0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(12):5993–5997

doi: 10.1210/jc.2004-0613

Relationship between Ghrelin and Energy Expenditure in Healthy Young Women

DAVID H. ST-PIERRE, ANTONY D. KARELIS, KATHERINE CIANFLONE, FLORENCE CONUS, DIANE MIGNAULT, REMI RABASA-LHORET, MAXIME ST-ONGE, ANDRÉANNE TREMBLAY-LEBEAU, AND ERIC T. POEHLMAN

Unité Métabolique (D.H.S.-P., A.D.K., F.C., D.M., R.R.-L., M.S.-O., A.T.-L., E.T.P.), Département de Nutrition, Faculté de Médicine, Université de Montréal, Montréal, Quebec, Canada H3C 3J7; and Mike Rosenbloom Laboratory for Cardiovascular Research (K.C.), Department of Medicine, McGill University, Montréal, Quebec, Canada H3A 1A1

Ghrelin is a novel peptide that has been isolated from human and rat stomach tissues. Despite its known stimulatory effects on appetite and eating behavior, little information is available regarding its relationship with energy expenditure in normal-weight humans. To address this issue, we examined the relationship between serum ghrelin and resting metabolic rate (RMR), the thermic effect of food (TEF), fasting and postprandial respiratory quotient, physical activity level, peak aerobic capacity ($VO_{2\ peak}$), energy intake, and psychological measures of feeding behavior. We recruited 65 young healthy women and determined RMR and TEF by indirect calorimetry after a 12-h fast. Physical activity was determined by a leisure time physical activity questionnaire; $VO_{2\ peak}$ was determined by bicycle ergometer test to exhaustion; energy intake was determined by a 24-h dietary recall; and food behavior was determined by a three-factor eating questionnaire. Our cohort showed a broad range of body mass index (range, 16.8–28.3 kg/m^2), RMR (range, 820-1550 kcal/d), TEF (range, 74.4–136.5 kcal/d), and percent body fat (range, 14.0–37.7%). We noted significant inverse correlations between ghrelin and RMR ($r = -0.350$, $P = 0.004$) and TEF ($r = -0.396$, $P = 0.001$). These inverse correlations persisted after statistical control for both fat-free mass and fat mass (ghrelin *vs.* RMR partial, $r = -0.284$, $P = 0.024$; and ghrelin *vs.* TEF partial, $r = -0.329$, $P = 0.01$) and insulin levels (ghrelin *vs.* RMR partial, $r = -0.255$, $P = 0.046$; and ghrelin *vs.* TEF partial, $r = -0.287$, $P = 0.024$) using partial correlation analysis. We also observed a significant inverse correlation between ghrelin and daily caloric intake ($r = -0.266$, $P = 0.032$), but ghrelin levels were not significantly correlated with fasting ($r = -0.002$), postprandial respiratory quotient ($r = -0.016$), leisure time physical activity ($r = 0.104$), $VO_{2\ peak}$ ($r = 0.138$), dietary disinhibition ($r = -0.071$), dietary restraint ($r = 0.051$), or feeling of general hunger ($r = -0.028$). These results suggest that higher levels of ghrelin are associated with low levels of resting and postprandial thermogenesis, which is independent of individual differences in fat-free mass and fat mass. Although speculative, serum ghrelin may play a role in the regulation of energy homeostasis by acting as a hormonal marker of increased energy efficiency. (*J Clin Endocrinol Metab* 89: 5993–5997, 2004)

OBESITY AND ITS associated metabolic disorders are among the leading causes of illness and mortality worldwide (1). Presently, the pathophysiological mechanisms that mediate these metabolic disorders are poorly understood. Ghrelin is a novel gut peptide that has been isolated from human and rat stomach tissues (2). There is evidence to suggest that ghrelin could be involved in energy homeostasis, specifically in the regulation of food intake and substrate oxidation (3–11). There is less information, however, on the relationship between ghrelin and energy expenditure in normal-weight humans. During the conduct of our experiment, we noted two investigations that reported a potential inverse relationship between ghrelin and energy expenditure (12, 13). Although speculative, these results raise the possibility that the metabolic effects of ghrelin may extend beyond the regulation of satiety and substrate oxidation and may serve as a biomarker of increased energy efficiency (*i.e.* lower energy expenditure) in humans.

