Stimulation of Thermogenesis by Carbohydrate Overfeeding

EVIDENCE AGAINST SYMPATHETIC NERVOUS SYSTEM MEDIATION

STEPHEN WELLE and ROBERT G. CAMPBELL, *Endocrine-Metabolism Unit, Monroe Community Hospital, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14603*

ABSTRACT Daily carbohydrate intake of seven men with normal weight was limited to 220–265 g/d for 6 d and then increased to 620–770 g/d for 20 d, while intake of protein, fat, and sodium remained constant. Carbohydrate overfeeding increased body weight by 4.8%, basal oxygen consumption (V_{O_2}) by 7.4%, BMR by 11.5%, and serum triiodothyronine levels by 32%. Overfeeding did not affect the thermic effect of a standard meal. Intravenous propranolol reduced the thermic effect of a meal by 22% during the base-line feeding period, and by 13% during carbohydrate overfeeding, but did not affect preprandial V_{O_2}. Overfeeding attenuated the rise in plasma glucose and FFA levels induced by infusion of norepinephrine, but had no effect on the increase in V_{O_2} induced by norepinephrine. Overfeeding did not alter 24-h urinary excretion of vanillylmandelic acid, supine plasma catecholamine levels (pre- and postprandial), blood pressure, or plasma renin activity, but increased peak standing plasma norepinephrine levels by 45% and resting pulse rate by 9%. Even though short-term carbohydrate overfeeding may produce modest stimulation of sympathetic nervous system activity in man, the increase in thermogenesis induced by such overfeeding is neither suppressed by beta adrenergic blockade nor accompanied by an increased sensitivity to the thermogenic effects of norepinephrine. These data do not support an important role for the sympathetic nervous system in mediating the thermogenic response to carbohydrate overfeeding.

INTRODUCTION

During experimental overfeeding, weight gain in many individuals falls short of what would be predicted from the amount of excess energy consumed (1). Since absorption of nutrients is not impaired by overfeeding (2), this phenomenon must be related to increased energy expenditure (thermogenesis) during periods of overnutrition. This adaptive increase in metabolic rate was initially referred to as "luxuskonsumption", and currently is often called dietary or adaptive thermogenesis. The mechanisms of dietary thermogenesis in man remain obscure, although an increase in lean body mass (LBM)[1] and triiodothyronine (T_3) production during overfeeding may contribute to an elevated metabolic rate (3, 4).

Recent studies (5–8) suggest that dietary thermogenesis in rats is related to increased sympathetic nervous system activity (norepinephrine turnover), and increased thermogenic responsiveness to norepinephrine. To determine if a similar mechanism is present in man, we have examined the effect of acute beta adrenergic blockade, which eliminates dietary thermogenesis in rats and pigs (5, 9), on resting metabolic rate (RMR) before and after carbohydrate overfeeding, and have examined the effect of overfeeding on the thermic response to norepinephrine. The subjects were overfed with carbohydrate because previous studies have suggested that the carbohydrate in the diet may be more important than other nutrients in mediating the sympathetic nervous system response to dietary alterations (10–12).

In order to estimate the effect of overfeeding on sympathetic nervous system activity, we measured urinary excretion vanillylmandelic acid (VMA) and plasma norepinephrine levels, both of which fall during caloric restriction in obese subjects (12, 13). The effect of overfeeding on blood pressure, pulse rate, and

Address all correspondence to Dr. Welle.

Received for publication 20 August 1982 and in revised form 6 December 1982.

[1] *Abbreviations used in this paper*: LBM, lean body mass; RMR, resting metabolic rate; VMA, vanillylmandelic acid; T_3, triiodothyronine.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/83/04/0916/10 $1.00*
Volume 71 April 1983 916–925

plasma renin activity also was examined, since stimulation of sympathetic nervous system activity by overfeeding is a potential factor in hypertension of obese subjects.

METHODS

Seven men, 19–36 yr old, were admitted to the Clinical Research Center for 26 d. At admission, their mean body weight was 69 kg (range 58–89 kg), which was 95% (83–113%) of ideal body weight (14). Mean daily intake during the first 6 d of the study was 9.12 MJ (megajoules) (Table I). Protein contributed ~15% of total energy, fat, 40%; and carbohydrate, 45%, as calculated from standard food tables (15). Caffeine intake was limited to no more than 3 cups of coffee/d or the equivalent, and was kept constant in each individual. Sodium intake was maintained at 150 meq/d throughout the study. The first meal each day consisted of 10 kcal/kg ideal weight of a liquid meal (Ensure, Ross Laboratories Div., Abbott Labs, [Columbus, OH]) that contained 14% of energy as protein, 31% as fat, and 55% as carbohydrate.

During the final 20 d of the study, this basic diet was supplemented with solutions of partially hydrolyzed corn starch (Polycose, Ross Laboratories Div.) to bring mean total energy intake to 16.69 MJ/d (Table I). The total intake of nutrients other than carbohydrate remained constant, but their proportion of total energy fell (8% protein, 22% fat), whereas the proportion of energy as carbohydrate increased to 70%. The daily ration of Polycose was divided into three equal parts, one given at the midday meal (12–2 p.m.), one at the evening meal (5–7 p.m.), and one at night (8–11 p.m.).

Throughout the study, physical activity was kept constant. Two subjects lifted weights each day, an activity they had initiated before the study. No other subject did anything more strenuous than walking. Two subjects smoked cigarettes, one 20/d and the other 10/d. No smoking was allowed for 12 h before any test.

The effect of the standard morning meal on thermogenesis was measured on six occasions: on days 3 and 4 of the base-line diet, and on days 6, 12, 18, and 19 of overfeeding (±1 d for each test). On day 3 of the base-line diet and day 18 of overfeeding, normal saline was infused during the test; on day 4 of the base-line diet and day 19 of overfeeding, propranolol was infused. Norepinephrine infusions were done on day 6 of the base-line diet and on day 21 of overfeeding. Flexible catheters were inserted into antecubital veins, for blood sampling and intravenous infusions, ~30 min before each test, and kept patent with normal saline.

TABLE I
Daily Intake (Mean and Range)

	Base-line diet	Overfeeding
Energy, *MJ*	9.12 (8.25–9.88)	16.69 (14.86–18.45)
Protein, *g*	82 (74–89)	82 (74–89)
Fat, *g*	97 (88–105)	97 (88–105)
Carbohydrate, *g*	245 (222–266)	698 (621–771)
Sodium, *meq*	150	150

These values are based on data from standard food tables (15), except for the increase in carbohydrate intake during overfeeding, which was all in the form of partially hydrolyzed corn starch.

