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Effect of Exogenous Cholecystokinin (CCK)-8 on Food Intake and Plasma CCK, Leptin, and Insulin Concentrations in Older and Young Adults: Evidence for Increased CCK Activity as a Cause of the Anorexia of Aging

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Healthy aging is associated with reductions in appetite and food intake—the so-called anorexia of aging, which may predispose to protein-energy malnutrition. One possible cause of the anorexia of aging is an increased satiating effect of cholecystokinin (CCK). To investigate the impact of aging on the satiating effects of CCK, 12 young and 12 older healthy subjects received 25-min iv infusions of saline (control) and CCK-8, 1 ng/kg per min or 3 ng/k per min, on 3 separate days before a test meal. Older subjects ate less than young subjects, and food intake was suppressed 21.6% by CCK-8, compared with the control day ($P < 0.05$). The suppression of energy intake by CCK-8 in older subjects was twice that in young subjects (32 ± 6% *vs.* 16 ± 6% SEM, $P < 0.05$) and was related to plasma CCK-8 concentrations, which were higher at baseline ($P < 0.05$) and increased more during CCK-8 infusions in older than young subjects ($P < 0.01$). The extent of suppression of food intake per given rise in plasma CCK-8 concentrations did not differ between the two age groups ($P = 0.35$). Endogenous CCK concentrations were higher at baseline in older subjects ($P < 0.001$) and decreased during the CCK-8 but not control infusions ($P < 0.01$), suggesting that CCK suppresses its own release. Plasma leptin concentrations were not affected by CCK infusion, whereas postprandial insulin concentrations were lowered and the peak postprandial glucose concentration was delayed but not affected by CCK-8 infusion. Because older people retain their sensitivity to the satiating effects of exogenous CCK and plasma endogenous CCK concentrations are higher in older people, increased CCK activity may contribute to the anorexia of aging. (*J Clin Endocrinol Metab* 86: 5830–5837, 2001)

HEALTHY AGING IS associated with a progressive decrease in appetite and food intake (1), which has been termed the anorexia of aging (2). This may predispose to pathological weight loss and protein-energy malnutrition, which is present in up to 20–65% of the hospitalized elderly and 5–85% of nursing home residents in the United States, and represents a major cause of morbidity and mortality (2). The cause(s) of the physiological anorexia of aging have not been established.

An important mediator of satiety signals is thought to be the release of cholecystokinin (CCK) from the small intestine in response to the presence of nutrients, particularly fat and protein (3). In young adult humans, iv infusion of CCK induces a dose-dependent suppression of food intake (4–9). In rats there is evidence that CCK-induced satiety may be mediated by the release of gastric stores of leptin (10). The effect of CCK administration on plasma leptin concentrations in humans has not been evaluated.

Most (11–13) but not all (14) studies have reported higher plasma CCK concentrations in healthy older adults than young adults. In rodents there is evidence that sensitivity to the satiating effects of CCK increases with aging (15). Reduced appetite and food intake in older humans may, therefore, be partly because of increased circulating CCK concentrations combined with enhanced or preserved sensitivity to the satiating effects of CCK.

Aging is associated with an impairment of glucose tolerance (16). The potential role of CCK requires clarification. Exogenous CCK-8 has been reported to decrease serum insulin concentrations in young men (17, 18) but to increase serum insulin and reduce the glycemic response to a meal in postmenopausal women (19). The effect of CCK in older men is unknown.

The aims of this study were to determine whether aging modifies the effects of iv administration of CCK-8 on appetite, food intake, blood glucose and plasma endogenous CCK, insulin, and leptin concentrations. The major hypothesis was that the suppression of appetite and energy intake by CCK-8 infusion would be greater in older than young subjects.

Experimental Subjects

Twelve healthy older subjects, mean age 71.2 ± 1.3 yr (range 67–83 yr) and 12 healthy young subjects, mean age

Abbreviations: CCK, Cholecystokinin; CCK > 12, endogenous CCK; CV, coefficient of variation; HD, higher dose; LD, lower dose.

22.6 ± 1.2 yr (range 18–33 yr), 6 men and 6 women in each age group, were recruited by advertisement. There was no difference in mean body weight or body mass index (24.1 ± 0.7 kg/m^2; older *vs.* 23.5 ± 0.8 kg/m^2; young, $P = 0.6$) between the two groups. In all subjects mean energy intake was > 4182 kJ/d (1000 kcal/d) as assessed by a 3-day food diary (20). All subjects were healthy, unrestrained eaters [score < 11 for "cognitive restraint" on the Three Factor Eating Questionnaire (21)], nonsmokers, and without a history of gastrointestinal disease or diabetes mellitus. None was taking medications known to influence appetite or gastrointestinal motility. The study was approved by the Ethics Committee of the Royal Adelaide Hospital, under the Clinical Trial Notification Scheme of the Therapeutic Goods Administration of the Australian Government. Subjects gave written, informed consent.

Materials and Methods

Protocol

Each subject underwent three studies on separate days, in random order. At 0830 h, after ~12 h of overnight fasting, an iv cannula was inserted into each arm for blood sampling and treatment infusion. Starting 30 min later (t = 0), isotonic (0.9%) saline (40 ml/h) was infused iv for 125 min. At t = 90 min a preload [similar to that used by Lieverse *et al.* (22)] consisting of 125 g banana blended with 150 ml low-fat (0.1%) milk and 150 ml water (744 kJ; 21% protein, 2% fat, 75% carbohydrate) was consumed within 3 min. From t = 100–125 min, iv infusions of either 50 ml saline or CCK-8 (Sincalide; Squibb Diagnostics, Montréal, Canada) lower dose (LD;1 ng/kg per min) or CCK-8 higher dose (HD; 3 ng/kg per min), both in 50 ml saline, were administered. At t = 110 min, subjects were offered a standardized high-carbohydrate, low-fat meal prepared in excess of what they would normally eat and invited to eat as much as they wished. The rate of ingestion and the total amount of food consumed were quantified (20). At regular intervals visual analog scales were administered to assess appetite ratings and venous blood samples collected in ice-chilled dipotassium EDTA tubes containing 400 KIU aprotinin (Trasylol) per milliliter of blood. Plasma was separated by centrifugation within 30 min of collection and stored at −70 C until assay. After leaving the laboratory, subjects recorded their food intake for the rest of the study day (until midnight). Subjects were told that the main aim of the study was to determine the effect of CCK infusion on circulating hormone concentrations. The assessment of food intake was not emphasized.

