Temporal Relationship of Tissue Somatostatin-like Immunoreactivity to Metabolic Changes in Genetically Obese and Diabetic Mice

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SUMMARY

Somatostatin-like immunoreactivity (SRIF-LI) content in 2 N acetic acid extracts of hypothalamus, gastric antrum, and pancreas was measured in genetically obese (C57BL/6J *ob/ob* and *db/db*) and diabetic (C57BL/KsJ *db/db* and *ob/ob*) mice and normal littermate controls from 5 to 24 wk to determine the relationship of previously reported changes to the development of metabolic abnormalities. Hypothalamic SRIF-LI concentration was similar in control, diabetic, and obese mice at all ages and increased progressively with age in all groups. Gastric antrum SRIF-LI was similar in all groups of mice at all ages. Obese mice gained weight progressively and showed moderate hyperglycemia and marked hyperinsulinemia from 5 wk of age. Pancreatic SRIF-LI content in obese (C57BL/6J) animals was similar to that in lean littermate controls, but pancreatic SRIF-LI concentration (expressed by weight or protein content) was decreased until 18 (6J *ob/ob*) and 10 (6J *db/db*) wk. Diabetic (C57BL/KsJ) mice showed a similar metabolic pattern until 10 wk with no change in pancreatic SRIF-LI content or concentration. Thereafter a progressive fall in serum insulin and a marked rise in serum glucose was associated with increasing pancreatic SRIF-LI content and concentration. These studies suggest that the genetically hyperphagic syndromes are unassociated with any change in hypothalamic or gastric SRIF-LI; that pancreatic SRIF-LI increases occur in response to, rather than as the cause of, relative hypoinsulinemia; and that the genetic background of the mice (KsJ or 6J) rather than the mutant gene (*db* or *ob*) determines the defect in carbohydrate metabolism and the pancreatic SRIF-LI response. **DIABETES *29*: 717–723, September 1980.**

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Somatostatin (SRIF), the hypothalamic inhibitor of pituitary growth hormone release, is present in high concentration in the pancreas,[1] where it has been shown immunohistochemically to be localized in D-cells of the islets of Langerhans in close apposition to the A- and B-cells.[2] Synthetic SRIF has been shown to inhibit basal and stimulated secretion of insulin and glucagon by the pancreas in vivo and in vitro under a variety of experimental situations.[3] Stimulation of insulin[4] and glucagon[5] release following administration of anti-SRIF serum suggests that pancreatic SRIF may exert a tonic inhibitory effect on the A- and B-cells, acting as a local (paracrine) regulator of islet hormone homeostasis.[6] To clarify the physiologic relationship between SRIF, insulin, and glucagon, pancreatic SRIF content and release has previously been studied under conditions of abnormal insulin and glucagon regulation. Increased pancreatic[7–10] and islet[7,8,11] SRIF-like immunoreactivity (SRIF-LI) and increased islet D-cell number[12–17] have been demonstrated in established primary insulin-deficiency states such as the spontaneously diabetic mouse,[10,16,17] in insulin-dependent human diabetes,[13] and in insulin deficiency induced by streptozotocin[7–9,11–14] or alloxan.[8,15] Conversely, a decrease in pancreatic[18,19] and islet[19,20] SRIF-LI, together with a paucity of D-cells,[16] has been reported in the obese mouse, which exhibits hyperphagia, hyperinsulinemia, and mild hyperglycemia.

However, these studies, utilizing models of established hypo- or hyperinsulinemia, do not provide information as to the relationship of the D-cell changes and resultant alterations in pancreatic SRIF-LI content to the development of hyperglycemia or alterations in insulin secretion. Two models of spontaneous diabetes in the mouse permit such a longitudinal study. The autosomal recessive *db* gene mutant on the 4 chromosome in homozygous C57BL/KsJ (KsJ) mice results in a diabetic syndrome characterized initially by obesity and insulin resistance but later (at ±2 mo of age) by pancreatic B-cell atrophy, insulinopenia, and severe hyperglycemia. The autosomal recessive *ob* gene mutant on the 6

chromosome in homozygous C57BL/6J (6J) mice causes a hyperphagic syndrome characterized by progressive obesity with marked hyperinsulinemia and mild hyperglycemia.[21] The genetic background on which the mutations are maintained is of critical importance in the expression of the syndrome. Both *ob* and *db* homozygous genes maintained on the KsJ background produce the diabetic syndrome while both genes on the 6J background produce the obesity syndrome.[21]

