Leptin Response and Body Weight Regulation in Humans

Linda J. McCARGAR

*Department of Agricultural, Food and Nutritional Science,
4-10 Agriculture Forestry Centre, University of Alberta,
Edmonton, AB T6G 2P5, Canada*

Summary The recent discovery of the positional cloning of the mouse obese (*ob*) gene has resulted in renewed attention to the scientific study of obesity and body weight regulation. It was further discovered that the *ob* gene encodes for a circulating hormone, *ob* protein or leptin, secreted by adipose tissue. In the *ob/ob* mouse model which becomes very obese, there is a complete leptin deficiency. As such, if leptin is administered to these animals, food intake and body weight decrease significantly. The *db/db* mouse also becomes obese, but it has high levels of circulating leptin. Thus it is believed that this animal model cannot respond to leptin because of an altered leptin receptor. Because humans appear to have serum leptin levels in proportion to the level of body fat mass it is speculated that "leptin resistance" may also be involved, at least in part, in the development of human obesity. However, it is also proposed that for "normal" body weight regulation, leptin may act like a lipostat in response to changes in energy balance and fat stores and that exogenous leptin could possibly reset the lipostat. Serum leptin levels were measured in three groups of women: those who had lost $\geq 20\%$ of their body weight and maintained the weight loss; those who lost $\geq 20\%$ of their body weight and regained it; and weight stable controls who had never dieted. The maintainers and controls had similar leptin levels at 10.2 ± 7.0 and 7.5 ± 3.8 ng/ml respectively. Those who had regained the weight had higher leptin levels than the other two groups at 20.8 ± 8.4 ng/ml ($p < 0.05$). Body mass index (BMI) was also significantly higher in this group ($p < 0.001$) at 34.1 ± 6.4 compared to 24.0 ± 2.0 in the maintainers and 21.3 ± 2.5 in the controls. Leptin has also been shown to influence resting metabolic rate (RMR). Thus, serum leptin levels were measured in women who were similar in height, weight and age but had a low ($\leq 85\%$ predicted) or high ($> 100\%$ predicted) RMR. No relationship between leptin and RMR was observed. There appear to be many unanswered questions about the mechanisms of action of leptin in humans.

Key Words: leptin, body weight, energy balance, *ob* gene, adipose tissue

The purpose of this paper is to provide an overview of the potential role of leptin in body weight regulation. Since its discovery in 1994, there have been more than 600 publications exploring its physiological and biochemical role [1]. This review article delineates essentially the significance of leptin in weight with particular reference to humans.

In 1953, Kennedy proposed the Lipostatic Theory [2] suggesting that humoral signals generated by the fat cells in proportion to the level of body fat provide negative feedback to the brain areas that control food intake and energy expenditure. Thus if fat cells start to decrease, this would stimulate food intake. If fat cells start to increase, this would stimulate energy expenditure. Five years later, Hervey was the first to demonstrate the presence of a hormone that regulated body weight through an interaction with the hypothalamus [3]. This was shown by experiments involving rats which used a parabiosis technique where two animals are physically joined at the level of the skin and subcutaneous tissues, such that they share cross-circulation of about 2%. Lesions to the ventromedial hypothalamus (VMH) rendered that area of the brain insensitive to satiety signals and the rats became obese. However the other parabiotic pair starved to death because its hypothalamus (that did not have a VMH lesion) responded to satiety signals and it resulted in hypophagia.

For the next two decades there were extensive investigations of two animal models of genetic obesity—the *ob/ob* and the *db/db* mice. These autosomal recessive single-gene mutations, cause animals to experience hyperphagia and severe obesity. Coleman, who also utilized the parabiosis technique, reported that the *ob* gene encodes for a negative feedback hormone, and that the *db* gene encodes for the receptor of this hormone [4]. Therefore animals with a recessive mutation at the *ob* locus, become obese because they cannot make the adiposity negative feedback signal. They are essentially deficient in the hormone. Animals with a recessive mutation at the *db* locus become obese because they cannot respond to the signal. That is, they are resistant to the hormone.

