Elevated Insulin Levels Contribute to the Reduced Growth Hormone (GH) Response to GH-Releasing Hormone in Obese Subjects

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We have recently presented experimental evidence indicating that insulin has a physiologic inhibitory effect on growth hormone (GH) release in healthy humans. The aim of the present study was to determine whether in obesity, which is characterized by hyperinsulinemia and blunted GH release, insulin contributes to the GH defect. To this aim, we used a simplified experimental protocol previously used in healthy humans to isolate the effect of insulin by removing the interference of free fatty acids (FFAs), which are known to block GH release. Six obese subjects (four men and two women; age, 30.8 ± 5.2 years; body mass index, 36.8 ± 2.8 kg/m^2 [mean ± SE]) and six normal subjects (four men and two women; age, 25.8 ± 1.9 years; body mass index, 22.7 ± 1.1 kg/m^2) received intravenous (IV) GH-releasing hormone (GHRH) 0.6 μg/kg under three experimental conditions: (1) IV 0.9% NaCl infusion and oral placebo, (2) IV 0.9% NaCl infusion and oral acipimox, an antilipolytic agent able to reduce FFA levels (250 mg at 6 and 2 hours before GHRH), and (3) euglycemic-hyperinsulinemic clamp (insulin infusion rate, 0.4 mU · kg^{-1} · min^{-1}). As expected, after placebo, the GH response to GHRH was lower for obese subjects versus normals (488 ± 139 *v* 1,755 ± 412 μg/L · 120 min, $P < .05$). Acipimox markedly reduced FFA levels and produced a mild reduction of insulin levels; under these conditions, the GH response to GHRH was increased in both groups, remaining lower in obese versus normal subjects (1,842 ± 360 *v* 4,871 ± 1,286 μg/L · 120 min, $P < .05$). In both groups, insulin infusion yielded insulin levels usually observed under postprandial conditions and reduced circulating FFA to the levels observed after acipimox administration. Again, the GH response to GHRH was lower for obese subjects versus normals (380 ± 40 *v* 1,075 ± 206 μg/L · 120 min, $P < .05$), and in both groups, it was significantly lower than the corresponding response after acipimox. In obese subjects, as previously reported in normals, the GH response to GHRH was inversely correlated with the mean serum insulin ($r = -.70$, $P < .01$). In conclusion, our data indicate that in the obese, as in normal subjects, the GH response to GHRH is a function of insulin levels. The finding that after both the acipimox treatment and the insulin clamp the obese still show higher insulin levels and a lower GH response to GHRH than normal subjects suggests that hyperinsulinemia is a major determinant of the reduced GH release associated with obesity.

THERE IS EXPERIMENTAL evidence that free fatty acids (FFAs) and insulin participate in the physiological control of growth hormone (GH) release. We have previously shown in healthy humans that the pharmacologic blockade of lipolysis by acipimox enhances the GH response to GH-releasing hormone (GHRH), mediated by the acute reduction of circulating FFA levels and the removal of their negative feedback on GH release.[1] More recently, we observed that when FFA levels are decreased by an insulin infusion yielding circulating insulin levels of 150 to 200 pmol/L (commonly observed during the day), the GH response to GHRH is significantly lower than that observed after acipimox,[2] indicating that, insulin per se, independently of FFA, exerts an inhibitory effect on GH release.

Obesity is characterized by high circulating FFA and insulin levels,[3,4] and blunted GH release.[5,6] A GH response to GHRH similar to that observed in normal subjects can be restored after pharmacologic blockade of lipolysis by acipimox,[6] suggesting that the excess of FFA has a crucial role in determining the altered GH release associated with obesity. However, when lipolysis is blocked to a similar extent by acipimox in normal and obese subjects, the GH response to GHRH still remains higher in the former compared with the latter group.[6] This may reflect the effect of circulating insulin levels, which, albeit slightly reduced by acipimox in both groups, still remain higher in the obese compared with normal subjects, notwithstanding the similarly low FFA levels. To elucidate the role that elevated insulin levels may play, independently of FFA, in the pathogenesis of the reduced GH release associated with obesity, in obese subjects we performed the same protocol previously used to study the FFA/GH/insulin interplay in normal subjects.[2] In the present study, the GH response to GHRH of obese subjects was evaluated at low and similar FFA levels obtained either by acipimox administration or by insulin infusion and compared with that of normal control subjects under the same conditions.

