Leptin reverses declines in satiation in weight-reduced obese humans[1–3]

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ABSTRACT

Background: Individuals who are weight-reduced or leptin deficient have a lower energy expenditure coupled with higher hunger and disinhibition and/or delayed satiation compared with never-weight-reduced control subjects. Because exogenous leptin inhibits feeding in congenitally leptin-deficient humans, reduced leptin signaling may reduce the expression of feeding inhibition in humans.

Objective: The objective was to test the hypothesis that reduced leptin signaling may reduce the expression of feeding inhibition (ie, blunt satiation) in humans by examining the effects of leptin repletion on feeding behavior after weight loss.

Design: Ten obese humans (4 men, 6 women) were studied as inpatients while they received a weight-maintaining liquid-formula diet. Satiation was studied by measuring intake and ratings of appetite-related dispositions 3 h after ingestion of 300 kcal of the liquid-formula diet. The subjects were studied at each of 3 time periods: *1*) while they maintained their usual weight ($Wt_{initial}$) and then after weight reduction and stabilization at 10% below initial weight and while they received 5 wk of either *2*) twice-daily injections of placebo ($Wt_{-10\%placebo}$) or *3*) "replacement doses" of leptin ($Wt_{-10\%leptin}$) in a single-blind crossover design with a 2-wk washout period between treatments. Energy expenditure was also measured at each study period.

Results: Both energy expenditure and visual analog scale ratings that reflect satiation were significantly lower at $Wt_{-10\%placebo}$ than at $Wt_{initial}$ and $Wt_{-10\%leptin}$.

Conclusion: The results are consistent with the hypothesis that the absence of leptin signaling after weight loss may blunt the expression of feeding inhibition in humans. *Am J Clin Nutr* 2012;95:309–17.

INTRODUCTION

More than 60% of US adults are overweight [BMI (weight in kg/height^2 in m) >25] or obese (BMI >30) and are at risk of adiposity-related morbidities, such as diabetes mellitus and hyperlipidemia (1, 2). Obesity's intractable nature is reflected in the 75–95% recidivism rate to obesity among the formerly obese (3, 4). After weight loss, there is a decline in energy expenditure that reflects both the loss of metabolically active tissue plus an additional 300–400 kcal/d, which may be termed "adaptive thermogenesis" (5–7), and is largely attributable to increased skeletal muscle work efficiency (8–10). In addition, individuals maintaining a reduced weight have decreased sympathetic nervous system tone and circulating concentrations of leptin, thyroxine, and triiodothyronine and increased parasympathetic nervous system tone. Thus, metabolic, autonomic, and neuroendocrine systems act in conjunction to favor weight regain after otherwise successful weight loss (9). Decreased energy expenditure after weight loss would have little consequence if it were easy to sustain a corresponding reduction in energy intake to maintain a reduced body weight. As anyone who has attempted to sustain weight loss can attest, this is not the case (4, 11, 12).

The metabolic, hormonal, and autonomic nervous system profiles of weight-reduced individuals are remarkably similar to those of leptin-deficient humans and rodents (13–15). Most of the metabolic, autonomic, and neuroendocrine physiology favoring weight gain via decreased energy expenditure is "reversed" by the administration of leptin to weight-reduced and leptin-deficient individuals (9, 16, 17). In contrast, there is little or no effect of leptin when administered to leptin-sufficient lean or obese humans, whose body weight has not been deliberately altered (18). In addition to being hypometabolic (16, 19–23), leptin-deficient humans and rodents are also hyperphagic and demonstrate reduced satiation and, to a lesser degree, increased hunger, thereby creating the optimal biological conditions that promote weight gain in leptin-deficient states (24).

The difficulty in sustaining weight loss, ie, in adjusting energy intake to meet decreased energy expenditure, suggests that the decline in perceptions of fullness that occur during dynamic weight loss and result in overeating to reach satiation (25–27) may persist, even after otherwise successful weight reduction. The similarities between the neuroendocrine, autonomic, metabolic, and behavioral changes of weight-reduced individuals and congenitally leptin-deficient individuals suggest that the reductions in circulating and central nervous system leptin concentrations that occur after weight loss (loss of fat mass) are sensed via hypothalamic neurons as the decrease in an inhibitory signal, thereby facilitating energy restoring actions (28, 29).

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We have suggested that leptin acts as part of a food intake inhibitory control system that prevents us, teleologically, from engaging in continual unrestricted foraging at the expense of reproduction. The importance of inhibitory control in reduced weight maintenance is illustrated by fMRI[4] studies showing that individuals who are successful in maintaining a reduced weight (a state associated with decreased activity of inhibitory control areas) have greater activity in brain areas (frontal regions and primary and secondary visual cortices) involved in inhibitory control in response to food cues than do obese or never-obese control subjects (32). Specifically, we examined the effects of administration of "replacement" doses of leptin to subjects whose circulating leptin concentrations were reduced by virtue of diet-induced weight loss. We hypothesized that weight-reduced subjects would demonstrate reduced satiation that would be "reversed" by leptin repletion as is predicted based on the observed effects of weight loss and leptin repletion on brain neural activity in response to food (33–35). The primary outcome variables of this study are thus those related to satiation.

SUBJECTS AND METHODS

Subjects

Ten obese [BMI, weight (kg)/height^2 (m) $>$ 30] subjects (4 men and 6 women) with a mean ($\pm$SEM) age of 33.6 $\pm$ 2.8 y were recruited by advertisement between January 2003 and August 2005. Subjects were admitted to the General Clinical Research Center at Columbia University Medical Center and remained as inpatients throughout the study. All subjects had been stable at their maximal lifetime weights for $\geq$6 mo before admission, were in good health, and were taking no medications. The protocol was approved by the Institutional Review Board of The New York Presbyterian Medical Center and is consistent with guiding principles for research involving humans (36). Written informed consent was obtained from all subjects. Subject characteristics are presented in **Table 1**.

Protocol

This protocol (**Figure 1**), which allows the study of subjects at their initial weight and then while they receive either placebo or leptin after weight loss, was described previously (37). Briefly, the subjects were fed a liquid-formula diet [40% of energy as fat (corn oil), 45% as carbohydrate (glucose polymer), and 15% as protein (casein hydrolysate); caloric density = 1.25 digestible kcal of energy/g], plus vitamin and mineral supplements, in quantities sufficient to maintain a stable weight (defined as an average daily weight variation of $<$10 g/d for $\geq$2 wk). This weight plateau is designated as $Wt_{initial}$. Each subject's aerobic fitness was measured by bicycle ergometry on admission. Supervised exercise (treadmill walking or stationary bicycling) was performed 3 times weekly at specified intensities and durations that were adjusted to maintain each subject's anaerobic threshold at their initial level throughout the study (9, 16).