Measurements

Appetite. Sensations of hunger, fullness, and nausea were assessed using 10-cm linear visual analog scales (20, 23). The t = 90 score (immediately before preload) was used as the baseline. These questionnaires have been validated in young (24) and older subjects (Chapman, I. M., unpublished data).

Energy intake

The total amount (grams) of food consumed during the meal (food offered: 375 ml low-fat iced coffee; 300 ml unsweetened orange juice; 100 g tomato, onion, and garlic pasta sauce mixed with 200 g (uncooked weight) white pasta; one whole-meal dinner roll; one white dinner roll; two slices of whole-meal bread; two slices of white bread; one slice of light cheddar cheese; eight slices of tomato and cucumber; one sachet of margarine; 200 g low-fat artificially sweetened strawberry yogurt; 150-g lime-flavored jelly; and 400 g tropical fruit salad in heavy syrup). The meal duration (minutes) and the rate of food intake (kilojoules per minute) were calculated. Food intake from the meal was analyzed using the DIET/1 Nutrient Calculation software (Xyris Software, Queensland, Australia) to determine both energy intake (kilojoules) and macronutrient composition (percent protein, percent fat, and percent carbohydrate) of the meal (20).

Assays

For each hormone, all of the samples for each subject were analyzed in the same assay.

CCK

CCK-8. CCK-8 was measured by RIA as described by Santangelo *et al.* (25). Standards (synthetic sulfated CCK-8, Sigma, St. Louis, MO) were prepared in charcoal-stripped plasma and extracted in 66% ethanol along with the samples. Extracts were dried under N_2 and resuspended in assay buffer (50 mM phosphate, 10 mM EDTA, 2 g/liter gelatin, pH 7.4). Antibody (C2581, lot 105H4852, Sigma) was added at a dilution of 1/17,500, and sulfated CCK-8 ^{125}I-labeled with Bolton and Hunter reagent (74 Tbq/mmol, Amersham International, Amersham Pharmacia Biotech, Bucks, UK) was used as tracer. Incubation was for 3 d at 4 C. The antibody-bound fraction was separated using dextran-coated charcoal containing gelatin (0.015 g gelatin, 0.09 g dextran, 0.15 g charcoal in 30 ml assay buffer). The interassay coefficient of variation (CV) at 50 pmol/liter was 9.5% and intraassay CV was 9%. Sensitivity was 1 pmol/liter.

Endogenous CCK (CCK > 12). A RIA technique was used to measure CCK peptides containing > 12 amino acid residues (CCK >12) as previously described (26). The antibody (1703) was raised in a rabbit by immunization with 30% CCK bound to all COOH terminal CCK-peptides containing at least 12 amino acid residues. The detection limit of the assay was 1 pmol/liter, interassay CV 11–26%, and the intraassay CV 4–11%.

Insulin

Plasma insulin was measured using the Imx microparticle enzyme immunoassay (Abbott Laboratories, Diagnostic Division, Dainabot, Tokyo, Japan) (26). The detection limit of the assay was 1.0 mU/ml. The interassay coefficients of variation were 4.5% at 8.3 mU/ml and 3.4% at 40.4 mU/ml.

Leptin

Plasma leptin was measured using the DSL ACTIVE Human Leptin ELISA immunoassay kit (Diagnostics Systems Laboratories, Inc., Webster, TX). The detection limit of the assay was 0.05 ng/ml, intraassay CVs 4.4% at 4.8 ng/ml and 1.5% at 46.3 ng/ml, and interassay CVs 4.9% at 4.7 ng/ml and 4.2% at 37.9 ng/ml.

Data analysis

Results are given as mean ± SEM. Comparisons between the young and older groups in the energy and macronutrient content of the previous diet, restraint score, and body mass index were performed using unpaired *t* test because these data were normally distributed. The data were analyzed using Statview version 5.0 (Abacus Concepts Inc., Berkeley, CA). Baseline scores for hunger, fullness, and nausea and fasting concentrations of blood glucose and plasma CCK-8, CCK >12, insulin and leptin, and differences in mean energy intake (kilojoules), meal duration, rate of eating, and macronutrient content of the buffet meal and energy intake for the remainder of the day were analyzed by repeated-measures, two-way ANOVA, with age and treatment as the factors. The effects of the preload (t = 90–100 min), iv infusions of saline (control), CCK-8 LD and CCK-8 HD (t = 100–110 min), and buffet meal (t = 140–185 min) on absolute ratings of hunger, fullness and nausea, plasma concentrations of CCK-8 and CCK >12, insulin, and leptin were analyzed using repeated-measures three-way ANOVA, with time, age, and treatment as the factors. When a significant interaction among factors was observed, contrasts were used to test preplanned hypotheses of interest enabling paired comparisons among the three study days. The ANOVAs were performed using SuperANOVA version 1.11 (Abacus Concepts Inc.). Relationships between energy intake (kilojoules and percent suppression) and plasma CCK >12 and CCK-8 concentrations were assessed by linear regression analysis using Statview version 5.0 (Abacus Concepts Inc.). A P value < 0.05 was considered significant.

Results

The studies were well tolerated. Energy intake from the usual diet was ~30% less in older than young subjects (7114 ± 293 *vs.* 9819 ± 828 kJ/d; $P < 0.01$), with no difference in the proportion of carbohydrate, fat, or protein eaten. There was no difference in restraint scores between older and young subjects (6.3 ± 0.8 *vs.* 4.3 ± 0.9; $P = 0.11$).

Appetite

There were no significant treatment × age or treatment × time interactions for any rating in either age group.

Hunger

Hunger ratings were less in older than young subjects at baseline (4.0 ± 0.3 *vs.* 6.1 ± 0.4 cm, F = 9.45; $P < 0.01$) and throughout the study days (effect of age; F = 12.80 $P < 0.01$). Hunger decreased during the study days (effect of time; F = 61.88; $P < 0.001$), with no effect of treatment (F = 0.48; $P = 0.62$), but a greater decrease in the older subjects (time × age interaction; F = 8.41; $P < 0.001$). There was a treatment × time × age interaction (F = 2.14; $P < 0.05$); before the meal (t = 110 min), hunger ratings were less during the HD than the LD (F = 9.46; $P < 0.01$) and control (F = 11.06; $P < 0.01$) infusions in the young than older subjects.

