Dexamethasone Treatment Induces Long-Lasting Hyperleptinemia and Anorexia in Old Rats

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Aging brings poor adaptation to stress, the causes of which remain unclear. We previously reported impairment of nitrogen metabolism in glucocorticoid-treated old rats due to profound anorexia. Here we investigated whether leptin, a satiety hormone, was implicated in impaired adaptation to stress. Plasma glucose and insulin levels, which are known to modulate leptin secretion, were also studied. Adult (3 months, n = 18) and aged (24 months, n = 18) rats were treated with dexamethasone (DEX) (1.5 mg/kg/d, intraperitoneal [IP] injection) for 3, 5, and 7 days. Results were compared with ad libitum (n = 12) and pair-fed groups, receiving intraperitoneal saline injection, for each age (n = 6 per group). Transitory anorexia was observed in adult rats (day 3 to day 5), whereas anorexia persisted in aged rats until day 7. This anorexia was associated ($r = -.65$, $P < .05$) with an elevated constant hyperleptinemia. In contrast, hyperleptinemia was moderate and reverted rapidly to basal values by day 5 in adult rats. The time course of plasma insulin and glucose levels was similar in old and adult rats, except for marked hyperglycemia noted in aged animals. In old stressed rats, DEX treatment induces an anorexia, which is concomitant to an increase in serum leptin levels. Thus, leptin may be implicated in the poor adaptation to stress of aged compared with adult rats.

THE INCREASE IN life span and aging of the population have sharpened interest in aging studies. The elderly display heightened sensitivity to aggression[1] and thereby an altered response to stress, which may be a factor of morbidity and mortality. After trauma, sepsis, or surgery, elderly patients are unable to recover their preinjury nutritional status rapidly and completely.[2-4] The mechanism of this altered response is multifactorial[5] and is still unclear. Glucocorticoid secretion is increased during stress,[6-9] and this hormone plays a pivotal role in metabolic adaptation to stress.[10] Administering pharmacologic doses of dexamethasone (DEX), we[11] and others[12-14] clearly demonstrated an abnormality of response to glucocorticoid in old compared with adult rats: Dardevet et al[12] showed that after withdrawal of glucocorticoids, adult rats rapidly restored their muscle protein mass within 3 days, whereas recovery was delayed to 7 days in old animals. In adult rats, recovery resulted from a decrease in proteolysis and an increase in protein synthesis,[12] whereas in old rats, only an increase in protein synthesis occurred.[12-14] In a recent study,[11] we demonstrated that response to chronic injection of DEX was delayed in aged rats compared with adults, nitrogen balance decreasing from day 1 in adults, but only from day 4 in old rats. However, the adult rats rapidly recovered a normal food intake after the fourth day of treatment, whereas a profound anorexia and negative nitrogen balance developed and persisted in elderly stressed rats. Using pair-fed controls, we demonstrated that this marked anorexia was the main factor accounting for the negative nitrogen balance in these animals. The cause of this spectacular DEX-induced anorexia in old rats remains unknown.

Leptin is a recently discovered 167 amino acid protein, mainly produced by white adipose tissue, transcribed from the *ob* gene.[15] This hormone limits food intake and increases energy expenditure.[16,17] It is thus a major sensor of body adiposity, and it regulates the central process of appetite control. In addition, De Vos et al[18] have established that glucocorticoid administration induces *ob* gene expression in rodents. Overall, these findings suggest that a dysregulation of leptin secretion might be implicated in the long-lasting anorexia observed during the stress response induced by glucocorticoids in old rats. The aim of this study was therefore to test this hypothesis by comparing leptin plasma levels in adult and old rats as a function of duration of treatment by DEX, a highly reproducible model of hypercatabolism,[19] and to establish whether anorexia is linked to leptinemia. In addition, since glycemia[18,20] and insulinemia[21-23] are known to modulate leptin secretion, possible relationships between these 2 parameters and leptinemia were also sought.