SUBJECTS AND METHODS

Subjects and Experimental Procedures

Six obese subjects (four men and two women; age, 30.8 ± 5.2 years; body mass index, 36.8 ± 2.8 kg/m^2 [mean ± SE]) and six normal control subjects properly matched for sex and age (four men and two women; age, 25.8 ± 1.9 years, $P > .05$ *v* obese subjects; body mass index, 22.7 ± 1.1 kg/m^2, $P < .05$ *v* obese subjects) were studied after provision of written informed consent. The normal control subjects were from a previous study.[2] The protocol of the current study was approved by the Ethics Committee of the Istituto Scientifico San Raffaele. All subjects were in good health and had a normal routine laboratory examination, normal endocrine function, and normal glucose tolerance after an oral glucose load (75 g) and were taking no medication.

According to a randomized crossover protocol, at 1-week intervals, all obese and normal control subjects underwent three GHRH tests (0.6 μg/kg intravenously [IV], corresponding to the 50-μg dose reported in Lanzi et al,[2] at 1:00 PM) during (1) IV 0.9% NaCl infusion (12:00 noon to 3:00 PM), with placebo administered orally at 7:00 and 11:00 AM, (2) IV 0.9% NaCl infusion (12:00 noon to 3:00 PM), with acipimox

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(Pharmacia Upjohn, Milan, Italy) 250 mg administered orally at 7:00 and 11:00 AM, and (3) 0.4 mU · kg^{-1} · min^{-1} euglycemic insulin clamp started at 11:00 AM in obese subjects and 12:00 noon in normal controls and continued until 3:00 PM, with placebo administered orally at 7:00 and 11.00 AM. The rate of insulin infusion was chosen to obtain steady-state serum insulin levels comparable to those reached physiologically in obese and normal subjects during the day, ie, under postprandial conditions.[4] In obese subjects, the insulin infusion was started earlier (at 11:00 AM), because when it was started at 12:00 noon as in normal subjects, it was not consistently able to decrease plasma FFA levels by the time of GHRH injection (1:00 PM) as required by the protocol.

All subjects were fasted overnight, and the tests were performed in the recumbent position. Blood samples for evaluation of plasma FFA, serum insulin (immunoreactive insulin [IRI]), and serum GH were drawn at −120 minutes (only in obese subjects during insulin clamp), −60 minutes, and immediately before GHRH administration (time 0) and at 10, 15, 30, 45, 60, and 120 minutes after, via an indwelling catheter inserted into a forearm vein at least 1 half-hour before beginning the sampling period. The euglycemic insulin clamp was performed as previously reported.[7]

Assays

Plasma FFA levels were measured by a spectrophotometric method adapted to the Cobas-Fara 2 instrument (Roche, Basel, Switzerland) using kits supplied by Italfarmaco (Milano, Italy). Intraassay and interassay coefficients of variation (CVs) were 2.3% and 3.1%, respectively. Serum IRI levels were measured by radioimmunoassay (RIA) using kits supplied by IncStar (Stillwater, MN). The minimum sensitivity of the assay was 13 pmol/L, and the intraassay and interassay CVs were 3.9% and 8.9%, respectively. Serum GH levels were measured by RIA using kits supplied by Farmos Diagnostic (Turku, Finland). The minimum sensitivity of the assay was 0.2 μg/L, and the median intraassay and interassay CVs for a GH concentration of 0.2 to 50 μg/L were less than 9% and 10%, respectively. Blood glucose levels were measured by a glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA).

Calculations and Statistical Analysis

Each variable is expressed as the mean ± SE at each time point. The integrated GH response to GHRH (GHΔ area under the curve 0 to 120 minutes) was calculated by the trapezoidal method. Statistical analysis for intragroup comparisons was performed by Student's *t* test for paired data. Intergroup comparisons were performed by Student's *t* test for unpaired data. Pairwise linear regression analysis was performed between GHΔ areas and mean serum IRI levels. *P* values less than .05 are considered statistically significant.

