Chronic leptin treatment does not prevent the development of obesity in transgenic mice with brown fat deficiency

A. Hamann¹, B. Büsing¹, C. Kausch¹, J. Ertl², G. Preibisch², H. Greten¹, S. Matthaei¹

¹ Department of Medicine, University of Hamburg, Hamburg, Germany

² Hoechst AG, TA Metabolic Diseases, Frankfurt/Main, Germany

Summary With the exception of *ob/ob* mice, circulating plasma leptin is elevated in all other obese rodents as well as in obese humans, suggesting that leptin resistance rather than leptin deficiency is a characteristic feature of obesity. The exact molecular mechanisms leading to leptin resistance and the applicability of exogenous leptin to overcome resistance to the anorectic effect of the hormone, are insufficiently characterized. The aim of this study was to investigate whether chronic leptin administration could prevent the development of obesity and its associated disorders in transgenic mice with toxigene mediated ablation of brown adipose tissue (BAT). Daily injections of leptin were started at the age of 6 weeks, when body weight, food intake and plasma leptin levels of transgenics were not different from control mice. Over the next 6 weeks, leptin treated transgenics showed the same excessive body weight gain as trans-

genic mice injected with saline. Leptin treatment was furthermore not able to prevent the development of hyperphagia, hyperglycaemia, hyperinsulinaemia and hyperlipidaemia in transgenic mice. In contrast, control mice injected with leptin had significantly lower body weight, food intake and plasma triglycerides than those treated with saline. In summary, leptin treatment was not able to prevent the development of obesity and its associated abnormalities in transgenic mice with BAT deficiency. This data suggests that intact BAT function is of critical importance for leptin's effect on food intake and energy expenditure, and that primary dysfunction of BAT is associated with leptin resistance, even when hyperleptinaemia is not yet present. [Diabetologia (1997) 40: 810–815]

Keywords Leptin, obesity, brown fat, transgenic mice, insulin resistance, hyperphagia, leptin resistance.

The high prevalence of obesity and its association with the cardiovascular risk factors diabetes mellitus, dyslipidaemia and hypertension represents a major health problem for the industrialized world. Numerous studies have documented that the dysregulation of energy homeostasis and the susceptibility to obesity results from a complex interplay between genetic

and environmental factors. However, the exact mechanisms involved in this process are insufficiently understood.

Brown adipose tissue (BAT) has been proposed to play an important role in the regulation of energy balance [1, 2]. BAT is distinguished by the unique presence of uncoupling protein (UCP), a mitochondrial proton transporter which uncouples oxidative metabolism from ATP synthesis. This capacity permits BAT to expend calories unrelated to the performance of work, the net result being the generation of heat [3, 4]. BAT activity is stimulated by the sympathetic nervous system during cold exposure (non-shivering thermogenesis) and during the ingestion of excess calories (diet-induced thermogenesis) [1, 2, 5]. The hypothesis that BAT activity might provide a cellular and molecular explanation for protection

Received: 11 November 1996 and in final revised form: 8 April 1997

Corresponding author: Dr. A. Hamann, Medizinische Kernklinik und Poliklinik, Universitäts-Krankenhaus Eppendorf, Martinistraße 52, D – 20246 Hamburg, Germany

Abbreviations: BAT, Brown adipose tissue; UCP, uncoupling protein; DTA, diphtheria toxin A-chain; CO₂, carbon dioxide; w/w, by weight; PBS, phosphate-buffered saline.

from obesity was proven by the creation of transgenic mice with brown fat deficiency [6]. This was accomplished using a suicide DNA transgenic vector in which regulatory elements of the UCP gene were used to drive brown fat specific expression of diphtheria toxin A-chain (DTA). Transgenic UCP-DTA mice have markedly diminished functional brown fat mass and therefore reduced energy expenditure. As transgenic mice age, they show excessive weight gain compared to control littermates and develop marked obesity. The progress of the obese phenotype is associated with severe insulin resistance and non-insulin-dependent diabetes mellitus (NIDDM) with both receptor and post-receptor components [7]. Dyslipidaemia and hyperphagia are further characteristic features of UCP-DTA mice, while corticosterone and thyroid hormone are within the normal range [6–8]. Feeding of a “Western diet” with high fat content leads to a synergistic effect on the development of obesity and its accompanying disorders in transgenic mice [8].