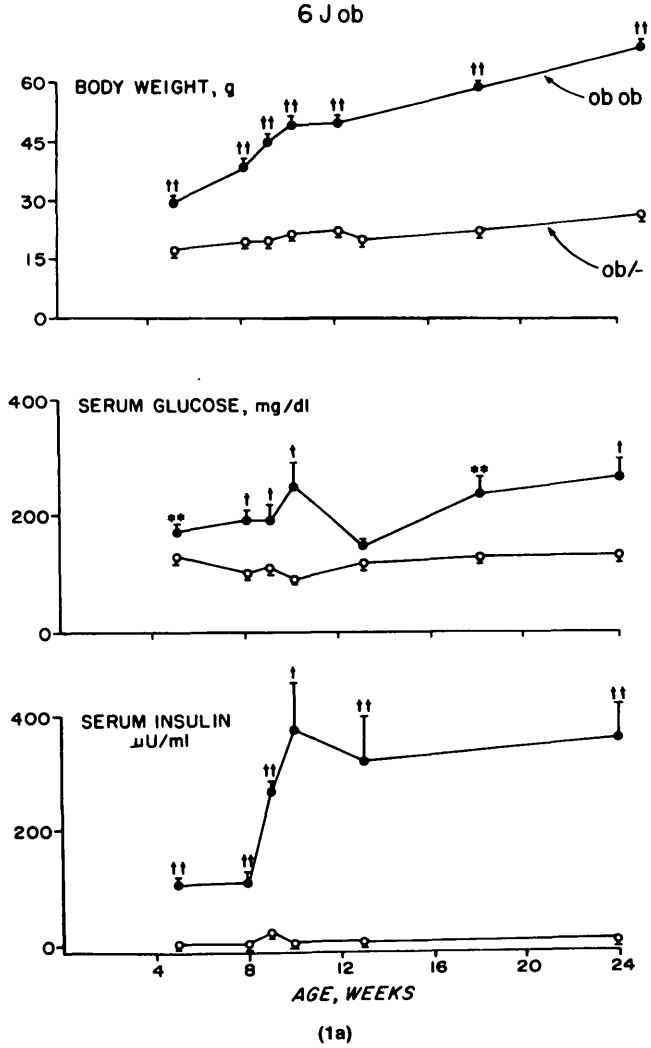
A primary hypothalamic defect has been suggested in *ob* and *db* mutants since both are infertile, hyperphagic, and exhibit abnormalities in thermoregulation.[21] SRIF is present in high concentration in the ventromedial hypothalamus,[22,23] a locus involved in the regulation of food intake and a possible site of the abnormality.[21] Alterations of hypothalamic SRIF in obesity and diabetes, however, remain to be documented.

The aims of the present studies were (1) to determine the temporal relationship of pancreatic SRIF-LI changes to the development of insulinopenic diabetes in homozygote KsJ *db* mice and of hyperinsulinemic obesity in 6J *ob* homozygote mice; (2) to determine whether the changes in pancreatic SRIF-LI are the result of the mutant gene (*ob* or *db*) or of the genetic background (KsJ or 6J); and (3) to determine whether an alteration of hypothalamic SRIF-LI occurs during the development of the disorders to provide evidence for the proposed abnormality of hypothalamic function.