THE DISCOVERY OF LEPTIN

The more recent discovery of leptin has added new information to the understanding of body fat/weight regulation. The positional cloning of the mouse *ob* gene was reported by Zhang and colleagues from Rockefeller University in 1994 [5]. They observed that the *ob* gene encodes for a 16 kDa circulating protein synthesized in adipose tissue. This was originally called *ob* protein and then was later renamed leptin, from the Greek word leptos, which means 'thin' [5]. The authors also reported that mutations of the *ob* gene in the *ob/ob* mouse result in absolute leptin deficiency. This is similar to Coleman's observations 25 years earlier. Subsequently there were reports that clearly showed that administration of leptin to *ob/ob* mice reverses the obesity [6–8] with acute decreases in food intake and longer term decreases in body fat. This lead to significant interest and

excitement regarding the importance of these observations for enhancing the understanding of human obesity.

A leptin receptor was soon identified and characterized and reported to be expressed in the central nervous system [9, 10]. The *db* mutation resides within the leptin receptor gene, and it was confirmed that the *db/db* mice do not respond to leptin administration because they have leptin resistance due to a receptor defect [9, 10]. Again, this is similar to Coleman's findings 25 years earlier. Leptin receptor isoforms are widely distributed throughout the body. The receptor has been found in the brain, choroid plexus, liver, lungs, heart, kidney, testes, spleen and adipose tissue [11, 12]. The brain appears to be a key target for leptin action. Campfield *et al.* demonstrated that leptin administration in the lateral ventricle of the brain of *ob/ob* and lean mice, decreased food intake and changed energy balance [8] and a high affinity for leptin binding has been shown in rat hypothalamic membranes. Also, leptin administration decreases hypothalamic neuropeptide Y (NPY), a potent stimulator of appetite and food intake [13]. Overexpression of NPY in *ob/ob* and *db/db* mice has been reported. NPY also has been shown to decrease thermogenesis and increase plasma insulin and corticosterone levels [14], supporting its possible links with leptin. Another system, the melanocortin system in the hypothalamus of the brain is also involved in energy homeostasis. A mutation of the melanocortin receptor gene can lead to obesity and there are receptors for leptin on the neurons that produce melanocortins. When rats were administered leptin, they ate significantly less; but when a specific group of melanocortin receptors (Melanocortin-4, MC4) was blocked, leptin no longer affected body weight or food intake [15].

HUMAN LEPTIN

With regards to humans, there is a strong positive correlation between serum leptin and percentage body fat [16, 17]. Strong agreement between studies confirms this. Thus, although it was originally believed that human obesity could be related to leptin deficiency, this does not appear to be the case. The exception may be that approximately 5% of obese humans have leptin deficiency [16, 17]. Congenital leptin deficiency has been reported in two first cousins, a boy (2 yr old weighing 29 kg) and a girl (8 yr old weighing 86 kg) who had severe obesity and hyperphagia, along with undetectable levels of leptin [18]. For the majority of individuals who are in energy balance or weight stable, leptin reflects the current amount of triglyceride stored in adipose tissue. For those in negative energy balance, it has been reported that a 10% decrease in body weight resulted in a 53% decrease in leptin levels [17]. Conversely, a 10% increase in body weight has been shown to increase leptin levels by 300% [19]. The changes in body weight and leptin occur in the same direction. It appears that a relatively small change in weight can result in a large change in serum leptin, suggesting that there are other

signals besides fat stores influencing this protein. It has been questioned if it also responds to energy intake.

