Assessment of the Primary Adrenal Cortical and Pancreatic Hormone Basal Levels in Relation to Plasma Glucose and Age in the Unstressed Ames Dwarf Mouse (43931)

KURT E. BORG, HOLLY M. BROWN-BORG, AND ANDRZEJ BARTKE[1]
Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, Illinois 62901-6512

Abstract. **Peripheral glucose concentrations in mammals are maintained within very narrow limits to provide a continuous, uninterrupted supply of this nutrient to tissues. Numerous factors have been shown to influence and/or regulate glucose levels. One such influence is growth hormone (GH) produced by the pituitary somatotrophs. Several animal models of hyposomatotropism are available in which GH secretion or actions are suppressed due to genetic abnormalities. One such model, the Ames dwarf mouse (*df/df*), has arisen from an autosomal recessive mutation in which GH-, prolactin- (PRL), and thyroid-stimulating hormone (TSH)-producing cell types of the anterior pituitary fail to develop. The current investigation examined the effects of GH deficiency on glucose, insulin, and corticosterone levels using male and female *df/df* mice and their normal (*Df/−*) littermates. Additionally, old and young females of both genotypes were used to determine whether aging and GH deficiency interact to influence insulin, corticosterone or glucose levels in these animals. Plasma samples collected from unstressed animals (normal, *df/df*; young [5 months], old [17–19 months]; male, female) were used. Glucose levels were lower ($P < 0.05$) in *df/df* than in *Df/−* mice regardless of sex and age. A sex difference in *Df/−* animals was evident—young and old females had significantly lower levels of glucose when compared with young *Df/−* males. Plasma insulin was elevated ($P < 0.05$) in old *df/df* females compared with young *df/df* and *Df/−* females. Young *Df/−* males had the highest insulin levels compared with all genotype and age groups. This observation paralleled results from glucose measurements. Corticosterone levels were highest in young *Df/−* females and lowest in young *Df/−* males, with *df/df* animals falling between these values. Plasma corticosterone levels in old *Df/−* females did not differ from the values measured in dwarfs. The present findings indicate that glucose and factors affecting glucose levels are altered in the *df/df* mouse. These results provide new insights into the roles GH may play in glucose metabolism and perhaps also in adiposity which is a common characteristic of *Df/−* aged females from this line of mice.** [P.S.E.B.M. 1995, Vol 210]

The regulation of circulating glucose levels is a negative feedback system that primarily involves the endocrine pancreas. Elevated plasma glucose, in response to postprandial metabolism, results in a secretion of insulin from the β cells of the pancreas to reduce plasma glucose levels. Conversely, as blood glucose levels fall during periods of fasting or exercise, pancreatic α cells respond by secreting glucagon to mobilize glycogen stores in the liver, which

[1] To whom requests for reprints should be addressed at Department of Physiology, School of Medicine, Southern Illinois University, Carbondale, IL 62901-6512.

Received December 20, 1994. [P.S.E.B.M. 1995, Vol 210]
Accepted May 22, 1995.

0037-9727/95/2105-0000$10.50/0
Copyright © 1995 by the Society for Experimental Biology and Medicine

liberates glucose into the periphery (1–3). Glucocorticoids, such as cortisol in humans and corticosterone in rodents, have integrated roles in the glucose regulatory pathway of the endocrine pancreas. Glucocorticoids stimulate gluconeogenesis by upregulating key enzymes in this pathway in hepatocytes (4). In opposition to the anabolic actions on the liver, glucocorticoids are catabolic in actions targeting skeletal muscle and adipose tissue. Such actions inhibit glucose uptake by these tissues, and thereby make more glucose available to tissues and organs, such as the brain, that have an obligatory need for steady nutrient supply.

The Ames dwarf (*df/df*) is a mouse model of pituitary dwarfism caused by an autosomal recessive mutation that was first described by Schaible and Gowen (5). The pituitary of *df/df* animals, similar to the pituitary of the Snell dwarf mouse (*dw/dw;* 6), is lacking acidophils, which are the source of growth hormone (GH) and prolactin (PRL) in normal animals (7, 8). In addition, *df/df* and *dw/dw* animals are hypothyroid (9, 10) as a result of thyroid-stimulating hormone (TSH) insufficiency and probably also lack of GH, which is needed for normal thyroid development (11). A mutation that causes these pituitary anomalies in *dw/dw* mice is at the *Pit-1* locus (12). In the *df/df* mice, a nonallelic mutation on a different chromosome interferes with *Pit-1* expression. Neither *Pit-1* mRNA nor protein is detectable in *df/df* pituitaries (12). *Pit-1* is a POU-domain pituitary transcription factor responsible for binding to cis-active elements (13, 14) which permits differentiation of somatotrophs, lactotrophs, and thyrothrophs during development (12, 15, 16) in addition to activating GH and PRL genes (17–20; for overview on GH gene regulation, see Ref. 21). Therefore, differentiation of these particular pituitary cell types does not occur in the *df/df* animal.

Somatic actions of GH include hypertrophy, hyperplasia or both in response to tissue differentiation, cellular proliferation and protein synthesis in target tissues (22). Such effects can be exerted by direct actions of GH on target tissues or via the mediator of GH action, insulin-like growth factor-I (IGF-I) (23). Examples of target tissues for GH action include bone (24), cartilage (25), muscle (26), adipose tissue (27), liver (28), immune (29), and reproductive systems (for review see Ref. 30).