MATERIALS AND METHODS

Animal Procedures

A total of 96 male Sprague Dawley rats, supplied by Iffa Credo (l'Arbresles, France), were used. They were 3 months old (370 ± 4 g, n = 48) or 24 months old (629 ± 38 g, n = 48). After their arrival in our animal facilities, the rats received a standard diet (17% protein, 3% fat, 59% carbohydrates, 21% water, fiber, vitamins, and minerals: UAR A04, Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France) and water ad libitum. They were kept in a controlled environment (constant temperature 24°C and a light cycle of 12 hours on/12 hours off). After 10 days of acclimatization in individual standard cages followed by 5 days in individual metabolic cages, the rats in each age category were randomly assigned into 3 treated groups (n = 6 in each group) and 1 untreated group (n = 12): G3, G5, G7, and G0, respectively, for adults and G3′, G5′, G7′, and G0′ for old rats. As previously described, experimental stressed groups G3, G5, G7, G3′, G5′, and G7′ received 1.5 mg/kg/d of DEX (Soludecadron, MSD, Chibret, France) by intraperitoneal (IP) injection for 3, 5, and 7 days, respectively.[11,19]

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Submitted September 28, 2000; accepted March 12, 2001.

Supported by a research fellowship from the European Society of Parenteral and Enteral Nutrition.

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0026-0495/01/5009-0026$35.00/0

doi:10.1053/meta.2001.25803

Because treatment by DEX induces anorexia, pair-fed control groups (PF, n = 6 in each group) were studied simultaneously for each treated group: G3PF/G3′PF, G5PF/G5′PF, and G7PF/G7′PF *versus*, respectively, G3/G3′, G5/G5′, and G7/G7′. They were managed as DEX-treated animals, but were injected with an isovolumic solution of 0.9% NaCl instead of DEX. G0 and G0′ groups received no treatment and were fed ad libitum throughout the study. G0PF and G0′PF were obtained by random division of G0 and G0′ groups, respectively.

During experimentation, body weight and food intake were recorded daily from day 0 to day 7. On days 4, 6, or 8, 24 hours after the last DEX injection (8:00 AM), rats were anesthetized and killed by decapitation for blood collection. Animal care and experimentation complied with the rules of our institution and 2 of us (M-P. V. and L.C.) are authorized by the French Ministry of Agriculture and Forestry to use animal models of stress.

Sample Collection

Blood was collected on sodium heparinized tubes and was centrifuged (1,500*g*, 10 minutes, +4°C). Plasma was then stored at −80°C until assayed.

Assays

Leptinemia (ng/mL) was measured by radioimmunoassay using a "rat leptin RIA kit" (Linco Research, MO). The sensitivity limit of the method was 0.5 ng/mL and coefficient of variation (CV) for reproductibility was less than 4.5%. Glycemia (mmol/L) was determined by an enzymatic assay (CV < 2.9%) involving glucose oxidase using the Hitachi 911 analyzer (Mannheim, Germany). Plasma insulin concentrations (ng/mL) were determined by radioimmunoassay using a "rat insulin RIA kit" (Linco Research) with a sensitivity limit of 0.1 ng/mL and a CV less than 4.5%.

Statistics

Data are represented as mean ± SEM. For food intake, measured daily in live animals, an analysis of variance (ANOVA) on repeated measurements with 1 factor (duration of treatment) was performed in either adult or old rats, with estimation of missing values. For the other parameters, a 2-way ANOVA was performed in either adult or old rats to discriminate the effect of DEX itself (d) and duration of treatment (D) as main factors (see the Results section for more information). The effects of these factors were analyzed by the Newman-Keüls test. The PCSM software (Deltasoft, Grenoble, France) was used. Differences at P less than .05 were considered significant.

RESULTS

During DEX treatment, body weight of adult rats was significantly reduced by 12% in the G3 and G5 groups (G3, G5 *v* G0, $P < .01$) and by 23% in the G7 group (G7 *v* G0, $P < .01$). In old rats, a significant decrease in body weight of 10%, 15%, and 18% in the G3′, G5′, and G7′ groups, respectively, *v* G0′ untreated rats was observed ($P < .01$).

Transitory anorexia was induced by DEX treatment in adult rats from day 3 to day 5 (G3, G5 *v* G0, $P < .01$ and .05, respectively). In old DEX-treated rats, anorexia occurred from day 3 and worsened until the end of treatment (G3′, G5′, G7′ *v* G0′, $P < .01$) (Fig 1). In PF groups (Table 1), anorexia induced in either adult or old rats seemed to decrease leptinemia, glycemia, and insulinemia compared with G0 and G0′PF groups, and no effect of duration was observed. Moreover, these effects of anorexia were contrary to those observed in the corresponding DEX-treated group (Table 1).

