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Clinical Implications of Thermal Therapy in Lifestyle-Related Diseases

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Systemic thermal therapy, such as taking a warm-water bath and sauna, induces systemic vasodilation. It was found that repeated sauna therapy (60°C for 15 min) improved hemodynamic parameters, clinical symptoms, cardiac function, and vascular endothelial function in patients with congestive heart failure. Vascular endothelial function is impaired in subjects with lifestyle-related diseases, such as hypertension, hyperlipidemia, diabetes mellitus, obesity, and smoking. Sauna therapy also improved endothelial dysfunction in these subjects, suggesting a preventive role for atherosclerosis. In animal experiments, sauna therapy increases mRNA and protein levels of endothelial nitric oxide synthase (eNOS) in aortas. In normal-weight patients with appetite loss, repeated sauna therapy increased plasma ghrelin concentrations and daily caloric intake and improved feeding behavior. In obese patients, the body weight and body fat significantly decreased after 2 weeks of sauna therapy without increase of plasma ghrelin concentrations. On the basis of these data, sauna therapy may be a promising therapy for patients with lifestyle-related diseases. Exp Biol Med 228:1245–1249, 2003

Key words: thermal therapy; sauna; lifestyle-related diseases; endothelial function

Systemic thermal therapy, such as taking a bath and sauna, induces systemic vasodilation. In congestive heart failure (CHF), clinical symptoms such as muscle fatigue, heaviness in the limbs, edema, appetite loss, and constipation are often observed due to increased peripheral vascular resistance and reduced peripheral perfusion. We therefore applied thermal therapy to patients with CHF. We found that 60°C sauna therapy for 15 min improved acute hemodynamics in patients with CHF, including cardiac index, mean pulmonary wedge pressure, systemic and pulmonary vascular resistance, and cardiac function (1). Subsequently, we examined the effects of repeated sauna therapy on clinical symptoms in patients with CHF and found that repeated sauna therapy significantly improved clinical symptoms and cardiac function (2–4). We then investigated the vascular endothelial function and cardiac function to clarify the mechanisms, since vascular endothelial function had been reported to be impaired in CHF (5). Two-week sauna therapy significantly reduced brain natriuretic peptide concentrations and improved endothelial function in CHF patients (4). Furthermore, we clarified that one of the molecular mechanisms by which repeated sauna therapy improved endothelial function was increase in mRNA and protein of endothelial nitric oxide synthase (eNOS) (6). Many studies indicate that vascular endothelial function also is impaired in patients with lifestyle-related diseases, such as hypercholesterolemia, hypertension, diabetes mellitus (DM), smoking habit, and obesity (7, 8). We therefore applied sauna therapy to patients with lifestyle-related diseases to examine whether the effects obtained in CHF patients were also observed in patients with lifestyle-related diseases. In addition, we investigated the influences of repeated sauna therapy on food intake–related hormones, leptin and ghrelin, from the observation that repeated sauna therapy improved quality of life, especially appetite loss, in CHF patients.

In the first half of this review, improvement effects of endothelial function by sauna therapy in patients with lifestyle-related diseases are discussed. The effects of sauna therapy on the concentration of leptin and ghrelin and body weight are then stated.

Vascular Endothelial Function

Endothelium, a monolayer covering the intimal surface, plays a pivotal role in maintaining vasomotor tone, coagulation and fibrinolysis, and vascular structure and modulating inflammatory response and oxidative stress. Endothelial cells secrete many vasoactive substances, including nitric oxide (NO), prostacyclin, endothelial-derived hyperpolarizing factors, endothelin, thromboxane, growth factors and cytokines, and others (9, 10). Endothelial function is thought to be determined by their balance. Among those substances, NO is well investigated and characterized and

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induces vasodilation and inhibits platelet aggregation, expression of adhesion molecules, and proliferation of smooth muscle cells. These biologic actions of NO are antiatherosclerosis. Therefore, decreased NO production and increased NO degradation are believed to induce atherosclerosis, probably resulting in cardiovascular diseases (8). Endothelial function is commonly measured as the vasomotor response to pharmacologic or physical stimuli such as acetylcholine, metacholine, bradykinin, and shear stress, because the endothelium-dependent vasodilator response may serve as surrogate for the bioavailability of NO. For the evaluation of coronary endothelial function, the assessment of the change in epicardial coronary artery diameter using quantitative coronary angiography and of the change in Doppler flow velocity for coronary resistance vessels in response to intracoronary administration of acetylcholine is used (11, 12). A noninvasive technique using high-resolution ultrasound to assess flow-mediated dilation of the brachial artery is used to estimate endothelial function of the peripheral artery (13).