With respect to metabolic effects, GH can act on adipocytes to stimulate lipolysis (31), can stimulate anabolism in certain tissues (26, 31) and affect electrolyte balance (32). Actions of GH on carbohydrate metabolism are complex and involve both acute and delayed effects (22). In the acute situation, GH can induce insulin release from the pancreas producing temporary hyperinsulinemia (33). Delayed effects, however, are antagonistic to the effects of insulin, since GH is diabetogenic in nature. This is evidenced by acromegalic patients who are hyperglycemic and insulin resistant (34).

Investigations in rodent models have established age-related changes in GH secretion which can contribute to major physiological alterations that occur with aging (35). Such changes involve reductions in GH pulse amplitude and pituitary GH content (36). Although basal GH levels remain unchanged in the face of lower GH pulse amplitude, other studies indicate that this effect is capable of inducing a progressive reduction in IGF-I levels (37, 38). Taken together, these findings imply that the waning of GH and IGF-I levels is a hallmark of the aging process in mammals. In humans, most recent evidence has demonstrated decreased GH secretion and decreased pituitary responsiveness to GRF administration in aged men (39).

With this information in mind, a study was undertaken to evaluate hormonal factors influencing regulation of blood glucose levels in the Ames dwarf mouse compared with normal littermates. The second objective was to determine whether aging and GH deficiency interact to influence aspects of glucose regulation examined in the present study. This was accomplished by measurements of glucose, insulin, and the glucocorticoid corticosterone in young and old female mice, and in young male mice of both *df/df* and *Df/−* genotypes. This allowed comparisons between young and aging *Df/−* mice, in which GH can be expected to decline with age, and between normal and dwarf mice, which are devoid of GH throughout the aging process.

Materials and Methods

Animals. Young male and female Ames dwarf (*df/df*) and normal (*Df/−*) littermate mice (approximately 5 months old) and old Ames *df/df* and *Df/−* littermate females (approximately 17–19 months of age) were used (n = 9–11/group). Due to the requirements of previous and concurrent experiments in our laboratory, old *df/df* males were unavailable for inclusion in this study. The animals were housed under a controlled lighting regimen (12:12-hr light:dark cycle) and temperature (22° ± 2°C). Animals were given free access to food (Lab Diet; Purina Mills Inc., St. Louis, MO) and tap water up to the time of termination. The line of *df/df* mice used in these studies is not well defined genetically and has not been systematically inbred. These mice, however, are fairly homogeneous because they were derived from a very limited number of mating pairs (Bartke, unpublished). Animals were produced either by mating *df/df* males × *Df/−* females (having *df/df* sires; i.e., were carriers of the *df* allele) or by mating *Df/−* males × *Df/−* females that had known *df/df* sires.

Animals were separated from normal housing con-

ditions (n = 5/cage for normals; n = 6/cage for dwarfs) to limit the maximum number of animals per cage to three. Care was taken not to mix animals that had not been housed together previously. At the time of segregation, animals were moved to a separate room to provide a quiet and controlled environment to facilitate collection of blood for measurement of basal levels of corticosteroids in the absence of environmental stressors. The morning (0830 to 0900 hr) after segregation, trunk blood was collected following decapitation within 30 sec of the initial disturbance of the cage. This method of assessing basal levels of adrenal corticosteroids has been used previously by our laboratory (40). A one-tenth volume of 6% EDTA was used as an anticoagulant. Plasma was collected following centrifugation for 20 min at 3000*g* and frozen at −20°C until time of assay for corticosterone, glucose, and insulin concentrations.

Hormone Assays. *Corticosterone.* Plasma corticosterone concentrations were determined in duplicate in a single assay using an [^{125}I]corticosterone radioimmunoassay kit specifically designed to measure corticosterone in rat and mouse plasma (ICN Biomedicals, Inc., Costa Mesa, CA) as per manufacturer's suggestions with some modifications. Briefly, plasma samples were diluted 1:400 in steroid diluent provided in the RIA kit. Fifty microliters of each corticosterone standard (six points; 25–1000 ng/ml) or diluted unknown plasma samples were added to 12 × 75 mm borosilicate glass tubes. To each standard and unknown, 100 μl of [^{125}I]corticosterone (~10,000 cpm) and 100 μl of anti-corticosterone antibody were added. Tubes were incubated for 2 hr at room temperature. Following incubations, 250 μl of precipitant solution was added and all tubes except total count tubes were centrifuged at 1000*g* for 15 min. Supernatant fluid was aspirated and tubes counted in a gamma counter. Intra-assay coefficient of variation (CV) was 8.5%, and sensitivity, expressed as 90% of maximum binding, was 0.45 ng/tube.