All tests were done in the morning after the subject had fasted overnight.

RMR was measured for 60 min before the standard morning meal (except on days 6 and 12 of overfeeding when preprandial RMR was measured for only 30 min), and for 120 min after the meal. At −30 min relative to the meal, after the first 30 min of RMR measurements, the saline or propranolol infusion was started. Propranolol (Inderal, Ayerst Laboratories, New York) was given as a 3-mg priming dose, followed by 80 μg/min for the remainder of the test. Saline was given in the same volumes that were used as a vehicle for propranolol. Neither propranolol nor saline was administered in tests done on days 6 and 12 of overfeeding. Blood samples were taken at −30, 0, 60, and 120 min relative to the meal for plasma glucose, insulin, catecholamine, and plasma renin activity levels.

Norepinephrine (Levophed bitartrate, Breon Laboratories, Inc., Sterling Drug, Inc., New York) was infused for a 60-min period after RMR was measured for 30 min. (In one subject the first norepinephrine infusion was terminated early due to blood pressure increasing to 180/96, and no norepinephrine infusion was done after overfeeding; blood pressure did not exceed 160/90 in the other subjects.) RMR was then measured during, and for 30 min after, infusion of 0.1 μg/kg per min of norepinephrine. Blood samples were taken at −30, 0, 30, 60, and 90 min relative to the start of the infusion for plasma glucose, insulin, FFA, norepinephrine, and plasma renin activity levels.

During the tests, measurements of basal oxygen consumption (V_{O_2}) and carbon dioxide expiration (V_{CO_2}) were made by continuous indirect calorimetry. A transparent hood was placed over the head and shoulders of the subjects and air was drawn through it at a fixed rate of 50 liters/min, which was measured by a calibrated (±1%) flowmeter (Fischer and Porter, Warminster, PA). Differences in CO_2 and O_2 concentrations between ambient air and air exhausted from the hood were measured ~70% of the time, and ambient air was measured the rest of the time. The analyzer (Kipp & Zonen Delft, Holland) is based on the measurement of differences in heat conductivity between gases of different composition. The system was calibrated by burning ethanol in the hood. Protein metabolism was estimated from mean daily 24-h nitrogen excretion, determined by the Kjeldahl method, by using one mean value for the base-line diet and another for the overfeeding period, and with the assumption that 6.25 g of protein was metabolized/g of urinary nitrogen (16). The rate of thermogenesis was calculated according to the formula: x kJ = $113N_u + [5\ (V_{CO_2} - 4.89\ N_u/V_{O_2} - 6.04\ N_u) + 16.1]\ [V_{O_2} - 6.04\ N_u]$, where N_u is urinary nitrogen excretion in grams, and V_{O_2} and V_{CO_2} are expressed in liters. This equation is based on the assumption that the energy equivalent of one liter of O_2 is 18.7 kJ for protein oxidation, 19.6 kJ for fat oxidation, 21.1 kJ for carbohydrate oxidation, and 29.6 kJ for lipogenesis from glucose (16–18).

Glucose levels of fresh plasma were measured by autoanalyzer (Beckman Instruments, Inc., Fullerton, CA). Heparinized plasma aliquots were frozen for later determination of FFA levels by a colorimetric procedure (19). EDTA-plasma was frozen for later determination of insulin (Cambridge Nuclear Corp., Billerica, MA) and plasma renin activity (Becton Immunodiagnostics, Becton-Dickinson & Co., Orangeburg, NY) levels by commercial radioimmunoassay kits. EGTA-plasma for norepinephrine levels was preserved with reduced glutathione and frozen until it was assayed by a radioenzymatic procedure (20). T_3 levels of −30-min serum samples were measured by radioimmunoassay (Clinical Assays, Inc., Div. Travenol Laboratories, Inc., Cambridge, MA).

Before the norepinephrine infusions, the effect of standing on blood pressure (n = 6), catecholamine levels (n = 6), and plasma renin activity (n = 5) was evaluated. Blood pressure and pulse rate were measured and blood samples were taken at −5, 0, 3, 5, and 10 min. Subjects were supine from −30 to 0 min, then stood at 0 min. Blood pressure and pulse rate also were measured each morning before breakfast while the subjects were supine.

Body weight was measured to the nearest 10 g every morning. LBM was estimated by ^{40}K whole-body counting (21), done twice in the base-line period and twice during the final week of overfeeding (except in one subject who had only a single determination before and after overfeeding). All urine was collected daily into a container with 20 ml of 6 N HCl and aliquots were assayed within 2 wk for VMA concentration (22), except in the first subject studied.

Data were first analyzed by repeated-measures analysis of variance (23). Post hoc testing was then done with paired t tests only if the probability of the F ratio of the appropriate main effect or interaction was <0.05. Data are reported as mean±SEM.

RESULTS

There was a small decrease (0.9±0.1 kg) in body weight during the base-line period; overfeeding produced an initial rapid weight gain and then slower weight gain (Fig. 1). The final mean weight was 3.3 kg above the mean weight at the end of the base-line feeding period (2.4±0.3 kg above admission weight). LBM increased in five of seven subjects; the mean LBM rose slightly from 62.3±3.9 to 63.7±4.4 kg ($P < 0.10$).

Basal V_{O_2} was 7.4% higher during the final week of overfeeding than basal V_{O_2} during the base-line period (Table II). After adjustment for the elevated respiratory exchange ratio (0.85±0.01 during base-line diet and 0.98±0.01 during overfeeding) and reduced protein metabolism (72±7 g/d during base-line diet and 46±3 g/d during overfeeding) associated with overfeeding, this translated into an 11.5% increase in BMR.

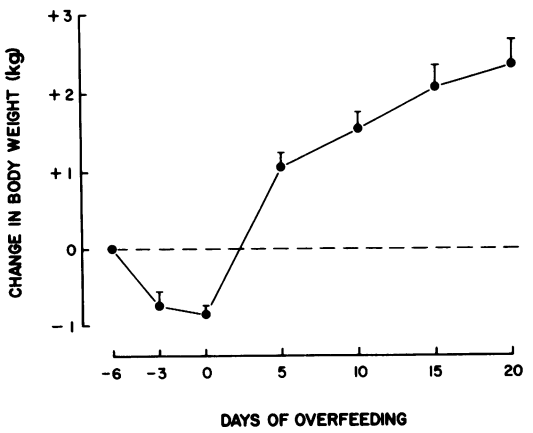

FIGURE 1 Mean (±SEM) changes in weight relative to admission weight during base-line diet (days −6 to 0) and during 20 d of carbohydrate overfeeding.