After completion of studies (described below) at $Wt_{initial}$, the subjects were provided 800 kcal energy/d of the same liquid-formula diet until they had lost $\sim$10% of $Wt_{initial}$. The duration of the weight-loss phase ranged from 36 to 62 d. Once 10% weight loss had been achieved, intake was adjusted upward until subjects were again weight stable as described above. Subjects were then randomly assigned to receive twice daily (0800 and 2000) subcutaneous injections of saline (weight plateau is designated as $Wt_{-10\%placebo}$) or recombinant human leptin (A-100, Amgen Inc; metreleptin, Amylin Pharmaceuticals Inc). This period is designated as $Wt_{-10\%leptin}$. The initial leptin doses were 0.08 mg/kg fat mass per dose in men and 0.14 mg/kg fat mass per dose in women (38). Circulating leptin concentrations at 0800 were measured weekly in subjects receiving leptin, and the dosages were adjusted until circulating leptin concentrations were similar to those measured at 0800 at $Wt_{initial}$ (18, 38). After completion of studies at $Wt_{-10\%placebo}$ or $Wt_{-10\%leptin}$, the subjects underwent a 2-wk washout period during which they received no injections. They were then crossed over to receive either leptin or placebo injections. Subjects were unaware of the order of testing and remained on a diet isocaloric to that initially shown necessary to maintain a 10% reduced body weight throughout the leptin or placebo arms of the study.

Tests of ingestive behavior

Eating-behavior tests were conducted at the New York Obesity Research Center, Ingestive Behavior Laboratory, which consists of a testing room measuring 2.4 $\times$ 3.7 m and equipped with a universal eating monitor as described previously (39–44). At each of the 3 phases ($Wt_{initial}$, $Wt_{-10\%placebo}$, and $Wt_{-10\%leptin}$), the subjects were tested twice in each of 2 paradigms designated as ad libitum and fixed-formula meals. The main purpose of the ad libitum formula meal was to assess the effects of the treatments on intake, whereas the main purpose of the fixed-formula meals was to assess the effects of the treatments on appetite-related feelings, which vary with intake and can be more precisely measured by keeping intake constant (*see* **Table 2**). The intake of the fixed-formula meal was also set high to ensure that appetite-related sensations were measured over their full potential range of consumption. Because it turned out that intake did not vary across treatments and because preliminary analysis showed that there was no treatment $\times$ meal-type interaction, the data from the 2 meal types were pooled for the final analysis, except for the comparison between the 2 meal types. The repetitive measures at each phase, as well as the redundancies of measurement built into the protocols, were previously established as was the validity of these protocols (39–44).

On all test days, subjects ate 300 kcal of the liquid-formula diet 3 h before testing (0900). Testing began at noon, and questionnaires were administered immediately before and immediately after the test meal (*see* below). On ad libitum days, the subjects were instructed to "eat until you feel comfortably satisfied." The total intake was recorded before and after the container was weighed, coupled with measurement of the total quantity of formula placed in the container. On the fixed-meal days, the food was delivered into the cup inside the opaque box. The subjects were instructed to stop eating when they heard a tone, fill out a 2-page questionnaire, and then return to eating.

[4] Abbreviations used: fMRI, functional magnetic resonance imaging; TEE, 24-h energy expenditure; $Wt_{initial}$, usual weight was maintained; $Wt_{-10\%leptin}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of leptin; $Wt_{-10\%placebo}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of placebo.

TABLE 1
Characteristics of the 10 obese subjects (4 men, 6 women) by group[1]

	$Wt_{initial}$	$Wt_{-10\%placebo}$	$Wt_{-10\%leptin}$
Weight (kg)	128.1 ± 12.1	109.2 ± 9.6†	107.5 ± 9.4†
FFM (kg)	65.6 ± 4.9	59.8 ± 4.4†	59.1 ± 4.1†
FM (kg)	62.5 ± 7.9	49.5 ± 4.5†,‡	48.5 ± 5.7†
TEE (kcal/d)	3335 ± 215	2600 ± 177†,‡	2915 ± 146†
TEE (kg FFM/d)	51.5 ± 1.9	44.0 ± 1.5†‡	51.9 ± 2.3
Leptin (ng/mL)	57.0 ± 14.7	45.8 ± 12.4†,‡	61.4 ± 15.1

[1] All values are means ± SEMs. Data between study periods were compared by repeated-measures ANOVA.†Significantly different from $Wt_{initial}$, $P < 0.001$.‡Significantly different from $Wt_{-10\%leptin}$, $P < 0.01$. FFM, fat-free mass; FM, fat mass; TEE, 24-h energy expenditure; $Wt_{initial}$, usual weight was maintained; $Wt_{-10\%leptin}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of leptin; $Wt_{-10\%placebo}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of placebo.

Ratings were made by marking 150-mm lines anchored by "not at all" and "most imaginable." Questions related to the primary outcome variable of this study, namely satiation, were as follows: "How full do you feel," "How do you feel about how much you have eaten?," and "How do you feel about switching to another food?" (*see* Table 2 for more detailed analyses of each anchor). In addition, to determine whether feelings of satiation and not feelings of discomfort were responsible for the effects of leptin or weight reduction, the subjects completed similar ratings regarding their overall feeling of well-being at the time of testing and as a result of eating small and large quantities of food. These ratings were "How sick do you feel?" as an indicator of discomfort, as opposed to satiation, as a contributor to meal termination and "How much stomach discomfort are you experiencing?" as an indicator of where discomfort was occurring, if it occurred. These ratings were not related to the primary outcome variable and were not expected to change as a result of leptin administration or weight loss. Similarly, subject liking of the liquid formula was rated based on the question "How much do you like what you are consuming?". Like the ratings of sickness, this rating of liking can be considered as a background or control variable that was included to avoid errors due to subject discomfort or changes in likeability of the formula as a result of weight loss or prolonged hospitalization (45).