Fullness

Fullness ratings were similar in older and young subjects at baseline (F = 0.04; $P = 0.85$) and during the studies (effect of age, F = 0.55; $P = 0.47$). Fullness increased throughout the study days (effect of time; F = 66.94; $P < 0.001$), with no effect of treatment (F = 1.87; $P = 0.17$) or time × age or treatment × time × age interactions.

Nausea

Ratings of nausea were similar during fasting in older and young subjects (F = 0.03; $P = 0.86$). There was no effect of age (F = 0.28; $P = 0.60$), treatment (F = 0.54; $P = 0.53$), or time (F = 1.40; $P = 0.25$) and no time × age or treatment × time × age interactions.

Food intake

Intake on the 3 study days (Fig. 1A) was 41% lower in the older than young subjects (age; F = 8.04; $P < 0.01$). In older subjects the duration of eating was less and they ate more slowly (data not shown). CCK-8 infusion had dose-dependent suppressive effects on both the duration (F = 7.84, $P < 0.01$) and rate (F = 10.42; $P < 0.001$) of eating, with no treatment × age interaction for either. Consequently, CCK-8 infusion was associated with a suppression of energy intake (treatment; F = 24.68; $P < 0.001$); LD and HD infusions produced 7.7% and 35.2% (mean 21.6%), respectively, suppression of energy intake, compared with the control infusion. Energy intake was significantly lower during HD than both control (F = 44.12; $P < 0.001$) and LD (F = 28.11; $P < 0.001$) but not during the LD than control infusion (F = 1.80; $P = 0.19$). There was no treatment × age interaction ($P = 0.51$).

When energy intake was expressed as a percentage of the control day intake (Fig. 1B), HD suppressed food intake more than LD (treatment; F = 19.32; $P < 0.001$), and CCK-8 suppressed intake more in older than young subjects (effect of age, F = 5.70; $P < 0.05$), with suppression by 16% and 48% (mean 32%) on the LD and HD days, respectively, in the older subjects. The comparable values in young subjects were an increase of 3% and a decrease of 34% (mean 15.5% suppression). There was no treatment × age interaction (F = 0.12; $P = 0.74$). The effects of age and treatment on weight (grams) of food consumed were similar to those on energy intake.

There were no differences between age groups in intake of carbohydrate (F = 3.62; $P = 0.07$), fat (F = 2.92; $P = 0.10$), or protein (F = 1.73; $P = 0.20$) as a percentage of the total energy intake, and no effect of treatment on these percentages (F = 0.61, $P = 0.53$; F = 0.39, $P = 0.68$; F = 0.51, $P = 0.54$). There were no treatment × age interactions for any macronutrients.

Older subjects ate approximately 35% less than young subjects during the remainder of the study days (mean: 3874 ± 242 *vs.* 5986 ± 586 kJ, F = 8.30; $P < 0.01$), with no effect of treatment (F = 0.76; $P = 0.72$) or treatment × age interaction (F = 1.05; $P = 0.35$).

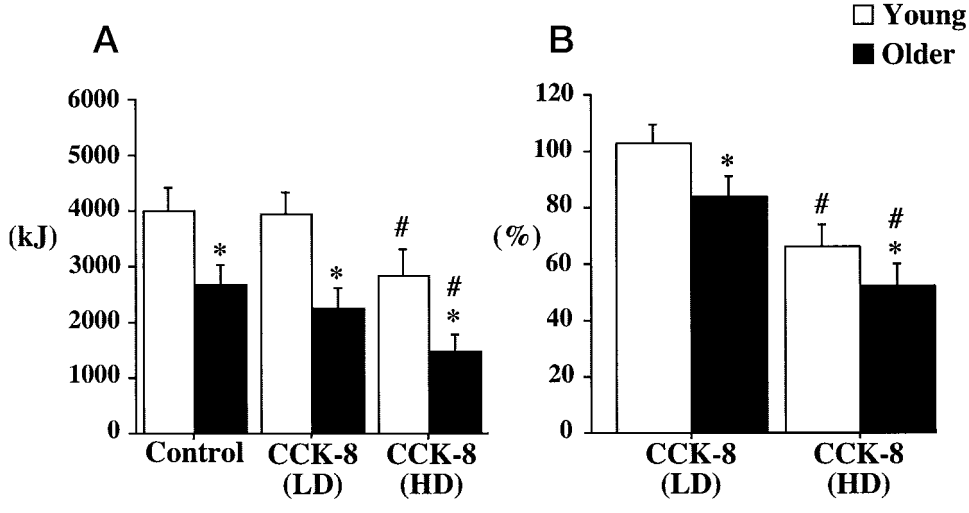

FIG. 1. A, Mean (± SEM) energy content (kilojoules) of meal consumed during iv control, CCK-8 LD, and CCK-8 HD; B, food intake (± SEM) (percentage of intake during control infusion) during iv CCK-8 LD and HD infusions. Effect of age, *, $P < 0.01$ young > older for (a) and †, $P < 0.05$ young > older for (b); effect of treatment, #, $P < 0.05$ LD and control > HD for (a) and LD > HD for (b).

Plasma CCK-8 concentrations

Plasma CCK-8 concentrations (Fig. 2, A, B, and C) were higher in the older than young subjects at baseline (mean of 3 d; 4.9 ± 1.6 *vs.* 1.2 ± 0.1 pmol/liter, $P < 0.05$) and throughout the 3 study days (mean of baseline-170 min; 17.3 ± 1.5 *vs.* 8.2 ± 0.9 pmol/liter, effect of age, F = 5.08; $P < 0.05$), including the control day (9.6 ± 1.3 *vs.* 3.7 ± 0.5 pmol/liter).