TABLE II

Effect of Carbohydrate Overfeeding on Mean (±SEM) BMR and Serum T_3 Levels in Seven Normal Men

	Base-line period	Final week of overfeeding	P
Basal V_{O_2}, *ml/min STP*	242±14	260±12	<0.01
BMR, *kJ/min*	4.86±0.27	5.42±0.26	<0.001
Serum T_3 concentration, *ng/dl*	111±8	146±7	<0.001
BMR/LBM *kJ h^{-1} kg LBM^{-1}*	4.69±0.09	5.15±0.14	<0.01

V_{O_2} and BMR are based on mean of three determinations during each period. T_3 and LBM are based on mean of two determinations during each period.
P is according to paired t test.

(In this paper, BMR refers to the RMR measured before any drug infusion or meals.) All points relating BMR to weight after overfeeding were above the regression line relating BMR to weight before overfeeding (Fig. 2). The ratio of BMR to LBM was increased by 10%, and serum T_3 levels were increased by 32% at the end of overfeeding (Table II).

The thermic effect of the standard meal was not altered by overfeeding (Table III and Fig. 3). Both preprandial and postprandial V_{O_2} were elevated by overfeeding (*overfeeding* main effect, $F(1,6) = 61.1$, $P < 0.001$), and the increase in V_{O_2} after the meal was highly significant (*time* main effect, $F(5,30) = 59.6$,

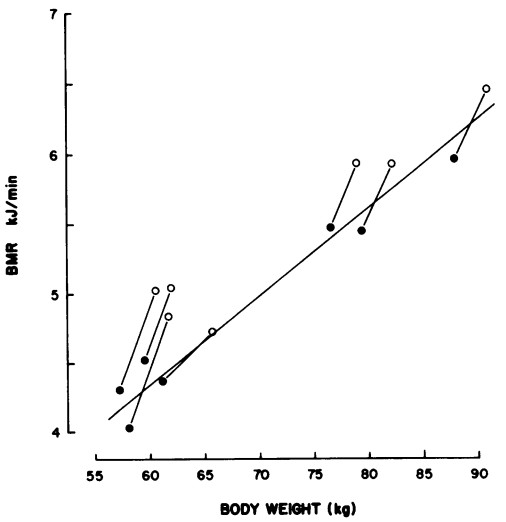

FIGURE 2 Effect of carbohydrate overfeeding on relationship between body weight and BMR in seven normal volunteers. Each point represents the mean of three determinations done either before the start of overfeeding (●) or in the third week of overfeeding (○).

TABLE III

Effect of Propranolol on Basal and Postprandial VO_2 (ml/min STP)

	Time relative to meal					
			min			
	Basal preinfusion	Basal postinfusion	0–30	30–60	60–90	90–120
Base-line diet						
saline	238±15	241±14	278±13	296±18	302±20	301±16
propranolol	241±14	240±13	263±13°	282±15	288±16°	301±18
Overfed						
saline	254±11	266±12	300±10	320±16	330±16	334±16
propranolol	256±13	254±12°	289±11	309±16°	307±18°	320±16°

Basal preinfusion data was obtained during 30-min period before start of saline or propranolol infusion, which was started 30 min before the meal.

° $P < 0.05$, compared with values at corresponding times of saline infusion in the same dietary condition.

$P < 0.001$). Propranolol had no effect on preprandial VO_2, but it slightly reduced postprandial VO_2 (Table III; *drug* × *time* interaction, $F(5,30) = 5.54$, $P < 0.001$). The critical finding was that there was no *overfeeding* × *drug* or *overfeeding* × *drug* × *time* interaction in the analysis of VO_2 ($P \geq 0.20$), indicating that the rise in VO_2 associated with overfeeding was not affected by propranolol. Propranolol reduced the total thermic effect of the meal, defined as total RMR during the 2-h period after the meal minus the BMR (kJ/2 h), by 22% during the base-line period and by 13% during the final week of overfeeding (*drug* main effect, $F(1,6) = 8.25$, $P < 0.03$; Fig. 3).

During norepinephrine infusions, mean plasma norepinephrine concentrations reached ~2,000 pg/ml

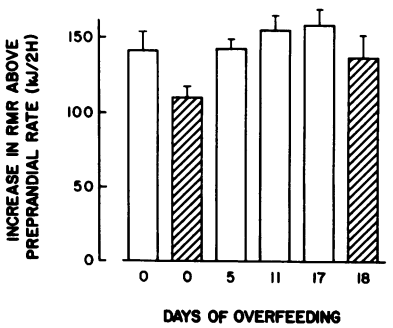

FIGURE 3 Mean (±SEM) increase in RMR during 2-h period after ingestion of morning meal (10 kcal/kg ideal body weight) before overfeeding (0 d of overfeeding), and at various times during overfeeding. Open bars represent effect of meal when saline (0 and 17 d of overfeeding) or nothing (5 and 11 d of overfeeding) was infused; cross-hatched bars represent effect of meal when propranolol was infused. Increase in RMR is based on assumption that RMR would have remained at the preprandial levels if no meal was consumed.

(Fig. 4) both before and after carbohydrate overfeeding. The increase in plasma levels of FFA (*overfeeding* × *time* interaction, $F(4,20) = 3.96$, $P < 0.02$) and glucose (*overfeeding* × *time* interaction, $F(4,20) = 10.8$, $P < 0.001$) induced by norepinephrine were diminished by carbohydrate overfeeding (Fig. 4). Before overfeeding, FFA levels increased 799±108 μeq/liter at 30 min of the norepinephrine infusion, whereas after overfeeding they increased only 473±78 μeq/liter ($P < 0.05$). Increases in FFA concentrations at 60 and 90 min were not affected by overfeeding. Because basal FFA levels were suppressed by carbohydrate overfeeding, the 30-min change in FFA was not inhibited by overfeeding if it was expressed as a percentage increase (193% before overfeeding and 183% after overfeeding). Basal glucose levels were not affected by overfeeding, but the rise in glucose levels during norepinephrine administration was reduced (22±2, 21±2, and 8±2 mg/dl at 30, 60, and 90 min of control test; 14±2, 12±3, and −4±2 mg/dl at corresponding times after overfeeding, $P < 0.05$). Basal insulin levels were slightly increased by carbohydrate overfeeding (*overfeeding* main effect, $F(1,5) = 130$, $P < 0.001$), but there was no significant change in insulin concentrations during norepinephrine infusion, either before or after carbohydrate overfeeding (Fig. 4). The respiratory exchange ratio (VCO_2/VO_2) was initially increased by norepinephrine, then it fell below basal values (*time* main effect, $F(3,15) = 95.3$, $P < 0.001$; Fig. 4). The respiratory exchange ratio was elevated by carbohydrate overfeeding (*overfeeding* main effect, $F(1,5) = 15.9$, $P < 0.02$), but the changes in respiratory exchange ratio induced by the norepinephrine infusion were not altered. Norepinephrine infusions increased VO_2 by 16% before overfeeding, and by 13% after overfeeding (Fig. 5). The changes