Among the other animal models of obesity, rodents with single gene mutations are most extensively studied. In *ob/ob* mice, a point mutation in the *obese* gene results in a phenotype of massive early onset obesity, hyperphagia, NIDDM, defective thermoregulation and infertility [9]. Leptin, the protein encoded by the *obese* gene, is truncated and inactive in *ob/ob* mice. In all other rodent models as well as in humans, leptin is synthesized exclusively in adipose tissue and secreted into the blood [10, reviewed in 11]. Systemic administration of recombinant leptin to *ob/ob* mice reduces fat mass, food intake, hyperglycaemia and hyperinsulinaemia [12–15]. With the exception of *ob/ob* mice as a model of absolute leptin deficiency, leptin mRNA and circulating plasma leptin are generally elevated in obesity [10, 11, 16, 17]. As in humans and several other animal models, this observation has also been made in UCP-DTA mice [16, 18]. The various effects of leptin are mediated by specific receptors expressed at high levels in the hypothalamus, but also in several other non-neuronal tissues [19, 20]. Taken together, these findings suggest an important role for leptin in the regulation of energy balance, as leptin could signal the magnitude of fat stores to the brain and thereby have an impact on food intake and energy expenditure. However, elevated endogenous plasma leptin in overweight rodents and humans does not lead to a normalization of the obese phenotype, for instance by sufficient reduction of energy intake. Therefore, resistance to leptin action appears to be a common feature of rodent and human obesity.

The concept of additional exogenous leptin administration to prevent or overcome leptin resistance and thereby to induce weight loss in obese patients, is very tempting. However, the feasibility of this therapeutic approach remains to be proven in obese humans and

in a suitable obese animal model, too. Like obese humans, transgenic UCP-DTA mice with primary BAT deficiency are neither leptin deficient, as *ob/ob* mice are, nor do they have a hypothalamic defect, as *db/db* mice and several other rodent models of obesity have. Therefore, in the present study we investigated whether leptin treatment of UCP-DTA mice could prevent the development of obesity and some of its accompanying metabolic disorders. Transgenic UCP-DTA mice and their control littermates received daily intraperitoneal injections of recombinant leptin, starting at an age when hyperleptinaemia in transgenics as the presently known indicator of leptin resistance was not yet detectable. The results of our studies suggest an important role of intact BAT function for the biologic effects of leptin.

Materials and methods

Animals. Transgenic FVB/N mice, bearing the toxigene construct, were bred as heterozygotes in our animal facility. Non-transgenic FVB/N littermates served as controls. Only female mice were used throughout the experiment. The “Principles of laboratory animal care” (NIH publication No. 85–23, revised 1985) were followed, and approval for the experiments was obtained from the state government in Hamburg, Germany. Mice were weaned at the age of 19 days and subsequently housed two per cage in plastic cages until the age of 12 weeks, when they were killed by CO₂ narcosis. Animals were maintained on a 12:12 h light-dark cycle under controlled temperature and humidity. Water and food were available ad libitum. Mice were fed mouse chow (Purina No. 5008, Ralston Purina, St. Louis, Mo., USA), containing 6.5 % (w/w) fat, 47 % (w/w) carbohydrates and 23.5 % (w/w) protein. Food intake and body weight were measured weekly. Food was weighed at the beginning and end of each week, and the differences were assumed to represent grams of food eaten per week. Cages were examined carefully for spillage of food and no significant amounts were detected. Starting at the age of 4 weeks, all mice received daily intraperitoneal injections of saline (PBS, pH 7.4, 5 µl/g). Starting at the age of 6 weeks, one group of control and transgenic mice each received daily injections of recombinant murine leptin dissolved in PBS (5 µg/g body weight), while another group of control and transgenic mice each continued to receive daily injections of PBS. All injections were performed shortly before the beginning of the dark cycle at 18.00 hours. To prepare recombinant leptin, the murine *ob* cDNA was expressed in *Escherichia coli* as N-terminal fusion to a His₆-tag. The protein was purified by metal affinity, reversed phase and gel permeation chromatography to greater than 95 % purity. The endotoxin content was less than 0.5 IU per mg of leptin (limulus amebocyte lysate assay).

