACTGAC-3′ (reverse); and mouse cyclophilin, 5′-TCTGTGCACGATTGTC-3′ (forward) and 5′-TCA-GTCTGACGATG-3′ (reverse).

Fig. 1. Expression of apolipoprotein AIV (ApoAIV), cholecystokinin (CCK), and CCK receptors in the small intestine and epididymal fat tissues of ApoAIV/CCK-knockout (KO), ApoAIV-KO, CCK-KO, and wild-type (WT) mice. Duodenal CCK1R and 2R in 4 genotypes (A), duodenal CCK in ApoAIV-KO mice (B), jejunal CCK1R and 2R in four genotypes (C), jejunal ApoAIV in CCK-KO mice (D), CCK1R and 2R in epididymal fat tissue (E), and brown adipose tissue (F). Small intestine, epididymal fat tissue, and brown fat tissue in animals fed a chow diet were collected after a 5-h fast. Data are means ± SE for 5 animals per group. *Values represent significant differences relative to the WT mice (P < 0.05).
Body weight, food intake, and energy expenditure. Body weight was measured with a top-loading balance (±0.01 g; Adventurer SL, Ohau, Pine Brook, NJ). Before the start of data collection for meal patterns, food intake, and energy expenditure of ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice after 20 wk of a LFD or HFD, all mice (n = 5–8 per group) were acclimatized to individual metabolic cages in an Oxymax system (Columbus Instruments, Columbus, OH) for 3 days. Mice had free access to either a powdered LFD or HFD. Food intake and energy expenditure were recorded at 15-min intervals for 2 days using the manufacturer’s software.

Fat absorption. Following our published protocols (32, 48), cohorts of ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice (n = 5 per group) were fed a LFD or a HFD for 18 wk. They were then fed the HFD mixed with sucrose polybehenate (Research Diets) for 4 days, and their fecal pellets were collected for analysis on the final day. Fatty acids in the 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. 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.

Lipid uptake by adipocytes. Intraduodenal infusion of dietary lipids was performed to determine lipid uptake by adipose tissue. For intraduodenal lipid infusions, ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice (n = 5 per group) maintained on a LFD for 16 wk received a continuous intraduodenal infusion of a lipid emulsion containing 4 μmol/h butter fat and labeled with [9,10-3H]oleic acid, 1 μCi/0.3 ml and [14C]cholesterol, 0.2 μCi/0.3 ml (Perkin Elmer, Boston, MA) 24 h after recovery from the duodenal cannulation. Because maximum transport of lymphatic lipids is observed at 2 h postinfusion (49), BAT, and ingunal and epididymal adipose tissue following the 2-h infusion was collected on dry ice. In accordance with our published protocols (37, 49), tissue lipids were extracted using the Folch method (20), and radioactivity in the plasma and tissues was measured by liquid scintillation counting.

Locomotor Activity. Home cages were placed in SmartFrame stainless steel cage rack frames (Hamilton-Kinder, Poway, CA). Infrared photobeam interruption sensors mounted in the frames detected each animal’s movements. Activity counts in the form of beam interruptions were recorded for 4 days.

Plasma parameters. Plasma insulin and leptin levels were determined using commercial ELISA kits (Millipore, St. Charles, MO). All samples were processed according to the manufacturer’s protocols. Briefly, 10-μl plasma samples with 1% dipetylpyridine IV (DPPIV) inhibitor were added to each well of a microtitrater plate precoated with anti-peptide monoclonal antibodies, and the detection antibody was added to the captured molecules. After incubation, absorbance was measured with a microplate reader (Synergy HT; BioTek Instruments, Richmond, VA), and the final concentrations were calculated using standards provided with the ELISA kits. Triacylglycerol and cholesterol in the plasma were determined using Randox triglyceride kits (Antrim, UK) and Infinity cholesterol kits (Thermo Electron, Noble Park, Victoria, Australia), respectively. Plasma glucose was determined using a Freestyle glucometer (Abbott Diabetes Care, Alameda, CA).

Data analysis and statistical analysis. All values are presented as means ± SE. Parametric statistical analyses, one-way ANOVA, and two-way ANOVA were performed using GraphPad Prism (version 6.0, San Diego, CA), followed by a Sidak multiple comparisons test. All differences were considered significant if the P < 0.05.