Caro *et al.* have suggested that leptin may act like a lipostat [20], not unlike the previously proposed Set Point Theory of body weight regulation. Leptin is believed to regulate body fat either by altering food intake (satiety signal) or energy expenditure (thermogenic signal). Thus, a change in energy balance may cause leptin to deviate from its current set point. If this is due to increased energy intake, leptin will suppress appetite and stimulate thermogenesis. If there is an increased energy expenditure, leptin will stimulate food intake and decrease thermogenesis. Either situation will return the individual to their previous body fat/weight levels. Only if fat stores change significantly and this is sustained for a long time, will a new set point be established. Acute changes in leptin levels in response to energy intake have been reported. With fasting, humans have a dramatic decrease in serum leptin [21]. One day of excessive overeating with no weight change, resulted in a 40% increase in serum leptin [19]. Therefore there may be short term and long term regulation related to leptin. That is, changes in fat stores will ultimately affect fasting serum leptin levels over the long term, but that short term regulation occurs based on energy flux. It is logical that a satiety signal would be expected to be a short term mechanism. Human leptin levels are highest between 12:00 midnight to 4:00 am, presumably giving a signal not to eat. Human leptin levels are lowest between 12:00 noon and mid-afternoon, presumably giving a signal to eat [22].

There appears to be gender differences in serum leptin levels, in that women have higher levels than men, independent of body fat stores [23]. This same gender difference has also been shown in children [24] and in non-caucasian races [25]. In individuals with Prader-Willi Syndrome (PWS), the leptin body fat association was significantly stronger than controls [26]. PWS is an autosomal dominant genetic disorder in which an insatiable appetite and severe obesity are classic features. It is speculated that if a leptin disorder is involved in human obesity, it may be even greater in individuals with PWS.

MECHANISMS OF LEPTIN RESISTANCE

What needs to be clearly shown is that leptin does respond to both energy flux and changes in fat stores to maintain an equilibrium around a certain set point. Further investigation related to obesity is required to determine whether there is a defect in leptin action that results in enhanced fat deposition. There may be a leptin resistance syndrome analogous to insulin resistance. As stated previously, obese humans as a group, are not leptin deficient. Leptin levels in obese individuals will be approximately 30 ng/ml reflecting a high level of body fat. A lean individual will have leptin levels of approximately 7 ng/ml (typical range: 3–35 ng/ml).

Circulating leptin is a 146 amino acid protein and therefore it cannot pass the

blood brain barrier without a transporter [27]. A transporter has yet to be identified, but it is speculated that a short form of the leptin receptor may function as a transporter. It may be that there is a defect which inhibits leptin transport into the brain. As reviewed by Caro *et al.* [20], other possible mechanisms include antibodies or antagonists to leptin or increased leptin binding proteins may result in decreased free leptin. There also may be a leptin receptor defect (analogous to the *db/db* mouse model), a signaling defect or a transducer defect. Researchers are investigating all possibilities with significant efforts.

LEPTIN AND RESTING METABOLIC RATE

Leptin appears to regulate body fat by altering food intake and/or energy expenditure. The energy expenditure side of the equation has been evaluated in different contexts. The relationship between leptin and resting metabolic rate (RMR) has been investigated by many researchers and a direct relationship has not been shown. In a study of RMR in 30 women who had a history of chronic dieting, there was no difference in leptin levels in women with a high versus a low metabolic rate and no relationship between the two variables was observed [28]. A study by Kennedy *et al.* [23] also concluded that there was no correlation between fasting leptin levels and RMR or insulin induced thermogenesis in men and women ($n=116$). In another study of 61 men and women there was no relationship between circulating leptin levels and either RMR or energy expenditure after a standard meal [29]. It is suggested that if leptin is a short term regulator of thermogenesis, perhaps RMR is not a sensitive enough indicator, as it remains relatively fixed over long periods of time. A short term response may be more detectable with variables related to the sympathetic nervous system. This was recently shown whereby there was a strong positive correlation between muscle sympathetic nervous system activity (MSNA) and serum leptin levels (MSNA = microelectrode is inserted into the muscle) [30]. It has also been reported that sympathoadrenal activity, determined by 24 h urinary norepinephrine excretion is reduced in obese individuals, and is associated with weight gain and central adiposity [31].