Old rats displayed higher leptin plasma levels than adults at the basal state (14.3 ± 4.3 and 1.9 ± 0.9 ng/mL in G0′ and G0, respectively). Leptin level increased (3-fold) in adult rats after 3 days of treatment with DEX (G3 *v* G0, $P < .05$) and returned to basal values by day 5 (Fig 1). In old rats, DEX induced a 3-fold increase in leptinemia by day 3 (G3′ *v* G0′, $P < .01$), and plasma leptin remained at the same high level until the end of the experiment (G5′, G7′ *v* G0′, $P < .01$ and $P < .05$, respectively) (Fig 1). In addition, a negative correlation was found only in aged rats between the calculated variation of food intake from day 0 to day of sacrifice and leptinemia at the day of sacrifice ($r = -.65$, $P < .05$, Fig 2). This correlation was not observed for adult rats ($r = -.42$, not significant [NS]).

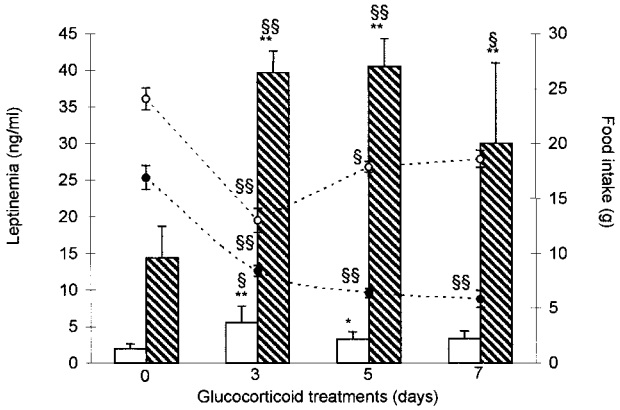

Fig 1. Plasma leptin levels (ng/mL) and food intake (g) of adult/old ad libitum fed rats (G0/G0′ groups) and DEX (1.5 mg/kg/d)-treated rats (G3/G3′, G5/G5′, G7/G7′ groups). Adult and old rats received 1.5 mg/kg of DEX for 3, 5, or 7 days by IP injection. Values are mean ± SEM for n = 6 rats in each group. For leptinemia, a 2-way ANOVA analysis was performed in adult (□) and old (▨) rats to discriminate between effects of dexamethasone (d), duration of treatment (D) and their interaction (d.D). For adult rats, a significant effect of d ($P < .0001$), D ($P < .01$), and d.D ($P < .002$) was noted and for old rats, a significant effect of d ($P < .0001$) was observed. For food intake, a 1-way ANOVA on repeated measurements was performed in either adult (○) or old (●) rats. Comparison of means was performed with the Newman-Keüls test: * $P < .05$ or ** $P < .01$ *v* PF control (values in Table 1), § $P < .05$ or §§ $P < .01$ *v* untreated rats.

In adult rats, glycemia was slightly increased (1.5 times basal values) by DEX treatment at day 5 (G5 *v* G0, $P < .05$) and returned to basal level by day 7 (Fig 3). In aged rats, a marked hyperglycemia (3 times basal values) occurred at day 5 (G5′ *v* G0′, $P < .01$) with a return to basal values at day 7 (Fig 3). No correlation was found between glycemia and leptinemia in adult or in old rats.

Insulinemia significantly increased from day 3 to day 7 in both adult (G3 *v* G0, $P < .05$ and G5, G7 *v* G0, $P < .01$) and old rats (G3′, G5′, G7′ *v* G0′, $P < .01$) (Fig 4). No correlation was found between insulinemia and leptinemia ($r = .32$ and $r = .60$) or insulinemia and glycemia ($r = .43$ and $r = .48$) both in adult and old rats, respectively.

DISCUSSION

Profound anorexia in response to DEX treatment is a characteristic of aged rats as reported in the recent literature.[11] Interestingly, a similar effect has been observed in other models of stress, for example after endotoxin administration.[24] Here, to

Table 1. Effects of Pair Feeding on Leptinemia, Insulinemia, Glycemia of Adult and Old Rats Compared With Corresponding Dex-Treated Groups