To investigate this possibility, we examined the relationship between ghrelin and energy expenditure with resting metabolic rate (RMR) and thermic effect of food (TEF). We also considered the relationship between ghrelin and several indices of food intake behavior and physical activity levels in a well-characterized population of healthy normal-weight young women.

Subjects and Methods

Subjects

Sixty-five normal-weight healthy young women were recruited to participate in this study. Subjects were recruited by announcements in the University of Montréal area (Montréal, Quebec, Canada). The ethnic make-up of our cohort consisted of 59 Caucasians, three Arabs, and three Blacks. The inclusion criteria for participation were female sex and age 18–35 yr. Exclusion criteria for participation were pregnancy, acute illness, diagnosis of eating disorders, diabetes, and hypertension or dyslipidemia. Thirty women (46.1%) used oral contraceptives. Four women (6.1%) had amenorrhea. One woman (1.5%) was a smoker, but she was instructed not to smoke 24 h before testing.

Overview of the protocol

The University of Montréal Ethics Committee approved this study. After reading and signing the consent form, volunteers participated in the following testing sequence. A blood sample was collected in the

Abbreviations: RMR, Resting metabolic rate; TEF, thermic effect of food; $VO_{2\ peak}$, peak aerobic capacity.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Unité Métabolique when subjects arrived at 0800 h after an overnight fast (12 h). Thereafter, RMR and TEF were measured. Subjects were then served a light lunch, after which body composition measurements were performed. Physical activity, dietary questionnaires, and a measure of peak aerobic capacity ($VO_{2\,peak}$) were performed in the afternoon.

Blood samples

Venous blood samples were collected, centrifuged at 3900 × *g* for 10 min at 4 C, and stored at −80 C for subsequent analysis of ghrelin. Serum immunoreactive total ghrelin levels were measured in duplicates with a commercial RIA procedure using ^{125}I-labeled bioactive human ghrelin as a tracer and a rabbit polyclonal antibody raised against full-length peptides (Phoenix Pharmaceuticals, Belmont, CA). Insulin levels were determined by electrochemiluminescence ECLIA adapted for Elecsys 1010 analyzer with the Insulin Elecsys (Reference no. 12017547) kit (Medicorp, Montréal, Quebec, Canada).

RMR

RMR was measured by indirect calorimetry using the ventilated hood technique (SensorMedics δ Track II; Datex-Ohmeda, Helsinki, Finland). Measurements of gas concentrations were then used to determine RMR using the equation of van Weir (14). Subjects were instructed to fast and drink only water for 12 h before testing, consume no alcohol and restrain from smoking for 24 h before testing, restrain from physical activity for 24 h before testing, and keep physical activity to a minimum the morning of the test. Women were tested in the follicular phase of the menstrual cycle. Measurements were performed while subjects were lying in a supine position. Measurements were performed during 40 min; the first 10 min were considered as an acclimatization period, and the last 30 min were used for analyses. The temperature of the room was maintained at an average of 22 C. The intraclass correlation for RMR was 0.921 and was determined using test-retest condition in 20 volunteers.

TEF

TEF was measured during 105 min after ingestion of 10 kcal/kg of body weight of ENSURE PLUS (Abbott, Abbott Park, IL; 1.5 kcal/ml, 61% carbohydrates, 24% lipids, 15% proteins). TEF was calculated as the difference between the energy expenditure after a meal minus RMR, and values are expressed in kilocalories per 105 min.

Body composition measurements

Body weight (kg) was measured using an electronic scale (Balance Industrielles Montréal Inc., Montréal, Quebec, Canada). Body mass index was calculated as body weight (kg)/height (m^2). Fat-free mass, fat mass, and percent fat mass were evaluated by dual-energy x-ray absorptiometry using a LUNAR Prodigy system, version 6.10.019 (General Electric Lunar Corporation, Madison, WI). Intra-class coefficient correlation for test-retest (n = 20) measures for fat mass and fat-free mass reached 0.999 and 0.998, respectively.