RESULTS

The results obtained in normal control subjects during the tests with acipimox and the insulin clamp have been reported previously.[2] In the present report, they are presented again for the sake of comparison to the data for obese subjects studied under the same conditions. Figure 1 shows plasma FFA, serum IRI, and serum GH levels before and after GHRH injection in obese subjects and normal controls. The tests with placebo are shown for both groups as a reference for the reader. For statistical analysis, only tests with acipimox and the insulin clamp were considered, since under these conditions, obese and normal control subjects showed similar plasma FFA levels and different circulating IRI levels. The mean plasma FFA, serum IRI, and GHΔ area during 2 hours after GHRH injection (0 to 120 minutes) are presented in Fig 2.

After acipimox, obese subjects and normal controls showed similarly low plasma FFA levels (0.05 ± 0.01 *v* 0.04 ± 0.01 g/L, NS), whereas the mean serum IRI level was higher in the obese compared with the normal controls (51 ± 22 *v* 12 ± 2 pmol/L, $P < .01$). The GH response to GHRH was lower in the former compared with the latter group (1,842 ± 360 *v* 4,871 ± 1,286 μg/L · 120 min, $P < .05$). Mean blood glucose levels (0 to 120 minutes) were similar in the two groups (obese *v* normal controls, 4.5 ± 0.03 *v* 4.4 ± 0.1 mmol/L, NS). Insulin infusion produced serum IRI levels of 350 to 500 pmol/L in obese subjects (420 ± 68 pmol/L, $P < .01$ *v* acipimox) and 150 to 200 pmol/L in normal controls (194 ± 19 pmol/L, $P < .01$ *v* acipimox and *v* obese subjects during insulin clamp). In both the obese and normal control subjects, insulin infusion reduced plasma FFA levels (0.04 ± 0.01 and 0.01 ± 0.01 g/L, respectively, NS) to the range observed in the two groups after acipimox. The GH response to GHRH was significantly lower for obese subjects versus normal controls (380 ± 40 *v* 1.075 ± 206 μg/L · 120 min, respectively, $P < .01$), and in both groups, it was significantly lower than the corresponding response after acipimox ($P < .01$). During the insulin clamp, mean blood glucose levels (0 to 120 minutes) were similar in obese subjects and normal controls (4.8 ± 0.02 *v* 4.6±0.1 mmol/L, NS). In both groups, they were slightly higher than the levels obtained after acipimox, and this difference was statistically significant due to the very low dispersion of data in the two tests ($P < .05$ in obese and $P < .01$ in normal controls).

In obese subjects, similar to the previous report in normal subjects,[2] at low FFA levels (ie, during tests with either acipimox or insulin infusion), a significant negative regression was observed between the mean serum IRI levels and the GH response to GHRH (GHΔ area, $r = -.70$, $P < .01$). No correlations were found between the mean blood glucose levels and the GH response to GHRH under the same experimental conditions.

DISCUSSION

Obesity is characterized by high circulating FFA and insulin levels and blunted GH release. GH release similar to that of normal subjects can be restored by an acute reduction of circulating FFA levels induced by acipimox, a pharmacologic agent able to block lipolysis.[6,8] However, when lipolysis is blocked to a similar extent by acipimox, the GH response to GHRH remains lower for obese subjects versus normals.[6] The data from the present study suggest that the reduced GH release observed in obese subjects also at low FFA levels may reflect circulating insulin levels that are significantly higher versus the levels in normal subjects. In fact, both after acipimox and during the insulin clamp, obese subjects at similarly low FFA and comparable glucose levels showed higher insulin levels and lower GH responsiveness to GHRH than normal controls. Furthermore, as in normals,[2] the GH response to GHRH of obese subjects was significantly lower during the insulin clamp versus after acipimox and was inversely related to circulating insulin levels. Also, blood glucose levels were slightly higher during the insulin clamp than after acipimox, but, in our opinion, the minimal (although statistically significant) changes observed across the tests render their impact on GH release irrelevant. It is also of note that no correlations were found