Glucose. Plasma glucose concentrations were analyzed using the quantitative glucose oxidase determination for plasma (Sigma Chemical Co., St. Louis, MO) as per manufacturer's suggestions with the following modifications. Glucose standards (six points; 0 to 4.0 mg/ml) were added to 12 × 75 mm borosilicate glass tubes in 250 μl of water. For unknowns, 12.5 μl of plasma were added to 237.5 μl water for single sample determinations. Two and one-half milliliters of a combined enzyme color reagent solution were added and all tubes were mixed and incubated for 45 min at room temperature in total darkness. At the end of the incubation period, colorimetric measurements were taken at 450 nm wavelength. Subsequent analysis showed complete linearity throughout the range of the standard curve (r^2 = 1.0). Sensitivity, as determined by the manufacturer is stated to be accurate above 0.25 mg/ml. Unknown plasma glucose concentrations ranged from 0.761 to 4.567 mg/ml.

Insulin. Insulin concentrations were determined in duplicate from 50 to 100 μl of plasma using a solid-phase [^{125}I]insulin RIA kit (Diagnostic Products Corp., Los Angeles, CA) that has been validated for use in rodent species (41). The intra- and interassay CVs were 6.2% and 2.5%, respectively. Sensitivity of the assay, defined as 90% of maximum binding was 2.3 μIU/ml plasma.

Statistical Analyses. Hormone concentrations, body weights, and age data were analyzed by analysis of variance (ANOVA) using the general linear models procedure (42). Main effects of the statistical model included genotype (*df/df,* normal), sex (male, female), and age (old, young). Dependent effects of the model were corticosterone, glucose, insulin, and body weight. All data were analyzed for homogeneity of variance using the F_{max} test (42). All data were normally distributed as determined by the PROC UNIVARIATE NORMAL procedure of SAS (42). In instances where the assumptions of homogeneity of variance could not be met, data were log or square root transformed to meet these assumptions. The Student-Newman-Keuls mean comparison test was employed in the general linear models procedure of SAS to identify differences in genotype, sex, and age at the $P < 0.05$ level.

Results

Body Weight and Age. Table I shows body weight and ages for animals used for the current study. Dwarf animals were, in most cases, one-third the size of *Df*/− animals of the same sex and age ($P < 0.05$). However, *df/df* do not merely stop body growth prematurely, but rather exhibit a much retarded growth rate: old *df/df* females were 56% heavier than their younger counterparts, yet only attained 44% of a "normal" body weight.

Table I. Body Weight and Age of Ames Dwarf and Normal Siblings by Sex

Animal	n	Body weight (g ± SE)	Age (days ± SE)
Young normal male	9	38.54 ± 1.78^a	155.9 ± 0.4
Young normal female	9	28.95 ± 1.05^b	152.8 ± 0.9
Old normal female	9	39.23 ± 2.39^a	521.6 ± 3.3
Young *df/df* male	9	12.91 ± 0.64^c	162.0 ± 0.0
Young *df/df* female	10	11.18 ± 0.46^c	162.0 ± 0.0
Old *df/df* female	11	17.42 ± 1.37^d	552.5 ± 5.4

Note. Values with different superscript letters within columns are significantly different at the $P < 0.05$ level.

Corticosterone. Young *Df/*− female mice had the highest plasma corticosterone concentrations of any group of animals studied (Fig. 1). Levels of corticosterone were more than 9-fold higher in young *Df/*− females than in young *Df/*− males ($P < 0.05$); the latter had the lowest levels of any group studied. However, as the animals aged, these levels decreased to a level numerically intermediate between those measured in the young females and the young males, but significantly lower than young female plasma corticosterone levels. Within the dwarf animals, neither sex nor age affected plasma corticosterone levels, which were comparable to levels found in old *Df/*− females.

Glucose. Plasma glucose concentrations were elevated in young *Df/*− males when compared with other genotype, sex, and age groups (Fig. 2). The young *Df/*− male group included three animals with glucose values 3- to 4-fold higher than values found in the other groups. When the mean for the young *Df/*− male group was calculated following exclusion of these animals based on the outlier identification procedures of Snedecor and Cochran (43), plasma glucose concentrations remained significantly higher ($P < 0.05$) in young phenotypically *Df/*− males when compared with all other groups examined. Young and old *Df/*− females had significantly lower levels of glucose when compared with *Df/*− males but higher than in all *df/df* animals, which had the lowest plasma glucose, ranging from 1.06 to 1.11 mg/ml.

Insulin. Results of insulin measurements indicated a similar pattern to that determined for glucose (Fig. 3); that is the highest levels of insulin were found in young *Df/*− males. The three young *Df/*− males identified as having elevated glucose levels also had elevated insulin concentrations that were 3- to 4-fold higher ($P < 0.05$) than those of any other group. As with the glucose data, insulin concentrations from these animals were excluded from the data based on the definition for outliers (43). Even with exclusion of these values, plasma insulin levels were higher in this group compared with all other genotype by sex classifications studied ($P < 0.05$). All other mice, regardless of genotype, age, and sex, were lower in plasma insulin and were not different from one another (Fig. 3).