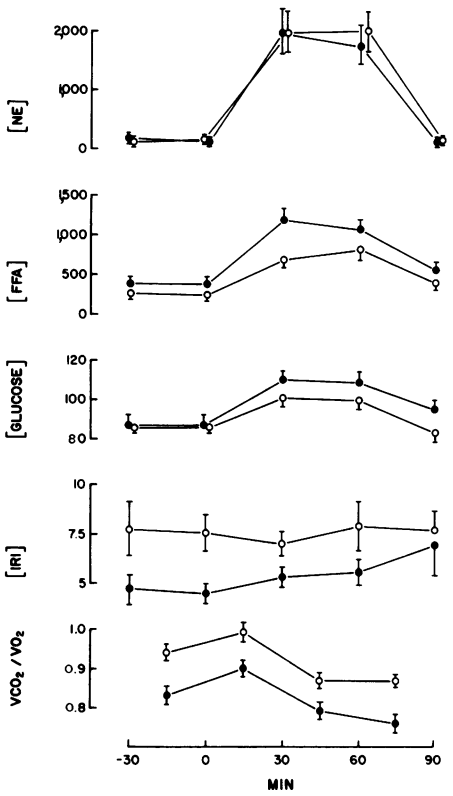

FIGURE 4 Mean (±SEM) plasma concentrations of norepinephrine (NE) in picograms per milliliter, FFA in microequivalents per liter, glucose in milligrams per deciliter, and immunoreactive insulin (IRI) in microunits per milliliter, and respiratory exchange ratio (V_{CO_2}/V_{O_2}) before (−30 and 0 min), during (30 and 60 min), and after (90 min) infusion of norepinephrine in six normal volunteers. ●, data obtained the day before the start of overfeeding; O, data obtained in the final day of overfeeding.

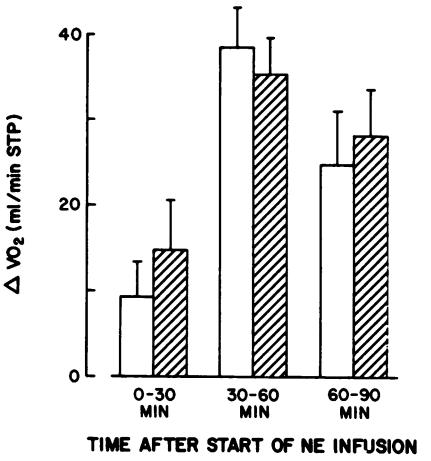

FIGURE 5 Mean (±SEM) increase in V_{O_2} after start of norepinephrine (NE) infusion in six normal volunteers on the day before start of overfeeding (open bars) and on final day of overfeeding (cross-hatched bars). Norepinephrine infusion was started at 0 min and ended at 60 min.

in V_{O_2} were significant (*time* main effect, $F(3, 15) = 20.6$, $P < 0.001$), but were not influenced by carbohydrate overfeeding (*overfeeding* × *time* interaction, $P > 0.5$).

There was no effect of overfeeding on basal plasma norepinephrine (Table IV) or epinephrine levels. Plasma norepinephrine, but not epinephrine, levels were increased by meals (*time* main effect, $F(2,12) = 10.8$, $P < 0.002$) by an average of 28% 2 h after the meal for all studies combined, but overfeeding did not alter this response (*overfeeding* × *time* interaction, $P > 0.5$). There was also a large increase in plasma norepinephrine levels during the first 10 min after standing (*time* main effect, $F(2,10) = 57.5$, $P < 0.001$), and this response was enhanced by overfeeding (*overfeeding* × *time* interaction, $F(2,10) = 4.44$, $P < 0.05$). The peak standing norepinephrine levels during overfeeding were 45% higher than those observed during the base-line diet (Table IV). Epinephrine levels were increased by standing (*time* main effect, $F(3,15) = 4.37$, $P < 0.03$), but overfeeding had no effect on this response (28±3 pg/ml supine and 112±47 pg/ml 10 min after standing in base-line test, compared with 34±12 and 66±18 pg/ml at corresponding times of test at end of overfeeding). VMA excretion was the same before and after overfeeding, either when all days of overfeeding were averaged (Table IV) or when data were analyzed in blocks of 5 d (*time* main effect, $P > 0.35$).

TABLE IV
Effect of Carbohydrate Overfeeding on Indices of Sympathetic Nervous System Activity (Mean±SEM)

	Base-line diet	Overfed	*P*
VMA excretion, *mg/24 h*	4.54±0.30	4.73±0.43	NS
Supine plasma norepinephrine, *pg/ml*	115±11	127±17	NS
Peak standing norepinephrine, *pg/ml*	264±17	384±41	<0.05
Peak postprandial norepinephrine, *pg/ml*	161±15	166±15	NS

VMA excretion represents mean of all values obtained during base-line periods and overfeeding. Plasma norepinephrine values in overfed column refer to data obtained during final week of overfeeding. Standing values were obtained during first 10 min after assumption of upright posture, after 30 min of remaining supine. Postprandial values were determined during 2 h after meal onset. *P* refers to probability of chance difference according to paired *t* test. There were no statistically significant differences in VMA excretion and supine or postprandial norepinephrine levels in earlier weeks of overfeeding.