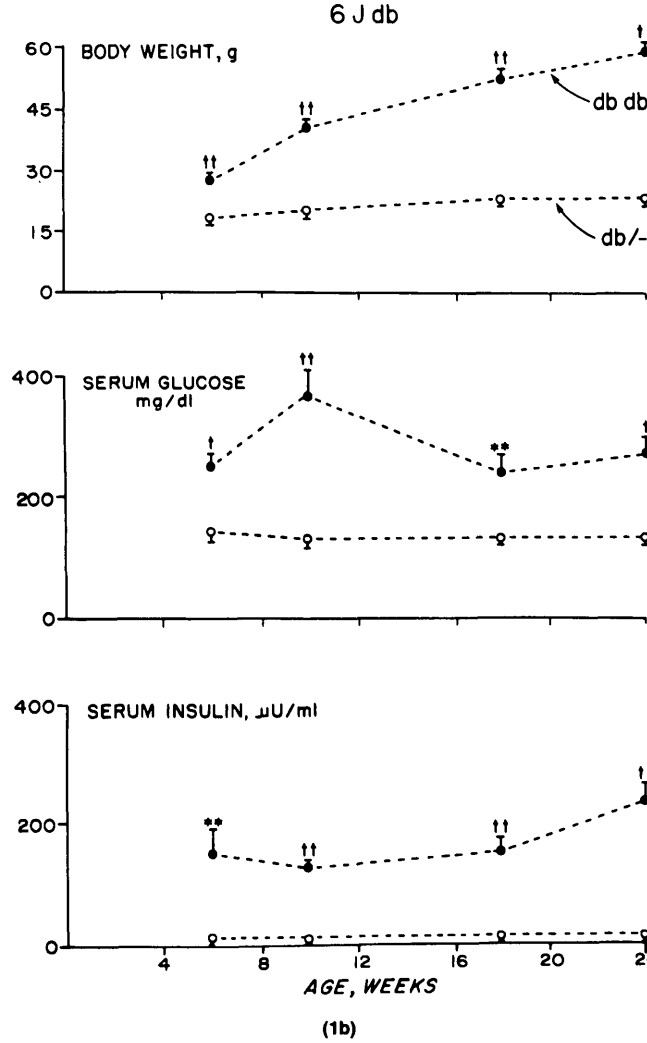
MATERIALS AND METHODS

C57BL/6J *ob/ob* (obese) and C57BL/KsJ *db/db* (diabetes) female mice and normal littermate controls were obtained from the Jackson Laboratory (Bar Harbor, Maine). C57BL/6J *db/db* and C57BL/KsJ *ob/ob* female mice and normal littermate controls were obtained from mutant research colonies maintained at the Jackson Laboratory. Animals were transported at 5–6 wk of age and were then maintained under constant conditions of temperature and light-dark cycle receiving Wayne laboratory chow and tap water ad libitum until they were killed. Blood samples were obtained by retroorbital puncture on the day before sacrifice for serum glucose and insulin measurement. Obese or diabetic mice and littermate controls were killed in the fasted state at various ages from 5 to 24 wk by cervical dislocation. To minimize

FIGURE 1a–d. Morphologic and metabolic characteristics of mice demonstrating the obese syndrome [associated with the *ob/ob* (Figure 1a) or *db/db* (Figure 1b) genes on the C57BL/6J background] and the diabetes syndrome [due to the *db/db* (Figure 1c) or *ob/ob* (Figure 1d) genes on the C57BL/KsJ background] compared with normal heterozygote littermate controls. Results are expressed as the mean ± SEM. Ten animals were studied at each time point. P values: * <0.05, ** <0.02, † <0.01, †† <0.001.



(1a)



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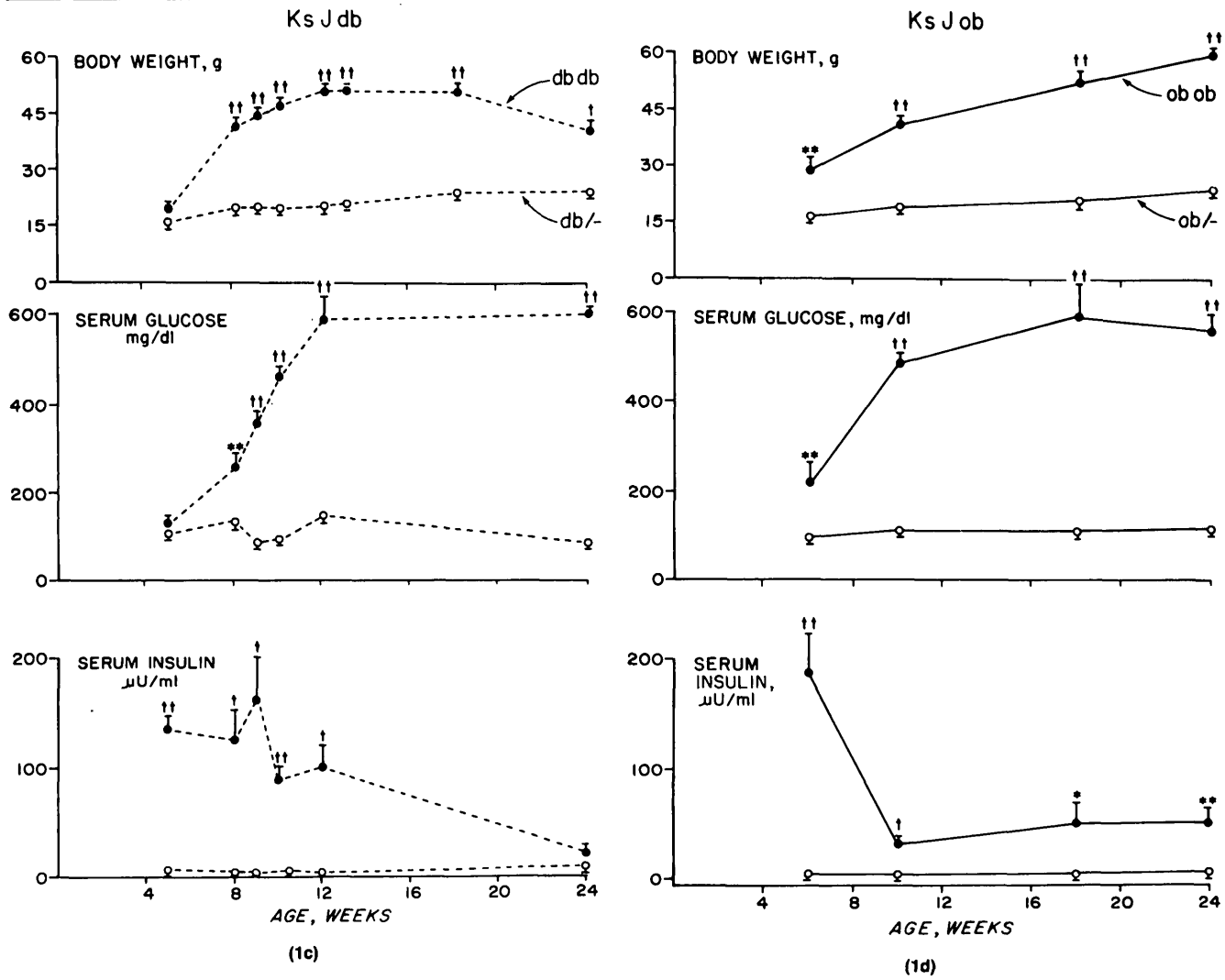