$VO_{2\,peak}$

Aerobic capacity was assessed on an ergocycle (Ergoline 900; Ergoline, Bitz, Germany), with an Ergocard (Medi Soft, Dinant, Belguim) cardiopulmonary exercise test station. Aerobic capacity was tested by a progressive test starting at 60 W, with an augmentation of 40 W every 3 min. Subjects were asked to maintain a constant speed, and the level of resistance on the wheel was adjusted to preserve a constant power output. O_2 and CO_2 were measured by a direct system using a face mask. $VO_{2\,peak}$ was achieved when the power output could no longer be maintained. Heart rate was monitored during all tests using a POLAR heart rate monitor S610 (Polar Electro Oy, Kempele, Finland). $VO_{2\,peak}$ was defined as the highest 30-sec average of oxygen consumption. Test-retest in 19 volunteers yielded an intraclass correlation coefficient of 0.956.

Leisure time physical activity

Energy expenditure in leisure time physical activity was evaluated by the Minnesota Leisure-Time Physical Activity Questionnaire (15). The participants were instructed to report whether or not they performed the activity in the last 12 months. The interviewer then asked the volunteer for the period, the frequency, and the duration of every activity performed. Calculations of energy expenditure were based on The Compendium of Physical Activities Tracking Guide, 2000 (16).

Energy intake

A 24-h dietary recall was used for evaluation of total daily energy intake. Portion sizes were evaluated using models of food serving size. Energy, protein, lipid, and carbohydrate intakes were calculated based on the corrected 2001b Canadian Nutrient File (Health Canada) originally published by Verdier and Beare-Rogers (17).

Eating behavior

Eating behavior was assessed by the self-administrated Three-Factor Eating Questionnaire of Stunkard and Messick (18). This 51-item questionnaire measures cognitive restrained eating (dietary restraint; *i.e.* the perception that one regularly and intentionally eats less than one desires). The second factor represents tendency toward disinhibition (an incidental inability to resist eating cues) on inhibition of dietary restraint and emotional eating. The third factor examines the subjective feeling of general hunger. The Three-Factor Eating Questionnaire has been validated as one accurate measure of cognitive concomitants of eating behavior (18, 19).

Statistical analyses

Statistical analyses were performed by SPSS for Windows (versions 11.5; SPSS Inc., Chicago, IL). Data are presented as mean ± SD. We verified the normality of the distribution of variables with a Kolmogorov-Smirnov test and found no significant deviation from normality. Pearson correlations were used to examine relationships between ghrelin and the variables of interest. Analysis of covariance was performed to examine the relationship between ghrelin and RMR and TEF after statistically controlling for fat-free mass and fat mass on RMR and TEF. $P = 0.05$ was considered statistically significant.

Results

Table 1 shows the physical characteristics of this cohort. This young cohort represented a broad range of normal body weights, body composition, and body habitus. Our intention was to recruit normal-weight young women within a relatively narrow age range to remove the confounding influences of age, eating disorders, high levels of body fat, insulin resistance, and generally large disparities of body habitus on metabolic complications.

Table 2 shows the major variables that were examined as potential correlates of serum ghrelin. We specifically determined measures for insulin and resting energy expenditure (RMR and TEF), substrate oxidation (fasting and postprandial respiratory quotient), estimated energy intake (24-h re-

TABLE 1. Physical characteristics of healthy young women

Physical characteristics	Healthy young women (n = 65)	
	Mean ± SD	Range
Age (yr)	22.8 ± 3.7	18–35
Height (m)	1.65 ± 0.05	1.53–1.82
Body mass (kg)	59.5 ± 7.4	41.6–77.9
BMI (kg/m^2)	21.99 ± 2.45	16.88–28.29
Fat mass (kg)[a]	15.46 ± 5.16	7.00–28.70
Fat-free mass (kg)	41.51 ± 3.40	32.58–49.47
Body fat (%)	25.49 ± 5.85	14.0–37.7

BMI, Body mass index.

[a] Fat mass and fat-free mass were measured by dual-energy x-ray absorptiometry.

