Endocrine, vol. 7, no. 1, 1–8, August 1997 0969-711X/97/7:1–8/$10.00

REVIEW

Regulation of Circulating Leptin in Humans

Bo Ahrén,[1] Hillevi Larsson,[1] Christer Wilhelmsson,[2] Birgitta Näsman,[2] and Tommy Olsson[3]

Departments of [1]Medicine, Lund University, Malmö, [2]Geriatrics and [3]Medicine, Umeå University, Umeå, Sweden

Leptin and the *Ob/Ob* Mouse

In the early 1950s, the *ob/ob* mouse was described *(1)*. Later extensive metabolic studies have shown that the syndrome of this mouse consists of massive obesity in conjunction with hyperphagia, insulin resistance, hyperinsulinemia, development of noninsulin-dependent diabetes mellitus (NIDDM), cold intolerance, and infertility *(2)*. Parabiosis studies in the 1950s and 1960s also revealed that the cause of the syndrome is the deficiency of a circulatory factor, rather than overproduction of a factor, since lean mice in parabiosis with obese mice were found to reduce the weight of the obese animals *(2–4)*. Finally, by the end of 1994, the gene coding for the factor lacking in the *ob/ob* mice was cloned *(5)* and in 1995 it was shown that administration of the protein product of this gene, leptin, normalized the hyperphagia, the massive obesity and the huge hyperinsulinemia in these animals *(6–8)*.

Leptin as the Adipocyte Hormone

Further studies have shown that leptin is exclusively expressed in the adipocytes *(9,10)* and released from these cells *(11,12)*. It has also been demonstrated that the rate of production of leptin is directly related to the degree of adiposity *(13)*, and it is now well established that administration of recombinant leptin reduces food intake and body weight *(6–8,14,15)*. The effects of leptin are thought to be mediated by cytokine receptor-like leptin receptors within the brain *(16–18)* and involves also other signals of importance in the regulation of food intake, like, for example, neuropeptide Y (NPY) *(19)*. However, leptin can also exert a food intake-suppressing effect in NPY deficient animals *(20)*. As recently reviewed, leptin is now considered a hormone from the fat tissue involved in the regulation of food intake and adiposity *(21)*. Therefore, factors regulating body weight and nutritional status may operate through changes in the circulating concentration of leptin.

Characterization of Circulating Leptin

During 1996, radioimmunological techniques for the determination of circulating leptin in humans have been developed *(22–24)*. These techniques have enabled studies to characterize circulating leptin and the factors involved in the regulation of circulating leptin in humans. It has been demonstrated that leptin binding proteins exist in the circulation, the total leptin binding capacity of which, however, is low, implying that the relative abundance of free leptin is higher in subjects with high circulating leptin *(25,26)*. The physiological importance of this finding is not yet known. One aspect is that compared to bound leptin, circulating free leptin may have a shorter half-life *(25)*, being approx 25 min in humans as a mean *(13)*, but then possibly shorter in subjects with hyperleptinemia. Another aspect is that the leptin-binding proteins might function to transport leptin across the blood–brain barrier, as has been suggested for cytokine-binding proteins *(27)*, which would imply that the relative rate of leptin transport is lower in subjects with hyperleptinemia. However, more studies are required to characterize the binding of leptin to proteins in the circulation.

Circulating Leptin and its Regulation by Body Weight

Circulating Leptin and Body Weight

Several studies have demonstrated that circulating leptin correlates closely to body mass index (BMI) over a wide range of BMI *(12,23,28–32)*. This is illustrated in Fig.1 showing the close relationship between BMI and plasma insulin in 157 healthy women of various ages. The *r*-value of this correlation was 0.65, which is within the same order as reported in other studies (Table 1). This implies that approx 50% of circulating leptin is governed by the degree of obesity.

Circulating Leptin and Fat Content

The underlying mechanism for subjects with high BMI having higher circulating leptin is related to the expression

Received December 16, 1996; Revised December 23, 1996; Accepted December 23, 1996.

Author to whom all correspondence and reprint requests should be addressed: Dr. Bo Ahrén, Department of Medicine, Malmö University Hospital, S-205 02 Malmö, Sweden. E-mail: bo.ahren@medforsk.mas.lu.se

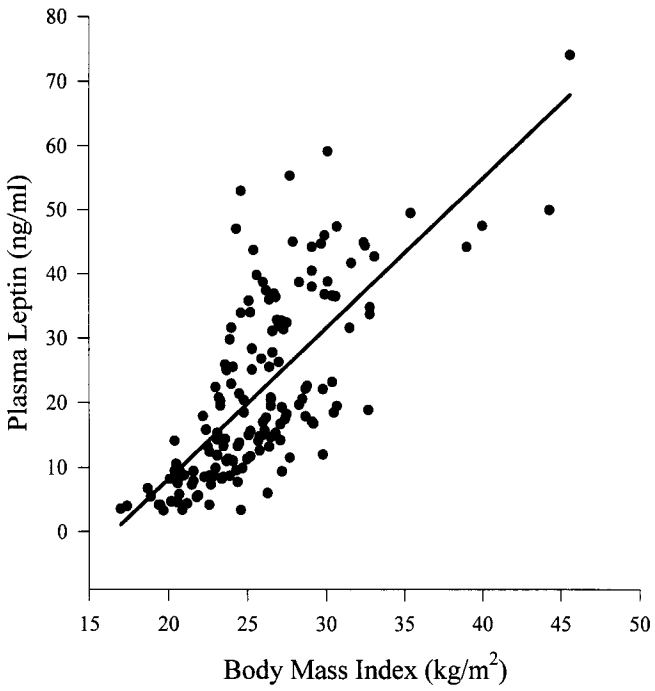

Fig. 1. Correlation between BMI and plasma leptin levels as determined by a sensitive radioimmunoassay *(22)* after an overnight fast in 157 nondiabetic, healthy women within the age range of 22–64 yr. $r = 0.65$, $p < 0.001$.