TEE

Whether an individual gains or loses weight depends on the balance between energy intake and expenditure. There would be little physiologic consequences of changes in energy intake if they were met by corresponding changes in energy expenditure. TEE was measured based on the number of kcal/d that the subjects required to maintain their body weight at $Wt_{initial}$ and $Wt_{-10\%placebo}$. As shown in Table 1, the subjects underwent changes in body composition (predominantly loss of fat mass) during leptin administration, and TEE was calculated as calories ingested per day adjusted for intercurrent changes in body composition (*see* Table 2 for a more detailed description of these calculations and of assessments of weight stability).

Leptin

Circulating plasma concentrations of leptin at 0800 (before any injection) were measured by ELISA (Diagnostic Systems Laboratories Inc) at each weight plateau.

Data processing and statistical analyses

Data regarding energy intake are presented as kcal of energy consumed. Ratings were quantified by measuring a subject's demarcated response's distance (in mm) from the left end of the line (ie, from a score of 0). The experiment had a repeated-measures design, with treatment as a trial/repeated factor. The dependent variables were the means collapsed across replicates and meal type.

Data regarding the subject's anthropometric measurements, energy expenditure, and circulating concentrations of leptin were analyzed by repeated-measures ANOVA. Data regarding ingestive behavior were analyzed by using a mixed model (SAS version 9.2, PROC MIXED; SAS Institute), with treatment as a fixed effect. We initially had replicates on the 2 types of meals

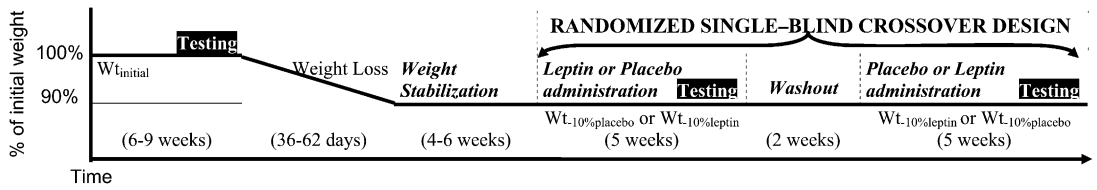

FIGURE 1. Schematic of protocol. Subjects were studied after stabilization at usual weight during a liquid-formula diet. They were then placed on a liquid-formula diet (800 kcal/d) until they had lost ~10% of $Wt_{initial}$ and were stabilized at that weight. Once weight was stable, subjects were randomly assigned in a single-blind crossover design to receive twice daily injections of either a placebo or leptin for 5 wk with a 2-wk washout period between treatments. Subjects were studied again at the end of $Wt_{-10\%placebo}$ and $Wt_{-10\%leptin}$, ie, while still receiving leptin or placebo injections. Leptin doses were calculated and titrated to restore circulating leptin concentrations at 0800 to pre–weight-loss concentrations. Subjects were inpatients throughout the study. $Wt_{initial}$, usual weight was maintained; $Wt_{-10\%leptin}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of leptin; $Wt_{-10\%placebo}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of placebo.

and conducted a preliminary analysis using both meal type, treatment, and their interaction, but when meal type × treatment proved not to be significant, we averaged across meal types by treatment, ending up with 3 observations per subject (one for each treatment total observations = 30). These treatment means were then analyzed by using PROC MIXED, with treatment as a fixed effect and subject as a random effect. Pairwise contrasts between the treatment means were tested by using the Estimate Statement. One-tailed tests were used for the preloss-placebo and leptin-placebo hypotheses, and 2-tailed tests were used for the leptin-preloss hypothesis. The covariance pattern of the residual matrix used in these analyses was determined by using the likelihood ratio statistic to compare an unstructured pattern with a compound symmetry pattern. The compound symmetry pattern was not rejected for any of the variables (46), except premeal sickness and premeal discomfort. Therefore, this pattern was selected for the covariance pattern of the residual matrix for all variables except the 2 aforementioned variables, for which the unstructured pattern was used. Comparisons of the effects of the 2 meal types were made by taking the means for each subject across all 6 test days for each meal type, regardless of treatment, and running a Student's *t* test (2-tailed) on the difference between the 2 means (one for each meal type) across the 10 subjects (df = 9).

Data are presented as means ± SEMs. The reason that statistical significance was prospectively defined as $P < 0.05$—one tailed for the $Wt_{-10\%placebo} - Wt_{initial}$ difference, and for the $Wt_{-10\%leptin} - Wt_{-10\%placebo}$ difference—was that the predicted effects were all unidirectional (47). As dictated by the known biochemistry and molecular physiology of leptin (48), changes in brain neuronal activity in response to food after weight loss and leptin repletion (33–35), and behavioral phenotypes of low leptin states (16, 19, 26, 27, 30, 31), subjects either would demonstrate delayed satiation after weight loss and increased satiation after leptin repletion or would show no significant effects of weight loss or leptin repletion. More specifically, knowledge of the physiology of alterations in neural signaling in response to food in low-leptin states, as well as the high recidivism rate after otherwise successful weight reduction and the feeding behavior of leptin-deficient or resistant rodents and humans, dictates the specific unidirectional hypothesis that low-leptin states are associated with decreased satiation (*see* Discussion). The $Wt_{-10\%leptin} - Wt_{initial}$ difference was expected to be zero, so those *P* values were 2-tailed. All comparisons with $P < 0.05$ are reported. Because, the primary hypothesis was a single contrast between leptin and placebo conducted on 4 variables, with each variable contributing unique scientific information, adjustment for multiple comparisons was not done as suggested by Rothman and others (49–51).

RESULTS

Body composition and energy expenditure

As dictated by the experimental design, weight, fat mass, and fat-free mass were significantly lower at $Wt_{-10\%placebo}$ and $Wt_{-10\%leptin}$ than at $Wt_{initial}$, and circulating leptin concentrations were significantly lower at $Wt_{-10\%placebo}$ than at $Wt_{initial}$ or $Wt_{-10\%leptin.}$ TEE was significantly lower at $Wt_{-10\%placebo}$ and at $Wt_{-10\%leptin}$ than at $Wt_{initial}$. TEE and TEE expressed per kg fat-free mass were significantly greater at $Wt_{initial}$ and $Wt_{-10\%leptin}$ than at $Wt_{-10\%placebo}$ (Table 1).

Food intake

Absolute intake did not differ across treatments, but absolute intake was greater during fixed-meal testing than during ad libitum testing in all study periods (Table 2 and **Table 3**).