FIGURE 1. Continued.

stress, animals were housed individually away from the work area and minimally handled before sacrifice. The brain was rapidly removed and the hypothalamic block, delineated by the optic chiasm rostrally, perihypothalamic sulci laterally, and the mamillary bodies caudally, was removed to the level of the roof of the third ventricle. The pancreas was carefully dissected free of omental fat and removed in its entirety. The gastric antrum was also removed. Tissues were immediately placed in preweighed vials containing ice-cold 2 N acetic acid and snap-frozen. After reweighing and boiling, the tissues were homogenized using a Polytron (Brinkmann) at a setting of 4 and an aliquot taken for protein estimation. The homogenates were centrifuged and the supernatant lyophilized before reconstitution in assay buffer (0.01 M phosphate, 0.15 M saline buffer, pH 7.8, to which 0.5 M disodium EDTA had been added) for SRIF radioimmunoassay (RIA). This extraction technique provides reproducible recoveries of added synthetic SRIF with almost total elimination of proteolytic activity as evidenced by a lack of ^{125}I-Tyr^{1} SRIF degradation.[24]

The SRIF RIA was performed as previously described[24] using rabbit anti SRIF-hemocyanin at a final dilution of 1:70,000, ^{125}I-Tyr^{1}-SRIF prepared by the lactoperoxidase-glucose/glucose oxidase method and purified by ion-exchange chromatography on CM52 (Whatman), and a second antibody separation technique. Glucose was measured on an AutoAnalyzer (Technicon) by the ferricyanide method. Insulin was measured by RIA as described.[25] Protein was measured by the method of Lowry.[26]

Pancreatic SRIF-LI content (expressed as ng SRIF-LI/organ) and SRIF-LI concentration in hypothalamus, pancreas, and gastric antrum (expressed as ng SRIF-LI/g wet wt or ng SRIF-LI/mg homogenate protein) were compared using Student's *t* test and, where appropriate, an analysis of variance.

RESULTS

Homozygous *ob* and *db* mutant mice on the KsJ or 6J background showed a marked increase in body weight compared with littermate controls beginning at 5 wk of age (Figures 1a–d). While 6J *ob* or *db* homozygotes gained weight up to 24 wk, KsJ *db* homozygotes (Figure 1c) tended to lose weight after 17 wk, although they remained considerably heavier than their littermate controls.

Both 6J *ob* and *db* homozygotes demonstrated the obesity syndrome (Figures 1a and b) with mild hyperglycemia at all ages from 5 wk (range 170–270 mg/dl) and marked hyperinsulinemia that tended to increase with age to a maxi-

mum of 220–390 μU/ml. KsJ *db* and *ob* homozygotes (Figures 1c and d) demonstrated a similar degree of hyperinsulinemia and hyperglycemia until 9–10 wk of age, when a fall in serum insulin levels (to ±50 μU/ml) was associated with a rapidly increasing hyperglycemia, which peaked at a level of ±600 mg/dl. The serum insulin levels, despite the fall, remained significantly ($P < 0.001$) above the values seen in normal littermate controls at all ages studied.