RESULTS

Gene expressions, body weight, and plasma parameters. Two types of CCK receptor have been described: CCK1R and CCK2R. Peripheral CCK1R are present in the enteric neurons of the duodenal mucosa, predominantly involved in secretory and mucosal functions; myenteric plexus, predominantly involved in the control of motor activity; and nodose ganglia (8, 58, 65). Peripheral CCK2R is found in the small intestine, and adipose tissues (3, 54, 85). To understand whether mice with absence of ApoAIV, CCK, or both have compensation of CCK1R and CCK2R to control energy homeostasis, the gene expression of CCK1R, CCK2R, CCK, and ApoAIV in the small intestine and adipose tissues was examined. Relative to WT mice, ApoAIV/CCK-KO and CCK-KO mice had comparable levels of CCK1R and CCK2R in the duodenum, jejunum, and epididymal fat tissues, except that ApoAIV/CCK-KO mice experienced a fivefold increase of CCK2R expression in the BAT (Fig. 1, A, C, E, and F). In addition, CCK-KO mice produced normal levels of jejunal ApoAIV compared with the control groups (Fig. 1B). Relative to their fasted control groups, ApoAIV-KO mice produced comparable levels of CCK1R and CCK2R in the small intestine, and adipose tissues, except for a fivefold increase of CCK2R expression in the jejunum (Fig. 1, A–E). Furthermore, ApoAIV-KO mice had normal levels of duodenal CCK (Fig. 1B). These findings suggest that the levels of CCK1R and CCK2R in BAT are upregulated in mice with absence of CCK or both ApoAIV and CCK, possibly due to the development of compensatory mechanisms.

ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice maintained on a LFD for 20 wk (n = 7 per group) had comparable body weight; comparable mass of brown adipose tissue (BAT) and epididymal fat; and comparable plasma levels of lipids, insulin, and leptin (Table 1). When animals were maintained on a LFD, CCK-KO mice had increased ingunal fat mass relative to ApoAIV/CCK-KO mice, but these were not statistically significant. In contrast, the ingunal fat mass of ApoAIV-KO mice was significantly greater than that of ApoAIV/CCK-KO mice (P < 0.05, Table 1). Furthermore, ApoAIV-KO and CCK-KO mice fed a LFD had epididymal fat pads of larger mass relative to LFD-fed WT mice (P < 0.05, Table 1). Before a HFD, initial body weights in different genotypes fed a LFD were comparable, except for increased body weight in ApoAIV-KO mice (Table 2). After 10 wk on a HFD, WT mice had significantly increased body weight gain.

| Table 1. Body weight, adipose tissue weights, and plasma parameters in animals after a 20-wk period of low-fat diet |
|----------------------------------|-----------------|-----------------|-----------------|
| BW, g (initial)                 | 24.48 ± 0.9     | 22.55 ± 0.5     | 26.87 ± 0.5*    | 22.81 ± 0.7     |
| BW, g (LFD)                     | 29.34 ± 0.7     | 28.57 ± 0.7     | 31.16 ± 0.6     | 29.16 ± 0.6     |
| BAT, g                         | 0.11 ± 0.0      | 0.10 ± 0.0      | 0.14 ± 0.0      | 0.18 ± 0.0      |
| Epidermidal fat, g              | 0.46 ± 0.0      | 0.35 ± 0.0      | 0.79 ± 0.1*     | 0.85 ± 0.1*     |
| Inguinal fat, g                 | 0.26 ± 0.0      | 0.17 ± 0.0      | 0.39 ± 0.1      | 0.31 ± 0.1      |
| Triacylglycerol, mg/dl          | 63.81 ± 6.2     | 40.02 ± 7.9     | 61.34 ± 13.0    | 45.68 ± 7.1     |
| Cholesterol, mg/dl              | 89.21 ± 5.6     | 55.53 ± 5.0*    | 88.18 ± 3.6     | 62.34 ± 9.9     |
| glucose, mg/dl                 | 151.85 ± 11.7   | 141.5 ± 10.8    | 157.00 ± 9.1    | 150.33 ± 6.1    |
| Insulin, ng/ml                  | 0.51 ± 0.1      | 0.44 ± 0.0      | 0.55 ± 0.1      | 0.57 ± 0.1      |
| Leptin, ng/ml                   | 2.34 ± 0.5      | 1.73 ± 0.4      | 1.98 ± 0.5      | 2.79 ± 0.6      |