Ratings

Meal type

Ratings of satiation (fullness, how much eaten and switching) and distress (sick and discomfort) were all significantly higher after subjects ate the fixed-formula meal than after they ate the ad libitum formula meal (Tables 2 and 3). Liking was not affected by meal type. Because no significant differences were found between meal types across treatments periods, the rest of the Results section is presented by variable with the data from the 2 meal types pooled (as described in the data analysis section above).

Fullness

Postmeal, but not premeal, ratings of fullness were higher at $Wt_{-10\%leptin}$ than at $Wt_{-10\%placebo}$, with differences that were significant averaged across both meal types. In addition, postmeal ratings of fullness were lower at $Wt_{-10\%placebo}$ than at $Wt_{initial}$.

How much eaten

Postmeal ratings of "how much have you eaten" were significantly lower at $Wt_{-10\%placebo}$ compared with ratings at $Wt_{initial}$ or $Wt_{-10\%leptin}$. Premeal ratings of this variable were significantly lower at $Wt_{-10\%placebo}$ than at $Wt_{initial}$, despite the fact that subjects received 300 kcal of the liquid-formula diet before all tests.

Amount eaten in relation to switching to another food

Postmeal ratings of the amount eaten in relation to switching to another food, after eating essentially the same amounts of food, were significantly higher after leptin administration than after $Wt_{-10\%placebo}$ and ratings at $Wt_{-10\%placebo}$ trended lower than at $Wt_{initial}$.

Palatability ratings

Whereas no significant difference in the rating of "liking" of the liquid-formula diet were found between subjects studied at $Wt_{-10\%leptin}$ and $Wt_{-10\%placebo}$, a significant decrease in the premeal liking rating for the $Wt_{-10\%placebo}$ condition compared with the $Wt_{initial}$ condition and a trend lower in that for the $Wt_{-10\%leptin}$ compared with the $Wt_{initial}$ condition. Irrespective of whether they were receiving leptin or placebo, the weight-reduced subjects, who had now received all nutrients as the liquid-formula diet for 5–9 mo, liked the formula less. This pattern did not persist in the postmeal ratings of liking.

Sickness ratings

There was significantly less reported premeal sickness at $Wt_{-10\%leptin}$ than at $Wt_{initial}$. Overall, mean sickness ratings were extremely low and not outside what has been encountered in

TABLE 2
Effect of meal type on ratings[1]

	Treatment									
	$Wt_{initial}$		$Wt_{-10\%placebo}$		$Wt_{-10\%leptin}$		All ad libitum		All fixed	
Variable	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Before the meal										
Fullness[2]	21.8	5.7	16.1	5.7	21.8	5.7	21.3	5.4	18.5	5.4
Switching[3]	31.2	7.0	20.2	6.9	21.5	6.9	26.3	6.6	22.3	6.6
How much eaten[4]	23.9	5.1	14.7	5.0	16.6	5.0	20.0	4.7	16.8	4.7
Liking	43.4	8.5	26.6	8.5	30.6	8.5	35.3	8.1	31.8	8.1
Sickness	3.6	1.3	5.0	2.8	2.0	1.0	2.8	1.2	4.2	1.9
Discomfort	8.7	4.2	11.5	5.5	4.3	2.3	7.4	4.9	9.0	3.6
After the meal										
Fullness[2]	110.8	6.9	93.1	6.9	107.7	6.9	92.2	6.1	115.6	6.1
Switching[3]	106.9	6.8	96.1	6.8	112.7	6.8	88.3	6.2	122.2	6.2
How much eaten[4]	97.8	6.9	85.5	6.9	97.3	6.9	75.9	6.4	111.2	6.4
Liking	38.5	11.1	43.5	11.1	54.4	11.1	47.7	10.6	43.3	10.6
Sickness	37.3	11.5	27.3	11.5	26.9	11.5	9.5	10.8	51.6	10.8
Discomfort	39.6	11.5	34.3	11.5	33.1	11.5	14.7	10.9	56.7	10.9
Energy consumed (kcal)	948.7	86.9	924.1	86.9	931.0	86.9	829.0	81.4	1040	81.4

[1] $Wt_{initial}$, usual weight was maintained; $Wt_{-10\%leptin}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of leptin; $Wt_{-10\%placebo}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of placebo.

[2] Ratings refer to subject response to the question "How full do you feel right now?" on a 150-mm scale that was anchored by "Not at all" and "Most imaginable."

[3] Ratings refer to subject response to the question "How do you feel about switching to another food?" on a 150-mm scale. The responses were "I've eaten so little I'd never switch" (0 mm), "I've eaten so much I'd always switch (150 mm)," and "I would usually switch at this amount (75 mm)."

[4] Ratings refer to subject response to "How do you feel about how much you have eaten?" with the left hand anchor (0 mm) "I've eaten so little I'd always eat more" and the right hand anchor (150 mm) "I've eaten so much I'd always eat less," and the middle anchor (75 mm) "at this amount, I would usually be comfortably satisfied."

other studies, with and without manipulations of testing conditions, from this laboratory (52, 53).

DISCUSSION

The major finding of this study is that leptin administration to weight-reduced subjects significantly increased satiation as reflected in postmeal feelings of fullness, the perception of how much food was eaten, and the perception of the amount eaten in relation to feelings that they were at the point in a meal at which they would ordinarily switch to another food. Further analysis of the effects of maintenance of reduced weight per se (ie, without leptin replacement) on these same variables showed effects of weight loss on feeding behavior related to satiation that were opposite those of leptin, in agreement with other studies that showed increased food craving (54) and decreased perception of how much food has been eaten in weight-reduced subjects (55). These changes in feeding behavior are entirely predictable from fMRI studies of subjects in a similar experimental paradigm (33) as well as from studies of feeding behavior in leptin-deficient states (24, 56, 57) and from the ~75–95% recidivism rate to previous levels of body fatness after otherwise successful weight reduction (3, 4). These findings suggest that the action of leptin, when administered to weight-reduced individuals, is actually to "reverse" some of the effects of maintaining a reduced body weight on feeding behavior. These effects are synergistic to those previously shown for energy expenditure (9).

The design of this study allowed for the examination of the effects of weight loss and leptin on eating behavior in weight-reduced individuals that was not confounded by changes in diet composition, exercise, lack of weight stability, while minimizing the effects of hedonic aspects of food. In contrast with Cameron et al (26), who recently reported significant increases in food "likeability" after weight loss, we found that ratings of "liking" the liquid formula were actually decreased by weight loss, regardless of treatment, which probably reflected dissatisfaction/boredom with the formula diet over time that does not seem to be affected by leptin. The disassociation of the effects of weight loss and leptin on "liking" and satiation is consistent with rodent studies, which indicated that "liking" and satiation are probably independently controlled (58, 59).