Table 1
Regression Coefficiency of the Correlation Between Body Mass Index and Circulating Leptin in Six Different Studies in Humans

Author (ref.)	Number of subjects	*r*-Value
Maffei et al. *(28)*	87	0.51
Considine et al. *(23)*	275	0.66
McGregor et al. *(24)*	75	0.77
Dagogo-Jack et al. *(29)*	27	0.76
Kolaczynski et al. *(12)*	77	0.68
Havel et al. *(32)*	38	0.81
Present study (Fig. 1)	157	0.65

and secretion of leptin from adipocytes *(10–12)*, since subjects with high BMI have higher fat mass. This has been verified by correlating circulating leptin to body fat content: the body fat content and circulating leptin are significantly correlated both when using underwater weight (r = 0.86; *28*), dual-energy X-ray absorptiometry ($r = 0.80$; 30), and impedance measurements (r = 0.82; 31) as determinants of body fat content. It was also recently demonstrated by measurements of adipose tissue leptin production in humans that the rate of production of leptin is directly related to the degree of adiposity *(13)*. High circulating leptin in obese subjects is therefore owing to both greater leptin production per unit of body fat and to increased production of leptin from the increased size of body fat mass *(13)*.

Circulating Leptin and Body Fat Distribution

It has been demonstrated that abdominal obesity is associated with a higher risk than lower body obesity of developing cardiovascular diseases through its close association with insulin resistance *(33,34)*. It is therefore of interest to ask whether not only total body fat content, but also the body fat distribution is of importance for circulating leptin. One study examined this question by correlating circulating leptin to the waist to hip circumference ratio and found a significant correlation between these parameters also after control for body fat content, ($r = 0.33$), i.e., in subjects with identical body fat content, the higher the circulating leptin, the higher the waist to hip circumference *(35)*. However, the same study also distinguished the abdominal distribution of fat by magnetic resonance imaging, and found that leptin correlated more to subcutaneous fat than to visceral fat. Furthermore, another study in premenopausal African-American women showed that after correction for influences of total body fat, circulating leptin did not correlate to visceral fat volume as determined by computed tomography *(30)*. We also found in 92 postmenopausal women, aged 58 yr, that plasma leptin correlated significantly with the waist to hip ratio. The r-value ($r = 0.25$, $p = 0.021$) was, however, lower than that for the correlation between plasma leptin and BMI (r = 0.72). Furthermore, the correlation was no longer significant when BMI was controlled for (r = –0.05, n.s.). Thus, it seems as if the distribution of fat only marginally, if at all, independently contributes to circulating leptin; the main contributor seems to be the total body fat content.

Several studies have thus demonstrated a close correlation between circulating leptin and the degree of obesity and body fat content, as determined by different measures. The implication of this relation remains to be established. One aspect is that in obesity, circulating leptin could be adaptively increased to reduce food intake and therefore aim to lower BMI. According to this view, subjects with high leptin are not in a nutritional steady state. Another view, however, is that subjects with obesity could have reduced leptin receptor sensitivity, which necessitates an increased circulating leptin to keep the body weight stable. If that were the case, however, some subjects with low BMI also would have high circulating leptin. Therefore, the homeostatic relation between leptin and body weight is still not established, and direct studies are now required regarding the leptin–leptin receptor interaction in lean and obese subjects.

Circulating Leptin and Changes in Body Weight

Since body fat content or BMI correlates with circulating leptin (*28,30,31,* Table 1), it is expected that circulating leptin is altered under conditions when body weight is changed. It has, for example, been shown that circulating leptin is reduced after 12 h of fasting in humans, and that the reduction progresses throughout a 36 h fasting period

to be rapidly restored after refeeding *(36)*. A similar fasting-induced reduction in circulating leptin has also been demonstrated in mice *(37)*. The mechanism of the fasting-induced reduction in circulating leptin is not known, although it seems to be a direct effect on the adipocyte, since a reduction in leptin during fasting in humans was shown to correlate to a reduction in adipocyte *ob* gene expression *(36)*. Also, the reduction in leptin during fasting correlated with an increase in ketones, suggesting a similar mechanism for fasting-induced hypoleptinemia and hyperketonemia. However, ketones by themselves do not seem to be the mediator of the hypoleptinemia, since infusion of β-OH-butyrate did not change circulating leptin *(36)*. Also during long-term weight reduction induced by restriction in dietary fat, plasma leptin is reduced *(32)*. In that study, the reduction in leptin correlated to the reduction in body weight, because after 8 mo on a low fat diet, 15 women who lost more than 7% of the body mass had a significant reduction in circulating leptin, whereas in 18 other subjects, who lost <7% of the body mass, plasma leptin did not change *(32)*. Furthermore, the reduction in leptin did not correlate to the content of fat in the diet, but rather to the reduction in body weight *(32)*. However, a dissociation between changes in body weight and circulating leptin has also been demonstrated. For example, as little as a 5% reduction in body weight has been shown to reduce circulating leptin by more than 50% *(23)*, and, conversely, a small increase in body weight by 10% increases circulating leptin by more than 300% *(38)*. These findings indicate that circulating leptin, in addition to its regulation by body weight or body fat content, also is regulated by a metabolic mechanism related to adipocyte metabolism. Since only 1 d of massive overfeeding has been found to increase circulating leptin by 40% without altering body weight *(38)*, it has been suggested that the triglyceride content of adipocyte is involved in the regulation of circulating leptin *(21)*.