Endothelial Dysfunction Caused by Lifestyle-Related Diseases

A wide variety of studies have shown that endothelial function is impaired in patients with lifestyle-related diseases, such as hypercholesterolemia, DM, hypertension, smoking, and obesity (8, 9). It is now accepted that coronary risk factors, including lifestyle-related diseases, probably provoke atherosclerosis through vascular endothelial dysfunction. In addition, the molecular mechanisms by which lifestyle-related diseases impair endothelial function have been revealed. Decreased protein expression of eNOS has been observed in DM (14), hypertension (15), and smoking (16). Reduced bioavailability of NO by reactive oxygen species also has been reported in DM, hypercholesterolemia, hypertension, and smoking (17, 18). In hypercholesterolemia, impaired signal transduction in activation pathway of eNOS has been found (19, 20).

Thermal Therapy Improves Endothelial Dysfunction Caused by Lifestyle-Related Diseases

We adopted sauna therapy as a thermal therapy for lifestyle-related diseases. Patients were placed in a 60°C sauna for 15 min using a far infrared–ray dry sauna system, followed by warmth with a blanket for an additional 30 min (2). In this condition, deep body temperature rises about 1°C and maintains during the treatment. To evaluate endothelial function we used a noninvasive ultrasound method. Endothelial function (percentage flow-mediated dilation [%FMD]) was impaired in 25 patients with at least one lifestyle-related disease, including hypertension (blood pressure > 140/90 mm Hg), hypercholesterolemia (total cholesterol level > 220 mg/dl), DM (fasting plasma glucose level > 126 mg/dl), obesity (body mass index > 25), and smoking, compared with 10 age- and gender-matched control subjects without any lifestyle-related disease (4.0% ± 1.7% vs 8.2% ± 2.7%, $P < 0.0001$, Table I) (21). In contrast, endothelium-independent vasodilation caused by nitroglycerin administration was not different between the two groups (18.7% ± 4.2% vs 20.4% ± 5.1%, NS). Two-week sauna therapy was performed in 25 risk patients without any modification of lifestyle-related diseases. The %FMD significantly increased from the baseline value (4.0% ± 1.7% to 5.8% ± 1.3%, $P < 0.001$). Interestingly, the body weight, blood pressure, and fasting plasma glucose significantly decreased after 2 weeks of sauna therapy (Table II). In a study using a hot tub for 30 min, 3-week therapy significantly decreased fasting plasma glucose and glycosylated hemoglobin levels in patients with Type II DM (22).

Possible Mechanisms by Which Thermal Therapy Improves Endothelial Dysfunction

As described here, several mechanisms underlying endothelial dysfunction caused by lifestyle-related diseases are proposed. To clarify the mechanisms of the effects improving endothelial dysfunction of repeated sauna therapy,

Table I. Clinical Characteristics of the Control and Risk Groups

	Control group (n = 10)	Risk group (n = 25)	P value
Age (years)	35 ± 8	38 ± 7	0.25
Hypercholesterolemia (%)	0/10	8/25 (32)	
Total cholesterol (mg/dl)	187 ± 12	214 ± 44	0.07
Hypertension (%)	0/10	8/25 (32)	
SBP (mmHg)	122 ± 11	128 ± 18	0.34
DBP (mmHg)	76 ± 8	77 ± 17	0.90
Diabetes mellitus (%)	0/10	3/25 (12)	
Fasting plasma glucose (mg/dl)	91 ± 7	99 ± 25	0.29
Smoking (%)	0/10	15/25 (60)	
Obesity (%)	0/10	9/25 (36)	
BMI	23.2 ± 1.8	25.6 ± 2.8	0.02
Resting arterial diameter (mm)	3.6 ± 0.4	3.9 ± 0.3	0.09
%FMD (%)	8.2 ± 2.7	4.0 ± 1.7	<0.0001
%NTG (%)	20.4 ± 5.1	18.7 ± 4.2	0.32

Note. BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure. Values are expressed as the mean ± SD.