Leptin, glucose, insulin, cholesterol and triglyceride determinations. Blood was collected from the tail vein or by cardiac puncture after killing. Plasma leptin was measured by radioimmunoassay with mouse leptin standards (Linco, St. Louis, Mo., USA). Whole blood glucose was measured by the glucose oxidase method with a reflectance glucometer (One Touch II; LifeScan, Milpitas, Calif., USA). Insulin was assayed by radioimmunoassay with rat insulin standards (Linco). Cholesterol (procedure 352) and triglycerides (procedure 339) were quantified using kits from Sigma (St. Louis, Mo., USA).

Body lipid determination. Carcasses were digested by alcoholic potassium hydroxide hydrolysis at 60 °C overnight with saponification of all fats, neutralization and then enzymatic determination of glycerol [21]. Body lipid was determined by multiplying moles of glycerol per mouse and the average relative molecular mass of a triglyceride molecule (860 g/mol).

Statistical analysis. All results are presented as means $\pm$ SEM of the number of values indicated in the legends to figures and tables. Statistical significance was assessed by unpaired Student's *t*-test and by ANOVA. Differences were accepted as significant at *p* less than 0.05.

Results

Body weight. Figure 1 illustrates the body weight gain within the different groups of mice. Mean body weights of the different groups were not significantly different at the age of 4 weeks, when monitoring and daily saline injections of the animals were started, and at the age of 6 weeks, when leptin treatment began. Between 6 and 12 weeks of age, daily leptin injections caused a weight loss of 0.5 g in control mice, while control animals injected with saline gained 2.9 g during the same time span ($p < 0.01$ for leptin vs saline). In transgenics, total weight gain over 6 weeks was 13.5 g in saline-injected mice and 13.4 g in leptin-treated mice, with no significant difference between both groups of transgenics at any individual time point between 6 and 12 weeks of age.

Total body lipid and plasma leptin. As depicted in Figure 2, lower body weight in control mice treated with leptin was associated with a 73% decrease in total body lipid content, when compared to control mice injected with saline (0.65 vs 2.36 g, $p < 0.0001$). The development of obesity in transgenic mice accompanied a ~ fivefold increase in body lipid compared to saline-treated controls. As with body weight, there was no significant difference in body lipid, whether transgenics were injected with saline (11.3 g) or leptin (10.7 g), respectively. The increase in body lipid in 12-week-old transgenic mice was accompanied by markedly higher plasma leptin levels with a 8.8-fold increase in those animals injected with saline ($p < 0.0001$) and a 9.8-fold increase in transgenics injected with leptin ($p < 0.0001$), both compared to saline-injected controls (Table 1). Significantly elevated leptin levels in the plasma of transgenic mice were already observed at the age of 9 weeks, but at both time points not markedly different between transgenics injected with leptin or saline. Importantly, no significant elevation in plasma leptin levels of transgenic mice was detected at the age of 6 weeks, when leptin injections began. Leptin treatment of control mice over 6 weeks led to a decrease in plasma leptin levels by 46% compared to 12-weeks-old saline-injected mice ($p < 0.05$), when measured approximately 20 h after the last leptin injection.

Fig. 1. Growth curves. Data represents mean $\pm$ SEM of weekly assessed body weight in control and transgenic mice receiving daily injections of either leptin or saline between the age of 6 weeks and 12 weeks ($n = 8-10$ in each experimental group). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs controls injected with saline. $p = \text{N.S.}$ for leptin-injected transgenics vs saline-injected transgenics at each time point

Fig. 2. Body lipid content. Data represents mean $\pm$ SEM of total body lipid from 12-week-old control and transgenic mice treated either with leptin or saline ($n = 8-10$). *, $p < 0.0001$ vs controls injected with saline. $p = \text{N.S.}$ for leptin-injected transgenics vs saline-injected transgenics