LEPTIN AND WEIGHT MAINTENANCE

Because leptin reflects current adiposity levels, when body weight increases, leptin should increase, when body weight decreases, leptin levels should decrease. Although some authors have suggested that a 10 kg decrease in weight results in approximately a 10 ng/ml decrease in leptin (1 kg = 1 ng change), the relationship is not that clear. Also, the timing of the requisite change in leptin is not well-established. In one of our studies of women who were weight maintainers (had lost >20% body weight and maintained it), weight regainers (had lost >20% body weight and regained it) or controls (who had been weight stable at a healthy

Table 1. Leptin levels after weight change.

Study 1 ¹	Regainers (n=9)	Maintainers (n=10)	Controls (n=10)
BMI (kg/m ²) [a vs. b+c***]	34.1±6.4 ^a	24.0±3.3 ^b	21.3±2.5 ^b
Leptin (ng/ml) [a vs. b+c**]	20.8±8.4 ^a Obese	10.2±7.0 ^b Previously obese	7.5±3.8 ^b Never obese
Study 2 ²	Regainers (n=14)	Maintainers (n=20)	Controls (n=17)
BMI (kg/m ²)	31.1-40.4	21-26.5	19.5-25.4
Leptin (ng/ml) [a vs. b****] [a vs. c****] [b vs. c*]	35.46±3.95 ^a	14.99±2.01 ^b	6.53±1.05 ^c

¹McCargar, L. *et al.* (unpublished observations). ²Reference 32. Different letters in the same row mean significantly different values. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

weight all their adult life), leptin levels were not different between maintainers and controls (Table 1; unpublished observations) and as expected levels were higher in regainers. In a similar study, Kissebah *et al.* [32] found the lowest leptin levels in the "never obese" group with significantly higher levels in the "previously obese" group who achieved and maintained a large weight loss and even higher levels in the obese group (Table 1). The main difference between these two studies was that in the Kissebah study the maintainers had kept the weight off for a minimum of one year, whereas in our study, the maintainers had kept the weight off for a minimum of two years; perhaps suggesting that an extended time period is required before leptin levels "normalize" and a new set point is established.

LEPTIN AS A TREATMENT FOR OBESITY ?

The potential for use of leptin for treating human obesity remains uncertain. Clinical trials are underway to assess this scientifically. Until a clear mechanistic defect of leptin is demonstrated in humans, providing more leptin to the system may be ineffective. It has been rationalized that leptin administration may trick the brain to think that fat stores are higher than they are, and perhaps stimulate mechanisms to support weight loss and re-set the lipostat over the long term. It is also not known however, whether the leptin signal to the hypothalamus can be overcome by hedonic qualities of a very palatable food supply [1].

SUMMARY

In summary, research supports the role of leptin in both satiety and thermogenic mechanisms. It appears to respond to energy flux (in the short term) and changes in fat stores (over the long term) and may act like a lipostat to monitor

these changes around a specific set point. Key issues remain unresolved, in terms of leptin action and human obesity and whether there is a defect in some step of leptin metabolism. Caro *et al.* have stated that "the discovery of a fat-melting hormone named leptin, raised the hopes of one-third of the North American population that there is a simple solution to cure their obesity" [20]. The hope is still there, however it appears that the solution is not so simple.

Supported in part by the Central Research Fund, University of Alberta; and the Canadian Fitness and Lifestyle Research Institute, Ottawa, ON.