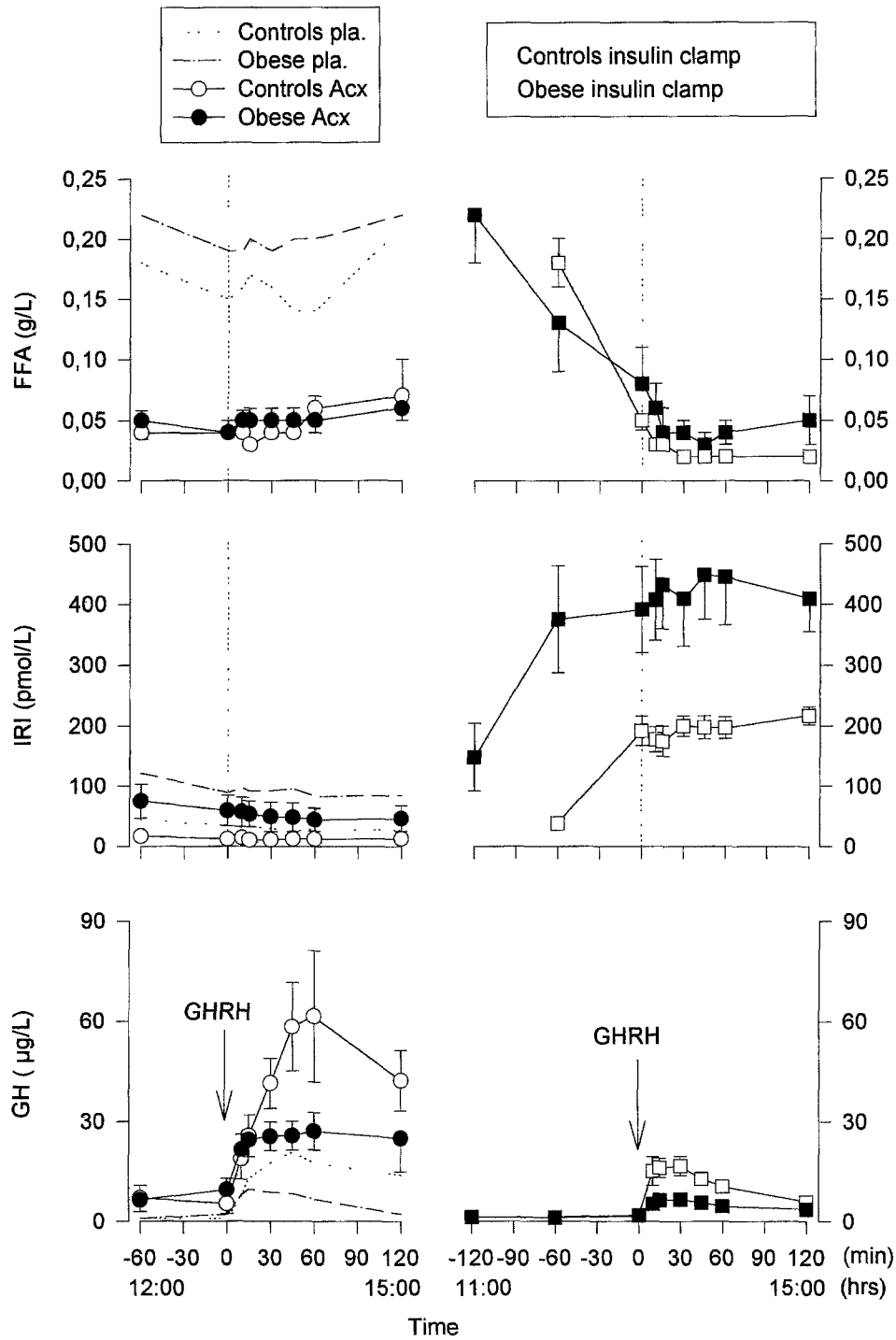

Fig 1. Plasma FFA, serum IRI, and serum GH levels in 6 obese subjects and 6 normal controls (1) after placebo administration and during 0.9% NaCl infusion, (2) after acipimox administration and during 0.9% NaCl infusion; and (3) after placebo administration and during hyperinsulinemic-euglycemic insulin clamp. Each time point represents the mean ± SE. Data after placebo and during NaCl infusion are presented only as a reference for the reader. Pla., placebo; Acx, acipimox; 15:00 h = 3:00 PM.