Table II. Changes in Clinical Parameters After 2 Weeks of Sauna Treatment in Patients with Lifestyle-Related Diseases

	Before sauna	After 2 weeks of sauna	*P* value
Body weight (kg)	75.2 ± 9.9	74.9 ± 9.9	<0.05
Heart rate	68 ± 10	68 ± 10	NS
Systolic blood pressure (mmHg)	128 ± 18	124 ± 17	<0.01
Diastolic blood pressure (mmHg)	77 ± 17	72 ± 16	<0.05
Hematocrit (%)	47.6 ± 2.9	47.2 ± 2.3	NS
Total cholesterol (mg/dl)	214 ± 44	208 ± 34	NS
Triglyceride (mg/dl)	268 ± 327	221 ± 159	NS
HDL cholesterol (mg/dl)	51 ± 11	50 ± 11	NS
Uric acid (mg/dl)	6.8 ± 1.8	6.6 ± 1.5	NS
Fasting plasma glucose (mg/dl)	99 ± 25	94 ± 16	<0.05
TBARS (m*M*)	2.8 ± 0.6	2.9 ± 0.6	NS
Resting arterial diameter (mm)	3.9 ± 0.3	3.9 ± 0.3	NS
Reactive hyperemia (%)	398 ± 170	352 ± 215	NS
%FMD (%)	4.0 ± 1.7	5.8 ± 1.3	<0.001
%NTG (%)	18.7 ± 4.2	18.1 ± 4.1	NS

Note. HDL cholesterol, high-density lipoprotein cholesterol; NS, not significant; TBARS, thiobarbituric acid reactive substances; %FMD, percentage of flow-mediated dilation; %NTG, percentage of nitroglycerin. Values are expressed as the mean ± SD.

we investigated the expression of eNOS protein and mRNA in hamsters using an experimental far infrared–ray dry sauna system (6). We determined the sauna condition in which their rectal temperatures raised about 1°C (39°C for 15 min). Four weeks of sauna therapy once a day significantly increased the expression of eNOS in the endothelium of coronary and aortas by immunohistochemistry and Western blot (Fig. 1). Reverse transcription polymerase chain reaction revealed that eNOS mRNA was significantly upregulated in aortas of hamsters after 4 weeks of sauna therapy. Concerning the possibility of reduced bioavailability of NO by reactive oxygen species, we clinically measured the concentrations of thiobarbituric acid reactive substances (TBARS) and found no changes after 2-week sauna therapy (Table II).

Food Intake–Related Hormone: Leptin and Ghrelin

Energy intake and body weight are tightly regulated at a consistent set point by control systems in the hypothalamus. These hypothalamic circuits receive feedback from peripheral signals (23). Discovery of the hormone leptin (24), which could regulate satiety, energy expenditure, and weight gain, added complexity to these relationships. Leptin is produced by adipocytes and is thought to feed back through the hypothalamic receptors to regulate weight gain and energy expenditure (25). It causes the increased energy expenditure and the decreased food intake (26). Ghrelin has been discovered as an orexigenic hormone secreted primarily by the stomach and duodenum (27, 28). Ghrelin is the natural ligand of the growth hormone secretagogue receptor and strongly stimulates growth hormone secretion. In addition, ghrelin is involved in energy homeostasis, acting as a peripheral signal stimulating food intake and promoting adiposity (29, 30). Ghrelin administration causes weight gain by increasing food intake and reducing fat utilization in rodents (31–33) and humans (34).

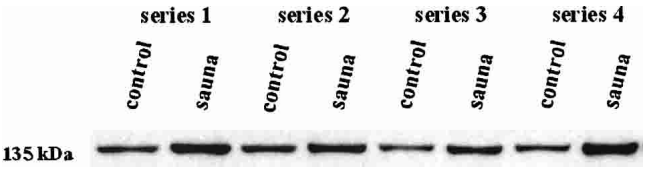

Figure 1. Western blot analysis for eNOS. eNOS expression was greater in the sauna group than in the control group in 4 independent experiments (6).