	Adult Rats (3 months)							
	G0	G0PF	G3	G3PF	G5	G5PF	G7	G7PF
Leptinemia (ng/mL)	1.9 ± 0.9	1.9 ± 0.9	6.0 ± 1.0	1.4 ± 0.4*	3.2 ± 0.4	1.0 ± 0.5†	3.3 ± 0.3	1.2 ± 0.4
Insulinemia (ng/mL)	1.2 ± 0.2	1.2 ± 0.2	9.3 ± 1.5	0.6 ± 0.2†	17.4 ± 2.7	0.8 ± 0.1*	14.3 ± 2.8	0.5 ± 0.1*
Glycemia (mmol/L)	9.6 ± 0.2	9.6 ± 0.2	10.5 ± 0.4	7.8 ± 0.5	13.8 ± 1.5	8.9 ± 0.3*	11.1 ± 3.6	8.6 ± 0.5
	Old Rats (24 months)							
	G0′	G0′PF	G3′	G3′PF	G5′	G5′PF	G7′	G7′PF
Leptinemia (ng/mL)	14.3 ± 4.3	14.3 ± 4.3	39.7 ± 3.0	4.8 ± 1.1*	40.5 ± 3.8	8.6 ± 2.0*	30.0 ± 11.0	11.6 ± 2.8*
Insulinemia (ng/mL)	1.4 ± 0.4	1.4 ± 0.4	15.4 ± 2.9	0.7 ± 0.1*	19.6 ± 2.6	1.1 ± 0.3*	13.9 ± 3.9	0.9 ± 0.3*
Glycemia (mmol/L)	8.6 ± 0.9	8.6 ± 0.9	10.3 ± 0.4	7.4 ± 0.4	26.1 ± 4.1	7.4 ± 0.4*	10.7 ± 1.6	7.8 ± 0.1

NOTE. Adult (G0, G3, G5, G7) and old rats (G0′, G3′, G5′, G7′) were treated during 0, 3, 5, or 7 days by DEX (1.5 mg/kg/d, IP), respectively. PF groups were studied for each treated group: G0PF/G0′PF, G3PF/G3′PF, G5PF/G5′PF, G7PF/G7′PF *v*, respectively, G0/G0′, G3/G3′, G5/G5′, G7/G7′. Values are mean ± SEM. A 2-way ANOVA was performed in either adult and old rats following by the Newman-Keüls test: *$P < .01$ *v* corresponding DEX-treated group. †$P < .05$.

investigate the origin of the profound anorexia in DEX-treated old rats, we examined the variations in plasma levels of leptin, a mediator involved in the regulation of satiety.

In our study, PF controls were used to discriminate between the effect of anorexia induced by treatment and the effect of DEX itself on the parameters measured. As expected,[25-27] a decrease in leptinemia, insulinemia, and glycemia was observed in the PF groups, while in the DEX-treated groups, these parameters were increased. This, therefore, enabled us to separate the effects of the anorexia itself, and so focus our study on the results for the treated and untreated groups only.

In untreated rats, plasma leptin levels were higher in old than in adult rats. Similar results have been reported by Li et al,[28] who supposed that they could be related to the higher fat mass in old rats and/or to an increase in *ob* ARNm expression in adipose tissue. Indeed, we found by absorptiometry measurement that untreated old rats had a higher body fat mass composition than untreated adults (34.1 ± 3.6 g and 10.9 ± 2.1 g, respectively, unpublished data). Thus, fat mass corresponds to 5% of body weight in old and 3% in adult rats. However, this fat mass difference may not be the only factor responsible for the difference in leptin levels with age.

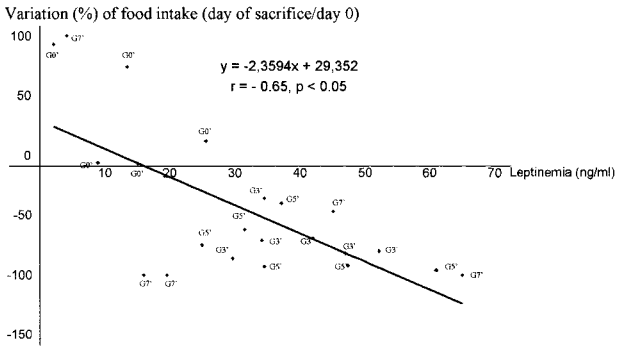

Fig 2. Correlation between variation of food intake (day of sacrifice/day 0) expressed in percent and leptinemia in old DEX-treated rats (G3′, G5′, and G7′ groups) and untreated old rats (G0′ group).