Hypothalamic SRIF-LI content in homozygous *ob* and *db* mutants on the KsJ or 6J background was similar to that in littermate controls (Figure 2). Hypothalamic SRIF-LI concentration increased significantly, as assessed by analysis of variance with increasing age in all the animals studied.

Gastric antral SRIF-LI content and concentration in homozygous *ob* and *db* mutants on the KsJ or 6J backgrounds were similar to values in littermate controls and did not change with age (Figure 3).

In *ob* and *db* homozygotes on the 6J background, total pancreatic SRIF-LI was similar to that in nonobese littermate controls (Figures 4a and b). Pancreatic SRIF-LI concentration, expressed per g wt or mg protein (data not shown), was decreased in homozygous obese mice until 10 (6J *db/db*) or 18 wk (6J *ob/ob*) compared with lean controls (Figures 4a and b).

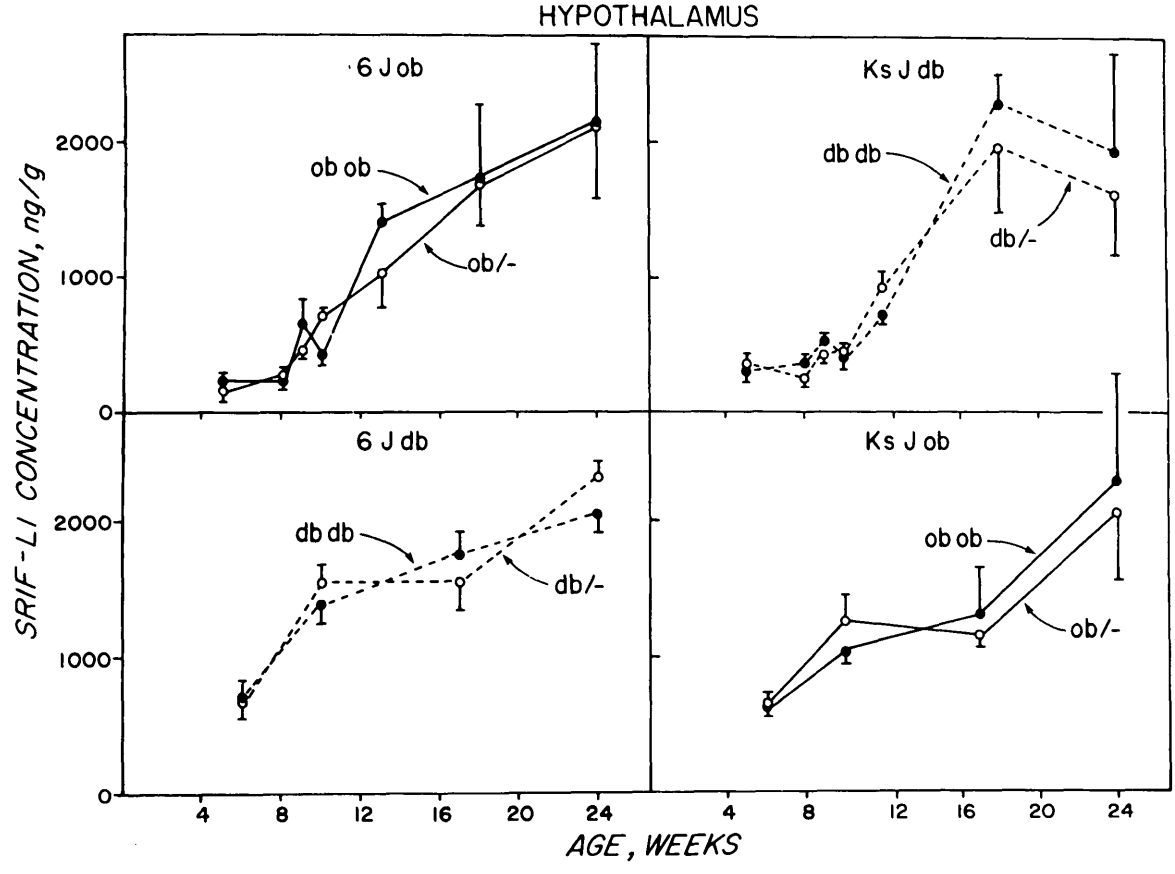
In *db* and *ob* homozygotes on the KsJ background, pancreatic SRIF-LI was increased beginning at 10 wk of age compared with littermate controls, whether expressed as total organ content or concentration relative to tissue weight (Figures 4c and d) or protein (data not shown).

DISCUSSION

This longitudinal study of KsJ and 6J mice with homozygous *ob* and *db* mutant genes confirms the importance of the genetic background in the development of the metabolic abnormality occurring in these animals.[21] 6J mice with the homozygous *ob* or *db* gene develop the "obesity" syndrome while KsJ mice with the homozygous *db* or *ob* gene develop the "diabetes" syndrome, with relative insulinopenia from 8 to 10 wk of age.