Supine systolic and diastolic pressures after overnight fasting (mean of 5-d blocks) were unaffected by overfeeding (*overfeeding* main effect, $P > 0.20$), but supine pulse rate increased gradually from 59.9±1.9 beats/min during the final 5 d of the base-line diet to 65.2±3.0 beats/min during the final days of overfeeding (*overfeeding* main effect, $F(4,24) = 2.96$, $P < 0.05$). The standard morning meal increased mean pulse rate (compared with 0-min values) by 11 beats/min (*time* main effect, $F(3,18) = 18.1$, $P < 0.001$) and mean systolic pressure by 6 mmHg (*time* main effect, $F(3,18) = 10.6$, $P < 0.001$), and decreased mean diastolic pressure by 5 mmHg (*time* main effect, $F(3,18) = 11.2$, $P < 0.001$). Propranolol did not alter the effect of the meal on blood pressure, but mean pulse rate increased after the meal by only 4 beats/min during propranolol infusion (*drug* × *time* interaction, $F(3,18) = 6.45$, $P < 0.004$). Overfeeding did not alter the changes in blood pressure or pulse rate associated with the meal. Standing reduced mean systolic pressure by 11 mmHg at 10 min (*time* main effect, $F(3,15) = 6.01$, $P < 0.01$), increased mean pulse rate by 20 beats/min (*time* main effect, $F(3,15) = 22.8$, $P < 0.001$), and did not affect diastolic blood pressure. Carbohydrate overfeeding did not alter these responses to standing. During the norepinephrine infusion, mean systolic pressure rose from 107±5 mmHg at 0 min to 142±5 mmHg at 60 min of the base-line test and from 113±3 mmHg to 137±4 mmHg after overfeeding (*time* main effect, $F(4,20) = 136$, $P < 0.001$). Diastolic pressure increased from 70±3 mmHg at 0 min to 85±3 mmHg during both norepinephrine infusions (*time* main effect, $F(4,20) = 27.3$, $P < 0.001$).

Plasma renin activity was increased (peak response 68%) by standing (*time* main effect, $F(1,4) = 14.8$, $P < 0.02$) and by the standard morning meal (Table V; time main effect $F(3,18) = 7.21$, $P < 0.003$). Norepinephrine infusion did not significantly increase plasma renin activity. Overfeeding did not alter basal plasma renin activity or responses to standing, feeding, and norepinephrine infusion (*overfeeding* main effects, $P > 0.6$). Propranolol reduced mean preprandial plasma renin activity by 27% (for all tests combined) and postprandial plasma renin activity by 35% (*drug* × *time* interaction, $F(3,18) = 26.1$, $P < 0.001$), but the effect of propranolol was not altered by overfeeding (*drug* × *time* × *overfeeding* interaction, $P > 0.5$; Table V).

DISCUSSION

Subjects who are overfed a cumulative excess of at least 20,000 kcal (84 MJ) typically exhibit increased thermogenesis, but the mechanism for this phenomenon remains open to question (1, 2, 24, 25). Recently, there has been much interest in the hypothesis that dietary thermogenesis is related to increased sympathetic nervous system activity. Young and Landsberg (6, 7) and Rappaport et al. (8) have shown that the overfeeding of sucrose increased norepinephrine turnover in heart and other tissues in rats. Rothwell and Stock (5) reported that overfeeding approximately doubled the increase in V_{O_2} induced by norepinephrine injections in rats, and that acute administration of the beta adrenergic antagonist propranolol reduced the V_{O_2} of overfed rats to levels observed in normally fed rats. Similarly, Dauncey and Ingram (9) found that acute propranolol administration inhibited thermogenesis in pigs fed ad lib., but not in pigs fed a restricted diet. These findings suggest that dietary thermogenesis is related to some combination of increased

TABLE V

Effect of Propranolol on Pre- and Postprandial Plasma Renin Activity during Overfeeding

	Time, *min*			
	−30	0	60	120
Base-line diet				
Saline	0.76±0.16	0.77±0.18	1.52±0.31*‡	1.12±0.22*
Propranolol	0.89±0.22	0.60±0.15*	1.08±0.30	0.79±0.26
Overfed				
Saline	0.82±0.14	0.82±0.16	1.45±0.28*‡	1.45±0.26*‡
Propranolol	0.84±0.13	0.66±0.14*	0.90±0.20	0.86±0.23

Values are expressed as nanograms angiotensin I generated per milliliter of plasma per hour at 37°C at pH 7.4; mean±SE. Propranolol infusion was started after −30-min blood sample, and standard morning meal was given after 0-min blood sample.

* $P < 0.05$, compared with −30 min-value using the paired t test.

‡ $P < 0.05$, compared with 0 min-value using the paired t test.

sympathetic nervous system activity and increased thermogenic sensitivity to norepinephrine.

Norepinephrine also stimulates thermogenesis in man (26–28), although not as much as in small mammals (29), and this effect is prevented by beta adrenergic blockade (28). Propranolol reduces RMR in obese women on weight-maintenance diets, but not obese women on low energy diets (30). Moreover, the catecholamine precursor L-dopa prevents the fall in RMR associated with carbohydrate restriction in obese subjects (31). These data suggest that the increase in RMR associated with human obesity (32, 33) is related to increased sympathetic nervous system activity.

In the present study, the effect of beta adrenergic blockade was studied in the postprandial state, because food intake stimulates sympathetic nervous system activity (10, 11), and therefore, sympathetic nervous system-mediated dietary thermogenesis should be greater after a meal than after an overnight fast. The dose of propranolol used in the present study, which abolishes the increase in thermogenesis associated with the marked catecholamine secretion caused by glucoprivation (34), had no effect on preprandial V_{O_2} but reduced the postprandial V_{O_2} by ~5%. However, the effect of propranolol on V_{O_2} was the same before and during overfeeding. This finding does not support the hypothesis that overfeeding stimulates sympathetic nervous system-mediated thermogenesis in man.

Meal-induced thermogenesis is related to the energy requirements for protein, glycogen, triglyceride, and urea synthesis, and for active absorption of nutrients (18), although it is not clear whether these processes can account for all of the postprandial increase in RMR. We have previously suggested that sympathetic nervous system stimulation may contribute to the thermic effect of carbohydrate (10, 11). Rothwell et al. have reported that acute propranolol administration abolished the rise in V_{O_2} induced by a meal in rats, suggesting a beta adrenergic-sympathetic mechanism for the thermic effect of feeding (35). In the present study, propranolol reduced the thermic response to a meal by ~20%, which suggests a small sympathetic component to meal-induced thermogenesis in man. However, without further control experiments it is impossible to determine if this effect is due to a gradual effect of propranolol on base-line RMR rather than a specific effect on the thermic effect of feeding.