Food intake. Food intake was determined weekly starting at the age of 4 weeks. Until the age of 6 weeks, when mice began to be treated with leptin or continued to be injected with saline, daily food intake was not significantly different between any of the four experimental groups (data not shown). Leptin treatment of control mice caused a significant decrease in cumulative food intake, as animals

Table 1. Plasma leptin levels

	Control		Transgenic	
	Saline	Leptin	Saline	Leptin
Plasma leptin (ng/ml)				
6 weeks	3.44 ± 0.78	3.16 ± 0.58	3.81 ± 0.56	3.65 ± 0.40
9 weeks	3.40 ± 0.37	2.97 ± 0.31	24.6 ± 4.86 ^b	20.5 ± 2.41 ^b
12 weeks	4.13 ± 0.54	2.24 ± 0.51 ^a	36.3 ± 7.88 ^b	40.5 ± 5.07 ^b

Data represent mean ± SEM from 6, 9 and 12-week-old control and transgenic mice treated with saline or leptin ($n = 8-10$). ^a $p < 0.05$; ^b $p < 0.0001$ vs controls of the same age treated with saline. $p = N.S.$ for all measurements in leptin-injected transgenics vs saline-injected transgenics of the same age

consumed a mean amount of 133.9 g chow between the age of 6 and 12 weeks, compared to 149.8 g eaten by controls injected with saline, representing a 10.6% decrease ($p < 0.02$). Saline-treated transgenics consumed a mean amount of 190.7 g between the ages of 6 and 12 weeks, thus exhibiting significant hyperphagia of 27.3% compared to controls ($P = 0.001$). Leptin treatment of transgenic mice did not prevent the development of hyperphagia, as animals in this group ate a mean amount of 194.2 g chow during the same time interval, representing a 29.6% increase compared to controls ($p < 0.002$ vs leptin-injected transgenics).

Blood glucose and plasma insulin. As shown in Table 2, blood glucose levels were not significantly different between transgenics and controls at the age of 6 weeks. When mice were 9 weeks old, both groups of transgenic mice injected with saline or leptin revealed significantly elevated glucose levels compared to both treated control groups ($p < 0.05$ in each case). Compared to saline-treated controls, a statistically significant increase was only seen in saline-treated transgenics ($p < 0.05$). Neither at 9 weeks nor at 12 weeks were glucose levels significantly affected by chronic leptin administration, when compared to mice of the same genotype. However, 12-week-old transgenics treated with saline or leptin had higher glucose levels than either of the control groups ($p < 0.01$ in each case). At the age of 6 weeks, no significant differences in plasma insulin levels were determined between controls and transgenics (Table 2). At the age of 9 and 12 weeks, hyperinsulinaemia was present in transgenics with an approximate fourfold increase in plasma insulin both in transgenic mice treated with saline and with leptin, when compared to saline-injected controls ($p < 0.01$ at the age of 9 weeks, $p < 0.05$ at the age of 12 weeks). An effect on plasma insulin levels by leptin compared to saline treatment was not observed between controls, or between transgenics.

Triglycerides and cholesterol. In order to evaluate whether leptin treatment had any effect on plasma

Table 2.

	Control		Transgenic	
	Saline	Leptin	Saline	Leptin
Blood Glucose (mmol/l)				
6 weeks	7.23 ± 0.31	7.34 ± 0.19	7.62 ± 0.39	7.90 ± 0.47
9 weeks	7.07 ± 0.16	6.74 ± 0.28	7.74 ± 0.26*	7.97 ± 0.40
12 weeks	7.83 ± 0.31	8.05 ± 0.23	9.82 ± 0.57**	10.0 ± 0.32***
Plasma Insulin (mmol/l)				
6 weeks	17.4 ± 2.61	16.1 ± 1.69	19.7 ± 2.00	18.1 ± 2.61
9 weeks	12.8 ± 1.54	11.2 ± 1.62	45.2 ± 10.5**	49.0 ± 12.0**
12 weeks	22.4 ± 1.95	17.8 ± 4.04	89.8 ± 25.0*	94.1 ± 25.0*

Data represent mean ± SEM from 6, 9 and 12-week-old control and transgenic mice treated with saline or leptin ($n = 8-10$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$ vs controls of the same age injected with saline. $p = N.S.$ for all measurements in leptin-injected transgenics vs saline-injected transgenics of the same age