TABLE 2. Energy expenditure characteristics of healthy young women

Variable	Healthy young women (n = 65)	
	Mean ± SD	Range
LTA (kcal/d)	335 ± 191	36–952
Energy intake (kcal/d)	2381 ± 718	1145–4033
RMR (kcal/d)	1219 ± 157	820–1550
TEF (kcal/105 min) (n = 63)	108 ± 14	74–136
RQ (fasting)	0.821 ± 0.047	0.73–0.93
RQ (postprandial) (n = 63)	0.895 ± 0.038	0.81–0.99
Dietary disinhibition score	5.4 ± 3.1	0–13
Dietary restraint score	9.3 ± 3.8	1–18
General feeling of hunger score	5.2 ± 2.7	1–13
VO_{2peak} (ml/kg·min) (n = 64)	37.65 ± 6.27	25–56.5
Insulin (μU/ml)	4.33 ± 1.71	1.2–7.9
Ghrelin (pg/ml)	785 ± 260	128–1360

The maximal scores for dietary disinhibition, dietary restraint, and general feeling of hunger are 16, 21, and 14, respectively.

RQ, Respiratory quotient; LTA, leisure time physical activity.

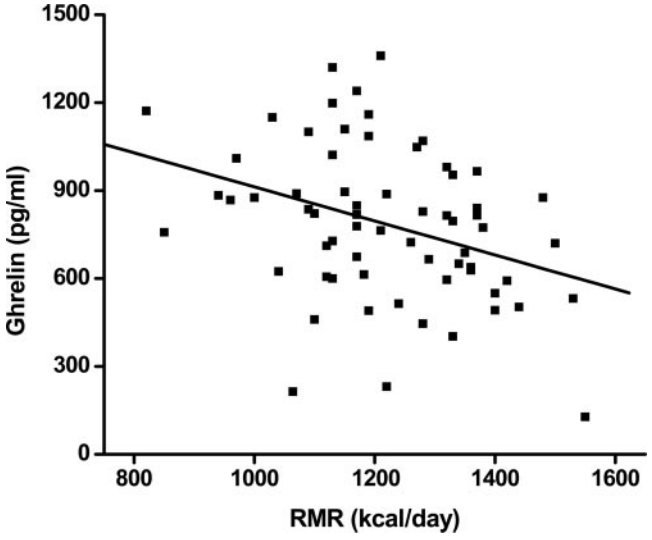

FIG. 1. Correlation between serum ghrelin and RMR. The *line of best fit* is indicated. The partial r value remained significant after statistical control for fat-free mass and fat mass (r = −0.284, P = 0.024) and insulin levels (r = −0.255, P = 0.046) using analysis of covariance.

call), indices of physical activity energy expenditure (physical activity questionnaire), $VO_{2\,peak}$, and dietary behavioral characteristics.

Figures 1 and 2 examine the relationships between resting and postprandial energy expenditure with serum levels of ghrelin. Figure 1 shows the inverse correlation between ghrelin and RMR (r = −0.350, P = 0.004). We also examined this relationship after statistical control for body composition and insulin levels using partial correlation analysis. The partial correlation between ghrelin and RMR remained significant after statistical control for both fat-free mass and fat mass (r = −0.284, P = 0.024) and insulin levels (r = −0.255, P = 0.046).

Figure 2 shows the inverse correlation between ghrelin and TEF (r = −0.396, P = 0.001). We examined whether this relationship persisted independently of differences in body composition and insulin levels. We found a significant partial correlation coefficient between ghrelin and TEF after statistical control for fat mass and fat-free mass (r = −0.329, P = 0.01) and insulin levels (r = −0.287, P = 0.024). Collectively, these analyses suggest that the inverse relationships between serum ghrelin and RMR and TEF are independent of the influences of body composition and insulin levels.