A hypothalamic defect has been postulated to play a role in determining the hyperphagia seen in the "obesity" and "diabetes" syndromes.[21] Previous studies of hypothalamic SRIF-LI in KsJ *db/db* and 6J *ob/ob* mice at a single point in time have resulted in conflicting data, with an elevation in content being found in both groups in one study[19] and no change in content in either group being found in others.[10,27] In the present study, no alteration in hypothalamic SRIF-LI was seen at any age in any of the groups of mice. Our results do not support a role for hypothalamic SRIF in the development of the abnormality of appetite control in these animals and also suggest that hypothalamic SRIF is unaffected by the metabolic disturbances. In the diabetic Chinese hamster, a decrease in hypothalamic SRIF-LI (expressed per tissue weight) has been found, although total

FIGURE 2. Hypothalamic SRIF-LI concentration in obese (6J *ob* and *db*) and diabetic (KsJ *db* and *ob*) mice and normal littermate controls, studied from the age of 5 wk. Results are expressed as mean ± SEM. Tissue samples from 10 animals were studied at each time point. Analysis of variance revealed a significant increase in SRIF-LI concentration with age in all groups. P values: 6J *ob* <0.005, KsJ *db* <0.001, 6J *db* <0.0005, and KsJ *ob* <0.025.



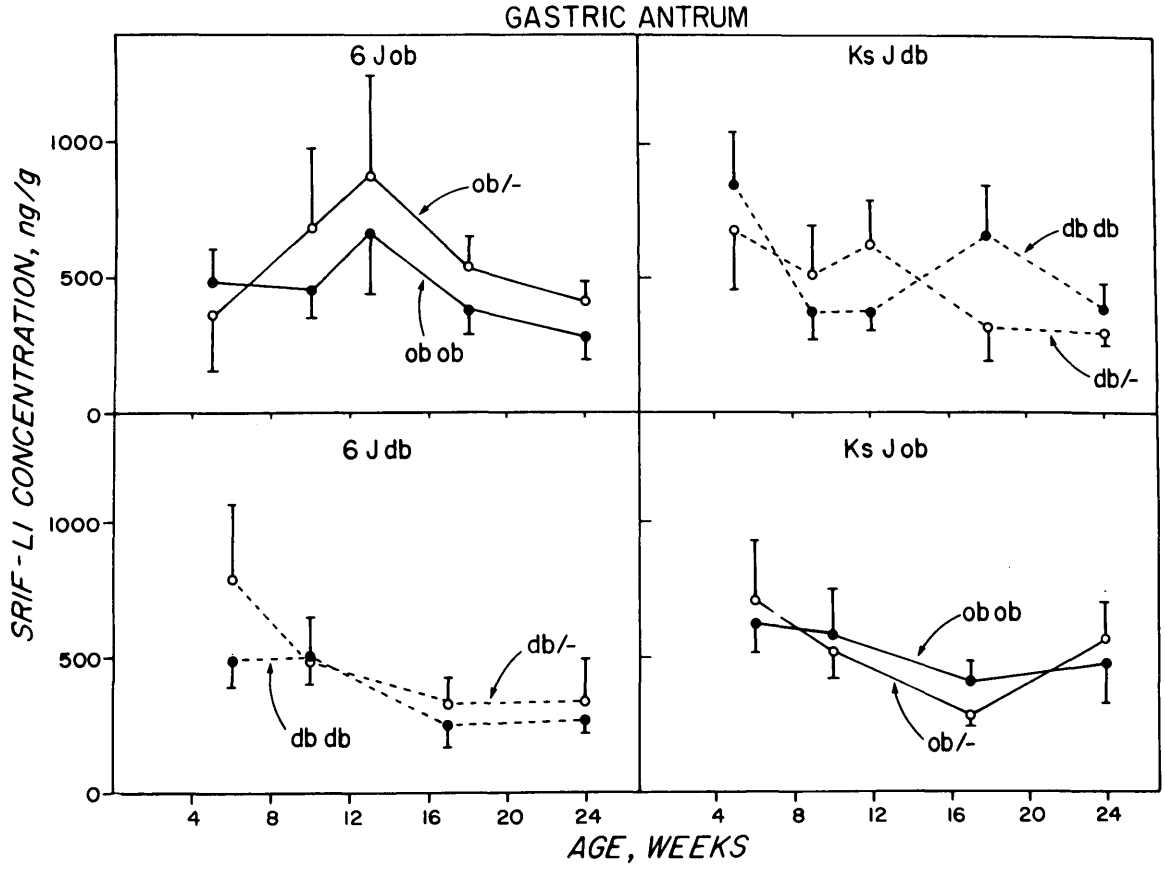


FIGURE 3. Gastric antrum SRIF-LI concentration in obese (6J *ob* and *db*) and diabetic (KsJ *db* and *ob*) mice and normal littermate controls, studied from the age of 5 wk. Results are expressed as mean ± SEM. Tissue samples from 10 animals were studied at each time point.