Values represent means ± SE. ApoAIV, apolipoprotein AIV; CCK, cholecystokinin; BW, body weight; BAT, brown adipose tissue; WT, wild type; KO, knockout; LFD, low-fat diet. Plasma and tissues in ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice (n = 7 per group) were collected after a 5-h fast after a 20-wk LFD. *Significant difference (P < 0.05) compared with LFD-treated WT controls.
Table 2. Body weight, adipose tissue weights, and plasma parameters in animals after a 20-week period of high-fat diet

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ApoAIV/CCK-KO</th>
<th>ApoAIV-KO</th>
<th>CCK-KO</th>
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<tr>
<td>BW, g (initial)</td>
<td>24.1 ± 0.9</td>
<td>24.0 ± 0.4</td>
<td>26.9 ± 0.5*</td>
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<td>BW, g (HFD)</td>
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<td>29.7 ± 0.8*</td>
<td>36.5 ± 1.9</td>
<td>33.3 ± 1.5</td>
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<tr>
<td>BAT, g</td>
<td>0.22 ± 0.0</td>
<td>0.15 ± 0.0</td>
<td>0.23 ± 0.0</td>
<td>0.18 ± 0.0</td>
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<tr>
<td>Epididymal fat, g</td>
<td>1.54 ± 0.1</td>
<td>0.81 ± 0.1*</td>
<td>1.36 ± 0.1</td>
<td>1.33 ± 0.2</td>
</tr>
<tr>
<td>Inguinal fat, g</td>
<td>0.80 ± 0.1</td>
<td>0.39 ± 0.1*</td>
<td>0.66 ± 0.1</td>
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<tr>
<td>Triglycerol, mg/dl</td>
<td>66.66 ± 11.9</td>
<td>39.41 ± 6.5*</td>
<td>42.99 ± 5.2</td>
<td>50.46 ± 3.0</td>
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<tr>
<td>Cholesterol, mg/dl</td>
<td>149.36 ± 23.4</td>
<td>87.90 ± 20.6</td>
<td>139.5 ± 18.0</td>
<td>128.45 ± 9.9</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>157.00 ± 9.1</td>
<td>175.83 ± 6.1</td>
<td>147.3 ± 6.6</td>
<td>126.85 ± 4.9</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>0.96 ± 0.1</td>
<td>0.43 ± 0.1*</td>
<td>0.79 ± 0.2</td>
<td>0.62 ± 0.2</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>17.57 ± 2.7</td>
<td>7.26 ± 1.3*</td>
<td>18.49 ± 2.9</td>
<td>10.51 ± 3.7</td>
</tr>
</tbody>
</table>

Values represent means ± SE. HFD, high-fat diet. Plasma and tissues in ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice (n = 8 per group) were collected after a 5-h fast after a 20-wk HFD. *Significant difference (P < 0.05) compared with HFD-treated WT controls.

(10.34 ± 1.1 g), relative to LFD-fed WT mice (5.08 ± 1.0 g, Fig. 2A, P < 0.05). Relative to WT mice, ApoAIV/CCK-KO mice showed significantly reduced body weight and white fat mass, including both epididymal and inguinal fat mass, body weight gain, as well as significant reduction in plasma triacylglycerol, insulin, and leptin after chronic consumption of a HFD for 20 wk (Table 2 and Fig. 2, P < 0.05). In contrast, relative to WT mice, ApoAIV-KO and CCK-KO mice had similar body weight, body weight gain, fat mass, and levels of plasma lipids, glucose, insulin, and leptin, except for an increase in epididymal fat mass (Table 2 and Fig. 2, A and B). When compared with ApoAIV-KO mice, ApoAIV/CCK-KO mice exhibited significantly decreased body weight, epididymal fat mass, and plasma leptin (P < 0.05, Table 2). The HFD-fed ApoAIV/CCK-KO mice had body weight gain comparable to the LFD-fed ApoAIV/CCK-KO mice (Fig. 2, A and B). These findings indicate that ApoAIV/CCK-KO mice are resistant to gaining body weight and epididymal fat mass in response to chronic consumption of a HFD.