Discussion

To our knowledge, this is the first investigation to address the major components involved in maintenance of glucose levels in the unstressed Ames dwarf mouse. Although lacking three important hypophyseal cell types (somatotrophs, lactotrophs, and thyrotrophs [10]), other pituitary cell types, namely the gonadotrophs and corticotrophs, are present and functional in the Ames dwarf. Luteinizing hormone produced by the gonadotropes is present in the circulation of the *df/df* mouse, albeit at lower levels than in *Df/*− animals (44). Based on corticosterone values in the current investigation, one can infer that ACTH is actively released from pituitary corticotrophs to induce adrenal cortical activity. Such a conclusion confirms previously published information on the structure of the adrenals in pituitary dwarf mice (45). Corticosterone concentrations were as great or greater in male and female *df/df* animals when compared with two of three groups of *Df/*− animals examined. The pituitary-

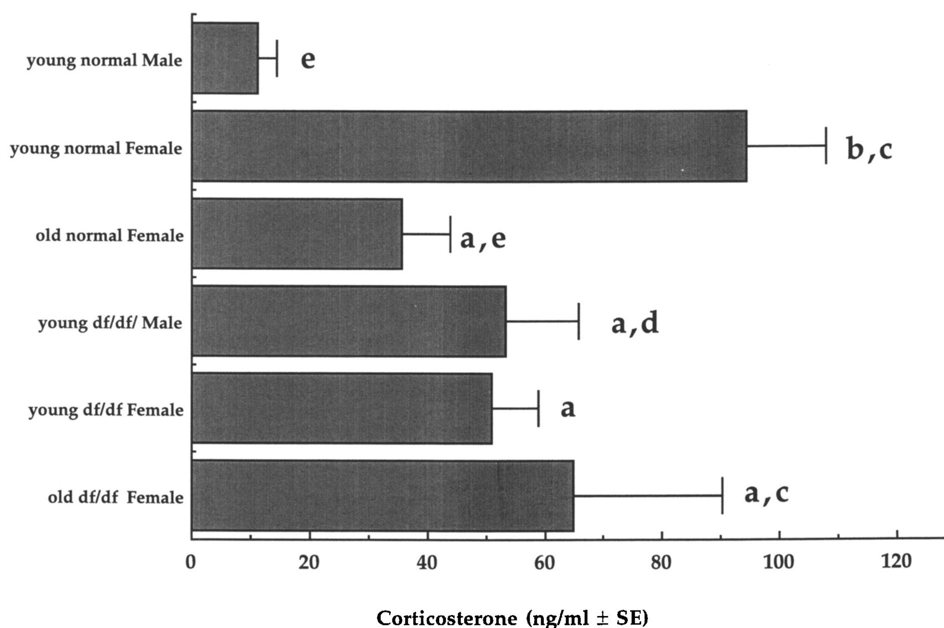

Figure 1. Plasma corticosterone concentrations (mean ± SE) in dwarf and normal animals by age group. Bars lacking a common letter designation are different ($P < 0.05$).

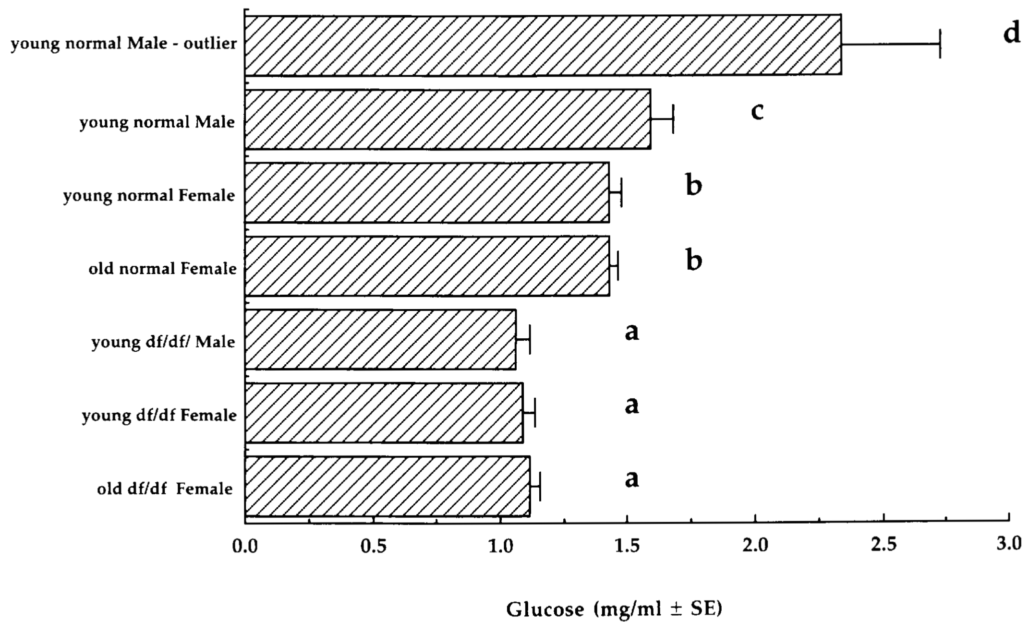

Figure 2. Plasma glucose concentrations (mean ± SE) in dwarf and normal animals by age group. Bars lacking a common letter designation are different ($P < 0.05$). Young normal male-outlier group contains values from all males in this group, including three animals statistically identified as outliers. The young normal male group is a mean of this group excluding values from these three outlier animals.