Circulating Leptin and Obesity

Since obese subjects have high levels of leptin *(12,23,24,28–32)* and high *ob* gene expression *(39,40)* it has been assumed that human obesity, at least most cases, exhibit leptin resistance rather than leptin deficiency *(21,41)*. Human obesity therefore seems to show similarities to the obesity in the *db/db* mouse, which has been shown to exhibit a defect leptin receptor *(16)* rather than the obesity in the *ob/ob* mouse, exhibiting defect expression of the *ob/ob* gene *(5)*. If leptin resistance is involved as a mechanism of human obesity, subjects with high BMI should have a higher ratio of leptin to BMI, since they need to "overcompensate" the leptin production in relation to the requirement for a given BMI. This was indeed verified in one recent study *(42)* and confirmed in our material of 157 women, as shown in Fig. 2. This figure shows that the ratio of plasma leptin to BMI increases by increasing BMI. Therefore, it may be concluded that obesity is accompanied

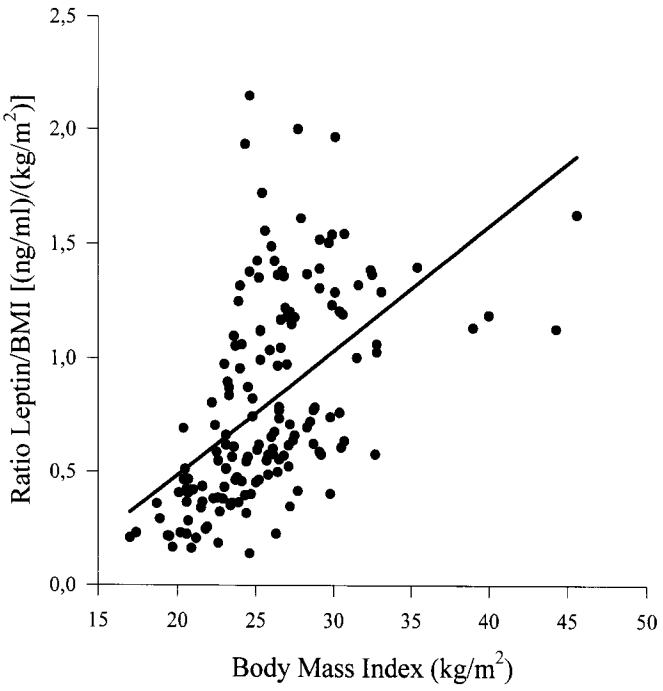

Fig. 2. Correlation between BMI and the ratio of plasma leptin as determined by a sensitive radioimmunoassay *(22)* to BMI after an overnight fast in 157 nondiabetic, healthy women within the age range of 22–64 yr. $r = 0.53$, $p < 0.0001$.

by leptin resistance and high circulating leptin levels, making an analogy to insulin in NIDDM appropriate. As theoretically discussed by Caro et al. *(21)*, the resistance of leptin in obesity might reside at various places in the chain of events from leptin secretion through leptin action and effector mechanisms. For example, leptin resistance might occur in the transportation of leptin in the circulation or across the blood–brain barrier, the latter in accordance with a recent finding that subjects with high leptin levels have a lower capacity to transport leptin to the brain *(43)*. Leptin resistance might also reside in the leptin receptors themselves or their signal transduction, or in the effector system, involving, for example, NPY *(19)*. More studies are, however, required to further increase our understanding of the role of leptin in obesity, and also to examine whether obese subjects respond with reduced body weight if exogenous leptin was administered.

In this context, individual subjects with an extreme relation between BMI or obesity and circulating leptin might be of interest. Recently, two obese subjects with very low circulculating leptin were described *(44)*. However, in spite of extensive characterization of these subjects, no clear differentiation of these subjects from other obese subjects was possible.

Circulating Leptin and its Regulation by Other Factors

Since the regression between body weight or body fat content and circulating leptin, although high, is only within

the range of 0.7–0.8 (Table 1), the r^2 is around 0.5–0.6, indicating that body weight or body fat content may explain only approx 50% of the circulating leptin. Therefore, other factors are also involved in its regulation, which is supported by the findings that the degree of change in body weight during fasting or overfeeding is different from the degree of change in circulating leptin *(23,38)*.

Influence of Gender on Circulating Leptin

As initially shown in mice *(45)* and later verified in humans *(29,46)*, it has been demonstrated that a gender difference exists in the circulating leptin levels in that higher levels are found in females than in males. In one study in humans, it was shown that this difference was independent of BMI and age *(29)*, and in the other study, it was revealed that the difference in circulating leptin between 34-yr-old men and 30-yr-old women persisted after control for body fat *(46)*. Furthermore, plasma leptin was found not to be altered after administration of estrogen and progesterone in postmenopausal women, suggesting that the gender difference in plasma leptin is not the result of the reproductive hormones *(46)*. Thus, the mechanism underlying the gender difference in circulating leptin is still unknown. We have confirmed the gender difference in plasma leptin by examining the relationship between BMI and plasma leptin in men and women at the ages of 28 and 64 yr (Fig. 3). We found that in both genders, a correlation between BMI and plasma leptin exists, but that the slopes of the curves for the relationships are different. Therefore, for a given BMI, plasma leptin is higher in women than in men.

Influence of Age on Circulating Leptin

In mice, we recently demonstrated that plasma leptin was higher in animals at the age of 11 mo than in younger animals at the age of 2 mo, suggesting an age-dependent influence on circulating leptin *(37)*. However, in humans, such an influence does not seem to exist. Thus, as is seen in Fig. 3, there was no difference in the relationship between BMI and circulating leptin in women or men at age 28–64 yr; the difference in circulating leptin between these ages being explained by differences in gender and BMI.