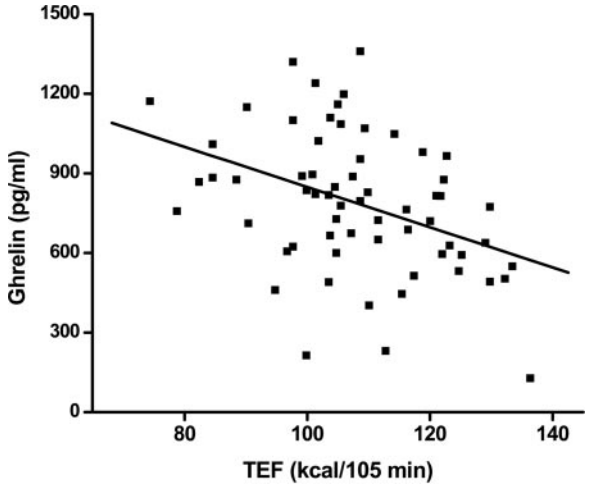

FIG. 2. Correlation between serum ghrelin and TEF. The *line of best fit* is indicated. The partial r value remained significant after statistical control for fat-free mass and fat mass (r = −0.329, P = 0.01) and insulin levels (r = −0.287, P = 0.024) using analysis of covariance.

Because of the known relationship between ghrelin and appetite, we also examined the relationship between ghrelin and RMR and TEF after statistically controlling for the effects of daily energy intake. We noted that energy intake showed a significant inverse relationship with ghrelin levels (r = −0.266, P = 0.032; data not shown). When analysis of covariance was performed using daily energy intake as the covariate, we still noted a significant partial correlation between ghrelin and both RMR (r = −0.343, P = 0.006) and TEF (r = −0.367, P = 0.003). Ghrelin levels, however, were not significantly correlated with total fat mass (r = −0.138), fat-free mass (r = −0.209), body mass index (r = −0.083), percent body fat (r = −0.085), fasting (r = −0.002), postprandial (r = −0.016) respiratory quotient, leisure time physical activity (r = 0.104), $VO_{2\,peak}$ (r = 0.138), and eating behavior (dietary disinhibition, r = −0.071; dietary restraint, r = 0.051; feeling of general hunger, r = −0.028).

Discussion

The discovery of the gastric peptide ghrelin in 1999 (2) has stimulated much interest in its role as a regulatory factor in energy homeostasis. Administration of this peptide has been reported to stimulate appetite, reduce fat oxidation, and induce weight gain in animal models (4, 20). The role of ghrelin in the regulation of energy homeostasis in humans, however, is less clear.

During the conduct of our experiments, two different investigative teams reported an inverse relation between ghrelin and RMR in lean, obese, and hyperthyroid subjects (12, 13). These findings raised the possibility that the effects of ghrelin may extend beyond the regulation of satiety and substrate oxidation and potentially serve as a biomarker of increased energy efficiency (*i.e.* lower energy expenditure) in humans.

Our results confirm the results of these investigators (12, 13), and the present investigation extends previous findings by examining the relationship between ghrelin and RMR, TEF, substrate oxidation, and measures of physical activity and eating behavior in a well-characterized large cohort of normal-weight healthy young women. This experimental design reduces the confounding influence of overweight or obese conditions on major outcome variables by focusing the analyses on a relatively homogenous, nonobese population of the same sex who are within a narrow age range. We noted significant inverse relationships between ghrelin and RMR ($r = -0.350$, $P = 0.004$) and TEF ($r = -0.396$, $P = 0.001$) when energy expenditure values were expressed on an absolute basis (*i.e.* unadjusted for body composition). However, because RMR, TEF, and ghrelin are influenced by body composition (21–27), insulin levels (28), and the daily energy intake, a valid question is whether these inverse relationships persist after taking into account differences in body composition, insulin levels, and caloric intake among individuals. Thus, we performed partial correlation analysis to examine these relationships independent of differences in fat-free mass and fat mass, insulin levels, and caloric intake. The partial correlation coefficients were lowered slightly (ghrelin *vs.* RMR partial, $r = -0.284$, $P = 0.024$; and ghrelin *vs.* TEF partial, $r = -0.329$, $P = 0.01$) when corrected for fat mass and fat-free mass (ghrelin *vs.* RMR partial, $r = -0.255$, $P = 0.046$; and ghrelin *vs.* TEF partial, $r = -0.287$, $P = 0.024$), when corrected for insulin (ghrelin *vs.* RMR partial, $r = -0.343$, $P = 0.006$; and ghrelin *vs.* TEF partial, $r = -0.367$, $P = 0.003$), and when corrected for caloric intake, but they remained significant nonetheless. Thus, our tentative conclusion is that the significant relationship between ghrelin and measures of resting and postprandial energy expenditure are independent of variations in body composition, insulin levels, and daily energy intake.