Fat absorption and lipid transport to adipocytes. ApoAIV-KO and CCK-KO mice fed a LFD have comparable or reduced fat absorption (36, 37, 75). To investigate whether reduced fat absorption in the small intestine results in reduced fat mass in ApoAIV/CCK-KO mice, the efficiency of dietary fatty acid absorption in ApoAIV/CCK-KO and WT mice was measured using our Olestra method (32). When maintained on a LFD for 18 wk, ApoAIV/CCK-KO mice (83.9 ± 3.3%, Fig. 3A) displayed total fat absorption comparable to WT mice (90.2 ± 1.2%). Specifically, ApoAIV/CCK-KO mice experienced significantly lower absorption of stearic acid relative to WT mice (P < 0.05, Fig. 3B). Following a HFD for 18 wk, ApoAIV/CCK-KO mice (97.0 ± 1.4%, Fig. 3C) showed total fat absorption comparable to WT mice (94.61 ± 1.2%). There was no significant difference in the absorption of various fatty acids between WT and ApoAIV/CCK-KO mice (Fig. 3D). These findings suggest that the absence of both ApoAIV and CCK does not alter total fat absorption in mice, apart from impaired absorption of long-chain saturated fatty acids, such as stearic acid, when they are maintained on a LFD.

To determine whether impaired intestinal lipid transport to adipocytes results in reduced fat mass in ApoAIV/CCK-KO mice, different cohorts of ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice were used for the determination of lipid uptake in adipocytes. Relative to WT mice (29.40 ± 0.8 g), ApoAIV/CCK-KO and CCK-KO mice fed a LFD had lower body weight (26.40 ± 0.3 g and 25.20 ± 0.7 g, respectively) and comparable fat mass (Fig. 4, A and B). In addition, ApoAIV-KO mice had similar body weight (30.40 ± 0.9 g) and fat mass (Fig. 4, A and B). Since maximum transport of lymphatic lipids is observed at 2 h postinfusion (49), radioactive lipids in the tissues after 2-h infusion were counted in the present experiment. Following a 2-h infusion of dietary lipids, ApoAIV/CCK-KO, ApoAIV-KO, and CCK-KO mice showed reduced levels of radioactive triacylglycerol-derived fatty acid uptake by epididymal fat (Fig. 4C, P < 0.05). In contrast, the KO mice displayed comparable levels of [14C]cholesterol derived fatty acid uptake by inguinal fat and BAT (Fig. 4C). The KO mice experienced similar uptake of [14C]cholesterol by white adipose tissues and BAT (Fig. 4D). These findings indicate that the absence of ApoAIV and CCK only influences triacylglycerol transport from the small intestine to epididymal fat depots when dietary lipids are infused into the duodenum.

Energy expenditure, respiratory quotient, and food intake in ApoAIV/CCK-KO mice. To investigate the effect of endogenous ApoAIV and CCK on the regulation of energy homeostasis, ApoAIV/CCK-KO, ApoAIV-KO, and CCK-KO mice were monitored after a 20-wk consumption of a LFD or HFD. Relative to the control group on a LFD, ApoAIV/CCK-KO, ApoAIV-KO, and CCK-KO mice fed a LFD had comparable hourly energy expenditure and average energy expenditure (Figs. 5, A and B, 6, A and B, and 7, A and B). The LFD-fed ApoAIV/CCK-KO and CCK-KO mice exhibited a reduced
respiratory quotient (RQ) in the dark period (Figs. 5, C and D, and 7, C and D), suggesting that these KO mice utilized more fatty acids as energy substrates. In contrast, there was no significant difference in the RQ between ApoAIV-KO and WT mice (Fig. 6, C and D). When maintained on a LFD, ApoAIV/CCK-KO and CCK-KO mice exhibited reduced hourly food intake with respect to their control group at some time points, while ApoAIV-KO mice experienced increased food intake at some time points (Figs. 5E, 6E, and 7E). Relative to the WT control, no significant difference in total daily food intake was found in these KO mice (Figs. 5F, 6F, and 7F). Thus the deficiency of ApoAIV, CCK, or both ApoAIV and CCK genes does not alter daily total energy intake and expenditure in animals maintained on a LFD.

Fig. 3. Fat absorption in ApoAIV/CCK-KO and WT mice. Total fat absorption (A) and fatty acid profiles (B) in fecal pellets of animals fed a low-fat diet (LFD) and total fat absorption (C) and fatty acid profiles (D) in fecal pellets of animals fed a HFD were determined using gas chromatography. Fecal pellets were collected on the 4th day after animals started to receive a 5% fat or 20% fat diet mixture with 5% Olestra and again during the 18th week on LFD or HFD. Data are means ± SE for 5 animals per group. *Values represent significant differences relative to the WT mice (P < 0.05).