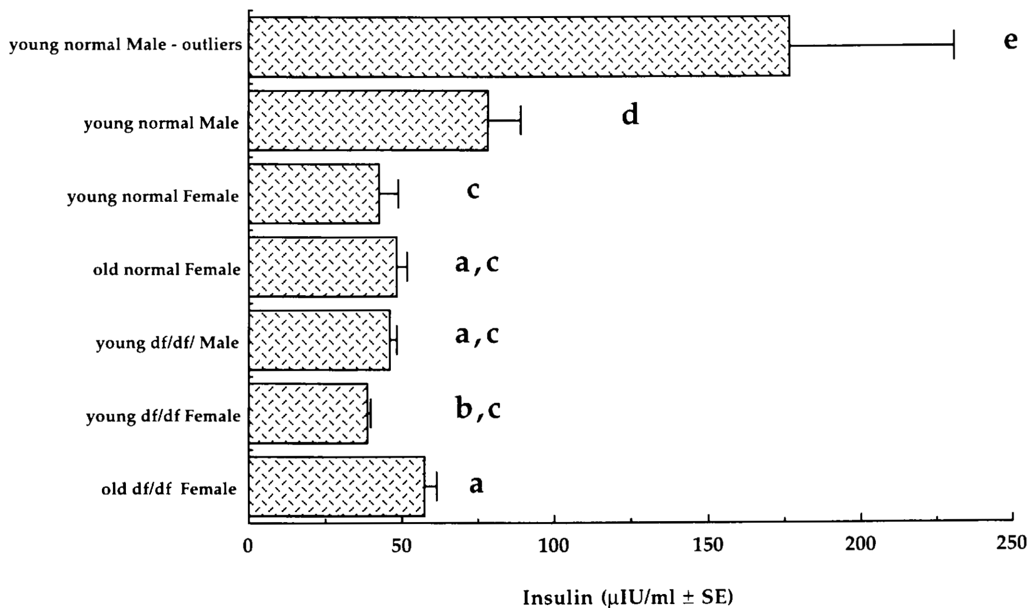

Figure 3. Plasma insulin concentrations (mean ± SE) in dwarf and normal animals by age group. Bars without a common letter designation are different ($P < 0.05$). Young normal male-outlier group contains values from all males in this group, including three animals statistically identified as outliers. The young normal male group is a mean of this group excluding values from these three outlier animals.

adrenal cortical axis in *dw/dw* mice is believed to be normal with respect to corticotroph function (45). This results in a normal adrenal response to ACTH despite the lack of maturational changes in the adrenal cortical X-zone that are seen in *Df/*− animals of similar genetic background. This may explain similarities observed among *df/df* animals and *Df/*− old females and *Df/*− young males in the current study.

There are numerous physiological manifestations associated with the aging process in mammals. With respect to reproduction and growth, dysfunction of the neuroendocrine activity of the hypothalamus reduces gonadotropin-releasing hormone (GnRH) and growth hormone–releasing factor (GHRF) release in aged animals (46). As a result of the reduced gonadotropin secretion secondary to decreased GnRH release, go-

nadal function is depressed leading to acyclicity in female rats and a reduction in testosterone biosynthesis in male rats (46). Consequences of aging are directly analogous to impairment of testicular function in mice (47), and direct evidence of *in vitro* fertilization experiments suggest spermatozoa from aged male mice are inferior, having lowered fertilizing ability (48).

With the decrease in estradiol and GH associated with acyclicity in aged females rats (46), one would expect a decrease in lipid turnover/metabolism, given the lipolytic effect of GH on adipocytes (31). This could potentially explain the appearance of most *Df/−* aged littermate females in our dwarf colony, which are extremely obese beginning at approximately 18 to 24 months of age. This condition is typically an accumulation of intra-abdominal adipose tissue (Borg, Brown-Borg, unpublished observations). However, excessive adiposity is not noticeable in *Df/−* males of similar age. Thus, *Df/−* females in this line are typically over 10 g heavier than *Df/−* males of comparable age (Borg, Brown-Borg, unpublished observations); this is striking as male mice are generally larger than females. The failure of males to exhibit obesity could perhaps be a result of testosterone which is present in males that maintain a functional hypothalamo-pituitary-testis axis. This androgen would be available for aromatization to estradiol, thus maintaining GH levels and preventing the reduction in lipolytic potential that is observed in the *Df/−* female. Although estrogen and testosterone unquestionably affect GH secretion in humans (49), there are some suggestions that a direct effect of nonaromatizable androgen on GH also exists (50). In the rat, there appears to be an androgen-mediated amplification of hypothalamic GHRF secretion and pituitary GH secretion (51) that is sexually dimorphic (52).

As a consequence of the dwarf condition, plasma glucose is reduced regardless of age or sex. This would be expected given the gluconeogenic properties of GH on liver function. However, plasma insulin did not differ between young *df/df* animals and young or old *Df/−* females. This may be a result of partial compensation of glucocorticoid maintenance of glucose at levels that require "normal" insulin secretion. Conversely, animals with normal GH levels were presented with higher glucose levels. This may be a direct result of GH action, which would then increase insulin levels to offset these glucose values, particularly in *Df/−* male mice.