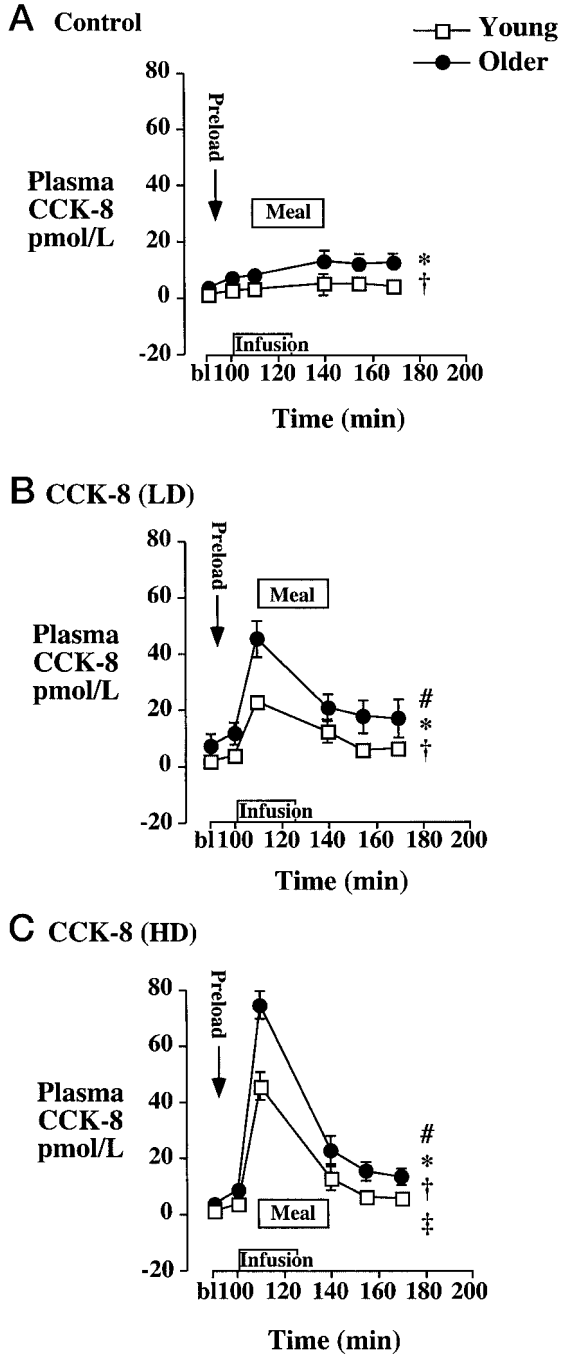

FIG. 2. Mean (± SEM) plasma CCK-8 concentrations (pmol/liter) at baseline (bl); after the preload (t = 100 min); during iv infusions of (A) saline, (B) CCK-8 LD, and (C) CCK-8 HD (t = 100–125 min); and following the buffet meal (t = 140–170 min). Effect of age, *, $P < 0.05$, older > young; effect of treatment, #, $P < 0.001$ HD and LD > control and †, $P < 0.05$ HD > LD. Effect of time, $P < 0.001$.

There was a small rise in plasma CCK-8 as a result of ingestion of the preload (from t = 90 to t = 100 min, 3.1 ± 2.3 to 5.8 ± 0.9 pmol/liter; F = 5.44; $P < 0.05$). Plasma CCK-8 increased in a dose-dependent manner during CCK-8 infusions [effect of treatment (F = 30.84; $P < 0.001$), time (F = 125.89; $P < 0.001$), and treatment × time interaction (F = 69.12; $P < 0.001$)] and more in older than young subjects [(time × age; (F = 7.37; $P < 0.001$) and treatment × time × age; (F = 69.82; $P < 0.01$)], with CCK-8 concentrations of 8.3 ± 2.3, 44.7 ± 6.3 and 74.1 ± 6.0 pmol/liter after 10 min of the control, LD, and HD infusions in the older subjects, compared with 3.4 ± 1.1, 22.4 ± 2.4, and 45.4 ± 5.7 pmol/liter in the young subjects.

Relationship between the suppression of food intake and plasma CCK-8 concentrations

There was an inverse relationship between energy intake at the test meal and plasma CCK-8 concentrations immediately before the meal (R = −0.34, $P < 0.01$) and a positive relationship between the percentage suppression of energy intake on the LD and HD infusion days and the rise in plasma CCK-8 concentrations (t = 100–110) associated with these infusions (R = 0.30, $P < 0.05$). There was no difference between the slopes of these regression lines in young and older subjects (t = 0.89, $P = 0.35$), indicating that the extent of suppression of food intake associated with a given rise in plasma CCK-8 was comparable in young and older subjects.

Endogenous plasma CCK concentrations (CCK >12)

Plasma CCK >12 concentrations (Fig. 3, A, B, and C) were higher in the older than young subjects at baseline (3.8 ± 0.1 *vs.* 2.3 ± 0.1 pmol/liter, $P < 0.001$) and throughout the three study days (mean baseline to 170 min; 4.1 ± 0.1 *vs.* 2.8 ± 0.1 pmol/liter, $P < 0.001$). There was a small rise in CCK >12 concentrations associated with preload ingestion, from 3.1 ± 0.1 to 3.8 ± 0.2 pmol/liter (F = 10.34; $P < 0.05$). CCK >12 concentrations decreased during treatment infusions [3.4 ± 0.1 pmol/liter at 100 min to 2.9 ± 0.1 pmol/liter at 125 min (F = 10.01; $P < 0.001$)], reflecting a decrease during both CCK-8 infusions but not the control infusion (treatment × time, F = 3.82; $P < 0.01$). There was an inverse relationship between the change in plasma CCK >12 and the change in CCK-8 concentrations during CCK-8 LD and HD infusions (R = −0.3, $P < 0.05$). There were no treatment × age, time × age, or treatment × time × age interactions for CCK >12 concentrations.