During DEX treatment, a decrease of body weight is observed both in adult and old rats, as previously reported by Minet-Quinard et al.[11] Interestingly, with the same treatment, our results clearly demonstrated a transitory increase in leptin plasma concentrations in adult rats, which rapidly returned to basal values. In these animals, anorexia was also transitory (occurring from the beginning of treatment and disappearing on day 5). The relationship between leptin secretion and control of food intake is now well established, and the hypothalamus appears to be an important site of leptin action.[29] Indeed, a single intracerebroventricular injection of leptin reduces food intake at doses that have no effect when delivered peripherally.[29] Interestingly, the effect of leptin administration is

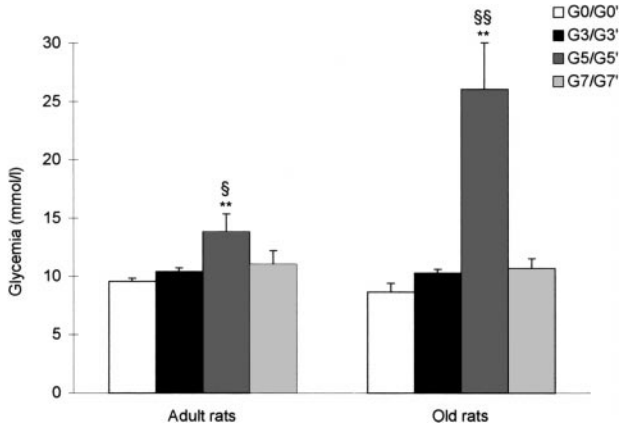

Fig 3. Plasma glucose (mmol/L) levels in adult and old rats for untreated groups (G0/G0′), and DEX (1.5 mg/kg/d)-treated groups (G3/G3′, G5/G5′, G7/G7′). Adult and old rats received 1.5 mg/kg of DEX for 3, 5, or 7 days by IP: injection. Values are mean ± SEM for n = 6 rats in each groups. A 2-way ANOVA analysis was performed to discriminate between effects of d, D, and d.D. For adult rats, a significant effect of d ($P < .0001$) and for old rats, a significant effect of d ($P < .0001$), D ($P < .0001$), and d.D ($P < .0001$) were observed. Comparison of means was performed with the Newman-Keüls test: * $P < .05$ and ** $P < .01$ *v* PF control (values in Table 1), § $P < .05$ or §§ $P < .01$ *v* untreated rats.