between the GH response to GHRH and blood glucose levels in both the obese and the normal subjects. In the absence of the interference of FFA and considering the role of blood glucose negligible under these experimental conditions, our data document a pathophysiological role for hyperinsulinemia in the reduced GH release associated with obesity. In fact, the insulin levels reached in this study are commonly observed during the day, eg, at nighttime or during fasting (acipimox) and under postprandial conditions (insulin clamp).[4] In this regard, by comparing the results of the present study and those of a

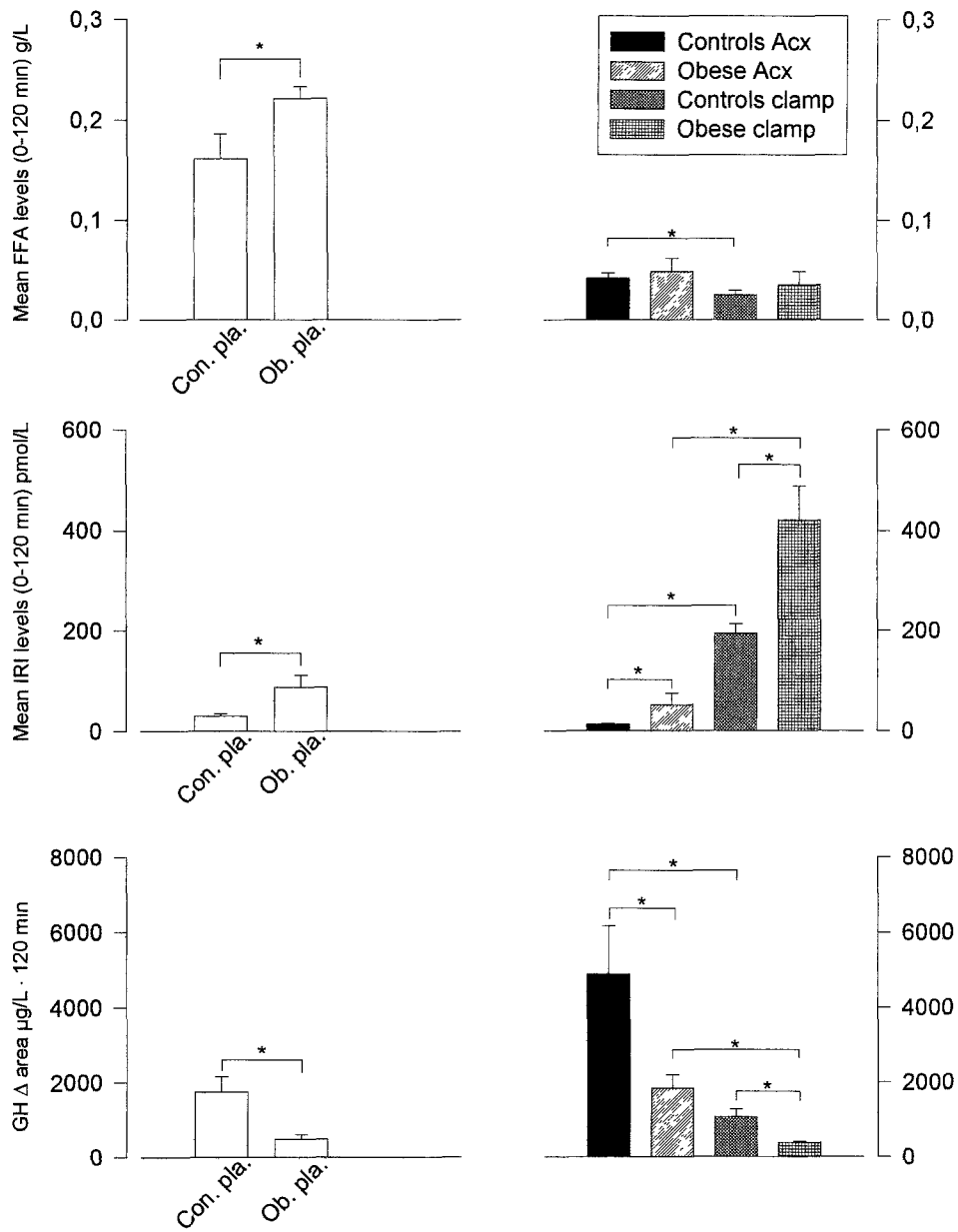

Fig 2. Mean plasma FFA and serum IRI levels and mean GHΔ area within the interval 0-120 minutes after GHRH injection in the 3 tests. Each value represents the mean ± SE. Acx, acipimox; pla., placebo; Con., normal control subjects; Ob., obese subjects. *$P < .05$ or less.