Fig. 4. Lipid uptake by adipocytes in ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice. Body weight (A), fat mass (B), uptake of [3H]triaclylglycerol-derived fatty acids (C), and cholesterol uptake (D) in animals fed a LFD. Five-hour fasted animals received an intraduodenal infusion of 4 μmol/h butter fat mixture, and tissues were collected at the end of the 2-h infusion. Data are means ± SE for 5 animals per group. *Values represent significant differences relative to the WT mice (P < 0.05).
After chronic consumption of a HFD, ApoAIV/CCK-KO mice had increased hourly energy expenditure relative to their controls (Fig. 8, A and B), while ApoAIV-KO and CCK-KO mice fed a HFD showed lower energy expenditure relative to WT mice at some time points during the dark period (Figs. 9, A and B, and 10, A and B). Additionally, the RQ in these KO mice was normal (Figs. 8, C and D, 9, C and D, and 10, C and D). These findings indicate that a HFD alters energy expenditure in the KO mice. After chronic consumption of a HFD for 20 wk, ApoAIV/CCK-KO, ApoAIV-KO, and CCK-KO mice had comparable meal size and total food intake relative to their control groups (Figs. 8, E and F, 9, E and F, and 10, E and F).

Locomotor activity in CCK/ApoAIV-KO mice. To investigate whether altered locomotor activity results in elevated or reduced energy expenditure in these HFD-fed KO mice, locomotor activity in ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice was monitored for 48 h after 19 wk of a HFD. Daily basic and cumulative movement in these KO mice were comparable to those movements in WT mice (Fig. 11, A–F). These findings suggest that HFD-fed mice without ApoAIV, CCK, or both ApoAIV and CCK genes have normal locomotor activity.

DISCUSSION

These experiments tested the hypothesis that HFD-induced ApoAIV and CCK control energy homeostasis. Peripheral and central administration of CCK or ApoAIV produces short-term satiating signals that do not alter total food intake (23–25, 30). ApoAIV-KO mice fed a chow diet have normal food intake and increased body weight (37, 75). In the present experiment, both food intake and body weight remained at normal levels in ApoAIV-KO mice. In addition, CCK-KO mice fed a LFD had normal total food intake and body weight results confirmed by previous studies (36, 50, 51). The present study showed that ApoAIV/CCK-KO mice fed a LFD for 20 wk had normal total

Fig. 5. Energy expenditure and intake in ApoAIV/CCK-KO and WT mice fed a LFD. Hourly energy expenditure (A), total energy expenditure (B), hourly respiratory quotient (RQ; C), RQ (D), meal pattern (E), and food intake (F). Data are means ± SE for 8 animals per group. *Values represent significant differences relative to the WT mice (P < 0.05).
food intake and body weight (Table 1). Surprisingly, a significant reduction of body weight was found in CCK-KO and ApoAIV/CCK-KO mice fed a LFD for 16 wk (Fig. 4). The discrepancy in body weight of CCK-KO or ApoAIV/CCK-KO mice (Table 1 and Fig. 4) might have been due to their different feeding time and ages. Chronic feeding of a HFD alters intestinal and hypothalamic production of CCK and ApoAIV (33, 44, 46, 60). Past studies have demonstrated that the increase in body weight of CCK-KO mice and ApoAIV-KO mice is less than that of the WT controls for 10–12 wk, although maintenance on a HFD or Western diet increases body weight in both genotypes (37, 48, 75). However, ApoAIV-KO and CCK-KO mice displayed normal food intake and body weight after chronic consumption of a HFD for 20 wk, while ApoAIV/CCK-KO mice exhibited normal food intake but reduced gain of body weight compared with their controls. Thus HFD-induced ApoAIV and CCK regulated the gain of body weight. Moreover, ApoAIV and CCK activity were not primary factors in controlling total food intake.