Blood glucose concentrations

Blood glucose concentrations (Fig. 4A) were higher in older than young subjects at baseline (5.2 ± 0.1 *vs.* 4.9 ± 0.1 mmol/liter; $P < 0.05$) and throughout the three study days (mean baseline to 170 min; 6.4 ± 0.1 *vs.* 5.7 ± 0.1 mmol/liter, $P < 0.01$). All fasting glucose concentrations were < 7 mmol/liter. Following nutrient ingestion, glucose concentrations increased (F = 49.38; $P < 0.001$), with no difference between the treatments (F = 0.34; $P = 0.71$). The increase in blood glucose was greater in the older than young subjects [time ×

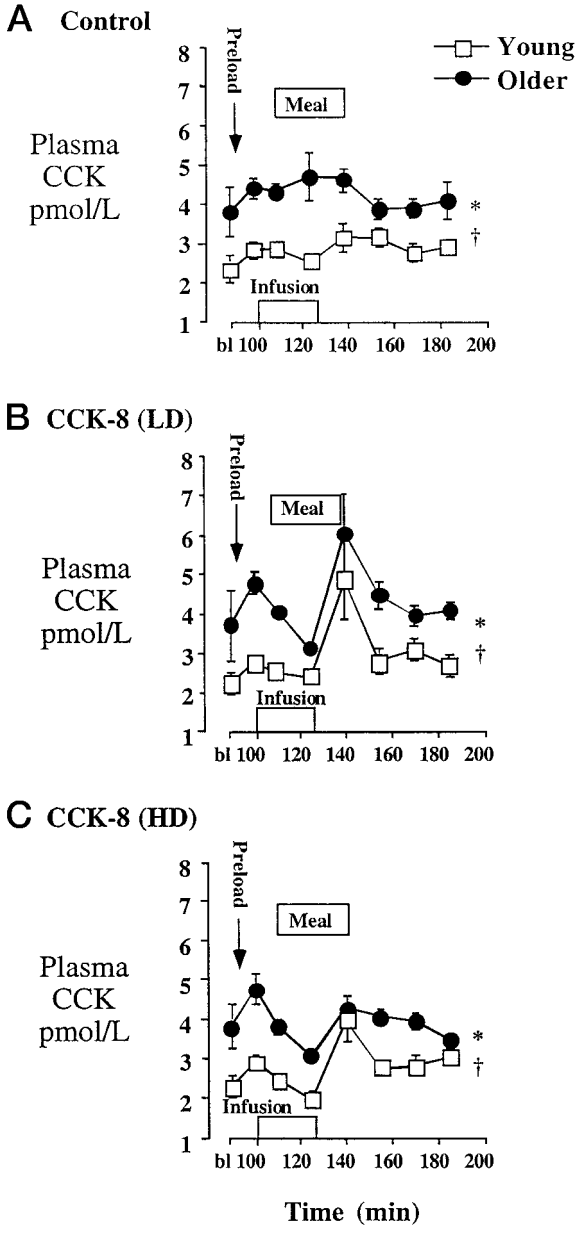

FIG. 3. Mean (±SEM) plasma concentrations of endogenous CCK (CCK > 12) at baseline (bl); after the preload (t = 100 min); during iv infusions of (A) saline, (B) CCK-8 LD, and (C) CCK-8 HD (t = 100–125 min); and following the buffet meal (t = 140–185 min). Effect of age, *, $P < 0.001$ older > young; effect of time, $P < 0.001$; treatment × time interaction, †, $P < 0.01$ greater decrease during HD and LD infusions than control.

age (F = 5.46; $P < 0.05$)] and tended to be less during the CCK-8 infusion than the control infusion in the elderly [treatment × time × age interaction (F = 2.03; $P = 0.06$)]. There were no significant treatment × age or treatment × time interactions (data not shown). The postprandial rise in blood glucose was delayed (time to peak; control, 153 ± 2.4 min; (LD), 158.8 ± 2.3 min; (HD), 161.3 ± 2.0 min; F = 3.40; $P < 0.05$) but not affected (mean peak; control, 7.1 ± 0.3 mmol/liter; CCK-8 (LD), 7.4 ± 0.3; CCK-8 (HD), 7.4 ± 0.2; F = 0.65; $P = 0.53$) by CCK-8 administration.

Plasma insulin concentrations

Plasma insulin concentrations (Fig. 4B) were lower in the older than young subjects at baseline (4.6 ± 0.3 *vs.* 5.6 ± 0.4 mU/liter; $P < 0.05$) and throughout the three study days (mean baseline to 170 min; 32.8 ± 2.3 *vs.* 57.9 ± 4.4 mU/liter, $P < 0.01$). Following nutrient ingestion, plasma insulin concentrations increased on all three study days (effect of time F = 49.38; $P < 0.001$)], but the magnitude of this increase was less in older than young subjects (time × age interaction F = 4.40; $P < 0.05$ [Fig. 4B]). There was a nonsignificant trend for plasma insulin to be lower on the CCK-8 infusion days (effect of treatment F = 2.89; $P = 0.07$) and the increase in plasma insulin was less on the HD than the LD day, with no difference between the control and either the LD or HD days [treatment × time interaction (F = 3.05; $P < 0.05$)]. There were no treatment × age or treatment × time × age interactions. CCK-8 treatment reduced plasma insulin concentrations after the meal (mean insulin at t = 140 min, control; 78.4 ± 7.8 mU/liter, LD; 77.5 ± 12.1 mU/liter, and HD; 49.1 ± 8.6; effect of treatment F = 5.26; $P < 0.01$), and there was a nonsignificant trend for this suppression to be more marked in the older subjects (age × treatment F = 0.99; $P = 0.38$). The postprandial rise in plasma insulin (peak minus t = 110 min) was strongly related to energy intake at the buffet meal (kilojoules) (R = 0.61, $P < 0.0001$). There was no effect of gender on the insulin response to CCK-8 (data not shown).

Plasma leptin concentrations

Baseline plasma leptin concentrations (Fig. 4C) were similar in the older and young subjects (8.8 ± 1.7 μg/liter *vs.* 6.8 ± 1.3 μg/liter; $P = 0.34$). Plasma leptin decreased throughout the three study days (effect of time; F = 3.22; $P < 0.05$), with no difference between young and older subjects (effect of age, F = 0.28; $P = 0.46$) and no effect of exogenous CCK-8 (effect of treatment, F = 0.21; $P = 0.55$). There were no significant interactions (age × treatment, time × age, treatment × time × age) for plasma leptin concentrations.

Discussion

The major observations of this study are that: 1) food (energy) intake was suppressed by iv CCK-8 infusion more in older than young subjects; 2) the magnitude of this suppression was related to the increase in plasma CCK-8 concentrations, which was greater in the older subjects; and 3) plasma concentrations of endogenous CCK were suppressed by CCK-8 infusion.