Table 3. Serum triglyceride and cholesterol levels

	Control		Transgenic	
	Saline	Leptin	Saline	Leptin
Triglycerides (mmol/l)	0.78 ± 0.06	0.52 ± 0.06 ^a	1.29 ± 0.17 ^a	1.39 ± 0.07 ^c
Cholesterol (mmol/l)	0.73 ± 0.05	0.73 ± 0.04	0.92 ± 0.08 ^a	0.93 ± 0.05 ^b

Data represent mean ± SEM from 12-week-old control and transgenic mice treated either with leptin or saline ($n = 8-10$). ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.0001$ vs controls injected with saline. $p = N.S.$ for all measurements in leptin-injected transgenics vs saline-injected transgenics

lipids in control and transgenic mice, we measured triglycerides and cholesterol in 12-week-old mice. As depicted in Table 3, leptin treatment of control mice caused a decrease in plasma triglycerides by 34% compared to saline-treated animals ($p < 0.05$). Saline-treated transgenics were characterized by an increase of 65% ($p < 0.05$), and leptin-treated transgenics by an increase of 78% ($p < 0.0001$) in plasma triglycerides. Cholesterol levels were not altered in control mice treated with leptin, both groups of transgenic mice exhibited an increase of 25% under saline-treatment ($p < 0.05$) and 27% under leptin treatment ($p < 0.01$) when compared to saline-injected control mice. Neither triglycerides, nor cholesterol were significantly different between saline- and leptin-treated transgenic mice.

Discussion

Absolute leptin deficiency in *ob/ob* mice is associated with a phenotype of massive obesity, hyperphagia and NIDDM [9, 11]. However, all other rodent models of obesity and obese humans are characterized by hyperleptinaemia rather than leptin deficiency [10, 16-18]. Overexpression of leptin mRNA in adipose

tissue and increased plasma leptin levels have also been demonstrated in UCP-DTA mice [16, 18]. In this transgenic mouse, expression of the DTA-chain selectively in BAT leads to primary BAT deficiency, reduced energy expenditure and, as animals age, the development of severe obesity [6]. Initial studies supported a critical role for BAT in the nutritional homeostasis of mice and characterized the UCP-DTA mouse as a new model for obesity and its major morbid complications [6–8]. The aim of this study was to investigate whether leptin treatment could prevent the development of obesity and its associated disorders in UCP-DTA mice. For this purpose, we started daily leptin injections at the age of 6 weeks, when body weight, food intake, leptin, glucose and insulin levels in transgenic mice were not significantly different from controls. Past the age of 6 weeks, body weight of transgenic mice increased markedly, and daily leptin administration did not prevent the development of obesity. In contrast, leptin injections caused a significantly lower body weight and body lipid level in non-transgenics, proving that the recombinant leptin used in our study was biologically active and applied at a sufficient dose. Furthermore, leptin-treated control mice consumed significantly less food during the 6-week-period. In transgenics, however, leptin treatment did not prevent the development of hyperphagia, which is typically observed in UCP-DTA mice past the age of 7–8 weeks [6–8]. Furthermore, leptin administration did not hinder the development of mild hyperglycaemia, hyperinsulinaemia and hyperlipidaemia in transgenic animals. Interestingly, triglycerides in leptin-treated control mice were significantly decreased, while cholesterol was unchanged. It remains to be determined whether this was due to the reduced food intake of these animals, or whether leptin could possibly have an effect on LPL activity and thereby directly influence lipid levels in the plasma.

In previous studies, the effect of chronic leptin treatment was investigated in *ob/ob* and *db/db* mice compared to lean control animals [12–14]. Leptin induced a dramatic decrease in body weight, food intake, blood glucose and plasma insulin in *ob/ob* mice with absolute leptin deficiency, while it had no effect in *db/db* mice with leptin resistance due to a genetic defect in the leptin receptor gene. In lean control mice, an effect of leptin on body weight comparable to what we describe herein, was observed [13]. With the exception of a limited study on a small number of animals with diet-induced obesity, treated with leptin over 3 days and losing slightly more weight than saline-injected mice [12], no further data on the applicability of leptin administration for the treatment or prevention of obesity has been published so far. Therefore, our data demonstrating resistance to exogenous leptin administration in an obese animal model without a hypothalamic lesion