Our glucose data are in complete agreement with a previous report (53) that nonfasting plasma glucose levels are higher in young male mice (4–5 months old) when compared with older animals (8–9 months old; 21–25 months old). Contrary to the results of Leiter *et al.* (53), who showed no change in insulin with age, our results demonstrate higher insulin levels in the young *Df/−* male group than in older animals. Even though earlier research provided evidence for absence of changes in insulin with age, additional evidence from the studies of Leiter *et al.* (53) demonstrated β-cell hypertrophy, increased insulin concentrations per milligram of pancreatic protein, higher insulin secretion rate in isolated pancreas, and lower peak glucose levels following oral and ip glucose challenges as animals age. Taken together, present and previous findings suggest that pancreatic dysfunction in the mouse is not a manifestation of the aging process. Thus glucose intolerance is not an inevitable consequence of old age in this species.

An observation parallel to the current investigation suggests that *df/df* individuals have greater longevity than *Df/−* animals (Borg, Brown-Borg, unpublished observations). These results may be comparable to a dietary restriction effect whereby reduction in neuroendocrine hormone secretion results in antiaging effects (54–56). Thus, the dwarf, which is partially lacking normal pituitary function, may have a reduced caloric need due to the absence of GH and TSH effects on metabolism and food intake.

The effects of glucocorticoids on GH secretion have been investigated extensively (for review, see Ref. 56). It is very apparent that chronic exposure to elevated glucocorticoids suppresses GH production (57, 58), whereas acute glucocorticoid administration can stimulate GH release (59). Effects of glucocorticoids on GH secretion occur at the genomic level via glucocorticoid receptor binding sites that are present on the GH gene (60, 61). In addition, glucocorticoids can stimulate GHRF release directly (62), reduce the sensitivity of somatotrophs to inhibitory effects of somatostatin by downregulating somatostatin receptors (63, 64), and abolish negative feedback effects of IGF-I on GH release (65). All these effects serve to increase GH release from the anterior pituitary. A conspicuous result from our experiments shows a sexual dimorphism in corticosterone levels. Corticosterone in young *Df/−* females was more than 9-fold higher than in *Df/−* males and still 3-fold higher than in old *Df/−* females. To our knowledge, such differences have not been noted previously, which adds to difficulties in interpretation. However, the elevation of corticosterone in young *Df/−* females in the current investigation may thus promote GH secretion to prevent the deposition of adipose tissue that is noted in older *Df/−* females, which have characteristically low corticosterone levels.

In summary, this study has demonstrated relationships between corticosterone and insulin, two factors which are involved in controlling peripheral glucose concentrations in the Ames dwarf mouse. Corticosterone levels in young and old *df/df* animals were intermediate to levels found in young *Df/−* males and

young *Df/−* females, indicating a lack of major alterations in adrenal cortical function in the dwarf condition. Among the female *df/df,* there was a lack of sexually dimorphic and age-related effects on glucose and corticosterone. However, glucose concentrations were suppressed in *df/df* animals in spite of nearly normal levels of insulin. This suggests that an impairment of glucose metabolism in *df/df* animals results in lower circulating glucose levels as an obvious side effect of the hyposomatotropic condition.

This research was supported by National Institutes of Health (NIH) NRSA 1F32 AI08745-01A1 (H.M.B.B.) and NIH Grant HD20033 (A.B.).