We found no increase in the thermic effect of a meal after overfeeding, as was reported by Miller et al. (36). However, Miller et al. fed subjects larger test meals during overfeeding, whereas we continued to give a standard meal. Since postprandial RMR was measured for only 2 h, which is not long enough to observe the full thermic effect of feeding, it is quite possible that we failed to measure a delayed increase in the thermic effect of feeding.

Miller et al. observed that the thermic effect of a meal was increased by overfeeding most dramatically when it was measured during exercise, but we have studied only resting subjects. Stock (37) has found that exercise potentiates the thermic effect of a meal after one day of overfeeding, but not after one day of fasting. Bray et al. (38) also found that exercise potentiates the thermic effect of a meal, but that overfeeding 4,000 kcal/d for 30 d did not magnify this effect.

The thermic effect of norepinephrine was unaffected by carbohydrate overfeeding. This observation is consistent with preliminary reports that the thermic effect of norepinephrine in man is not enhanced by overfeeding of mixed diets (39, 40) and is in marked contrast to the enhanced thermic effect of norepinephrine during overfeeding in rats (5). A possible explanation for the failure of overfeeding to enhance the thermic effect of norepinephrine in man is that the amount of brown adipose tissue in adult man is relatively small compared with the amount of brown adipose tissue in the rat. In the rat the dominant site of norepinephrine-induced thermogenesis is brown adipose tissue, although skeletal muscle may also be involved (41, 42).

Inhibition of lipolysis with nicotinic acid reduces the thermic effect of norepinephrine by 50% in man, indicating that FFA mobilization is an important factor in norepinephrine-induced heat production (26). In the present study, overfeeding with carbohydrate attenuated the increases in plasma FFA and glucose concentrations produced by norepinephrine, and therefore, may have partly inhibited the thermogenic response to norepinephrine. This inhibition of FFA and glucose responses may be due to the slightly higher insulin levels during overfeeding.

Since T_3 is a potent thermogenic hormone, and since overfeeding elevates T_3 production (4), it is tempting to speculate that overfeeding-induced changes in T_3 production mediate dietary thermogenesis. However, direct evidence that increased T_3 production mediates dietary thermogenesis is lacking. Acheson and Burger (43) reported that inhibition of T_3 production by iopanoic acid, which caused decreases in serum T_3 levels that are of the same order of magnitude as those observed with dietary restriction, did not affect RMR in euthyroid subjects. In view of the fact that thyroxine levels were increased by iopanoic acid (43), these data do not rule out a role for altered T_3 production in dietary thermogenesis.

Another factor that may contribute to dietary thermogenesis is increased mass of active tissue. Approximately one-third of the excess weight in spontaneous and experimental obesity is fat-free tissue (3). How-

ever, in the present study the percentage of increase in BMR was much greater than the percentage of change in either total body weight or LBM.

Neuronal reuptake is the primary mechanism for clearing released norepinephrine, and therefore plasma norepinephrine levels represent only the small fraction of norepinephrine that diffuses from synapses into the circulation. Estimation of norepinephrine release by infusion of tracer doses of tritiated norepinephrine has indicated that overfeeding increases release of norepinephrine into the circulation by 81% while increasing plasma norepinephrine levels by only 25% (44). Thus, the failure of overfeeding to increase basal norepinephrine levels or VMA excretion does not exclude the possibility of elevated norepinephrine turnover in this study. The fact that standing norepinephrine levels and supine pulse rate were increased by overfeeding suggests that there was in fact some stimulation of sympathetic nervous system activity during overfeeding in the present study. Nevertheless, the failure of propranolol to alter RMR suggests that any stimulation of sympathetic nervous system activity that may have occurred was not sufficient to have a significant effect on resting thermogenesis.

Little is known about the increase in plasma norepinephrine levels and plasma renin activity associated with meals. We have previously found that glucose ingestion increases norepinephrine levels, whereas protein or fat ingestion do not (10, 11). Thus the composition of the meal may be an important determinant of the sympathetic response. Since sympathetic nervous system stimulation increases plasma renin activity (45), it is conceivable that the plasma renin activity response to a meal is related to increased sympathetic nervous system activity. Plasma renin activity initially fell after propranolol administration and then tended to rise (albeit not a statistically significant rise) after the meal, which suggests that the meal-induced stimulation of plasma renin activity may not be fully explained by beta adrenergic stimulation. A possible factor in the meal-related increases in plasma renin activity and norepinephrine levels is an increase in blood flow to splanchnic organs, causing reduced pressure in other organs and stimulation of baroreceptors involved in norepinephrine and renin secretion. Another possible explanation is that postprandial insulin secretion increases norepinephrine levels (46) and plasma renin activity. One mechanism whereby insulin may increase plasma renin activity is by reducing plasma K^+ concentration (47).

The failure of overfeeding to increase blood pressure is not surprising in light of the fact that overfeeding does not increase blood pressure in normotensive rats (48), in spite of the fact that cardiac norepinephrine turnover is enhanced by overfeeding in these rats (6–8). However, spontaneously hypertensive rats do exhibit increased blood pressure during overfeeding (48), suggesting that individuals with a genetic tendency towards hypertension may be more susceptible to overfeeding-induced changes in blood pressure. It is interesting that the subject whose blood pressure rose markedly during the norepinephrine infusion had an increase in mean arterial pressure of ~10 mmHg during overfeeding, whereas none of the other subjects had increased blood pressure.

Insulin promotes renal sodium reabsorption (49), and therefore may cause plasma volume expansion and increased blood pressure. In the present study, basal insulin levels were elevated by ~50% as early as 5 d after the start of overfeeding, without an increase in blood pressure. However, basal insulin levels remained well within normal limits, even though they were increased by carbohydrate overfeeding. Perhaps a greater degree of hyperinsulinemia is needed to produce enough sodium retention to cause a detectable increase in blood pressure.

Caloric restriction reduces the blood pressure and plasma renin activity of obese patients, regardless of sodium intake (50). However, the fact that overfeeding did not alter blood pressure or plasma renin activity in the present study, together with the fact that the reduction in blood pressure and plasma renin activity associated with caloric restriction takes several weeks to occur (50), indicate that hyperphagia per se is not the cause of elevated blood pressure or plasma renin activity in many obese individuals.

The effects of carbohydrate overfeeding on postprandial glucose tolerance, insulin levels, and carbohydrate disposal in these subjects are discussed elsewhere (51). Briefly, we found that carbohydrate tolerance, carbohydrate oxidation, and conversion of carbohydrate to fat were all increased during overfeeding, whereas postprandial insulin levels were not increased.