hypothalamic content was unchanged.[28] In diabetes induced by streptozotocin in the rat, no changes in hypothalamic SRIF-LI have been demonstrated.[7,8] The increase of hypothalamic SRIF-LI seen with increasing age in all groups of mice studied has not been previously documented, and its physiologic relevance is unclear.

Gastric antral SRIF-LI content is increased by feeding[29] and an increase in content in hyperphagic animals would have been predicted. However, antral SRIF-LI concentration was similar in obese and diabetic mice to that in normal controls at all time periods examined. In a previous study, a decrease in antral SRIF-LI was demonstrated in KsJ *db/db* and 6J *ob/ob* mice[19] and in the diabetic Chinese hamster,[28] while an increase in antral content has been described in the streptozotocin-diabetic rat.[9] The discrepancy in results between the various studies is unexplained but may depend on differences in the methods of sacrifice and the degree of stress involved, tissue handling and techniques used to minimize tissue SRIF-LI degradation, and, finally, antiserum specificity.

In 6J *ob/ob* and *db/db* mice, no change was seen in total pancreatic SRIF-LI content (ng/pancreas) at any age, while pancreatic SRIF-LI concentration (ng/g) was decreased in 6J *ob/ob* (until 18 wk) and in 6J *db/db* mice (until 10 wk) compared with littermate controls. Several previous studies provide conflicting data. Patel et al. found no change in pancreatic SRIF-LI concentration in 6J *ob/ob* mice at 8 wk of age[19] but, in a separate study, found decreased pancreatic concentration in 11–12-wk-old animals.[18] On immunohistochemical staining of the pancreas, Baetens et al. found a decrease in SRIF-LI positive D-cell volume density in 10–12-wk-old 6J *ob/ob* and *db/db* mice.[16] In older (18–28 wk old) animals, Makino et al. demonstrated increased pancreatic SRIF-LI content and concentration,[10] a finding confirmed by Dolais-Kitabgi et al. in 6J *ob/ob* mice aged 2, 6, and 8 mo.[27] The decrease in pancreatic SRIF-LI concentration in 6J *ob* and *db* homozygotes found in the present study thus appears to be a relative rather than an absolute deficiency and is present from an early age, although at a time when obesity, hyperglycemia, and hyperinsulinemia have already become manifest.

In KsJ *db/db* and *ob/ob* mice, no change in pancreatic SRIF-LI was seen until 10 wk of age. Thereafter pancreatic SRIF-LI content and concentration was considerably increased in diabetic animals compared with normal controls. A similar increase in pancreatic SRIF-LI has been seen in KsJ *db/db* mice by Makino et al.[10] and immunohistochemically in KsJ *db/db* and *ob/ob* mice by Baetens et al.[16] and Leiter et al.[17] In streptozotocin- and alloxan-induced diabetes in the rat, an increase in pancreatic SRIF-LI has been seen immunohistochemically[12–15] and in extracts of whole pancreas[7–9] or islets;[7,8,11] an increased volume density of islet SRIF-LI positive cells has been observed immunohistochemically in human juvenile-onset diabetes.[13] A decrease in pancreatic SRIF-LI described in the diabetic Chinese hamster[28] and by Patel et al. in KsJ *db/db* mice aged 11–12 wk[18] was not seen in our studies.