We initially hypothesized that ghrelin would be inversely related to indicators of fasting and postprandial respiratory quotient as previously reported (3, 4). Our data does not support this hypothesis because we found no relationship between ghrelin and fasting ($r = -0.002$, P = not significant) or postprandial respiratory quotient ($r = -0.016$, P = not significant). Several factors may explain these divergent observations with previous investigators. First, species specificity between rats and mice and humans may contribute to differential effects of ghrelin on substrate oxidation. Second, chronic administration of active (*n*-octanoyl) ghrelin in rats or inactivation of the ghrelin gene in mice may induce diverging physiological observations when compared with the effects elicited by endogenous (*n*-octanoyl and des-*n*-octanoyl) ghrelin levels in humans. Third, intracerebroventricular administration in rats and hypothalamic deletion of the gene in mice may amplify specific effects of ghrelin in the brain, whereas these observations cannot be described with endogenous serum levels of the hormone in humans.

Our findings differ somewhat from those of Marzullo *et al.* (12) with respect to the relationship between ghrelin and body habitus variables. In contrast to their study, we found no significant relationships between ghrelin and serum, percent body fat, or body mass index. These nonsignificant correlations may partially reflect the more homogeneous sample population in the present study (*i.e.* normal-weight young women), whereas the subject population of Marzullo *et al.* (12) was lean and obese men and women.

To verify the orexigenic effects initially reported in rodents (4) and humans (20), we sought to evaluate the relationship between daily energy intake and measures of dietary behavior (dietary restraint, disinhibition, and feeling of general hunger) and ghrelin concentrations. We observed a significant inverse relationship between energy intake and ghrelin ($r = -0.265$, $P < 0.032$). However, no significant relationships were noted between the psychological measures of dietary behavior and ghrelin. Collectively, the inverse correlation between energy expenditure and energy intake and ghrelin may suggest a regulatory role for this gut peptide in energy balance. That is, ghrelin may be a signal for down-regulation of energy consumption and energy expenditure to maintain body weight in young healthy women.

Several limitations in our study should be noted. First, because of the correlative nature of our study, a cause and effect relationship cannot be assumed. Second, our results are limited to healthy nonobese young women. Third, ghrelin levels were not measured during the postprandial period, thus it is unclear whether postprandial levels of ghrelin correlate with postprandial levels of energy expenditure.

In conclusion, these results suggest that higher levels of ghrelin are associated with lower levels of both resting and postprandial thermogenesis, which are independent of individual differences in fat-free mass and fat mass in young healthy women. Although speculative, serum ghrelin levels may play a role in the regulation of energy homeostasis by acting as a hormonal marker of increased energy efficiency.

Acknowledgments

We acknowledge the contributions of Peptidec Technologies Ltd., Dr. Huy Ong and his research team, and Dr. Eric Doucet, Dr. May Faraj, and Dr. Jean-Francois Henry.

Received March 31, 2004. Accepted September 10, 2004.

Address all correspondence and requests for reprints to: Eric T. Poehlman, Ph.D., Unité Métabolique, Département de Nutrition, Faculté de Médicine, Université de Montréal, Pavillon Liliane de Stewart, 2405 Côte Ste Catherine, Montréal, Canada H3C 3J7. E-mail: eric.poehlman@umontreal.ca.

This work was supported in part by Canadian Institute of Health Research New Emerging Teams in Obesity (University of Montreal with University of Ottawa, Grant OHN-63279; and Laval University, Grant OHN-63276) and Canadian Institute of Health Research Operating Grant MOP 62976. E.T.P. is supported by a Canadian Institute of Health Research Chair in Nutrition and Metabolism.