It should be noted that the base-line diet was not a weight-maintenance diet. This was done intentionally to ensure that no subject would gain weight during this period, even with the relative inactivity associated with being housed in the Clinical Research Center. Because underfeeding decreases sympathetic nervous system activity (12, 13, 52, 53), it seemed appropriate to slightly underfeed the subjects in the base-line period to increase the probability of finding an increase in sympathetic nervous system-mediated thermogenesis during overfeeding. If it is assumed that the weight-maintenance requirement was 1.5 × BMR (54), then the subjects received an average of 87% of weight-maintenance energy during the base-line period, and 143% of weight-maintenance energy at the end of overfeeding. Since sodium intake was maintained at

150 meq/d during the base-line period, it is unlikely that there was any stimulation of sympathetic nervous system activity due to plasma volume contraction associated with the slight weight loss of 1–2% during the base-line period.

Ready access to a large variety of palatable foods may circumvent normal physiological regulation of energy intake in a large segment of the population, making spontaneous overfeeding and subsequent obesity the most prevalent nutritional disease in our society. Although overfeeding inevitably produces weight gain, the rate and magnitude of weight gain is influenced by dietary thermogenesis. The possibility that some individuals with a genetic tendency for obesity fail to increase thermogenesis during overfeeding therefore warrants investigation.

ACKNOWLEDGMENTS

We thank Sharon Stowe, Elaine King, Kirti Bhatt, and the staff of the Clinical Research Center for their technical assistance; Nancy A. Bezek for preparing the manuscript; and Dr. Gilbert Forbes for the ^{40}K counts.

This work was supported by grants from the National Institutes of Health (AM 20494 and RR 00044) and the U. S. Department of Agriculture (CRGO 8000547).

REFERENCES

1. Danforth, E., Jr., A. G. Burger, R. F. Goldman, and E. A. H. Sims. 1978. Thermogenesis during weight gain. *In* Recent Advances in Obesity Research: II, Proceedings of the 2nd International Congress on Obesity. G. Bray, editor. Newman Publishing Ltd. London. 229–236.
2. Goldman, R. F., M. F. Haisman, G. Bynum, E. S. Horton, and E. A. H. Sims. 1975. Experimental obesity in man: metabolic rate in relation to dietary intake. *In* Obesity in Perspective. G. A. Bray, editor. U. S. Government Printing Office, Washington, DC. 165–186.
3. Forbes, G. B., and S. L. Welle. 1983. Lean body mass in obesity. *Int. J. Obesity.* In press.
4. Danforth, E., Jr., E. S. Horton, M. O'Connell, E. A. H. Sims, A. G. Burger, S. H. Ingbar, L. Braverman, and A. G. Vagenakis. 1979. Dietary-induced alterations in thyroid hormone metabolism during overnutrition. *J. Clin. Invest.* **64:** 1336–1347.
5. Rothwell, N. J., and M. J. Stock. 1979. A role for brown adipose tissue in diet-induced thermogenesis. *Nature (Lond.).* **281:** 31–34.
6. Young, J. B., and L. Landsberg. 1977. Stimulation of the sympathetic nervous system during sucrose feeding. *Nature (Lond.).* **269:** 615–617.
7. Young, J. B., and L. Landsberg. 1979. Effect of diet and cold exposure on norepinephrine turnover in pancreas and liver. *Am. J. Physiol.* **236:** E524–E533.
8. Rappaport, E. B., J. B. Young, and L. Landsberg. 1982. Initiation, duration, and dissipation of diet-induced changes in sympathetic nervous system activity in the rat. *Metab. Clin. Exp.* **31:** 143–146.
9. Dauncey, M. D., and D. L. Ingram. 1979. Effect of dietary composition and cold exposure on non-shivering thermogenesis in young pigs and its alteration by the β-blocker propranolol. *Br. J. Nutr.* **41:** 361–370.
10. Welle, S. L., U. Lilavivathana, and R. G. Campbell. 1980. Increased plasma norepinephrine concentrations and metabolic rates following glucose ingestion in man. *Metab. Clin. Exp.* **29:** 806–808.
11. Welle, S. L., U. Lilavivat, and R. G. Campbell. 1981. Thermic effect of feeding in man: increased plasma norepinephrine levels following glucose but not protein or fat consumption. *Metab. Clin. Exp.* **30:** 953–958.
12. De Haven, J., R. Sherwin, R. Hendler, and P. Felig. 1980. Nitrogen and sodium balance and sympathetic nervous system activity in obese subjects treated with a low-calorie protein or mixed diet. *N. Eng. J. Med.* **302:** 477–482.
13. Jung, R. T., P. S. Shetty, M. Barrand, B. A. Callingham, and W. P. T. James. 1979. Role of catecholamines in hypotensive response to dieting. *Br. Med. J.* **1:** 12–13.
14. National Research Council. 1980. Recommended Dietary Allowances. National Academy of Sciences, Washington, DC. 9th Edition. 22.
15. Adams, C. F. 1975. Nutritive Value of American Foods in Common Units. United States Department of Agriculture, Washington, DC. Handbook No. 456.
16. Durnin, J. V. G. A., and R. Passmore. 1967. Energy, Work, and Leisure. Heinemann Educational Books Ltd., London. 15–18.
17. De Weir, J. B. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *J. Physiol. (Lond.).* **109:** 1–9.
18. Flatt, J. P. 1980. Energetics of intermediary metabolism. *In* Assessment of Energy Metabolism in Health and Disease. J. M. Kinney, editor. Ross Laboratories Div., Abbott Labs, Columbus, OH. 77–87.
19. Trout, D. L., E. H. Estes, Jr., and S. J. Friedberg. 1960. Titration of free fatty acids in plasma: a study of current methods and a new modification. *J. Lipid Res.* **1:** 199–202.
20. Passon, P. G., and J. D. Peuler. 1973. A simplified radiometric assay for plasma norepinephrine and epinephrine. *Anal. Biochem.* **51:** 618–631.
21. Forbes, G. B., F. Schultz, C. Cafarelli, and G. H. Amirhakimi. 1968. Effects of body size on potassium-40 measurements in the whole body counter (tilt-chair technique). *Health Phys.* **15:** 435–442.
22. Pizano, J. J., J. R. Crout, and D. Abraham. 1962. Determination of 3-methoxy-4-hydroxymandelic acid in urine. *Clin. Chim. Acta.* **7:** 285–291.
23. Health Sciences Computing Facility. 1979. BMDP-79 Biomedical Computer Programs. University of California Press, Los Angeles.
24. Garrow, J. S. 1978. Energy Balance and Obesity in Man. 2nd edition. Elsevier North-Holland, Inc., New York. 91–93.
25. Norgan, N. G., and J. V. G. A. Durnin. 1980. The effect of 6 weeks of overfeeding on the body weight, body composition, and energy metabolism of young men. *Am. J. Clin. Nutr.* **33:** 978–988.
26. Havel, R. J., L. A. Carlson, L. G. Ekelund, and A. Holmgren. 1964. Studies on the relation between mobilization of free fatty acids and energy metabolism in man: effects of norepinephrine and nicotinic acid. *Metab. Clin. Exp.* **13:** 1402–1411.
27. Jung, R. T., P. S. Shetty, W. P. T. James, M. A. Barrand, and B. A. Callingham. 1979. Reduced thermogenesis in obesity. *Nature (Lond.).* **279:** 322–323.
28. Steinberg, D., P. J. Nestel, E. R. Buskirk, and R. H. Thompson. 1964. Calorigenic effect of norepinephrine correlated with plasma free fatty acid turnover and oxidation. *J. Clin. Invest.* **43:** 167–176.