REFERENCES

1. Havel, P.J. (1998): Leptin production and action: Relevance to energy balance in humans. Editorial. *Am. J. Clin. Nutr.*, **67**, 355-356.
2. Kennedy, G.C. (1953): The role of depot fat in the hypothalamic control of food intake in the rat. *Proc. R. Soc. Lond.*, **140**, 578-592.
3. Hervey, G.R. (1958): The effects of lesions in the hypothalamus in parabiotic rats. *J. Physiol.*, **145**, 336-352.
4. Coleman, D.L. (1973): Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*, **9**, 294-298.
5. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. (1994): Positional cloning of the mouse obese gene and its human homologue. *Nature*, **372**, 425-432.
6. Pelleymounter, M.A., Cullen, M.J., Baker, M.B., Hecht, R., Winters, D., Boone, T., and Collins, F. (1995): Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*, **269**, 540-543.
7. Halaas, J.L., Gajiwala, K.S., Maffei, M., Cohen, S.L., Chait, B.T., Rabinowitz, D., Lallone, R.L., Burley, S.K., and Friedman, J.M. (1995): Weight reducing effects of the plasma protein encoded by the obese gene. *Science*, **269**, 543-546.
8. Campfield, A.L., Smith, F.J., Guisez, Y., Devos, R., and Burn, P. (1995): Recombinant mouse OB protein: Evidence for peripheral signal linking adiposity and central neural networks. *Science*, **269**, 546-549.
9. Chua, S.C.J., Chung, W.K., Wu-Peng, X.S., Zhang, Y., Lui, S.M., Tartaglia, L., and Leibel, R.I. (1996): Phenotypes of mouse diabetes and rat fatty due to mutation in the ob (leptin) receptor. *Science*, **271**, 994-996.
10. Devos, R., Richards, J.G., Campfield, L.A., Tartaglia, L.A., Guisez, Y., and Burn, P. (1996): OB protein binds specifically to the choroid plexus of mice and rats. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 5668-5673.
11. Tartaglia, L.A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., Richards, G.J., Campfield, L.A., Clark, F.T., and Deeds, J. (1995): Identification and expression: Cloning of a leptin receptor, OB-R. *Cell*, **83**, 1263-1271.
12. Lee, G.H., Proenca, R., Montez, J.M., Carroll, K.M., Darvishzadeh, J.G., Lee, J.I., and Friedman, J.M. (1996): Abnormal splicing of the leptin receptor in diabetic mice. *Nature*, **379**, 632-635.
13. Stephens, T.W., Basinski, M., Bristow, P.K., Bue-Vallesky, J.M., Burgett, S.G., Craft, L., Hale, J., Hoffman, J., Hsuing, H.M., Kriaciunas, A., MacKellar, W., Rostek, P.R., Jr., Schoner, B., Sloop, K., Smith, D., Tinsley, F.C., Zhang, S.Y., and Heiman, M. (1995): The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature*, **377**, 530-532.
14. Zarjevski, N., Cusin, I., Vettor, R., Rohner-Jeanrenaud, F., and Jeanrenaud, B. (1993): Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes in obesity. *Endocrinology*, **133**, 1753-1758.
15. Seeley, R.J., Yagaloff, K.A., Fisher, S.L., Burn, P., Thiele, T.E., van Dijk, G., Baskin, D.G., and Schwartz, M.W. (1997): Melanocortin receptors in leptin effects. *Nature*, **390**, 349.