Leptin deficiency may be a cause of obesity (35). However, common adult obesity is related to elevated leptin levels, possibly indicative of leptin resistance (36). Fasting plasma ghrelin concentrations are decreased in obesity and are negatively correlated with body mass index (37). These results raise the possibility that ghrelin and leptin are part of a dynamic feedback system in the regulation of body weight. Changes in plasma ghrelin and serum leptin concentrations might produce important differences in food intake and energy balance and play a significant role in the pathogenesis of obesity.

Food Intake Is Improved by Repeated Sauna Therapy

We often observed that appetite loss in patients with chronic heart failure was improved by sauna therapy (1–4, 21). We hypothesized that the improvement of appetite loss after sauna therapy may be associated with plasma ghrelin and serum leptin concentrations. The plasma ghrelin and serum leptin concentrations, daily caloric intake, feeding behavior, and body weight and body fat were investigated in normal-weight patients with appetite loss after 2 weeks of sauna therapy. Feeding behavior was evaluated by the presence of appetite, hunger, taste, pleasure in eating, and deliciousness. High scores indicate problems in feeding behavior (in submission). Consequently, we found that repeated sauna therapy increased plasma ghrelin concentrations and daily caloric intake and improved feeding behavior. However, serum leptin concentrations, body weight, and body fat did not change after repeated sauna therapy. These findings suggest that improvement of daily caloric intake and feeding behavior in normal-weight patients with appetite loss might be related to increased plasma ghrelin concentrations.

Effects of Repeated Sauna Therapy for Obese Patients

Obesity represents a global epidemic and is one of the leading causes of lifestyle-related diseases and death world-

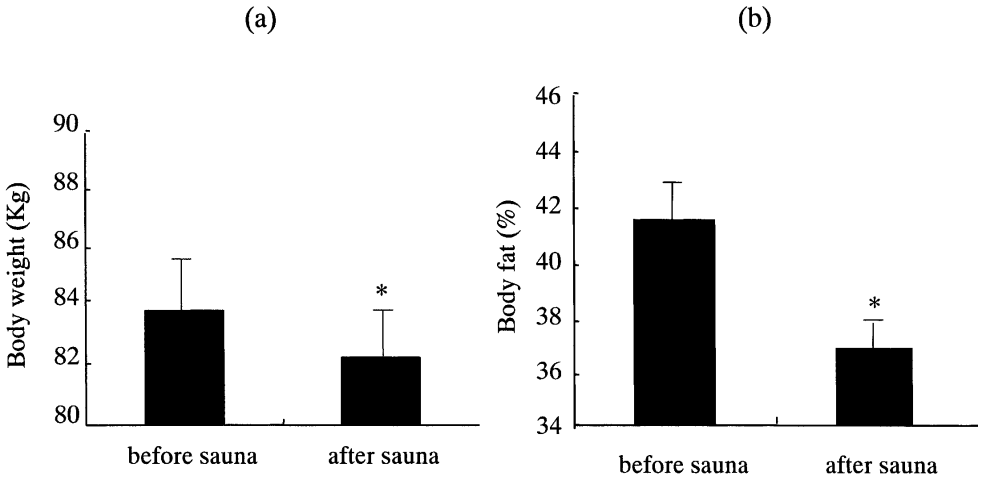

Figure 2. Body weight (a) and body fat (b) significantly decreased in 10 obese subjects after 2-week sauna therapy (*$P < 0.05$).

wide (38, 39). Hyperphagia, weight gain, and increased adiposity occur after continuous systemic ghrelin administration in experimental animals. Ghrelin stimulates appetite and food intake potently in humans. As such, ghrelin would be an important new target for the development of treatments for obesity. In obese patients, plasma ghrelin concentrations are low and serum leptin concentrations are high. We examined the effects of 2-week sauna therapy on plasma ghrelin and serum leptin concentrations and feeding behavior in 10 obese patients (body mass index > 30, mean age 46 ± 5 years, five men and five women) using a far infrared–ray dry sauna system. The patients took the same meals of 1800 cal/d during this period. Plasma ghrelin and serum leptin concentrations and feeding behavior did not change after 2 weeks of sauna therapy, unlike in the normal-weight group (in submission).