CCK and ApoAIV alter plasma levels of glucose and insulin in a hyperglycemia condition (21, 83). CCK binds with CCK1R to assist glucose uptake by the pancreas to elevate insulin secretion (35). ApoAIV decreases circulating glucose by enhancing glucose-induced insulin release and attenuating hepatic glucose production (40, 83). In the present experiments, CCK-KO, ApoAIV-KO, and ApoAIV/CCK-KO mice had comparable levels of basal glucose and insulin in the plasma when maintained on LFD. Consistent with previous reports (50, 83), CCK-KO and ApoAIV-KO mice had comparable basal levels of insulin and glucose when fed a HFD. In contrast, lower levels of basal insulin were found in ApoAIV/CCK-KO mice with chronic consumption of a HFD, possibly due to the absence of insulin induction by ApoAIV and CCK. Leptin secretion is highly correlated to fat mass and is increased when animals are fed a HFD (55, 92). When maintained on a LFD, ApoAIV/CCK-KO mice had comparable levels of plasma leptin compared with the other genotypes, although they had reduced mass of epididymal and inguinal fat.
tissues. Leptin is also produced by the stomach (76, 92). It remains unknown whether higher production of gastric leptin or slower leptin degradation in the LFD-fed ApoAIV/CCK-KO mice results in comparable levels of plasma leptin relative to the other genotypes. The present study showed that a HFD increased the mass of white adipose tissues, including epididymal and inguinal fat tissues, and leptin content in WT mice, a finding also presented in previous studies (55). In the current study, when maintained on a HFD, ApoAIV-KO and CCK-KO mice displayed normal fat mass and leptin. In contrast, ApoAIV/CCK-KO mice experienced a smaller increase of white adipose tissues and plasma leptin compared with the controls and the ApoAIV-KO mice. Because insulin reduces lipolysis and enhances uptake of fatty acids in white adipose tissues (18, 22, 72), a lower level of insulin might induce diminished fat mass in HFD-fed ApoAIV/CCK-KO mice relative to HFD-fed WT mice.

Pancreatic lipase hydrolyzes dietary triacylglycerol to fatty acids in the intestinal lumen, and the absorbed fatty acids are subsequently resynthesized to triacylglycerol in the mucosal cells (80). Fatty acids are packaged into chylomicrons in the small intestine and transported to the bloodstream (80). Upon entering the circulation, lipoprotein lipase hydrolyzes the triglycerides in chylomicrons to free fatty acids, and half of the fatty acids are directly transported to adipose tissue (26, 63). CCK physiologically stimulates pancreatic lipase secretion to increase fat absorption (10, 73). CCK-containing neurons are present in the small intestine, and CCK increases intestinal smooth muscle contraction (9, 41). CCK and gastrin activate CCK2R, whereas only CCK activates CCK1R (85). In the present and previous studies (39), CCK-KO mice had normal levels of CCK1R and CCK2R in the small intestine and the epididymal fat tissue. CCK-KO mice have a slower transit time of lipids in the small intestine and delayed lipid transport from the small intestine to the lymph (36, 84). When fed a LFD, CCK-KO mice have normal fat absorption and delayed lipid transport (36). After a HFD feeding, CCK-KO mice have normal or reduced fat absorption, depending on their age (36, 84).
Consistent with a previous report (36), LFD-fed CCK-KO mice had lower fatty acid uptake by epididymal fat and comparable uptake of fatty acids in the BAT and inguinal fat tissues. ApoAIV is required for chylomicron assembly and stabilizing expanding lipid interfaces (53, 87). In the previous and current experiments, ApoAIV-KO mice had normal levels of CCK and CCK1R in the small intestine and gallbladder (88, 91). In contrast, ApoAIV-KO mice had reduced expression of duodenal CCK (67). The ApoAIV-KO mice had upregulated levels of CCK1R in the nodose ganglia and upregulated levels of CCK2R in the jejunum, possibly due to the development of compensatory mechanisms (91). LFD-fed ApoAIV-KO mice have normal fat absorption and delayed chylomicron clearance (37, 38). The current study demonstrated that ApoAIV-KO mice fed a LFD had impaired fatty acid uptake by epididymal fat tissues. Because the effect of intestinal CCK2R in the regulation of lipid transport remains unknown, another experiment is required for the determination of lipid transport from the small intestine to epididymal fat tissue via a CCK2R-dependent pathway in ApoAIV-KO mice.