Diurnal Variation of Circulating Leptin

In a study of both lean and obese healthy subjects and in obese subjects with NIDDM, it was demonstrated that plasma leptin exhibits a diurnal rhythm, with an increase in its value starting at 11 PM and peaking at 2 AM *(47)*. We have recently confirmed this diurnal rhythm. Thus, in five healthy subjects, circulating leptin was higher at 11 PM and at 3 AM than at 6 AM (Fig. 4). The mechanism of this diurnal rhythm is not known. The profile resembles that of prolactin and TSH and the leptin peak precedes the peak in cortisol *(48–50)*. In fact, we found in our five subjects an inverse correlation between circulating leptin and circulating cortisol ($r = -0.45$, $p = 0.024$), suggesting a relationship between leptin and cortisol. Another possibility is that the elevated insulin during daytime through a delayed effect increases circulating leptin. However, more studies are required to understand this diurnal variation.

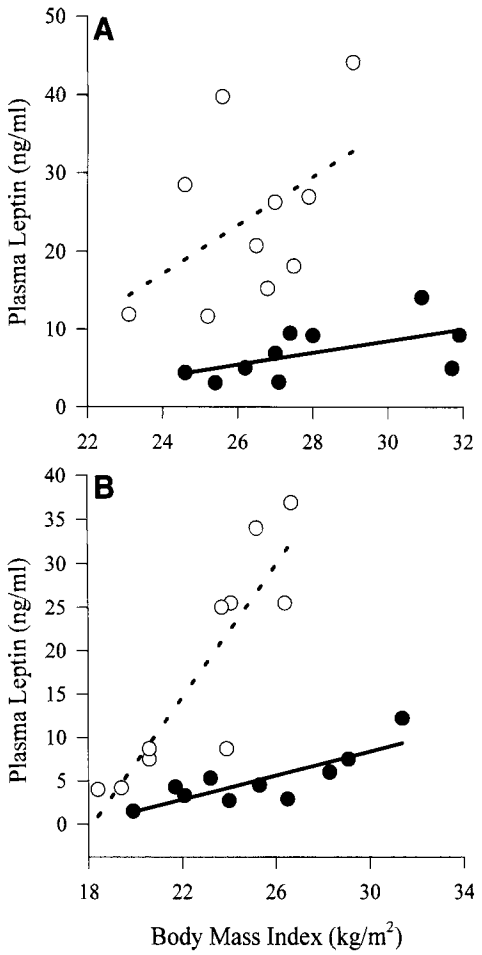

Fig. 3. Correlation between BMI and plasma leptin levels as determined by a sensitive radioimmunoassay *(22)* in 28- and 64- yr-old healthy males and females. There were 10 subjects in each group. **(A)** Age 64 yr, $r = 0.48$, $p = 0.01$, slope = 2.54; $r = 0.56$, $p < 0.001$, slope = 0.74. **(B)** Age 28 yr, $r = 0.87$, $p < 0.0001$, slope = 3.52, $r = 0.82$, $p < 0.0001$, slope = 0.69. ○ females, ● males.

Circulating Leptin and Insulin Sensitivity

Since circulating leptin correlates with total body fat *(13,28,30,31)* and since high BMI is associated with insulin resistance *(51–55)*, it has been of interest to examine whether circulating leptin correlates with insulin sensitivity. To that end, we examined a large number of postmenopausal women with the hyperinsulinemic, euglycemic clamp technique for determination of insulin sensitivity, and found expectedly that the measure for insulin sensitivity correlated negatively with plasma leptin ($r = -0.68$; *31*). Furthermore, another study determined insulin sensitivity by the use of the minimal model technique measuring the insulin sensitivity index (S_I) after bolus injections of glucose and insulin in premenopausal women, and, as expected, the S_I was found to correlate negatively with

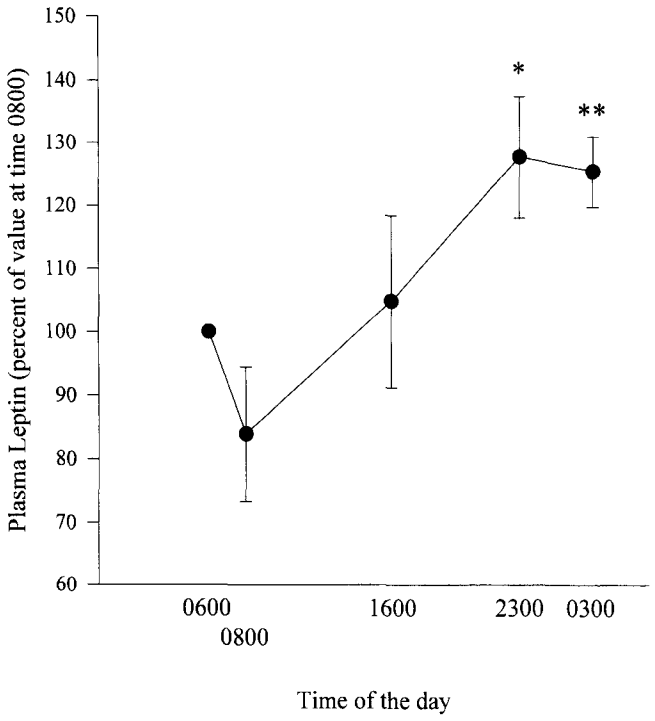

Fig. 4. Plasma leptin levels as determined by a sensitive radioimmunoassay *(22)* in five healthy volunteers at five different occassions during 1 d. Asterisks indicate the probability level of random difference vs the value at 8 AM as determined by paired *t*-test; *indicates $p < 0.05$, and **indicates $p < 0.01$.