previous report,[6] clear evidence is apparent. In obese subjects, the acute reduction of FFA levels by acipimox results in GH release similar to that of normal subjects after placebo, despite insulin levels that remain mildly elevated. In contrast, at similarly low FFA levels but higher insulin levels (as during the euglycemic insulin clamp), the GH response to GHRH of obese subjects remains significantly lower than that of normal subjects after placebo (comparison not shown). Taken together, these data suggest that the elevation of both FFA and insulin contributes to the reduced GH release associated with obesity. Particularly, high FFA levels seem to have a major role under conditions of mild hyperinsulinemia (eg, nighttime and fasting), while at higher circulating insulin levels (eg, under postprandial conditions), insulin action becomes predominant. Experimental data support a possible negative effect of peripheral insulin on GH release, occurring both at the level of the pituitary or (predominantly) the hypothalamus.[9-12] A thorough discussion on this topic can be found in a previous report from our group.[2]

In conclusion, our data indicate that in obese subjects, GH responsiveness to GHRH, among other factors including circulating FFA, is a function of circulating insulin levels. The finding that after both acipimox and the insulin clamp obese subjects still show higher insulin levels and a lower GH response to GHRH than normal subjects suggests that hyperinsulinemia (mild or severe depending on the time of day) plays a relevant role in the reduced GH release associated with obesity.

REFERENCES

1. Pontiroli AE, Lanzi R, Monti LD, et al: Effect of acipimox, a lipid lowering drug, on growth hormone (GH) response to GH-releasing hormone in normal subjects. J Endocrinol Invest 13:539-542, 1990

2. Lanzi R, Manzoni MF, Andreotti AC, et al: Evidence for an inhibitory effect of physiological levels of insulin on the growth hormone (GH) response to GH-releasing hormone in healthy subjects. J Clin Endocrionol Metab 82:2239-2243, 1997

3. Opie LH, Walfish PG: Plasma free fatty acid concentration in obesity. N Engl J Med 268:757-760, 1963

4. Golay A, Swislocki AL, Chen YD, et al: Effects of obesity on ambient plasma glucose, free fatty acid, insulin, growth hormone, and glucagon concentration. J Clin Endocrinol Metab 63:481-484, 1986

5. Veldhuis JD, Iranmanesh A, Ho KKY, et al: Dual defects in pulsatile growth hormone secretion and clearance subserve the hyposomatotropism of obesity in man. J Clin Endocrinol Metab 72:51-59, 1991

6. Pontiroli AE, Manzoni MF, Malighetti ME, et al: Restoration of growth hormone (GH) response to GH-releasing hormone in elderly and obese subjects by acute pharmacological reduction of plasma free fatty acids. J Clin Endocrinol Metab 81:3998-4001, 1996

7. Luzi L, Battezzati A, Perseghin G, et al: Combined pancreas and kidney transplantation normalizes protein metabolism in IDD uremic patients. J Clin Invest 93:1948-1958, 1994

8. Andreotti AC, Lanzi R, Manzoni MF, et al: Acute pharmacologic blockade of lipolysis normalizes nocturnal growth hormone levels and pulsatility in obese subjects. Metabolism 43:1207-1213, 1994

9. Schwartz MW, Figlewicz DP, Baskin DG, et al: Insulin in the brain: A hormonal regulator of energy balance. Endocr Rev 13:387-414, 1992

10. Baskin DG, Porte D Jr, Guest K, et al: Regional concentrations of insulin in the rat brain. Endocrinology 112:898-903, 1983

11. Baskin DG, Stein LJ, Ikeda H, et al: Genetically obese Zucker rats have abnormally low brain insulin content. Life Sci 36:627-633, 1985

12. Folli F, Bonfanti L, Renard E, et al: Insulin receptor substrate-1 (IRS-1) distribution in the rat central nervous system. J Neurosci 14:6412-6422, 1994