Fatty acids are used as an energy source in peripheral tissues, including BAT and muscle (13, 19, 70). Duodenal lipids enhance BAT thermogenesis via a CCK1R-dependent pathway in the small intestine (6). Peripheral administration of CCK acts on CCK1R and CCK2R to reduce whole-body energy expenditure (15, 34, 69). Central administration of CCK at lower doses decreases body temperature (59, 77). In contrast, higher doses of brain CCK elevate body temperature and energy expenditure (4, 79). CCK2R KO mice fed a LFD have increased energy expenditure (56). In the present study, when fed a LFD, ApoAIV-KO and CCK-KO mice had normal energy expenditure like CCK-KO and CCK1R-KO mice in the previous reports (51, 56). CCK-KO mice fed a LFD utilized more fatty acids as energy substrates than their control groups. A HFD stimulates peripheral release of CCK, reduces hypothalamic levels of CCK, and attenuates CCK signaling in the vagus nerves (16, 60, 78). Although HFD-fed CCK-KO mice had altered energy expenditure at some time points in present and previous results (36, 48), CCK-KO mice consumed a normal total daily energy expenditure compared with WT
mice. Chronic feeding of a HFD attenuates intestinal and hypothalamic production of ApoAIV (33, 46). ApoAIV-KO mice fed the HFD had normal total energy expenditure with lower hourly energy expenditure at some time points, suggesting that HFD-induced ApoAIV enhanced energy expenditure.

Blocking CCK1R or CCK2R in WT and CCK2R-KO mice changes locomotor activity (81, 86). However, CCK-KO mice fed a HFD for 10 wk have normal locomotor activity (48). The present experiments showed that ApoAIV-KO and CCK-KO mice displayed normal locomotor activity after chronic consumption of a HFD for 20 wk. These findings suggest that the deficiency of global endogenous CCK or ApoAIV did not alter locomotor activity.

Three factors may have contributed to the reduced body weight of HFD-fed ApoAIV/CCK-KO mice: 1) reduced fat absorption and lipid transport to adipocytes; 2) increased energy expenditure; and 3) elevated locomotor activity. First, the present study showed that ApoAIV/CCK-KO mice displayed normal fat absorption and attenuated lipid transport from the small intestine to white adipose tissues. Second, ApoAIV/CCK-KO mice fed a LFD had normal energy expenditure and utilized more fatty acids as energy substrates than the control group. In contrast, maintenance on a HFD for 20 wk caused a greater increase in energy expenditure in ApoAIV/CCK-KO mice than in WT mice. Thus enhanced energy expenditure in HFD-fed mice lacking CCK and ApoAIV resulted in attenuation of body weight and fat mass. Furthermore, ApoAIV/CCK-KO mice had upregulated CCK2R expression in the BAT, suggesting that both ApoAIV and CCK interact with CCK2R to regulate BAT thermogenesis. Since the role of CCK2R in the control of BAT thermogenesis remains unknown, further study of BAT thermogenesis control induced by ApoAIV and CCK via a CCK2R-dependent pathway is warranted. Third, ApoAIV/CCK-KO mice exhibited normal locomotor activity following chronic consumption of a HFD for 20 wk. This finding suggests that locomotor activity was not responsible for the elevated energy expenditure in the ApoAIV/CCK-KO mice.
In summary, this study demonstrated that when maintained on a LFD, animals with global deficiency of ApoAIV, CCK, or both exhibited normal food intake, fat absorption, and energy expenditure, except for impaired lipid transport. After a 20-wk maintenance on a HFD, ApoAIV/CCK-KO mice experienced a profound reduction of body weight and white adipose tissues, possibly due to elevated energy expenditure, impaired lipid transport, and lower insulin-induced adiposity, despite normal food intake, fat absorption, and locomotor activity.

**Perspectives and Significance**

Obese subjects have normal or higher levels of basal CCK or ApoAIV in the plasma than control subjects (7, 11, 42, 43). Plasma ApoAIV is increased in human obesity and attenuated after a short-term weight loss (43, 64). In view of the observations in the current study, increased energy expenditure, impaired lipid transport, and reduced insulin-induced adiposity in the global deficiency of ApoAIV and CCK are particularly interesting factors in the reduction of body weight and white adipose tissues. Further investigation should be conducted to determine whether attenuated levels of ApoAIV and CCK via a CCK2R-dependent pathway enhance energy expenditure and reduce body weight.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
Fig. 11. Locomotor activity in ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice fed a HFD. Basic movement (A) and average of basic movement (B) in ApoAIV/CCK-KO and WT mice. Basic movement (C) and average of basic movement (D) in CCK-KO and WT mice. ApoAIV/CCK-KO, ApoAIV-KO, CCK-KO, and WT mice were individually housed in home cages that were then placed in a SmartFrame system for 4 continuous days. Data are means ± SE for 6–8 animals per group.

AUTHOR CONTRIBUTIONS


REFERENCES

A novel, long-acting, mini-PEGylated cholecystokinin (CCK) agonist that...}