1. Unger RH, Aguilar-Parada E, Muller WA, Eisentrautt AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. J Clin Invest **49**:837–848, 1970.
2. Unger RH, Raskin P, Srikant CB, Orci L. Glucagon and the A cells. Rec Prog Horm Res **33**:477–517, 1977.
3. Unger RH, Dobbs RE, Orci L. Insulin, glucagon, and somatostatin secretion in the regulation of metabolism. Ann Rev Physiol **40**:307–343, 1978.
4. Hadley ME. Adrenal steroids. In: Hadley ME, Ed. Endocrinology. Englewood Cliffs, NJ: Prentice-Hall, pp 344–377, 1984.
5. Schaible R, Gowen JW. A new dwarf mouse. Genetics **46**:896, 1961.
6. Snell GD. Dwarf, a new mendelian recessive character of the house mouse. Proc Natl Acad Sci USA **15**:733–734, 1929.
7. Ortmann R. A study of some cytochemical reactions and of the hormone content of the adenohypophysis in normal and genetic dwarf mice. J Morph **99**:417–431, 1956.
8. Rennels EG, McNutt WC. The fine structure of the anterior pituitary of the dwarf mouse. Anat Rec **131**:591–592, 1958.
9. Elftman H, Wegelius O. Anterior pituitary cytology of the dwarf mouse. Anat Rec **135**:43–50, 1959.
10. Bartke A. Histology of the anterior hypophysis, thyroid and gonads of two types of dwarf mice. Anat Rec **149**:225–236, 1964.
11. Bartke A. The response of two types of dwarf mice to growth hormone, thyrotropin and thyroxine. Gen Comp Endocrinol **5**:418–426, 1965.
12. Li S, Crenshaw EB III, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG. Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit-1*. Nature **347**:528–533, 1990.
13. Nelson C, Crenshaw EB III, Franco R, Lira SA, Albert VR, Evans RM, Rosenfeld MG. Discrete cis-active genomic sequences dictate the pituitary cell type-specific expression of rat prolactin and growth hormone genes. Nature **322**:557–562, 1986.
14. Nelson C, Albert VR, Elsholtz HP, Lu LI-W, Rosenfeld MG. Activation of cell-specific expression of rat growth hormone and prolactin genes by a common transcription factor. Science **239**:1400–1405, 1988.
15. Ingraham HA, Chen R, Mangalam HJ, Elsholtz HP, Flynn SE, Lin CR, Simmons DM, Swanson L, Rosenfeld MG. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell **55**:519–529, 1988.
16. Theill LE, Karin M. Transcriptional control of GH expression and anterior pituitary development. Endocr Rev **14**:670–689, 1993.
17. Bodner M, Karin M. A pituitary-specific trans-acting factor can stimulate transcription from the growth hormone promoter in extracts of non-expressing cells. Cell **50**:267–275, 1987.
18. Mangalam HJ, Albert VR, Ingraham HA, Kapiloff M, Wilson L, Nelson C, Elsholtz H, Rosenfeld MG. A pituitary POU domain protein, Pit-1, activates both growth hormone and prolactin promoters transcriptionally. Gene Dev **3**:946–958, 1989.
19. Fox SR, Jong MTC, Casanova J. The homeodomain protein, Pit-1/GHF-1 is capable of binding to and activating cell-specific elements of both the growth hormone and prolactin gene promoters. Mol Endocrinol **4**:1069–1080, 1990.
20. Larkin S, Tait S, Treacy M, Martin F. Characterisation of tissue specific transacting factor binding to a proximal element in the rat growth hormone promoter. Eur J Biochem **191**:605–615, 1990.
21. Karin M, Castrillo J-L, Theill LE. Growth hormone gene regulation: A paradigm for cell-type-specific gene activation. Trends Genetics **6**:92–96, 1990.
22. Strobl JS, Thomas MJ. Human growth hormone. Pharmacol Rev **46**:1–34, 1994.
23. Casanueva FF. Physiology of growth hormone secretion and action. Endocr Metab Clin North Am **21**:483–512, 1992.
24. Isaksson OGP, Lindahl A, Nilsson AL, Isgaard J. Mechanism of the stimulatory effects of growth hormone on longitudinal bone growth. Endocr Rev **8**:426–438, 1987.
25. Howell DS, Dean DD. The biology, chemistry and biochemistry of the mammalian growth plate. In: Coe F, Favus MJ, Eds. Disorders of Bone and Mineral Metabolism. New York: Raven Press, pp 313–353, 1992.
26. Cuneo RC, Salomon F, Wiles CM, Hesp R, Sonksen PH. Growth hormone treatment in growth hormone deficient adults. I. Effects on muscle mass and strength. J Appl Physiol **70**:688–693, 1991.
27. Hauner H. Physiology of the fat cell, with emphasis on the role of growth hormone. Acta Paediatr **383**(Suppl):47–51, 1992.
28. Sundseth SS, Alberta JA, Waxman DJ. Sex specific growth hormone-regulated transcription of the cytochrome P450 2C11 and 2C12 gene. J Biol Chem **267**:3907–3914, 1992.
29. Gelato MC. Growth hormone-insulin-like growth factor I and immune function. Trends Endocr Metab **4**:106–110, 1993.
30. Spiteri-Grech J, Nieschlag E. The role of growth hormone and insulin-like growth factor I in the regulation of male reproductive function. Horm Res **38**(Suppl 1):22–27, 1992.
31. Crist DM, Peake GT, Loftfield RB, Kraner JC, Egan PA. Supplemental growth hormone alters body composition, muscle protein metabolism, and serum lipids in fit adults: Characterization of dose-dependent and response-recovery effects. Mech Ageing Dev **58**:191–205, 1991.
32. Pratt JH, Peacock M, Henry DP. Effect of recombinant human growth hormone on adrenocortical function, and on sodium and potassium homeostasis. Pharmacology **47**:36–42, 1993.
33. Jorgensen JOL, Moller N, Lauritzen T, Alberti KGMM, Orskov H, Christiansen JS. Evening versus morning injections of growth hormone (GH) in GH-deficient patients: Effects on 24-hour patterns of circulating hormones and metabolites. J Clin Endocrinol Metab **70**:207–214, 1990.
34. Molitch ME. Clinical manifestations of acromegaly. Endocr Metab Clin North Am **21**:597–614, 1992.
35. D'Costa AP, Ingram RL, Lenham JE, Sonntag WE. The regulation of mechanisms of action of growth hormone and insulin-like growth factor 1 during normal aging. J Reprod Fertil Suppl **46**:87–98, 1993.
36. Sonntag WE, Steger RW, Forman LJ, Meites J. Decreased pulsatile release of growth hormone in old male rats. Endocrinology **107**:1875–1879, 1980.
37. Florini JR, Roberts SB. Effect of age on blood levels of somatomedin-like growth factor. J Gerontol **35**:23–30, 1979.
38. Florini JR, Prinz PN, Vitiello MV, Hintz RL. Effect of rat age on serum levels of growth hormone and somatomedins. Mech Ageing Dev **15**:165–176, 1981.