It is reported that plasma ghrelin falls in response to food intake in non-obese subjects (40) but not in obese subjects (41). These results indicate that the responses of plasma ghrelin to food intake and repeated sauna therapy are different between non-obese and obese subjects. The low plasma ghrelin concentrations in obese patients may represent a physiological adaptation to the positive energy balance associated with obesity (37). The lack of response to plasma ghrelin concentrations after repeated sauna therapy in obese patients suggests that the sensitivity to circulating ghrelin may be decreased. Furthermore, the obese subjects, having sufficient energy stores, may have maximally suppressed ghrelin secretion, failing to respond to sauna therapy (41).

Interestingly, the body weight and body fat in obese patients significantly decreased after 2 weeks of sauna therapy (Fig. 2). The acceleration of appetite and abnormal feeding behavior such as eating a snack between meals and overeating did not appear. These results suggest that repeated sauna therapy decreased body weight and body fat in obese patients without increasing plasma ghrelin concentrations and decreasing serum leptin concentrations. We consider that repeated sauna therapy is useful in the treatment of obesity. We recently treated an interesting obese case in which sauna therapy was very effective. The body weight and body fat rapidly decreased after 10 weeks of sauna therapy with 1600 cal/d. The patient could not take any exercise because of both-knee joint pain in osteoarthritis. Body weight decreased from 117.5 kg to 100.0 kg and body fat decreased from 46% to 35% over 10 weeks. The therapy also had improving effects of mood such as anxiety, anger, and irritability. There was no acceleration of appetite or abnormal feeding behavior during the treatment.

Conclusions

We have applied sauna therapy, a thermal therapy, to lifestyle-related diseases and have found that repeated sauna therapy improves vascular endothelial function and reduces body weight. Since endothelial dysfunction represents an early stage of atherosclerosis, we think that sauna therapy could prevent atherosclerosis. Sauna therapy also has an advantage that it is applicable to subjects who are unable to exercise. We believe that sauna therapy may be a promising therapy for patients with lifestyle-related diseases to prevent cardiovascular diseases, especially in combination with diet therapy and exercise therapy.

1. Tei C, Horikiri Y, Park JC, Jeong JW, Chang KS, Toyama Y, Tanaka N. Acute hemodynamic improvement by thermal vasodilation in congestive heart failure. Circulation **91:**2582–2590, 1995.
2. Tei C, Tanaka N. Thermal vasodilation as a treatment of congestive heart failure: A novel approach. J Cardiol **27:**29–30, 1996.
3. Tei C. Thermal therapy for congestive heart failure: Estimation by TEI index. J Cardiol **37:**155–159, 2001.
4. Kihara T, Biro S, Imamura M, Yoshifuku S, Takasaki K, Ikeda Y, Otuji Y, Minagoe S, Toyama Y, *et al.* Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. J Am Coll Cardiol **39:**754–759, 2002.
5. Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM. Endothelium-dependent vasodilation is attenuated in patients with heart failure. Circulation **84:**1589–1596, 1991.
6. Ikeda Y, Biro S, Kamogawa Y, Yoshifuku S, Eto H, Orihara K, Kihara T, Tei C. Repeated thermal therapy upregulates arterial endothelial nitric oxide synthase expression in Syrian golden hamsters. Jpn Circ J **65:**434–438, 2001.