Studies in healthy, young subjects have demonstrated that acute administration of CCK-8 and -33 decreases food intake by 15–50% (4–9). This suppression is enhanced by simultaneous gastric distention (7), hence our use of a low-fat, oral banana "shake" preload to distend the stomach while minimally stimulating endogenous CCK release. Although we recognize that a banana shake is not consumed regularly by most people, the consumption of a nutrient preload before a main meal is certainly not unusual. It is likely that in some previous studies, the doses of CCK administered resulted in supraphysiological plasma CCK concentrations (4, 6, 8). This was also the case for the high-dose infusion in this study and

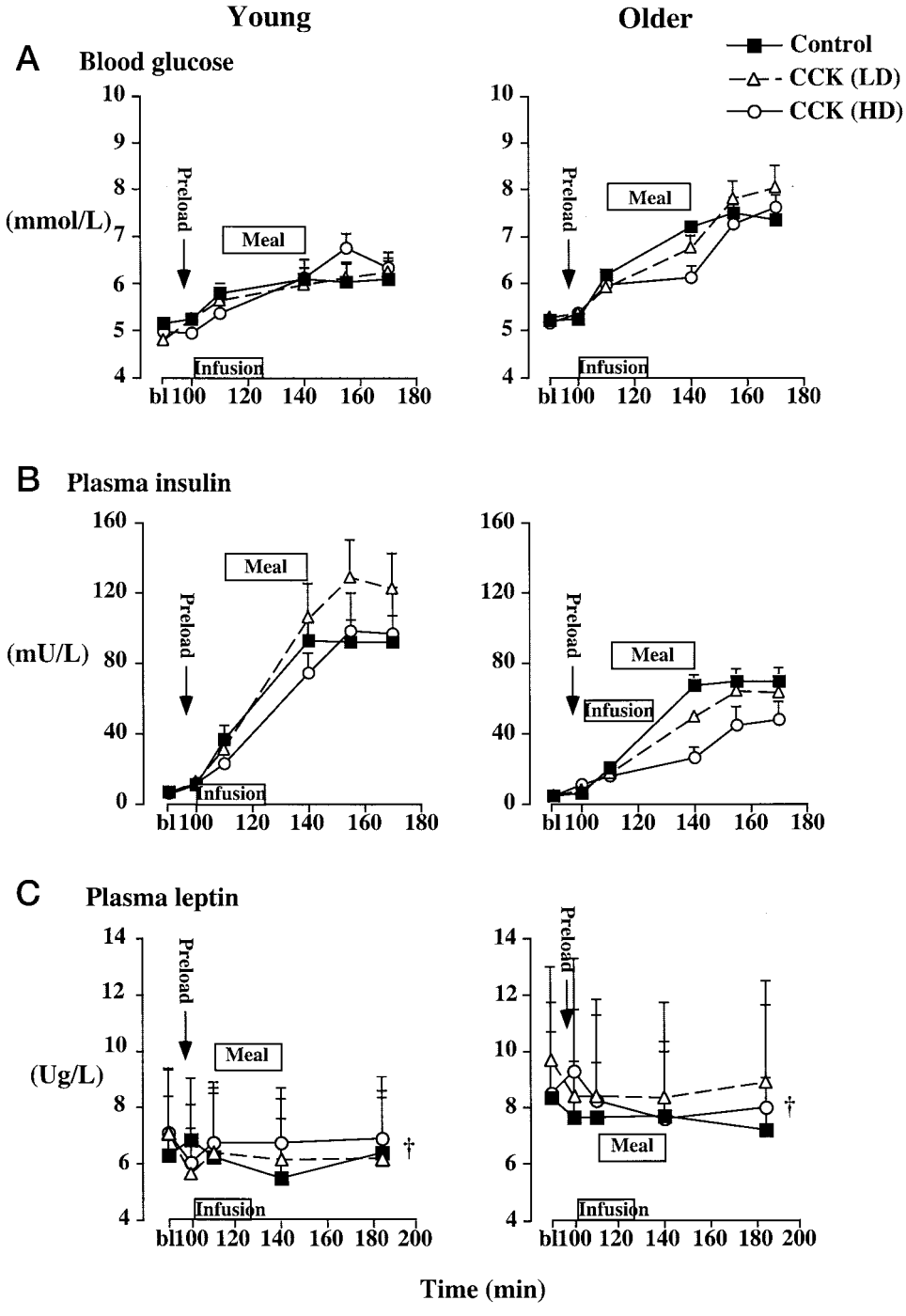

FIG. 4. Concentrations of (A) blood glucose, (B) plasma insulin, and (C) plasma leptin at baseline (t = mean of 0 and 90 min); after the preload; during iv infusions of saline, CCK-8 LD, and CCK-8 HD (t = 100–125 min); and following the buffet meal (t = 140–185 min) in 12 young and 12 older healthy subjects. Effect of age, *, $P < 0.01$ older > young for glucose and older < young for insulin; effect of time, *P*, < 0.001 increase in insulin and glucose, $P < 0.05$ decrease in leptin; effect of treatment, #, $P < 0.01$ postprandial insulin concentration (t = 140) LD and control > HD; treatment × time interaction, †, $P < 0.05$ increase in insulin HD < LD.

possibly also for the low dose, which resulted in peak plasma CCK-8 concentrations that were about twice those achieved after the test meal on the control day. There is persuasive evidence, however, that CCK is a physiological satiety hormone in both animals and humans. Intraperitoneal administration of CCK antagonists increases food intake in satiated animals (27, 28) and Lieverse *et al.* (22) demonstrated an 18.5% reduction in food intake using a dose of CCK-33 that produced plasma levels comparable to those observed following a meal (9). A trend toward increased food intake after administration of the CCK antagonist, loxiglumide, has been reported in healthy young humans (9, 29), and in young men, iv infusion of loxiglumide abolishes the suppression of energy intake produced by intraduodenal fat (30).

Exogenous CCK-8 suppressed food intake approximately twice as much in the older than the young subjects (32% *vs.* 15.5%). This is consistent with a previous report that older mice are more sensitive than young mice to the satiating effects of exogenous CCK-8 (15). Plasma CCK concentrations increased more during CCK-8 infusions in the older than young subjects, which is likely to explain the greater suppression of energy intake by CCK-8 infusions in the former

group. The suppression of energy intake at the buffet meal was related to the increase in plasma CCK-8 during CCK-8 infusion, with no evidence of a differential response between the two groups. This latter observation indicates that sensitivity to the suppressive effects of exogenous CCK-8 on appetite is preserved in the healthy elderly and, when considered together with the higher plasma CCK concentrations in healthy older than young subjects demonstrated in this and most previous studies (11–13), is consistent with the concept that increased endogenous CCK activity is of etiological importance in the physiological anorexia of aging. Investigation of the effect of CCK antagonists, such as loxiglumide, on food intake in the elderly is indicated to confirm this hypothesis.