should be significant for the understanding of leptin's role and mode of action in the regulation of energy homeostasis. One possible explanation for the marked resistance to leptin treatment in BAT-deficient mice could be that intact BAT thermogenesis is of critical importance for leptin's anorectic effect. Recently, it was demonstrated that a reduction in food intake could not fully account for the weight-reducing action of chronic leptin infusion in *ob/ob* and lean mice [22]. Furthermore, leptin treatment was shown to normalize oxygen consumption and body temperature in *ob/ob* mice [14]. These data suggest that increased thermogenesis in BAT contributes to the alteration in energy balance caused by leptin. This assumption is supported by another study, in which the administration of recombinant leptin resulted in a selective increase in sympathetic outflow to interscapular BAT, indicating activation of thermogenesis in BAT [23]. As BAT-deficient animals should not be capable of increasing thermogenesis sufficiently after leptin administration, they are consequently less responsive to leptin treatment than control mice.

Interestingly, chronic leptin administration also does not affect the development of hyperphagia in transgenic UCP-DTA mice. It has previously been demonstrated that rodents induced to overeat or tube-fed excess calories become obese and insulin resistant, but are not hyperphagic when returned to an ad libitum chow diet [24, 25]. Thus, it seems plausible that obesity alone does not cause hyperphagia, and that increased food intake in UCP-DTA mice is specifically a consequence of BAT ablation. This would suggest that control of appetite is linked to brown fat function. BAT may be required for the generation of a satiety signal, and when BAT is diminished or dysfunctional, the satiety signal is decreased, resulting in hyperphagia. Heat is produced by BAT as a by-product of uncoupled respiration [1–4]. Many years ago, Brobeck [26] proposed the thermostatic theory of appetite control which suggested that heat, generated as a direct consequence of ingesting calories (thermic effect of food), caused satiety. Afferent temperature-sensitive neurons located peripherally and centrally were thought to sense added heat and transmit negative signals to the feeding centres of the hypothalamus, thus decreasing food intake. A role for BAT was suggested when it was realized that it mediated a major portion of meal-induced thermogenesis in rodents [5]. Since UCP-DTA mice have a deficiency of BAT and a defect in meal-induced thermogenesis, the observation of hyperphagia in these mice is consistent with the thermostatic theory. However, BAT could also influence food intake through an as yet unknown mechanism, for example the secretion of another signalling molecule. In both cases, the absence of a leptin effect on food intake in BAT-deficient animals supports the hypothesis that BAT is involved in appetite regulation.

Besides massive obesity, *ob/ob* mice are characterized by other numerous metabolic and endocrine abnormalities [11, 27]. In contrast to *ob/ob* mice and more resembling the characteristic features of obese humans, UCP-DTA mice have normal linear growth and lean body mass, are fertile, and have normal levels of corticosterone and thyroid hormone [6–8]. Therefore, the apparent resistance to leptin in BAT-deficient mice does not involve all of its actions. BAT's important role in leptin action could be restricted to energy metabolism and not include other components of the endocrine system. This suggests that there could be potentially divergent pathways of leptin action and signalling, e.g. via different receptor isoforms or different post-receptor second messengers, thus mediating the various biological effects of the hormone.

Leptin resistance in UCP-DTA mice is already existent at the age of only 6 weeks, when obesity, hyperinsulinaemia and especially hyperleptinaemia are not yet present. This finding argues against the assumption that leptin resistance in transgenics could be solely associated with the presence of obesity and hyperleptinaemia. It cannot be excluded, however, that once obesity is established, hyperleptinaemia-associated leptin resistance contributes to the exacerbation of the phenotype and prevents weight loss. A possible molecular explanation for this could be high levels of leptin downregulating its own receptors, or a saturable transport system through which leptin enters the brain, as suggested by recent studies showing that the capacity of leptin transport is lower in obese individuals [28, 29]. These observations as well as our data support the view that the attempt to overcome leptin resistance with high doses of exogenous leptin may not be a successful treatment of obesity.