plasma leptin ($r = -0.42$, $p < 0.05$; *30*). In addition, when selecting groups with various insulin sensitivities, groups with low insulin sensitivity have been shown to exhibit higher circulating leptin *(56)*. Moreover, both in humans *(31,32)* and rodents *(37)*, fasting plasma insulin, which is an indirect measure of insulin sensitivity in subjects with normal glucose tolerance, has been shown to correlate with circulating leptin. Thus, both direct and indirect estimates of insulin sensitivity have shown that the more insulin-resistant a subjects is, the higher the circulating leptin. This is, however, expected in view of the close correlation between insulin resistance, i.e., low insulin sensitivity, and obesity, i.e., high BMI. Therefore, in the two studies examining insulin sensitivity directly, its relationship with circulating leptin has also been determined after control for the influence of BMI, and in both studies, this abolished the correlation *(30,31)*. It has also been shown that improvement of reduced insulin sensitivity in a group of obese subjects by means of the insulin sensitizer, troglitazone, did not affect circulating leptin *(57)*. Thus, it seems fairly well established that insulin sensitivity and circulating leptin correlates owing to the confounding influence of BMI and not to a direct influence of one parameter on the other.

Nevertheless, it has been demonstrated that experimentally induced insulin resistance is associated with a marked increase in plasma leptin *(58)*. Thus, in healthy, postmenopausal women, dexamethasone was given for 48 h, which reduces insulin sensitivity. This treatment was found to double plasma leptin levels. This increase was independent of changes in plasma insulin or insulin sensitivity, and therefore, most likely the result of an action of dexamethasone on leptin expression and secretion. This effect may either be directly at the adipocytes as inferred from a previous study in the rat in which corticosteroid treatment was found to increase *ob* gene expression rapidly *(59)*, or indirectly through influences on the central nervous system (cf *58*). Therefore, it might be concluded that insulin sensitivity does not *per se* influence circulating leptin.

Circulating Leptin and Insulin Secretion

In subjects with normal glucose tolerance, reduced insulin sensitivity is compensated with increased insulin secretion causing hyperinsulinemia to preserve normoglycemia *(51,60,61)*. The signal mediating this islet adaptation to reduced insulin sensitivity is not known, but might hypothetically involve leptin, since circulating leptin is increased in subjects with low insulin sensitivity. On the other hand, under in vitro conditions, it has been demonstrated that insulin stimulates the *ob* gene expression in adipocytes from both humans *(62)* and rodents *(63,64)* and insulin has also been shown to stimulate leptin secretion from human adipocytes in vitro *(12)*. This might imply that hyperinsulinemia resulting from increased insulin secretion would increase circulating leptin. To study whether a correlation exists between plasma leptin and insulin secretion, we recently examined insulin secretion by using the glucose-dependent arginine stimulation test in postmenopausal women with known circulating levels of leptin *(31)*. It was found that various parameters of insulin secretion, such as basal and maximal insulin secretion as well as the glucose sensitivity of the B-cells, correlated with plasma leptin, and that these correlations persisted also after control for BMI in the statistical analysis. Thus, insulin secretion and plasma leptin do seem to correlate with each other directly. Two different aspects for this correlation could be proposed. On the one hand, if leptin stimulates insulin secretion, leptin could be involved in the increased insulin secretion seen in obese people. Whether leptin stimulates insulin secretion has not yet been established, but is possible considering a recent demonstration of leptin receptors on insulin producing islet B-cells *(65)*. On the other hand, the correlation between insulin secretion and circulating leptin could also imply that insulin under physiological conditions increases the expression and secretion of leptin as inferred from the in vitro studies *(12,62–64)*. Although not finally excluded, this seems less likely, however, since a supraphysiological infusion of insulin over at least 4 h is required to increase circulating leptin in humans *(66–68)*, whereas a 2-h supraphysiological insulin infusion is insufficient, both during euglycemia *(12,29,31)* and hypoglycemia *(31)*. Therefore, the mechanism of the correlation between circulating leptin and insulin secretion remains to be estab-

lished. The relationship seems not, however, to be dependent on an acute correlation between circulating leptin and insulin secretion, since following iv administration of glucose, arginine, or tolbutamide, insulin secretion is increased but circulating leptin is not increased *(56,69)*.

Circulating Leptin and Glucose Intolerance

Since circulating leptin correlates to insulin secretion *(31)*, it is of interest to examine whether circulating leptin is altered in subjects with glucose intolerance and NIDDM, which are conditions associated with impaired insulin secretion *(61,70)*. A study comparing circulating leptin in postmenopausal age- and BMI-matched women with normal or impaired glucose tolerance showed, however, that their circulating leptin levels did not differ *(31)*. Similarly, in fully developed NIDDM, no difference from control subjects has been found for circulating leptin *(47,66)*. Therefore, no abnormalities in circulating leptin have so far been detected in association with glucose intolerance and diabetes.

Circulating Leptin and Other Agents

It has been shown that a variety of simple and complicated, multifactorial influences, such as fasting, feeding, insulin, dexamethasone, β_3-adrenoceptor agonists, streptozotocin-induced diabetes, and sympathetic nervous activation, affect *ob* gene expression or leptin secretion in vitro *(12,45,59,71–78)*. It is therefore likely that circulating leptin might be affected by a multitude of other factors than those described above. For example, it was recently demonstrated that both the β-adrenoceptor agonist, isoproterenol, as well as somatostatin reduce circulating leptin when infused in humans *(79)*. More studies are, however, required to examine the relationship between leptin and these other factors.