Subject premeal ratings of "How sick do you feel?" remained extremely low throughout the study (means consistently $\leq$5 mm with ranges generally from 0 to 25 mm on a 150-mm scale anchored at 0 mm with "not at all," The rating of "sickness" at $Wt_{-10\%leptin}$ was significantly lower than at $Wt_{initial}$ but always within this range. Because this small (mean $\pm$ SEM: -1.5 ± 0.6 mm) but significant difference reflects a data set with a very small variance and always at the extremely low end of the sickness rating scale, there was no indication that any subject actually felt unwell. All these values are well within what has been encountered in other studies, with and without manipulations of testing conditions, from this laboratory (52, 53). The postmeal rating of "sickness" at $Wt_{-10\%placebo}$ was not significantly different from that at $Wt_{initial}$. The differences in satiation

TABLE 3
Differences in ratings and in amount consumed between $Wt_{initial}$, $Wt_{-10\%placebo}$, and $Wt_{-10\%leptin}$ groups[1]

	Treatment differences											
	$Wt_{-10\%placebo} - Wt_{initial}$			$Wt_{-10\%\ (leptin\ -\ placebo)}$			$Wt_{-10\%leptin} - Wt_{initial}$			Fixed − ad libitum		
Variable	Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*	Mean	SEM	*P*
Before the meal												
Fullness	−5.7	5.9	0.174	5.8	5.9	0.172	0.0	5.9	0.993	−2.8	3.1	0.397
Switching	−12.7	7.1	0.046	1.5	7.1	0.420	−11.3	7.1	0.131	−2.9	4.6	0.553
How much eaten	−9.5	4.1	0.017	1.8	4.1	0.332	−7.6	4.1	0.081	−3.2	4.3	0.477
Liking	−16.3	6.7	0.013	2.8	6.7	0.342	−13.6	6.7	0.059	−3.2	5.8	0.587
Sickness	1.4	2.7	0.314	−2.9	2.8	0.164	−1.5	0.6	0.033	1.4	1.8	0.448
Discomfort	2.8	2.7	0.163	−7.2	4.1	0.057	−4.4	2.5	0.113	1.6	4.0	0.689
After the meal												
Fullness	−17.8	7.3	0.013	14.6	7.3	0.029	−3.1	7.3	0.675	23.4	9.9	0.042
Switching	−11.0	6.6	0.056	16.6	6.6	0.011	5.6	6.6	0.409	34.0	7.1	0.001
How much eaten	−12.3	6.3	0.034	11.8	6.3	0.039	−0.4	6.3	0.944	35.3	7.8	0.001
Liking	5.0	8.6	0.284	10.9	8.6	0.109	15.9	8.6	0.080	−4.4	7.1	0.553
Sickness	−10.0	6.3	0.066	−0.4	6.3	0.477	−10.4	6.3	0.118	42.1	15.0	0.020
Discomfort	−5.4	6.9	0.224	−1.1	6.9	0.438	−6.5	6.9	0.362	42.0	11.8	0.006
Energy consumed (kcal)	−24.5	74.6	0.373	6.9	74.6	0.464	−17.6	74.6	0.816	211.2	91.6	0.047

[1] $Wt_{initial}$, usual weight was maintained; $Wt_{-10\%leptin}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of leptin; $Wt_{-10\%placebo}$, after weight reduction and stabilization at 10% below initial weight and while subjects received twice-daily injections of placebo.

between leptin and placebo could not be attributed to differences in sickness ratings, because there was no significant difference in this rating between leptin and placebo. These results indicate that the fixed-formula meal met its goal of measuring reactions at the outer limit of tolerability.

This was the first study, to our knowledge, to demonstrate that leptin administration to human subjects whose leptin concentrations decreased after nonsurgical weight loss has effects on satiation similar to those observed after leptin administration to individuals with congenital leptin-deficiency (16, 17, 19) and in direct contrast with the lack of effect of leptin in human subjects at usual body weight (18). It fills an important "niche" in the sparse literature regarding the dependence of leptin effects on the nutritional context in which it is administered. In states of congenital leptin deficiency (hypoleptinemia, stable weight), leptin administration increases energy expenditure and decreases energy intake (16, 17, 19, 60), but has little or no effect when administered to lean or obese subjects at their "usual" weight (euleptinemia, stable weight) (18). During dynamic weight loss, circulating leptin concentrations are lower than in the same subjects maintaining the same weight (61). In this state of hypoleptinemia and ongoing weight loss due to caloric restriction, pegylated leptin administration increases satiation, while having few if any effects on neuroendocrine or autonomic function. Effects of pegylated leptin on energy expenditure in this design were variable and were calculated based solely on whether there was (62) or was not (63, 64) greater weight loss in leptin-treated than in control subjects on a fixed diet (ie, without direct measurement of 24-h energy expenditure). The differential effects of leptin at usual weight (no significant changes in energy intake or expenditure noted), during active weight loss (increased satiation, little if any effect on energy expenditure, autonomic function, and neuroendocrine function), and reduced weight maintenance or congenital leptin deficiency (increased satiation, energy expenditure, circulating concentrations of bioactive thyroid hormones, and increased sympathetic nervous system tone) are consistent with the view that leptin concentrations below a certain individualized "threshold" invoke a decrease in satiation and energy expenditure that would tend to favor weight regain, which persists to some degree beyond the period of dynamic weight loss, thereby opposing sustained reduced weight maintenance (5, 6, 65–67). Exogenous leptin administration "reverses" the decline in satiation in subjects undergoing dynamic weight loss or static reduced weight maintenance, but the increase in energy expenditure after leptin administration to reduced-weight-maintenance subjects is at least "blunted" during caloric restriction.

The declines in satiation and energy expenditure after weight loss, and the increase in these variables after leptin repletion, emphasize the biological bases for the difficulties in sustaining weight loss. The lack of a significant increase in energy intake in the setting of decreased satiation may reflect the loss of conditioned meal size control, which ordinarily would adjust intakes to the postingestive consequences in relation to current metabolic state based on the prolonged exposure to fixed meal sizes of the liquid-formula diet (68, 69). It is quite possible that this decreased satiation would, outside of the confines of this experimental design, be associated with an actual increase in food intake after weight loss. Regardless, the lack of decline in energy intake to match the decline in expenditure creates a positive energy balance favoring the regain of lost weight. The increase in satiation and energy expenditure in weight-reduced subjects after leptin administration supports the possibility of using stimulation of the leptin signaling pathway as a means to assist in sustaining weight loss.