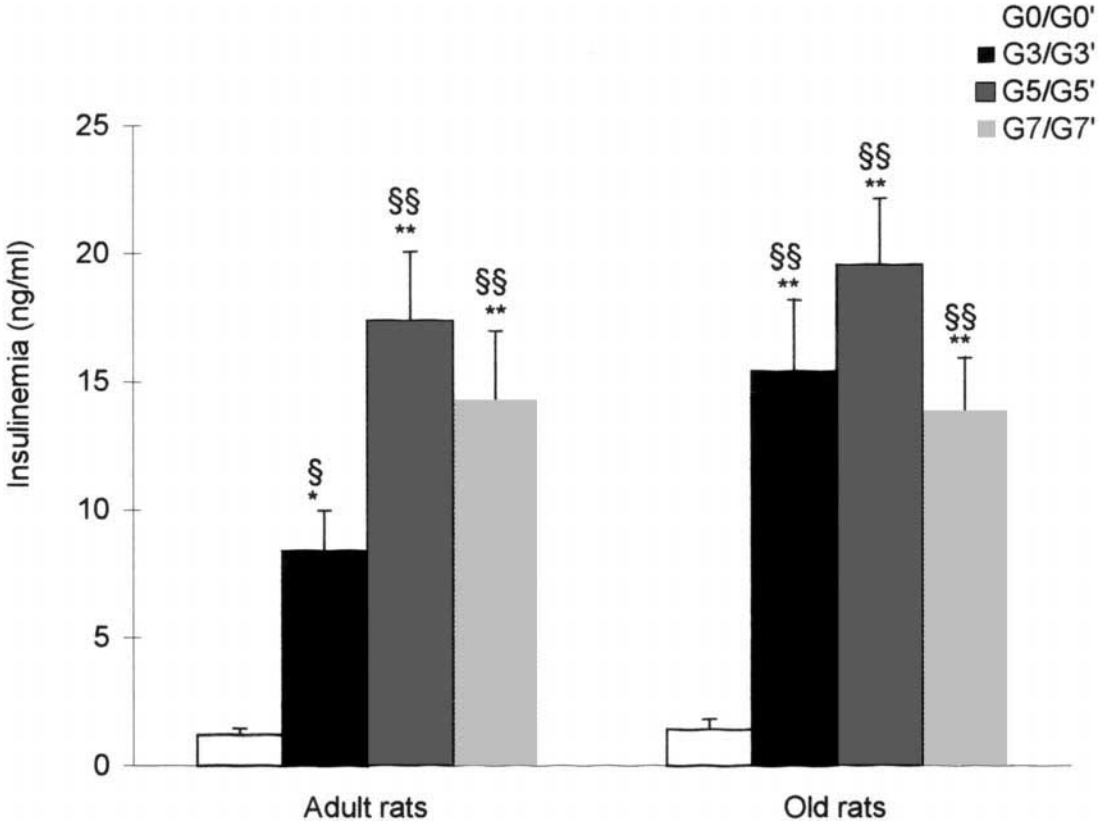

Fig 4. Plasma insulin (ng/mL) levels in adult and old rats for untreated groups (G0/G0′), and DEX (1.5 mg/kg/d)-treated groups (G3/G3′, G5/G5′, G7/G7′). Adult and old rats received 1.5 mg/kg of DEX for 3, 5, or 7 days by IP: injection. Values are mean ± SEM for n = 6 rats in each group. A 2-way ANOVA analysis was performed to discriminate between effects of d, D, and d.D. For adult and old rats, significant effects of d ($P < .0001$), D ($P < .0001$), and d.D ($P < .0001$) were observed. Comparison of means was performed with the Newman-Keüls test: * $P < .05$ and ** $P < .01$ *v* PF control (values in Table 1), § $P < .05$ and §§ $P < .01$ *v* untreated rats.

transitory because reduction of food intake persists until the adipose tissue mass has been depleted, after which food intake returns to normal.[30] This could explain the transitory decrease in food intake associated with the transitory increase in plasma leptin in adult rats. These data suggest that in adult stressed rats, anorexia was consecutive to hyperleptinemia, and that the normalization of leptinemia is associated with the improvement of both food intake and nitrogen balance.[11] Importantly, in old rats, the increase in leptinemia occurred also at day 3 and persisted throughout the treatment. In parallel, anorexia was irreversible and was accompanied by a large negative nitrogen balance.[11] Thus our data show for the first time that characteristic anorexia observed in stressed old animals is secondary to the increase in leptin.

Furthermore, we report a marked hyperglycemia at day 5 in old stressed rats, which disappeared at day 7 probably because of a hypometabolism. This hyperglycemia may reflect an insufficiency of insulin secretion or an attenuation of tissular sensitivity to insulin. In support of the first hypothesis, Kieffer et al[31] have demonstrated, in vitro, that leptin may be a direct inhibitor of insulin secretion. Thus, an increased leptin level would act as an inhibitor of insulin secretion. However, in our study, insulin levels were high in old rats in response to DEX and similar to values noticed in adult rats. Hence, our data suggest that in elderly stressed rats, leptin does not exert its negative feed-back effect on insulin secretion as previously described in adult rats.[31,32] According to Scarpace et al,[33] we hypothetize about a leptin-mediated resistance process in aged rats, which may explain the failure of leptin to regulate insulin secretion in aged rats. Therefore, hyperglycemia is probably induced by a decline in peripheral tissue sensitivity to insulin, *ie*, insulin resistance often described in old animals.[34,35] Also in favor of a leptin-dependent process, it has been shown that leptin may be able to impair the metabolic action of insulin, particularly on its stimulation of glucose transport and activation of glycogen synthase activity.[36] Moreover, leptin is known to interact with the transduction of insulin messages, particularly on insulin receptor substrate-1 (IRS-1) phosphorylation and insulin receptor autophosphorylation,[33] which may help install hyperglycemia. Nevertheless, it has been shown that molecular mechanisms of leptin transduction are common to the molecular pathways of insulin involving the PI3 kinase.[37] Thus, leptin may be implicated in muscular insulin resistance observed in type *2* diabetes.[37]

In conclusion, we demonstrate for the first time that in old DEX-treated rats, profound anorexia and hyperleptinemia are

long lasting, whereas adult rats are able to adapt to the stress and rapidly recover normal levels of food intake and leptinemia. Our data also suggest that this dysregulation of leptin homeostasis aggravates insulin resistance often described in aging and so help install hyperglycemia. However, the relationship between leptin and insulin regulation seems to be profoundly altered in elderly animals. This data should be confirmed in studies dealing with humans, and further research is required to understand the effective role of leptin in insulin resistance of the elderly.

ACKNOWLEDGMENT

We are indebted to P. Davot in our department for expert technical assistance.

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