The appearance of increased pancreatic SRIF-LI in KsJ

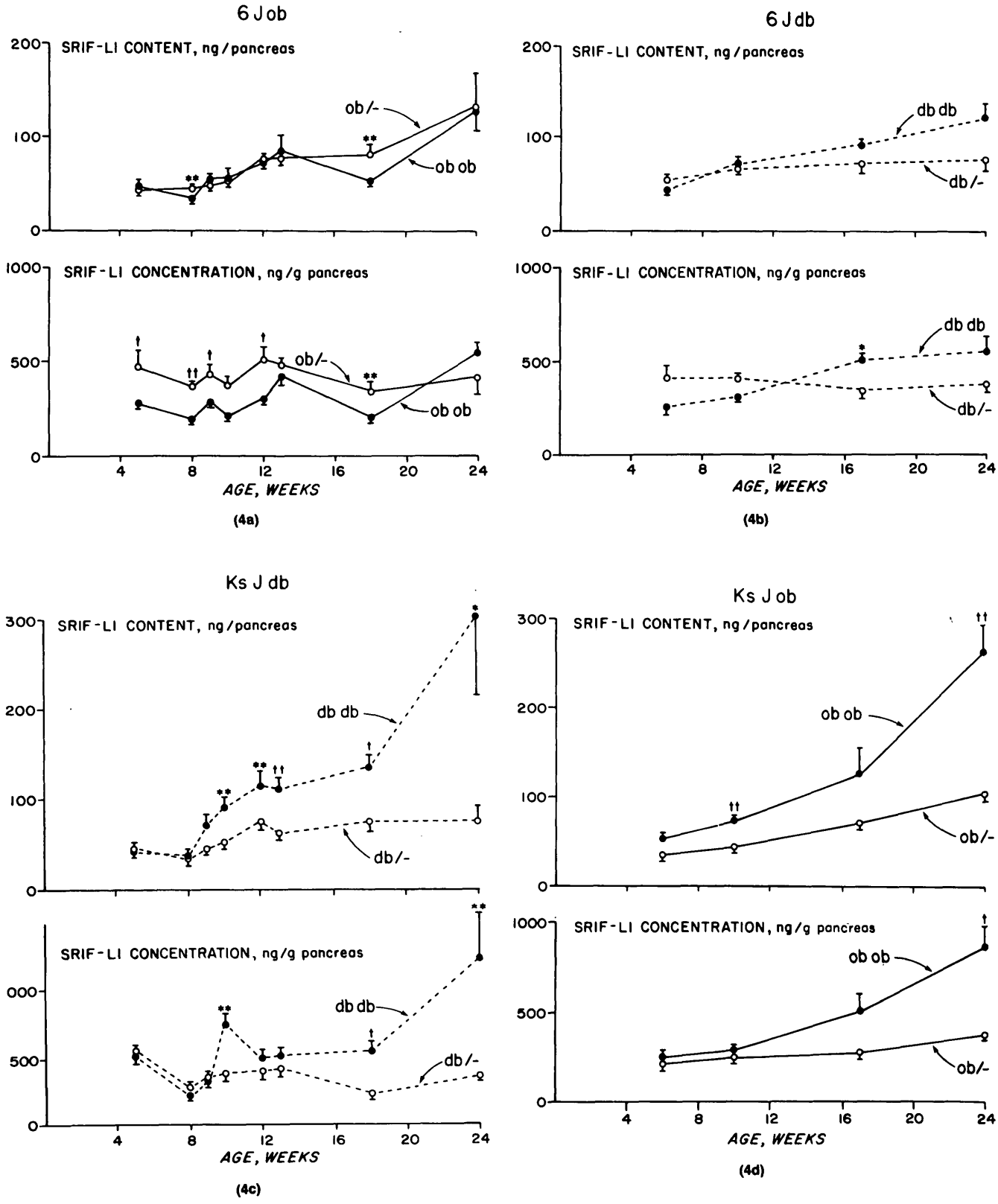


FIGURE 4a–d. Pancreatic SRIF-LI content (above) and concentration (below) in 6J *ob* (Figure 4a) and 6J *db* (Figure 4b) obese mice and in KsJ *db* (Figure 4c) and KsJ *ob* (Figure 4d) diabetic mice aged from 5 to 24 wk compared with normal littermate controls. Results are expressed as mean ± SEM. Tissue samples from 10 animals were studied at each time point. P values: * <0.05, ** <0.02, † <0.01, and †† <0.001.

db/db and *ob/ob* mice concomitant with the initial decrease in serum insulin levels, together with the continuous rise in pancreatic SRIF-LI as insulin secretion progressively fails, suggests that it is hypoinsulinemia or an associated metabolic abnormality other than hyperglycemia that is responsible. The fact that insulin replacement in streptozotocin-diabetic rats results in normalization of the pancreatic SRIF-LI elevation without totally correcting the hyperglycemia provides further support for this suggestion.[9] The elevation of pancreatic SRIF-LI in KsJ *db/db* and *ob/ob* mice in the presence of insulin levels that are still greater than in controls suggests that the D-cells exhibit a resistance to the effect of insulin. The present studies confirm previous suggestions that the genetic background of the mouse (KsJ or 6J) rather than the superimposed mutant *ob* or *db* gene is of critical importance in determining the metabolic sequelae, which include alterations in pancreatic SRIF-LI in addition to the well-recognized changes in insulin secretion and glucose homeostasis.

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