39. Corpas E, Harman SM, Pineyro MA, Roberson R, Blackman MR. GHRH 1-29 twice daily reverses the decreased GH and IGF-I levels in old men. J Clin Endocrinol Metab **75**:530–535, 1992.
40. Cecim M, Ghosh PK, Esquifino AI, Began T, Wagner TE, Yun JS, Bartke A. Elevated corticosterone levels in mice expressing human or bovine growth hormone genes. Neuroendocrinology **53**:313–316, 1991.
41. Snyder DL, Towne B. The effect of dietary restriction on serum hormone and blood chemistry changes in aging Lobund-Wistar rats. In: Snyder DL, Ed. Dietary Restriction and Aging. New York: Alan R. Liss, pp 135–146, 1989.
42. SAS. SAS User's Guide, Statistics. Cary, NC: Statistical Analysis Systems Institute, 1985.
43. Snedecor GW, Cochran WG, Eds. Statistical Methods (6th ed). Ames: Iowa State University Press, 1967.
44. Chandrashekar V, Bartke A. Induction of endogenous insulin-like growth factor-I secretion alters the hypothalamo-pituitary-testicular function in growth hormone-deficient adult dwarf mice. Biol Reprod **48**:544–551, 1993.
45. Shire JGM, Hambly EA. The adrenal glands of mice with hereditary pituitary dwarfism. Acta Pathol Microbiol Scand A **81**:225–228, 1973.
46. Meites J. Anti-ageing intervention and their neuroendocrine aspects in mammals. J Reprod Fertil **46**(Suppl):1–9, 1993.
47. Parkening TA, Collins TJ, Au WW. Paternal age and its effects on reproduction in C57BL/6NNia mice. J Gerontol **43**:B79–B84, 1988.
48. Parkening TA. Fertilizing ability of spermatozoa from aged C57BL/6NNia mice. J Reprod Fertil **87**:727–733, 1989.
49. Kerrigan JR, Rogol AD. The impact of gonadal steroid hormone action on growth hormone secretion during childhood and adolescence. Endocr Rev **13**:281–298, 1992.
50. Ulloa-Aguirre A, Blizzard RM, Garcia-Rubi E, Rogol AD, Link K, Christie CM, Johnson ML, Veldhuis JD. Testosterone and oxandrolone, a non aromatizable androgen, specifically amplify the mass and rate of growth hormone (GH) secreted per burst without altering GH secretory burst duration or frequency or the GH half-life. J Clin Endocrinol Metab **71**:846–854, 1990.
51. Wehrenberg WB, Giustina A. Basic counterpoint: Mechanisms and pathways of gonadal steroid modulation of growth hormone secretion. Endocr Rev **13**:299–308, 1992.
52. Jansson J-O, Eden S, Isaksson O. Sexual dimorphism in the control of growth hormone secretion. Endocr Rev **6**:128–150, 1985.
53. Leiter EH, Premdas F, Harrison DE, Lipson LG. Aging and glucose homeostasis in C57BL/6J mice. FASEB J **2**:2807–2811, 1988.
54. Meites J, Goya R, Takahashi S. Why the neuroendocrine system is important in the aging process. Exp Gerontol **22**:1–15, 1987.
55. Meites J. Aging: Hypothalamic catecholamines, neuroendocrine-immune interaction and dietary restriction. Proc Soc Exp Biol Med **195**:304–311, 1990.
56. Thakore JH, Dinan TG. Growth hormone secretion: The role of glucocorticoids. Life Sci **55**:1083–1099, 1994.
57. Von Werder K, Hane S, Forsham PH. Suppression of hypothalamo-pituitary-adrenal axis and growth hormone release with dexamethasone. Horm Metab Res **3**:171–173, 1971.
58. Nakagawa K, Mashimo K. Suppression of exercise-induced growth hormone release with dexamethasone. Horm Metab Res **5**:225–226, 1973.
59. Casanueva FF, Burguera B, Muruais C, Dieguez C. Acute administration of corticosteroids: A new and peculiar stimulus of growth hormone secretion in man. J Clin Endocrinol Metab **70**:234–237, 1990.
60. Rousseau GG, Eliard PH, Barlow JW, Lemaigre FP, Lafontaine DA, DeNayer P, Economidis I-V, Fromstecher P, Idziorek T, Marthy-Hartet M, Voz MLJ, Belayew A, Martial JA. Approach to the molecular mechanisms of the modulation of growth hormone gene expression by glucocorticoid and thyroid hormones. J Steroid Biochem **27**:149–158, 1987.
61. Slater EP, Rabenau O, Karin M, Baxter JD, Beato M. Glucocorticoid receptor binding and activation of a heterologous promoter by dexamethasone by the first intron of the human growth hormone gene. Mol Cell Biol **5**:2984–2992, 1985.
62. Michel D, Lefevre G, Labrie F. Dexamethasone is a potent stimulator of growth hormone-releasing factor-induced cyclic AMP accumulation in the adenohypophysis. Life Sci **35**:597–602, 1984.
63. Holl RW, Thorner MO, Leong DA. Intracellular calcium concentrations and growth hormone secretion in individual somatotropes: Effects of growth hormone-releasing factor and somatostatin. Endocrinology **122**:2927–2932, 1988.
64. Schonbrunn A. Glucocorticoids down-regulate somatostatin receptors on pituitary cells in culture. Endocrinology **110**:1147–1154, 1982.
65. Lamberts SWJ, Den Holder F, Hofland LJ. The interrelationship between the effects of insulin-like growth factor-I and somatostatin on growth hormone secretion by normal rat pituitary cells: The role of glucocorticoids. Endocrinology **124**:905–911, 1989.