The higher plasma CCK-8 concentrations observed in the older subjects during CCK-8 infusions probably reflects a lower volume of distribution and/or delayed clearance of CCK-8. The latter appears more likely. For lipophilic compounds cleared mainly by the liver, such as CCK-8 (31), clearance is more closely related to lean body mass than total body weight, whereas the opposite is so for volume of distribution (32); although our groups were matched for total body weight and CCK-8 was administered on a per kilogram total body weight basis, it is well recognized that lean body weight declines as a proportion of total body weight with age (32).

A recent study in rats suggests that gastric stores of the satiety hormone, leptin, may be involved in CCK-mediated suppression of food intake (10); within 15 min of ip CCK-8 administration, the leptin content of the gastric fundus decreased and plasma leptin increased. *In vitro* studies in rats also suggest that leptin and CCK may act synergistically, via direct stimulation of gastric vagal afferents, to inhibit food intake (33). Although we found no effect of CCK administration on plasma leptin concentrations, this does not exclude the possibility of an effect of CCK on leptin secretion because plasma leptin was measured under standardized conditions for only 10 min after the start of the CCK infusions.

To our knowledge, this is the first study to demonstrate that CCK inhibits its own release. The exogenous CCK-8, but not control, infusions were associated with a significant suppression of the plasma CCK fragments greater than 12 amino acids in length (CCK >12), and the increase in plasma CCK-8 in response to CCK-8 infusion was related to the fall in CCK >12. Cholecystokinin occurs in a number of forms, including CCK-4, -8, -21, -33, -38, -54, and -58 (34); fragments with >12 amino acid residues are the most abundant circulating, biologically active form (3). Our finding that CCK inhibits its own release is in contrast to that of Jebbink *et al.* (35) who, using the same assay for CCK >12, found no effect of iv CCK-8 infusion in a similar total dose to our high dose (79.8 ng/kg over 60 min *vs.* 75 ng/kg over 25 min in our study) on plasma CCK >12 after a high-fat meal in healthy young subjects. This may indicate that the autofeedback effects of CCK are relatively weak, so CCK-8 can overcome the secretory drive to endogenous CCK release produced by a low-fat, low-energy meal, as in the present study, but not a strong stimulus such as a high-fat meal.

We found that CCK-8 infusion reduced plasma insulin concentrations after the *ad libitum* meal. Several studies have examined the effect of exogenous CCK on the insulin and glucose responses to either a mixed meal or oral glucose, with seemingly contradictory results (17–19). Consistent with our observations, Schick *et al.* (17) found that CCK-9 (24 pmol/kg per hr for 45 min) inhibited the insulin response to an *ad libitum* meal as well as decreased the size of this meal. Similarly, Liddle *et al.* (18) reported that iv CCK-8 infusion reduced plasma insulin and glucose concentrations after oral glucose, by slowing gastric emptying. In contrast, Ahren *et al.* (19) recently reported that iv CCK-8 (24 pmol/liter per kilogram per hour for 90 min) increased plasma insulin and lowered glucose concentrations following a meal of fixed size in postmenopausal women, both with and without type 2 diabetes, and postulated that CCK may be of some benefit in the treatment of diabetes. These discrepant observations may potentially be attributable in part to the different insulin assays used. For example, we used an immunoassay, whereas Ahren *et al.* (19) used a RIA. We believe, however, that a more likely explanation is the use of *ad libitum vs.* fixed-size mixed meals in these studies. CCK potentiates amino acid-stimulated insulin secretion (36), so when the meal is of fixed size and contains protein, insulin levels are increased, whereas in the *ad libitum* mixed-meal studies, CCK exerts a satiating effect and so reduces meal size, favoring a reduction in insulin release (37). Consistent with this, we observed a strong positive relationship between meal size and postprandial insulin levels. Of note, although infusion of CCK-8 delayed the postprandial rise in blood glucose, probably because of its slowing of gastric emptying (18, 38), the peak glucose level was unaffected in this study. Nevertheless, the reduction in food intake produced by CCK, if sustained, might be of benefit in overweight people with type 2 diabetes mellitus.

In summary, exogenous CCK-8 is more satiating in older than young adults, and in older people plasma endogenous CCK concentrations are higher both in the fasted state and in response to a low-energy preload. Despite higher circulating CCK concentrations, older people retain their sensitivity to the satiating effects of exogenous CCK, suggesting that enhanced endogenous CCK activity may contribute to the anorexia of aging. Hence, there is a potential therapeutic role for CCK antagonists to increase food intake in the anorectic and/or malnourished elderly.

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References

1. **Wurtman JJ, Lieberman H, Tsay R, Nader T, Chew B** 1988 Calorie and nutrient intakes of elderly and young subjects measured under identical conditions. J Gerontol 43:B174–B180
2. **Morley JE** 1997 Anorexia of aging: physiologic and pathologic. Am J Clin Nutr 66:760–773