16. Considine, R.V., Sinha, M.K., Heiman, M.L., Kriaciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKee, L.J., and Bauer, T.L. (1996): Serum immuno-reactive-leptin concentrations in normal weight and obese humans. *N. Engl. J. Med.*, **334**, 292-295.
17. Maffei, M., Halaas, J., Ravussin, E., Pratley, R.E., Lee, G.H., Zhang, Y., Fei, H., Kim, S., Fallone, R., and Ranganathan, S. (1995): Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat. Med.*, **1**, 1155-1161.
18. Montague, C.T., Farooqi, I.S., Whitehead, J.P., Soos, M.A., Rau, H., Wareham, N.J., Sweter, C.P., Digby, J.E., Mohammed, S.M., Hurst, J.A., Cheetham, C.H., Earley, A.R., Barnett, A.H., Prins, J.B., and O'Rahilly, S. (1997): Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, **387**, 903-908.
19. Kolaczynski, J.W., Ohannesian, J., Considine, R.V., Marco, C.C., and Caro, J.F. (1997): Response of leptin to short term and prolonged overfeeding in humans. *J. Clin. Endocrinol. Metab.*, **81**, 4162-4165.
20. Caro, J., Sinha, M.K., Kolaczynski, J.W., Zhang, P.L., and Considine, R.V. (1996): Leptin: The tale of the obesity gene. *Diabetes*, **45**, 1455-1462.
21. Kolaczynski, J.W., Considine, R.V., Ohannesian, J., Marco, C., Opentanova, I., Nyce, M.R., Myint, M., and Caro, J.F. (1996): Responses of short term fasting and refeeding in humans: A link with ketogenesis but not ketones themselves. *Diabetes*, **45**, 1511-1515.
22. Sinha, M.K., Ohannesian, J.P., Heiman, M.L., Kriaciunas, A., Stephens, T.W., Magosin, S. A., Morco, C., and Caro, J.F. (1996): Nocturnal rise of leptin in lean, obese and non-insulin dependent diabetes mellitus subjects. *J. Clin. Invest.*, **97**, 1344-1347.
23. Kennedy, A., Gettys, T.W., Watson, P., Wallace, P., Ganaway, E., Pin, Q., and Garvey, T. (1997): The metabolic significance of leptin in humans: Gender-based differences in relationship to adiposity, insulin sensitivity and energy expenditure. *J. Clin. Endocrinol. Metab.*, **82**, 1293-1300.
24. Hassink, S.G., Sheslow, D.V., de Lancey, E., Opentanova, I., Considine, R.V., and Caro, J.F. (1996): Serum leptin in children with obesity: Relationship to gender and development. *Pediatrics*, **98**, 201-203.
25. Luke, A.H., Rotimi, C.N., Cooper, R.S., Long, A.E., Forrester, T.E., Wilks, R., Bennett, F.I., Ogunbiyi, O., Compton, J.A., and Bowsher, R.R. (1998): Leptin and body composition of Nigerians, Jamaicans, and US blacks. *Am. J. Clin. Nutr.*, **67**, 391-396.
26. Pietrobelli, A., Allison, D.B., Faith, M.S., Beccaria, L., Bosio, L., Chiumello, G., Campfield, L.A., and Heymsfield, S.B. (1998): Prader-Willi Syndrome: Relationship of adiposity to plasma leptin levels. *Obes. Res.*, **6**, 196-201.
27. Kishimoto, T., Taga, T., and Akiran, S. (1994): Cytokine signal transduction. *Cell*, **76**, 253-262.
28. Gingras, J., McCargar, L., and Harber, V. (1998): Metabolism and body image of female chronic dieters. *FASEB J.*, (abstr)#1189, A204.
29. Roberts, S.B., Nicholson, M., Staten, M., Dallal, G.E., Sawaya, A.L., Heyman, M.B., Fuss, P., and Greenberg, A.S. (1997): Relationship between circulating leptin and energy expenditure in adult men and women aged 18 years to 81 years. *Obes. Res.*, **5**, 459-463.
30. Snitker, S., Pratley, R.E., Nicolson, M., Tataranni, P.A., and Ravussin, E. (1997): Relationship between muscle sympathetic nerve activity and plasma leptin concentrations. *Obes. Res.*, **5**, 338-340.
31. Tataranni, P.A., Young, J.B., Bogardus, C., and Ravussin, E. (1997): A low sympathoadrenal activity is associated with body weight gain and development of central adiposity in Pima Indian men. *Obes. Res.*, **5**, 341-347.
32. Kissebah, A.H., Marks, J., James, R., and Leibel, R. (1996): Persistent elevation in plasma leptin level in ex-obese with normal body mass index (BMI). *Obes. Res.*, **4** (Suppl. 1), (abstr) #M27, 7S.