The demonstration in the current study of a significant increase in satiation after leptin administration to individuals who have already lost weight is significant to our understanding of the basic role of leptin in human physiology. Mechanistically, Sclafani and Ackroff (70) and Warwick and Weingarten (71) have suggested

that postmeal nutrient satiation with more concentrated nutrients decreases the reward value of food. fMRI studies have shown that leptin administration to leptin-deficient subjects increases activation of brain areas related to satiation and decreases activation of brain areas involved in reward on exposure to food-related stimuli (56, 57). This is consistent with the hypothesis that low leptin states result in both reduced satiation and an increased reward value of food. Similarly, studies in our laboratory of the fMRI responses to food compared with nonfood visual cues in fasted subjects, in the same experimental paradigm outlined in this study, suggest that low leptin states are associated with an increased emotional response to food and decreased activity in brain areas related to restraint (33).

These results are entirely consistent with a substantial body of evidence regarding appetite regulation by leptin, which include studies of basic leptin molecular physiology, leptin-mediated neuronal signaling, leptin-mediated behavioral changes, and even epidemiologic data that support the hypothesis that leptin repletion after weight loss will "reverse" the delay in satiation and the resultant overeating relative to energy expenditure that occurs both during and after weight loss (25–27). Physiologically, leptin inhibits the expression of the orexigenic peptides neuropeptide Y and agouti-related protein while stimulating the expression anorexiant peptides proopiomelanocortin/cocaine-amphetamine regulated transcript in the hypothalamus (48, 72, 73). Our previous fMRI studies indicate the effects of weight loss and leptin repletion on neural signaling in response to food compared with nonfood that is predicted from this physiology (33). More specifically, characteristic leptin-reversible fMRI changes suggest that weight-reduced subjects experience increased cognitive and emotional responses to food (ie, increased anticipated reward value, as reflected in activity in orbito-frontal and somatosensory cortices) coupled with decreased activity in brain areas associated with restraint and control of food intake (as reflected in activity in the parahippocampal and fusiform gyri) (33–35). The predicted behavioral consequence of these changes in neural signaling would be decreased satiation and decreased perception of the amount of food eaten (due to decreased restraint) and increased hunger (due to increased anticipated reward value) when leptin is decreased. As noted above, these behaviors are evident in observations that low leptin states such as leptin deficiency or weight loss are associated with decreased energy intake and in particular delayed satiation (16, 65). In the case of leptin deficiency, this hyperphagia is clearly alleviated by leptin repletion (19), but leptin repletion after weight loss has not been studied in this manner.

These biochemical, neuronal, and behavioral actions of leptin would logically contribute to the high rate of recidivism after otherwise successful weight loss. At usual weight, the average adult has been reported to gain approximately 0.2 to 1.0 kg/y (an average of ~4000 kcal stored energy/y) (74–77), despite the ingestion of between 800,000 and 950,000 kcal/y (75, 76), ie, energy intake and output are "balanced" to within ~ 0.5% over time. This balance clearly no longer operates after weight loss, because most individuals will regain all of their lost weight within a few years (3, 4). This weight regain must reflect a tendency to eat more (relative to energy expenditure) after weight loss. Absolute levels of energy intake after weight loss must either exceed or mimic those evident before weight loss in most subjects. There can be no partial decline in appetite after weight loss, because if individuals consumed any fewer calories than at usual weight, their weight regain would necessarily stop before reaching preweight loss levels.

In summary, leptin repletion in subjects after weight loss results in significant increases in satiation. The combination of decreased energy expenditure and reduced satiation in individuals attempting to sustain weight loss results in the optimal circumstances for the regain of lost weight (6, 78). These data, viewed in the context of our earlier studies (9) and the similarities of feeding behaviors and response to leptin of individuals maintaining a reduced body weight or who are leptin-deficient, support the hypothesis that the neural pathways controlling energy balance respond to the weight-reduced state—both behaviorally and bioenergetically—as a state of leptin-deficiency. The behavioral data from the current study are also directly relevant to the possible future use of medications that stimulate the leptin-signaling pathway as "weight-maintenance" drugs, especially when considered in the context of our other studies showing that leptin administration to subjects maintaining a reduced body weight results in increases in energy expenditure that offset those produced as a result of weight reduction (9) and that would otherwise not abate over time (5).

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REFERENCES

1. Ogden CL, Carroll M, Curtin L, McDowell M, Tabak C, Flegal K. Prevalence of overweight and obesity in the United States, 1999-2004. JAMA 2006;295:1549–55.
2. Wyatt SB, Winters K, Dubbert P. Overweight and obesity: prevalence, consequences, and causes of a growing public health problem. Am J Med Sci 2006;331:166–74.
3. Tsai AG, Wadden T. Systematic review: an evaluation of major commercial weight loss programs in the United States. Ann Intern Med 2005;142:56–66.
4. Wing RR, Hill J. Successful weight loss maintenance. Annu Rev Nutr 2001;21:323–41.
5. Rosenbaum M, Hirsch J, Gallagher D, Leibel R. Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. Am J Clin Nutr 2008;88:906–12.
6. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med 1995;332:621–8.
7. Tremblay A, Chaput J. Adaptive reduction in thermogenesis and resistance to lose fat in obese men. Br J Nutr 2009;102:488–92.
8. Goldsmith R, Joanisse D, Gallagher D, Simoneau J, Pavlovich K, Leibel R, Rosenbaum M. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. Am J Physiol Regul Integr Comp Physiol 2010;298: R79–88.

9. Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, Gallagher D, Mayer L, Murphy E, Leibel R. Low dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest 2005;115: 3579–86.
10. Rosenbaum M, Vandenborne K, Leigh J, Goldsmith R, Simoneau J, Hirsch J, Heymsfield S, Segal K, Leibel R. Effects of experimental weight perturbation on skeletal muscle work efficiency in human subjects. Am J Physiol Regul Integr Comp Physiol 2003;285:R183–92.
11. Klem ML, Wing R, McGuire M, Seagle H, Hill J. A descriptive study of individuals successful at long term maintenance of substantial weight loss. Am J Clin Nutr 1997;66:239–46.
12. Wing RR, Phelan S. Long-term weight maintenance. Am J Clin Nutr 2005;82(suppl):222S–5S.
13. Ozata M, Ozdemir I, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab 1999;84:3686–95.
14. Farooqi IS, Keogh J, Yeo G, Lank E, Ceetham T, O'Rahilly S. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 2003;348:1085–95.
15. Leibel RL. Molecular physiology of weight regulation in mice and humans. Int J Obes (Lond) 2008;32:S98–108.
16. Farooqi IS, Jebb S, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 1999;341:879–84.
17. Galgani JE, Greenway F, Wong M, Licinio J, Ravussin E. Leptin replacement prevents weight loss-induced metabolic adaptation in congenital leptin-deficient patients. J Clin Endocrinol Metab 2010;95: 851–5.
18. Heymsfield SB, Greenberg AS, Fujioka K, Dixon R, Kushner R, Hunt T, Lubina J, Patane J, Self B, Hunt P, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 1999;282:1568–75.
19. Farooqi IS, Matarese G, Lord G, Keogh J, Lawrence E, Agwu C, Sanna V, Jebb S, Perna F, Fontana S, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002; 110:1093–103.
20. MacLean PS, Higgins J, Jackman M, Johnson G, Fleming-Elder B, Wyatt H, Melanson E, Hill J. Peripheral metabolic responses to prolonged weight reduction that promote rapid, efficient regain in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol 2006;290:R1577–88.
21. Maclean PS, Higgins J, Johnson G, Fleming-Elder B, Donahoo W, Melanson E, Hill J. Enhanced metabolic efficiency contributes to weight regain after weight loss in obesity-prone rats. Am J Physiol Regul Integr Comp Physiol 2004;287:R1306–15.
22. Schwartz MW. Brain pathways controlling food intake and body weight. Exp Biol Med (Maywood) 2001;226:978–81.
23. Schwartz MW, Woods S, Porte D, Seeley R, Baskin D. Central nervous system control of food intake. Nature 2000;404:661–71.
24. Grill HJ, Schwartz M, Kaplan J, Foxhall J, Breinger J, Baskin D. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. Endocrinology 2002;143:239–46.
25. Keim NL, Stern J, Havel P. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. Am J Clin Nutr 1998;68:794–801.
26. Cameron JD, Goldfield G, Cyr M, Doucet E. The effects of prolonged caloric restriction leading to weight-loss on food hedonics and reinforcement. Physiol Behav 2008;94:474–80.
27. Doucet E, Imbeault P, St-Pierre S, Almeras N, Mauriege P, Richard D, Tramblay A. Appetite after weight loss by energy restriction and a low-fat diet-exercise follow-up. Int J Obes Relat Metab Disord 2000; 24:906–14.
28. Ahima RS. Revisiting leptin's role in obesity and weight loss. J Clin Invest 2008;118:2380–3.
29. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, Flier J. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382:250–2.
30. Doucet E, Cameron J. Appetite control after weight loss: what is the role of bloodborne peptides? Appl Physiol Nutr Metab 2007;32:523–32.
31. Doucet E, Imbeault P, St.-Pierre S, Almeras N, Mauriege P, Despres J, Bouchard C, Tremblay A. Greater than predicted decrease in energy expenditure during exercise after body weight loss in obese men. Clin Sci 2003;105:89–95.
32. McCaffery JM, Haley A, Sweet L, Phelan S, Raynor H, Del Parigi A, Cohen R, Wing R. Differential functional magnetic resonance imaging response to food pictures in successful weight-loss maintainers relative to normal-weight and obese controls. Am J Clin Nutr 2009;90:928–34.
33. Rosenbaum M, Sy M, Pavlovich K, Leibel R, Hirsch J. Leptin reverses weight loss–induced changes in regional neural activity responses to visual food stimuli. J Clin Invest 2008;118:2583–91.
34. DelParigi A, Chen K, Salbe A, Hill J, Wing R, Reiman E, Tataranni P. Persistence of abnormal neural responses to a meal in postobese individuals. Int J Obes Relat Metab Disord 2004;28:370–7.
35. DelParigi A, Chen K, Salbe A, Hill J, Wing R, Reiman E, Tataranni P. Successful dieters have increased neural activity in cortical areas involved in the control of behavior. Int J Obes (Lond) 2007;31:440–8.
36. American Physiological Society. Guiding principles for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol 2002;283:R281–3.
37. Rosenbaum M, Ravussin E, Matthews D, Gilker C, Feraro R, Heymsfield S, Hirsch J, Leibel R. A comparative study of different means of assessing long-term energy expenditure in humans. Am J Physiol 1996;270:R496–504.
38. Rosenbaum M, Murphy E, Heymsfield S, Matthews D, Leibel R. Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. J Clin Endocrinol Metab 2002;87:2391–4.
39. Kissileff H, Klingsberg G, VanItallie T. Universal eating monitor for continuous recording of solid or liquid consumption in man. Am J Physiol 1980;238:R14–22.
40. Kissileff HR. Ingestive behavior microstructure, basic mechanisms and clinical applications. Neurosci Biobehav Rev 2000;24:171–2.
41. Kissileff HR, Wentzlaff T, Guss J, Walsh B, Devlin M, Thornton J. A direct measure of satiety disturbance in patients with bulimia nervosa. Physiol Behav 1996;60:1077–85.
42. Kissileff H, Thornton J. Facilitation and inhibition in the cumulative food intake curve in man. In: Morrison A, Strick P, eds. Changing concepts of the nervous system. New York, NY: Academic Press, 1982: 585–607.
43. Kissileff HR, Thornton J, Becker E. A quadratic equation adequately describes the cumulative food intake curve in man. Appetite 1982;3: 255–72.
44. Nolan L, Guss J, Liddle R, Pi-Sunyer F, Kissileff H. Elevated plasma cholecystokinin and appetitive ratings after consumption of a liquid meal in humans. Nutrition 2003;19:553–7.
45. Peryam D, Pilgrim F. Hedonic scale method of measuring food preferences. Food Technol 1957;1957:9–14.
46. Brown H, Prescott R. Applied mixed models in medicine. 2nd ed. Chichester, United Kingdom: John Wiley & Sons, Ltd, 2006.
47. Zar J. Biostatistical analysis. 2nd ed. Englewood Cliffs, NJ: Prentice-Hall, Inc, 1984.
48. Korner J, Savontaus E, Chua S, Leibel R, Wardlaw S. Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. J Neuroendocrinol 2001;13:959–66.
49. Rothman KJ. No adjustments are needed for multiple comparisons. Epidemiology 1990;1:43–6.
50. Perneger TV. What's wrong with Bonferroni adjustments. BMJ 1998; 316:1236–8.
51. Thomas DC, Siemiatycki J, Dewar R, Robins J, Goldberg M, Armstrong B. The problem of multiple inference in studies designed to generate hypotheses. Am J Epidemiol 1985;122:1080–95.
52. Kissileff HR, Walsh B, Kral J, Cassidy S. Laboratory studies of eating behavior in women with bulimia. Physiol Behav 1986;38:563–70.
53. Kissileff HR, Carretta J, Geleibter A, Pi-Sunyer F. Cholecystokinin and stomach distention combine to reduce food intake in humans. Am J Physiol Regul Integr Comp Physiol 2003;285:R992–8.
54. Chaput JP, Drapeau V, Hetherington M, Lemieux S, Provencher V, Tremblay A. Psychobiological effects observed in obese men experiencing body weight loss plateau. Depress Anxiety 2007;24:518–21.
55. Rodríguez-Rodríguez E, Aparicia A, Bermejo L, Lopez-Sobalier M, Ortega R. Changes in the sensation of hunger and well-being before and after meals in overweight/obese women following two types of hypoenergetic diet. Public Health Nutr 2009;12:44–50.

56. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher P. Leptin regulates striatal regions and human eating behavior. Science 2007;317:1355.
57. Nearly MT, Batterham R. Gaining new insights into food reward with functional neuroimaging. Forum Nutr 2010;63:152–63.
58. Paulino G, Darcel N, Tome D, Raybould H. Adaptation of lipid-induced satiation is not dependent on caloric density in rats. Physiol Behav 2008;93:930–6.
59. Warwick ZS, Synowski S, Rice K, Smart A. Independent effects of diet palatability and fat content on bout size and daily intake in rats. Physiol Behav 2003;80:253–8.
60. Williamson DA, Ravussin E, Wong M, Wagner A, DiPaoli A, Caglayan S, Ozata M, Martin C, Walden H, Arnett C, et al. Microanalysis of eating behavior of three leptin deficient adults treated with leptin therapy. Appetite 2005;45:75–80.
61. Rosenbaum M, Nicolson M, Hirsch J, Murphy E, Chu F, Leibel R. Effects of weight change on plasma leptin concentrations and energy expenditure. J Clin Endocrinol Metab 1997;82:3647–54.
62. Hukshorn CJ, Meneheere P, Westerterp-Plantenga M, Saris W. The effect of pegylated human recombinant leptin (PEG-OB) on neuroendocrine adaptations to semi-starvation in overweight men. Eur J Endocrinol 2003;148:649–55.
63. Westerterp-Plantenga MS, Saris W, Hukshorn C, Campfield L. Effects of weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men. Am J Clin Nutr 2001;74:426–34.
64. Hukshom CJ, van_Dielen F, Buuman W, Westerterp-Plantenga M, Campfield L, Saris W. The effect of pegylated recombinant human leptin (PEG-OB) on weight loss and inflammatory status in obese subjects. Int J Obes Relat Metab Disord 2002;26:504–9.
65. Doucet E, St-Pierre S, Almeras N, Tremblay A. Relation between appetite ratings before and after a standard meal and estimates of daily energy intake in obese and reduced obese individuals. Appetite 2003; 40:137–43.
66. Sumithran P, Prendergast L, Delbridge E, Purcell K, Shulkes A, Kriketos A, Proietto J. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med 2011;365:1597–604.
67. Rosenbaum M, Hirsch J, Murphy E, Leibel R. The effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. Am J Clin Nutr 2000;71:1421–32.
68. Booth DA. Physiological regulation through learnt control of appetites by contingencies among signals from external and internal environments. Appetite 2008;51:433–41.
69. Booth DA. Learnt reduction in the size of a meal: measurement of the sensory-gastric inhibition from conditioned satiety. Appetite 2009;52: 745–9.
70. Sclafani A, Ackroff K. The relationship between food reward and satiation revisited. Physiol Behav 2004;82:89–95.
71. Warwick ZS, Weingarten H. Flavor-postingestive consequence associations incorporate the behaviorally opposing effects of positive reinforcement and anticipated satiety: implications for interpreting two-bottle tests. Physiol Behav 1996;60:711–5.
72. Jean A, Conductier G, Manrique C, Bouras C, Berta P, Hen R, Chamay Y, Bockaert J, Compan V. Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens. Proc Natl Acad Sci USA 2007;104:16335–40.
73. Nahon JL. The melanocortins and melanin-concentrating hormone in the central regulation of feeding behavior and energy homeostasis. C R Biol 2006;329:623–38.
74. Lewis CE, Jacobs D, McCreath H, Kiefe C, Schreiner P, Smith D, Williams O. Weight gain continues in the 1990s: 10-year trends in weight and overweight from the CARDIA study. Coronary Artery Risk development in Young Adults. Am J Epidemiol 2000;151:1172–81.
75. Du H, van der A D, Ginder V, Jebb S, Forouhi N, Wareham N, Halkjaer J, Tjønneland A, Overvad K, Jakobsen M, et al. Dietary energy density in relation to subsequent changes of weight and waist circumference in European men and women. PLoS ONE 2009;4:e5339.
76. Forouhi NG, Sharp S, Du H, van der A D, Halkjaer J, Schulze M, Tjønneland A, Overvad K, Jakobsen M, Boeing H, et al. Dietary fat intake and subsequent weight change in adults: results from the European Prospective Investigation in Cancer and Nutrition cohorts. Am J Clin Nutr 2009;90:1632–41.
77. Pietrobelli A, Allison D, Heshka S, Heo M, Wang Z, Berktay A, Laferrere B, Rosenbaum M, Aloia J, Pi-Sunyer X, et al. Sexual dimorphism in energy content of weight change. Int J Obes Relat Metab Disord 2002;26:1339–48.
78. Whybrow S, Hughes D, Ritz P, Johnstone A, Horgan G, King N, Blundell J, Stubbs R. The effect of an incremental increase in exercise on appetite, eating behaviour and energy balance in lean men and women feeding ad libitum. Br J Nutr 2008;100:1109–15.