Conclusions

Leptin is a hormone produced in and released from the adipocytes in relation to the size of the body fat. Leptin activates cytokine-like receptors in the central nervous system which reduces food intake. Leptin therefore seems to be the signaling pathway in a feedback system working to control body weight. Since circulating leptin may be determined by radioimmunoassay, factors affecting its circulating levels are possible to establish. The most important determinant of circulating leptin is body fat content. However, variations in body fat content explain only approx 50% of circulating leptin, making it important to establish also other factors of physiological impact on circulating leptin. Studies undertaken so far, i.e., mainly in 1996, have shown that circulating leptin is higher in women than in men, that circulating leptin undergoes a diurnal variation with higher levels during nighttime than during daytime, and that the level of circulating leptin correlates to insulin secretion. Figure 5 illustrates these influences. However, more studies are required to establish the impact of these other factors as well as the mechanisms underlying their influence on circulating leptin. It is also of importance to examine whether these other factors regulate the nutritional state and body weight through their influences on leptin. Finally, the exact role of leptin in nutrition and regulation of body weight and food intake needs to be carefully established. Considering the marked interest in this newly described peptide during the last year, we may expect fast development of new information within the near future.

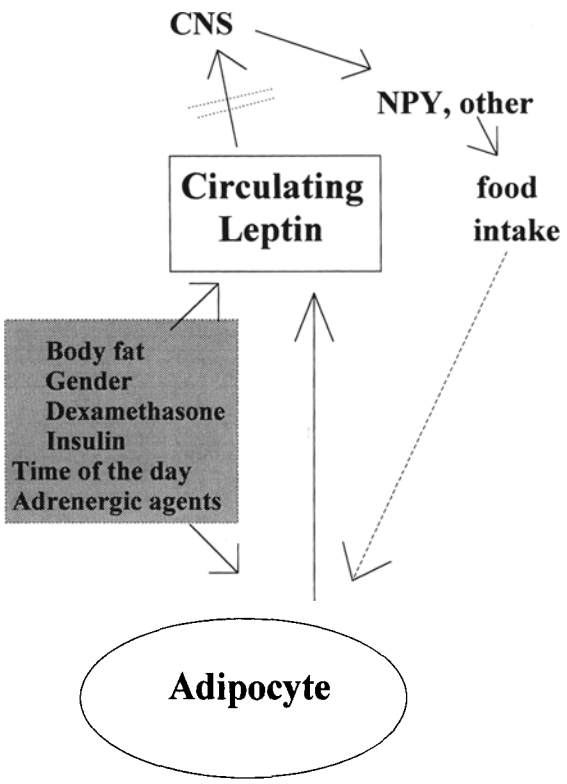

Fig. 5. Schematic summary of factors influencing circulating leptin as discussed in the text and the tentative feedback loop between the adipocyte and CNS linked by circulating leptin on one hand and food intake on the other hand.

Acknowledgments

The studies by the authors have been financially supported by the Swedish Medical Research Council (grant no 14X-6834), Ernhold Lundström, Albert Påhlsson, Crafoord and Novo Nordic Foundations, Swedish Diabetes Association, Malmö University Hospital, and the Faculty of Medicine, Lund University.

References

1. Ingalls, A. M., Dickie, M. M., and Snell, G. D. (1950). *J. Hered.*, **41,** 317–318.
2. Coleman, D. L. (1978). *Diabetologia* **14,** 141–148.
3. Hausberger, F. X. (1959). *Anat. Rec.* **130,** 313.
4. Coleman, D. L. (1973). *Diabetologia* **9,** 294–298.

5. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). *Nature* **372,** 425–432.
6. Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., and Collins, F. (1995). *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). *Science* **269,** 543–546.
8. Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., and Burn, P. (1995). *Science* **269,** 546–549.
9. Maffei, M., Fei, H., Lee, G. H., Dani, C., Leroy, P., Zhang, Y., Proenca, R., Negrel, R., Ailhaud, G., and Friedman, J. M. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 6957–6960.
10. Masazuki, H., Ogawa, Y., Isse, N., Satoh, N., Okazaki, T., Shigemoto, M., Mori, K., Tamura, N., Hosada, K., Yoshimasa, Y., Jingami, H., Kawada, T., and Nakao, K. (1995). *Diabetes* **44,** 855–858.
11. MacDougald, O. A., Hwang, C. S., Fan, H., and Lane, M. D, (1995). *Proc. Natl. Acad. Sci. USA* **92,** 9034–9037.
12. Kolaczynski, J. W., Nyce, M. R., Considine, R. V., Boden, G., Nolan, J. J., Henry, R., Mudaliar, S. R., Olefsky, J., and Caro, J. F. (1996). *Diabetes* **45,** 699–701.
13. Klein, S., Coppack, S. W., Mohamed-Ali, V., and Landt, M. (1996). *Diabetes* **45,** 984–987.
14. Weigle, D. S., Bukowski, T. R., Foster, D. C., Holderman, S., Kramer, J. M., Lasser, G., Lofton-Day, C. E., Prunkard, D.E., Raymond, C., and Kujiper, J. L. (1995). *J. Clin. Invest.* **96,** 2065–2070.
15. Levin, N., Nelson, C., Gurney, A., Vandlen, R., and De Sauvage, F. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 1726–1730.
16. Chen, H., Charlat, O., Tartaglia, L. A., Woold, E. A., Weng, X., Ellis, S. J., Lakey, J. D., Culpepper, J., Moore, K. J., Breitbart, R., Duyk, G. M., Tepper, R. I. and Morgenstern, J. P. (1996). *Cell* **84,** 491–495.
17. Lee, G. H., Proenca, R., Montez, J. M., Carrol, K. M., Proenca, R., Montez, J. M., Darvishzadeh, J. G., Lee, J. I., and Friedman, J. M. (1996). *Nature* **279,** 632–635.
18. Tartaglia, L. A., Dembski, M., Weng, X. U., Deng, N., Culpepper, J., Devos, R., Richards, G. J., Campfield, L. A., Clark, F. T., Deeds, J., Muir, C., Sanker, S., Moriarty, A., Moore, K. J., Smutko, J. S., Mays, G. G., Woolf, E. A., Monroe, C. A., and Tepper, R. I. (1995). *Cell* **83,** 1263–1271.
19. Schwartz, M. W., Baskin, D. G., Bukowski, T. R., Kuijper, J. L., Foster, D., Lasser, G., Prunkard, D. E., Porte Jr, D., Woods, S. C., Seeley, R. J., and Weigle, D. S. (1996). *Diabetes* **45,** 531–535.
20. Erickson, J. C., Clegg, K. E., and Palmiter, R. D. (1996). *Nature* **381,** 415–418.
21. Caro, J. F., Sinha, M. K., Kolaczynski, J. W., Zhang, P. L., and Considine, R. V. (1996). *Diabetes* **45,** 1455–1462.
22. Ma, Z. A., Gingerich, R. L., Santiago, J. V., Klein, S., Smith, H. C., and Landt, M. (1996). *Clin. Chem.* **42,** 942–946.
23. Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nuce, M. R., Ohannesian, J. P., Marco, C. C., McKee, L. J., Bauer, T. L., and Caro, J. F. (1996). *N. Engl. J. Med.* **334,** 292–295.
24. McGregor, G. P., Desaga, J. F., Ehlenz, K., Fischer, A., Heese, F., Hegele, A., Lämmer, C., Peiser, C., and Lang, R. E. (1996). *Endocrinology* **137,** 1501–1504.
25. Houseknecht, K. L., Mantzoros, C. S., Kuliawat, R., Hadro, E., Flier, J. S., and Kahn, B. B. (1996). *Diabetes* **45,** 1638–1643.
26. Sinha, M. K., Opentanova, I., Ohannesian, J. P., Kolaczynski, J. E., Heiman, M. L., Hale, J., Becker, G. W., Bowsher, R. R., Stephens, T. W., and Caro, J. F. (1996). *J. Clin. Invest.* **98,** 1277–1282.
27. Bonner, J. C., and Brody, A. R. (1995). *Am. J. Physiol.* **12,** L869–L878.
28. Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., Fei, H., Kim, S., Lallone, R., Ranganatahan, S., Kern, P.A., and Friedman, J. M. (1995). *Nature Med.* **1,** 1155–1161.
29. Dagogo-Jack, S., Fanelli, C., Paramore, D., Brothers, J., and Landt, M. (1996). *Diabetes* **45,** 695–698.
30. Dua, A., Hennes, M. I., Joffmann, R. G., Maas, D. L., Krakower, G. R., Sonnenberg, G. E., and Kissebah, H. (1996). *Diabetes* **45,** 1635–1637.
31. Larsson, H., Elmståhl, S., and Ahrén, B. (1996). *Diabetes* **45,** 1580–1584
32. Havel, P. J., Kasim-Karakas, S., Mueller, W., Johnson, P. R., Gingerich, R. L., and Stern, J. S. (1996). *J. Clin. Endocrinol. Metabol.* **81,** 4406–4413.
33. Kissebah, A., Vydelingum, N., Murray, R., Evans, D., Hartz, A., Kalkhoff, R. K., and Adams, R. W. (1982). *J. Clin. Endocrinol. Metab.* **54,** 254–260.
34. Kissebah, A. H., and Krakower, G. R. (1994). *Physiol. Rev.* **74,** 761–811.
35. Pratley, R. E., Tataranni, P. A., Snitker, S., Bogardus, C., and Ravussin, E. (1996). *Obes. Res.* **4,** 25S.
36. Kolaczynski, J. W., Considine, R. V., Ohannesian, J., Marco, C., Opentanova, I., Nyce, M. R., Myint, M., and Caro, J. F. (1996). *Diabetes,* **45,** 1511–1515.
37. Havel, P. J., Månsson, S., Gingerich, R. L., and Ahrén, B. (1997). *Am. J. Physiol.* **273,** R113–120.
38. Kolaczynski, J. W., Ohannesian, J., Considine, R. V., Marco, C. C., and Caro, J. F. (1996). *J. Clin. Endocrinol. Metab.* **81,** 4162–4165.
39. Hamilton, B. S., Paglia, D., Kwan, .Y. M., and Deitel, M. (1995). *Nature Med.* **1,** 953–956.
40. Lönnquist, F., Arner, P., Nordford, L., and Schalling, M. (1995). *Nature Med.* **1,** 950–953.
41. Hamann, A., and Matthaei, S. (1996). *Exp. Clin. Endocrinol. Diabetes,* **104,** 293–300.
42. Wei, M., Haffner, S. M., and Stern, M. P. (1996). *Obes. Res.* **4,** 44S.
43. Schwartz, M. W., Peskind, E., Raskin, M., Boyko, E. J., and Porte Jr, D. (1996). *Nature Med.* **2,** 589–593.
44. Fujioka, K., Patane, J., Manuak, C., and Staten, M. (1996). *Obes. Res.* **4,** 42S.
45. Frederich, R. C., Löllman, B., Hamann, A., Napolitano-Rosen, A., Kahn, B. B., Lowell, B. B., and Flier, J. S. (1995). *J. Clin. Invest.* **96,** 1658–1663.
46. Havel, P. J., Kasim-Karakas, S., Dubuc, G. R., Mueller, W., and Phinney, S. D. (1996) *Nature Med.* **2,** 949–950.
47. Sinha, M. K., Ohannesian, J. P., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Magosin, S., Marco, C., and Caro, J. F. (1996). *J. Clin. Invest.* **97,** 1344–1347.
48. Robyn, C. (1981). *Am. J. Physiol.* **241,** E355–E363.
49. Weitzman, E. D., Zimmerman, J. C., Czeisler, C. A., and Ronda, J. M., (1983). *J. Clin. Endocrinol. Metab.* **56,** 352–358.
50. van Coevorden, A., Laurent, E., Decoster, C., van Cauter, E., Kerkhof, M., Meve, P., and Mockel, J. (1989). *J. Clin. Endocrinol. Metab.* **69,** 177-185.
51. Bergman, R. N., Phillips, L. S., and Cobelli, C. (1981). *J. Clin. Invest.* **68,** 1456-1467.
52. Bogardus, C., Lillioja, S., Mott, D.M., Hollenbeck, C., and Reaven, G. (1985). *Am. J. Physiol.* **248,** E286–E291.
53. Bonadonna, R. C., and DeFronzo, R. A. (1991). *Diabetes Metab.* **17,** 112–135.
54. Olefsky, M., Kolterman, O. G., and Scarlet, J. A. (1982). *Am. J. Physiol.* **243,** E15–E30
55. Zunigo-Guajardo, S., Jimenez, J., Angel, A., and Zinman, B. (1986). *Metabolism 35,* 278–282.
56. Segal, K. R., Landt, M., and Klein, S. (1996). *Diabetes* **45,** 988–991.
57. Nolan, J. J., Olefsky, J. M., Nyce, M. R., Considine, R. V., and Caro, J. F. (1996). *Diabetes 45,* 1276–1278.
58. Larsson, H., and Ahrén, B. (1996). *J. Clin. Endocrinol. Metab.* **81,** 4428–4432.