References

1. **Kopelman PG** 2000 Obesity as a medical problem. Nature 404:635–643
2. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
3. **Wortley KE, Anderson K, Garcia K, Murray J, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox H, Yancopoulos GD, Wiegand SJ, Sleeman MW** 2004 Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc Natl Acad Sci USA 101:8227–8232
4. **Tschop M, Smiley DL, Heiman ML** 2000 Ghrelin induces adiposity in rodents. Nature 407:908–913
5. **Casanueva FF, Dieguez C** 2002 Ghrelin: the link connecting growth with metabolism and energy homeostasis. Rev Endocr Metab Disord 3:325–338
6. **Druce M, Bloom SR** 2003 Central regulators of food intake. Curr Opin Clin Nutr Metab Care 6:361–367
7. **Eisenstein J, Greenberg A** 2003 Ghrelin: update 2003. Nutr Rev 61:101–104

8. **Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M** 2001 Minireview: ghrelin and the regulation of energy balance–a hypothalamic perspective. Endocrinology 142:4163–4169
9. **Inui A** 2001 Ghrelin: an orexigenic and somatotrophic signal from the stomach. Nat Rev Neurosci 2:551–560
10. **Sainsbury A, Cooney GJ, Herzog H** 2002 Hypothalamic regulation of energy homeostasis. Best Pract Res Clin Endocrinol Metab 16:623–637
11. **St-Pierre DH, Wang L, Tache Y** 2003 Ghrelin: a novel player in the gut-brain regulation of growth hormone and energy balance. News Physiol Sci 18:242–246
12. **Marzullo P, Verti B, Savia G, Walker GE, Guzzaloni G, Tagliaferri M, Di Blasio A, Liuzzi A** 2004 The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. J Clin Endocrinol Metab 89:936–939
13. **Riis AL, Hansen TK, Moller N, Weeke J, Jorgensen JO** 2003 Hyperthyroidism is associated with suppressed circulating ghrelin levels. J Clin Endocrinol Metab 88:853–857
14. **van Weir JB** 1949 New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109:1–9
15. **Taylor HL, Jacobs Jr DR, Schucker B, Knudsen J, Leon AS, Debacker G** 1978 A questionnaire for the assessment of leisure time physical activities. J Chronic Dis 31:741–755
16. **Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett Jr DR, Schmitz KH, Emplaincourt PO, Jacobs Jr DR, Leon AS** 2000 Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc 32:S498–S504
17. **Verdier P, Beare-Rogers JL** 1984 The Canadian Nutrient File. J Can Diet Assoc 45:52–55
18. **Stunkard AJ, Messick S** 1985 The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 29:71–83
19. **Laessle RG, Tuschl RJ, Kotthaus BC, Pirke KM** 1989 A comparison of the validity of three scales for the assessment of dietary restraint. J Abnorm Psychol 98:504–507
20. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR** 2001 Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 86:5992
21. **Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML** 2001 Circulating ghrelin levels are decreased in human obesity. Diabetes 50: 707–709
22. **Rodriguez Ayala E, Pecoits-Filho R, Heimburger O, Lindholm B, Nordfors L, Stenvinkel P** 2004 Associations between plasma ghrelin levels and body composition in end-stage renal disease: a longitudinal study. Nephrol Dial Transplant 19:421–426
23. **Malik IA, English PJ, Ghatei MA, Bloom SR, MacFarlane IA, Wilding JP** 2004 The relationship of ghrelin to biochemical and anthropometric markers of adult growth hormone deficiency. Clin Endocrinol (Oxf) 60:137–141
24. **Bunt JC, Salbe AD, Tschop MH, DelParigi A, Daychild P, Tataranni PA** 2003 Cross-sectional and prospective relationships of fasting plasma ghrelin concentrations with anthropometric measures in Pima Indian children. J Clin Endocrinol Metab 88:3756–3761
25. **Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, Jorgensen JO** 2002 Weight loss increases circulating levels of ghrelin in human obesity. Clin Endocrinol (Oxf) 56:203–206
26. **Ravussin E** 1995 Low resting metabolic rate as a risk factor for weight gain: role of the sympathetic nervous system. Int J Obes Relat Metab Disord 19(Suppl 7):S8–S9
27. **Poehlman ET** 1993 Regulation of energy expenditure in aging humans. J Am Geriatr Soc 41:552–559
28. **Armellini F, Zamboni M, Mino A, Bissoli L, Micciolo R, Bosello O** 2000 Postabsorptive resting metabolic rate and thermic effect of food in relation to body composition and adipose tissue distribution. Metabolism 49:6–10

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.