Acknowledgements. We thank Bradford B. Lowell and Jeffrey S. Flier (Beth Israel Hospital, Boston, Mass., USA) for providing us with UCP-DTA mice. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to A.H. (DFG Ha 2093/2–1).

References

- Himms-Hagen J (1989) Brown adipose tissue thermogenesis and obesity. *Prog Lipid Res* 28: 67–115
- Hamann A, Matthaei S (1995) Obesity, thermogenesis and brown adipose tissue. *Diab Stoffw* 4: 321–326
- Nicholls D, Locke R (1984) Thermogenic mechanisms in brown fat. *Physiol Rev* 64: 1–64
- Klaus S, Casteilla L, Boillard F, Ricquier D (1991) The uncoupling protein UCP: a membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. *Int J Biochem* 23: 780–791
- Rothwell N, Stock M (1979) A role for brown adipose tissue in diet induced thermogenesis. *Nature* 281: 31–35
- Lowell BB, Susulic VS, Hamann A, et al. (1993) Development of obesity in transgenic mice following the genetic ablation of brown adipose tissue. *Nature* 366: 740–742
- Hamann A, Benecke H, Le Marchand-Brustel Y, Susulic VS, Lowell BB, Flier JS (1995) Characterization of the insulin resistance and NIDDM in transgenic mice with reduced brown fat. *Diabetes* 44: 1266–1273
- Hamann A, Flier JS, Lowell BB (1996) Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes and hyperlipidaemia. *Endocrinology* 137: 21–29
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 372: 425–432
- Maffei M, Halaas J, Ravussin E, et al. (1995) Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine* 1: 1155–1161
- Hamann A, Matthaei S (1996) Regulation of energy balance by leptin. *Exp Clin Endocrinol Diabetes* 104: 293–300
- Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995) Recombinant mouse ob protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269: 546–549
- Halaas JL, Gajiwala KS, Maffei M, et al. (1995) Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269: 543–546
- Pelleymounter MA, Cullen MJ, Baker MB, et al. (1995) Effects of the obese gene product on body weight regulation in *ob/ob* mice. *Science* 269: 540–543
- Stephens TW, Basinski M, Bristow PK, et al. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377: 530–532
- Frederich RC, Löllmann B, Hamann A, et al. (1995) Expression of ob mRNA and its encoded protein in rodents: impact of nutrition and obesity. *J Clin Invest* 96: 1658–1663
- Igel M, Kainulainen H, Brauers A, Becker W, Herberg L, Joost HG (1996) Long-term and rapid regulation of *ob* mRNA levels in adipose tissue from normal (Sprague Dawley rats) and obese (*db/db* mice, *fa/fa* rats) rodents. *Diabetologia* 39: 758–765
- Frederich RC, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS (1995) Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nature Medicine* 1: 1311–1314
- Tartaglia LA, Dembski M, Weng X, et al. (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263–1271
- Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC (1996) Defective STAT signaling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci USA* 93: 6231–6235
- Salmon D, Flatt J (1985) Effect of dietary fat content on the incidence of obesity among ad libitum fed mice. *Int J Obesity* 9: 443–449
- Levin N, Nelson C, Gurney A, Vandlen R, Sauvage FD (1996) Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc Natl Acad Sci USA* 93: 1725–1730
- Collins S, Kuhn CM, Petro AE, Swick AG, Chruncy BA, Surwit RS (1996) Role of leptin in fat regulation. *Nature* 380: 677 (letter)
- Hill JO, Dorton J, Sykes MN, Digirolamo M (1989) Reversal of dietary obesity is influenced by its duration and severity. *Int J Obesity* 13: 711–722
- Harris RBS, Martin RJ (1989) Changes in lipogenesis and lipolysis associated with recovery from reversible obesity in mature female rats. *Proc Soc Exp Biol Med* 191: 82–89
- Brobeck J (1948) Food intake as a mechanism of temperature regulation. *Yale J Biol Med* 20: 545–557
- Bray G, York D (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* 59: 719–809
- Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D (1996) Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nature Medicine* 2: 589–593
- Caro JF, Kolaczynski JW, Nyce MR, et al. (1996) Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 348: 159–161