3. **Peikin SR** 1989 Role of cholecystokinin in the control of food intake. Gastroenterol Clin North Am 18:757–775
4. **Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP** 1981 C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr 34: 154–160
5. **Pi-Sunyer FX, Kissileff HR, Thornton J, Smith GP** 1982 C-terminal octapeptide of cholecystokinin decreases food intake in obese men. Physiol Behav 29:627–630
6. **Stacher G, Steinringer H, Schmierer G, Schneider C, Winklehner S** 1982 Cholecystokinin octapeptide decreases intake of solid food in man. Peptides 3:133–136
7. **Muurahainen NE, Kissileff HR, Lachaussee J, Pi-Sunyer FX** 1991 Effect of a soup preload on reduction of food intake by cholecystokinin in humans. Am J Physiol 260:R672–R680
8. **Greenough A., Cole G, Lewis J, Lockton A, Blundell J** 1998 Untangling the effects of hunger, anxiety, and nausea on energy intake during intravenous cholecystokinin octapeptide (CCK-8) infusion. Physiol Behav 65:303–310
9. **Lieverse RJ, Jansen JB, Masclee AA, Lamers CB** 1995 Satiety effects of a physiological dose of cholecystokinin in humans. Gut 36:176–179
10. **Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau J-P, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le-Marchand-Brustel Y, Lewin MJ** 1998 The stomach is a source of leptin. Nature 394:790–793
11. **MacIntosh CG, Andrews JM, Jones KL, Wishart JM, Morris HA, Jansen JB, Morley JE, Horowitz M, Chapman IM** 1999 The effects of age on plasma cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) concentrations and their relation to appetite and pyloric motility. Am J Clin Nutr 69:999–1006
12. **Khalil T, Walker JP, Wiener I, Fagan CJ, Townsend Jr CM, Greeley Jr JR, Thompson JC** 1985 Effect of aging on gallbladder contraction and release of cholecystokinin 33 in humans. Surgery 98:423–429
13. **Masclee AA, Geuskens LM, Driessen WMM, Jansen JBMJ, Lamers CBHW** 1988 Effect of aging on plasma cholecystokinin secretion and gallbladder emptying. Age 11:136–140
14. **Berthelemy P, Bouisson M, Vellas B, Moreau J, Vaysse N, Albarede JL, Ribet A** 1995 Postprandial cholecystokinin secretion in elderly with protein-energy undernutrition. J Am Geriatr Soc 40:365–369
15. **Silver AJ, Flood JF, Morley JE** 1988 Effect of gastrointestinal peptides on ingestion in old and young mice. Peptides 9:221–225
16. **Ranganath L, Sedgewick L, Morgan L, Wright J, Marks J** 1998 The ageing entero-insular axis. Diabetalogia 41:1309–1313
17. **Schick RR, Schusdziarra V, Mossner J, Neuberger J, Schroder B, Segmuller R, Maier V, Classen M** 1991 Effect of CCK on food intake in man: physiological or pharmacological effect? Z Gastroenterol 29:53–58
18. **Liddle RA, Rushakoff RJ, Morita ET, Beccaria L, Carter JD, Goldfine ID** 1988 Physiological role for cholecystokinin in reducing postprandial hyperglycemia in humans. J Clin Invest 81:1675–1681
19. **Ahren B, Holst JJ, Efendic S** 2000 Antidiabetic action of cholecystokinin-8 in type 2 diabetes. J Clin Endo Metab 85:1043–1048
20. **Cook CG, Andrews JM, Jones KL, Wittert GA, Chapman IM, Morley JE, Horowitz M** 1997 Effects of small intestinal nutrient infusion on appetite and pyloric motility are modified by age. Am J Physiol 273:R755–R761
21. **Stunkard AJ, Messick S** 1985 The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 29:71–83
22. **Lieverse RJ, Jansen JB, Masclee AA, Rovati LC, Lamers CB** 1994 Effect of a low-dose of intraduodenal fat on satiety in humans: studies using the type A cholecystokinin receptor antagonist loxiglumide. Gut 35:501–505
23. **Sepple CP, Read NW** 1989 Gastrointestinal correlates of the development of hunger in man. Appetite 13:183–191
24. **Chapman IM, Goble EA, Wittert GA, Morley JE, Horowitz M** 1998 Effect of intravenous glucose and euglycemic insulin infusions on short-term appetite and food intake. Am J Physiol 274:R596–R603
25. **Santangelo A, Peracchi M, Conte D, Fraquelli M, Porrini M** 1998 Physical state of meal affects gastric emptying, cholecystokinin release and satiety. Br J Nutr 80:521–527
26. **Jansen JB, Lamers CB** 1983 Radioimmunoassay of cholecystokinin in human tissue and plasma. Clin Chim Acta 131:305–316
27. **Brenner L, Ritter RC** 1995 Peptide cholecystokinin antagonist increases food intake in rats. Appetite 24:1–9
28. **Ebenezer IS, De La Riva C, Baldwin BA** 1990 Effects of the CCK receptor antagonist MK-329 on food intake in pigs. Physiol Behav 47:145–148
29. **Drewe J, Gadient A, Rovati LC, Beglinger C** 1992 Role of circulating cholecystokinin in control of fat-induced inhibition of food intake in humans. Gastroenterology 102:1654–1659
30. **Matzinger D, Gutzwiller J-P, Drewe J, Orban A, Engel R, D'Amato M, Rovati L, Beglinger C** 1999 Inhibition of food intake in response to intestinal lipid is mediated by cholecystokinin in humans. Am J Physiol 277:R1718–R1724
31. **Hunter EB, Powers SP, Kost LJ, Pinon DI, Miller LJ, La Russo NF** 1990 Physicochemical determinants in hepatic extraction of small peptides. Hepatology 12:76–82
32. **Morgan DJ, Bray KM** 1994 Lean body mass as a predictor of drug dosage. Implications for drug therapy. Clin Pharmacokinet 26:292–307
33. **Wang YH, Tache Y, Sheibel AB, Go V-LW, Wei JY** 1997 Two types of leptin-responsive gastric vagal afferent terminals: an *in vitro* single unit study in rats. Am J Physiol 273:R833–R837
34. **Pirke KM, Kellner MB, Friess E, Krieg JC, Fichter MM** 1994 Satiety and cholecystokinin. Int J Eat Disord 15:63–69
35. **Jebbink MC, Jansen JB, Mooy DM, Schouten CM, Lamers CB** 1992 Evidence against the autocrine feedback regulation of cholecystokinin secretion in man. Peptides 13:287–290
36. **Rushakoff RJ, Goldfine ID, Carter JD, Liddle RA** 1987 Physiological concentrations of cholecystokinin stimulate amino acid-induced insulin release in humans. J Clin Endocrinol Metab 65:395–401
37. **Whitley HA, Humphreys SM, Samra JS, Campbell IT, Maclaren DP, Reilly T, Frayn KN** 1997 Metabolic responses to isoenergetic meals containing different proportions of carbohydrate and fat. Br J Nutr 78:15–26
38. **Kleibeuker JH, Beekhuis H, Jansen JMBJ, Piers DA, Lamers CBHW** 1988 Cholecystokinin is a physiologic hormonal mediator of fat-induced inhibition of gastric emptying. Eur J Clin Invest 18:173–177