59. De Vos, P., Saladion, R., Auwerx, J., and Staels, B. (1995). *J. Biol. Chem.* **270,** 15,958–15,961.
60. Kahn, S. E., Prigeon, R. L., McCulloch, D. S. K., Boyko, E. J., Bergman, E. N., Schwartz, M. W., Neifing, J. L., Ward, W.K., Beard, J. C., Palmer, J. P., and Porte Jr, D. (1993). *Diabetes* **42,** 1663–1672.
61. Larsson, H., and Ahrén, B. (1996). *Diabetologia* **39,** 1099–1107.
62. Wabitsch, M., Jensen, P. B., Blum, W. F., Christoffersen, C. T., Englaro, P., Heinze, E., Rascher, W., Teller, W., Tornqvist, H., and Hauner, H. (1996). *Diabetes* **45,** 1435–1438
63. Cusin, I., Sainsbury, A., Doyle, P., Rohner-Jeanrenaud, F., and Jeanrenaud, B. (1995). *Diabetes* **44,** 1467–1470.
64. Saladin, R., De Vos, P., Guerre-Millo, M., Leturque, A., Girard, J., Staels, B., and Auwerx, J. (1995). *Nature* **377,** 527–529.
65. Kieffer, T. J., Heller, R. S., and Habener, J. F. (1996). *Biochem. Biophys. Res. Commun.* **224,** 522–527.
66. Malmström, R., Taskinen, M. R., Karonen, S. L., and Yki-Järvinen, H. (1996). *Diabetologia* **39,** 993–996.
67. Utriainen, T., Malmström, R., Mäkimattila, S., and Yki-Järvinen, H. (1996). *Diabetes* **45,** 1364–1366.
68. Havel, P. J., Aoki, T. T., Grecu, E. O., Stern, J. S. and Kasim-Karakas, S. (1996). *Obes. Res.* **4,** 15S.
69. Ahrén B. and Larsson, H. (1996). *Diabetologia* **39,** A58.
70. Porte Jr, D. (1991). *Diabetes* **40,** 166–180.
71. Becker, D. J., Ongemba, L. N., Brichard, V., Henquin, J. C., and Brichard, S. M. (1995). *FEBS Lett.* **371,** 324–328.
72. Moinat, M., Deng, C., Muzzin, P., Assimacopoulos-Jeannet, F., Seydoux, J., Dulloo, A.G., and Giacobino, J.P. (1995). *FEBS Lett.* **373,** 131–134.
73. Trayhurn, P., Thomas, M. E. A., Duncan, J. S., and Rayner, D. V. (1995). *FEBS Lett.* **368,** 488–490.
74. Gettys, T. W., Harkness, P. J., and Watson, P. M. (1996). *Endocrinology* **137,** 4054–4057.
75. Mizuno, T. M., Bergen, H., Funabashi, T., Kleopoulos, S. P., Zgong, Y. G., Bauman, W. A., and Mobbs, C. V. (1996). *Proc. Natl. Acad. Sci. U.S.A.* **93,** 3434–3438.
76. Slieker, L. J., Sloop, K. W., Surface, P. L. K., Kriauciunas, A., Laquier, F., Manetta, J., Buevalleskey, J., and Stephens, T.W. (1996). *J. Biol. Chem.* **271,** 5301–5304.
77. Thompson, M. P. (1996). *Biochem. Biophys. Res. Commun.* **224,** 332–337.
78. Mantzaros, C., Qu, D., Frederich, R.C., Susulic, V.S., Lowell, B.B., Maratos-Flier, E., and Flier, J. S. (1996). *Diabetes* **45,** 909–914.
79. Donahoo, W. T., Jensen, D. R., Yost, T. J., and Eckel, R. H. (1996). *Obes. Res.* **4,** 39S.