29. Jansky, L. 1973. Non-shivering thermogenesis and its thermoregulatory significance. *Biol. Rev. Camb. Philos. Soc.* **48:** 85–132.
30. Jung, R. T., P. S. Shetty, and W. P. T. James. 1980. The effect of beta-adrenergic blockade on metabolic rate and peripheral thyroid metabolism in obesity. *Eur. J. Clin. Invest.* **10:** 179–182.
31. Shetty, P. S., R. T. Jung, and W. P. T. James. 1979. Effect of catecholamine replacement with levodopa on the metabolic response to semistarvation. *Lancet.* **I:** 77–79.
32. James, W. P. T., K. Bailes, H. L. Davies, and M. J. Dauncey. 1978. Elevated metabolic rates in obesity. *Lancet.* **I:** 1122–1125.
33. Ravussin, E., B. Burnand, Y. Schutz, and E. Jequier. 1982. Twenty-four hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. *Am. J. Clin. Nutr.* **35:** 566–573.
34. Welle, S. L., D. A. Thompson, and R. G. Campbell. 1982. β-adrenergic blockade inhibits thermogenesis and lipolysis during glucoprivation in man. *Am. J. Physiol.* **243:** R379–R382.
35. Rothwell, N. J., M. E. Saville, and M. J. Stock. 1982. Factors influencing the acute effect of food on oxygen consumption in the rat. *Int. J. Obesity.* **6:** 53–59.
36. Miller, D. S., P. Mumford, and M. J. Stock. 1967. Gluttony 2. Thermogenesis in overeating man. *Am. J. Clin. Nutr.* **20:** 1223–1229.
37. Stock, M. J., 1980. Effects of fasting and refeeding on the metabolic response to a standard meal in man. *Eur. J. Appl. Physiol. Occup. Physiol.* **43:** 35–40.
38. Bray, G. A., B. J. Whipp, and S. N. Koyal. 1974. The acute effects of food intake on energy expenditure during cycle ergometry. *Am. J. Clin. Nutr.* **27:** 254–259.
39. Katzeff, H., and E. Danforth, Jr. 1982. Norepinephrine sensitivity and energy expenditure in response to overnutrition in lean and obese man. *Clin. Res.* **30:** 245a. (Abstr.)
40. Bray, G. A., B. J. Whipp, S. N. Koyal, and A. L. Campfield. 1981. The effect of weight gain on the norepinephrine-induced stimulation of O_2 uptake in normal subjects. *Clin. Res.* **29:** 63a. (Abstr.)
41. Foster, D. O., and M. L. Frydman. 1978. Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of calorigenesis induced by noradrenaline. *Can. J. Physiol. Pharmacol.* **56:** 110–122.
42. Himms-Hagen, J. 1975. Role of the adrenal medulla in adaptation to cold. *Handb. Physiol.* **6**(Sect. 7, Endocrinology): 637–665.
43. Acheson, K. G., and A. G. Burger. 1980. A study of the relationship between thermogenesis and thyroid hormones. *J. Clin. Endocrinol. Metab.* **51:** 84–89.
44. Esler, M. 1982. Assessment of sympathetic nervous function in humans from noradrenaline plasma kinetics. *Clin. Sci.* **62:** 247–254.
45. Davis, J. O. 1973. The control of renin release. *Am. J. Med.* **55:** 333–350.
46. Rowe, J. W., J. B. Young, K. L. Minaker, A. L. Stevens, J. Pallotta, and L. Landsberg. 1981. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes.* **30:** 219–225.
47. Himathongkam, T., R. G. Dluhy, and G. H. Williams. 1975. Potassium-aldosterone-renin interrelationships. *J. Clin. Endocrinol. Metab.* **41:** 153–159.
48. Young, J. B., and L. Landsberg. 1981. Effect of oral sucrose on blood pressure in the spontaneously hypertensive rat. *Metab. Clin. Exp.* **30:** 421–424.
49. DeFronzo, R. A., 1981. Insulin and renal sodium handling: clinical implications. *In* Recent Advances in Obesity Research: III, Proceedings of the 3rd International Congress on Obesity. P. Bjorntorp, M. Cairella, A. N. Howard, editors. John Libbey & Company, London. 32–41.
50. Tuck, M. L., J. Sowers, L. Dornfield, G. Kledzik, and M. Maxwell. 1981. The effect of weight reduction on blood pressure, plasma renin activity, and plasma aldosterone levels in obese patients. *N. Engl. J. Med.* **304:** 930–933.
51. Welle, S. L., and R. G. Campbell. 1983. Improved carbohydrate tolerance and stimulation of carbohydrate oxidation and lipogenesis during short-term carbohydrate overfeeding. *Metab. Clin. Exp.* In press.
52. Sowers, J. R., L. A. Whitfield, R. A. Catania, N. Stern, M. L. Tuck, L. Dornfield, and M. Maxwell. 1982. Role of the sympathetic nervous system in blood pressure maintenance in obesity. *J. Clin. Endocrinol. Metab.* **54:** 1181–1186.
53. Young, J. B., and L. Landsberg. 1977. Suppression of the sympathetic nervous system during fasting. *Science (Wash. DC).* **196:** 1473–1475.
54. Payne, P. R., and J. C. Waterlow. 1971. Relative energy requirements for maintenance, growth, and physical activity. *Lancet.* **II:** 210–211.