7. Vogel RA. Coronary risk factors, endothelial function, and atherosclerosis: A review. Clin Cardiol **20:**426–432, 1997.
8. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: A marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol **23:**168–175, 2003.
9. Vapaatalo H, Mervaala E. Clinically important factors influencing endothelial function. Med Sci Monit **7:**1075–1085, 2001.
10. Behrendt D, Ganz P. Endothelial function: From vascular biology to clinical applications. Am J Cardiol **90**(10C):40L–48L, 2002.
11. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med **315:**1046–1051, 1986.
12. Hamasaki S, Higano ST, Suwaidi JA, Nishimura RA, Miyauchi K, Holmes DR Jr, Lerman A. Cholesterol-lowering treatment is associated with improvement in coronary vascular remodeling and endothelial function in patients with normal or mildly diseased coronary arteries. Arterioscler Thromb Vasc Biol **20:**737–743, 2000.
13. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, *et al*. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol **39:**257–265, 2002.
14. Perreault M, Dombrowski L, Marette A. Mechanism of impaired nitric oxide synthase activity in skeletal muscle of streptozotocin-induced diabetic rats. Diabetologia **43:**427–437, 2000.
15. Chou TC, Yen MH, Li CY, Ding YA. Alterations of nitric oxide synthase expression with aging and hypertension in rats. Hypertension **31:**643–648, 1998.
16. Su Y, Han W, Giraldo C, De Li Y, Block ER. Effect of cigarette smoke extract on nitric oxide synthase in pulmonary artery endothelial cells. Am J Respir Cell Mol Biol **19:**819–825, 1998.
17. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest **91:**2546–2551, 1993.
18. Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM. Vascular superoxide production by NAD(P)H oxidase: Association with endothelial dysfunction and clinical risk factors. Circ Res **86:**E85–E90, 2000.
19. Shimokawa H, Flavahan NA, Vanhoutte PM. Loss of endothelial pertussis toxin-sensitive G protein function in atherosclerotic porcine coronary arteries. Circulation **83:**652–660, 1991.
20. Feron O, Dessy C, Moniotte S, Desager JP, Balligand JL. Hypercholesterolemia decreases nitric oxide production by promoting the interaction of caveolin and endothelial nitric oxide synthase. J Clin Invest **103:**897–905, 1999.
21. Imamura M, Biro S, Kihara T, Yoshifuku S, Takasaki K, Otsuji Y, Minagoe S, Toyama Y, Tei C. Repeated thermal therapy improves impaired vascular endothelial function in patients with coronary risk factors. J Am Coll Cardiol **38:**1083–1088, 2001.
22. Hooper PL. Hot-tub therapy for type 2 diabetes mellitus. N Engl J Med **341:**924–925, 1999.
23. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. Nature **404:**661–671, 2000.
24. Zhang Y, Proenca R, Maffei M, Borone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature **372:**425–432, 1994.
25. Rohner JF, Cusin I, Sainsbury A, Zakrzewska KE, Jeanrenaud B. The loop system between neuropeptide Y and leptin in normal and obese rodents. Horm Metab Res **28:**642–648, 1996.
26. Ahima RS, Prabakaran D, Mantzoros C. Role of leptin in the neuroendocrinology of fasting. Nature **382:**250–252, 1996.
27. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature **402:**656–660, 1999.
28. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology **141:**4255–4261, 2000.
29. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. Nature **407:**908–913, 2000.
30. Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, *et al*. Ghrelin causes hyperphagia and obesity in rats. Diabetes **50:**2540–2547, 2001.
31. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. Nature **409:**194–198, 2001.
32. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, *et al*. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology **141:**4325–4328, 2000.
33. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA. Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab **86:**5992–5995, 2001.
34. Campfield LA, Smith FJ, Burn P. The OB protein (leptin) pathway: A link between adipose tissue mass and central neural networks. Horm Metab Res **28:**619–632, 1996.
35. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: The tale of an obesity gene. Diabetes **45:**1455–1462, 1996.
36. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, *et al*. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab **87:**240–244, 2002.
37. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes **50:**707–709, 2001.
38. Kopelman PG. Obesity as a medical problem. Nature **404:**635–643, 2000.
39. Lew EA. Mortality and weight: Insured lives and the American Cancer Society Studies. Ann Intern Med **103:**1024–1029, 1985.
40. Tschop M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R. Folwaczny C. Post-prandial decrease of circulating human ghrelin levels. J Endocrinol Invest **24:**RC19–RC21, 2001.
41. English PJ, Ghatei MA, Malik IA, Bloom SR, Wilding JPH. Food fails to suppress ghrelin levels in obese humans. J Clin Endocrinol Metab **87:**2984–2987, 2002.