Syndrome X–Like Alterations in Adult Female Rats Due to Neonatal Insulin Treatment

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Hypothalamic structures are decisively involved in the regulation of body weight and metabolism. In syndrome X, complex metabolic alterations are present, which in women are found to be associated with disturbances of reproductive function and altered androgen levels. In previous experiments in rats, it was shown that a temporary intrahypothalamic hyperinsulinism during early life predisposes to overweight and diabetogenic disturbances later in life, associated with disorganization of hypothalamic regulatory centers. To investigate the possible long-term consequences of elevated peripheral insulin levels during ontogenesis, the following experiment was performed. Newborn female Wistar rats were treated during neonatal life with daily subcutaneous injections of long-acting insulin ([IRI group] 0.3 IU on days 8 and 9 of life and 0.1 IU on days 10 and 11 of life), whereas control animals (CO) received daily NaCl injections. This temporary exposure to increased insulin levels during a critical developmental period resulted in an increased body weight gain including juvenile life and adulthood ($P < .01$), accompanied by hyperinsulinemia ($P < .01$), impaired glucose tolerance ($P < .05$), and increased systolic blood pressure in adulthood ($P < .025$). No significant alterations were detected either in cyclicity and fertility or in the levels of testosterone, androstenedione, or dehydroepiandrosterone (DHEA) in IRI rats. Morphometric evaluation of hypothalamic nuclei showed a reduced numerical density of neurons ($P < .025$) and a decreased neuronal volume density ($P < .025$) within the ventromedial hypothalamic nucleus (VMN) of the IRI rats, whereas the antagonistic lateral hypothalamic area (LHA) was morphometrically unchanged. Newborn offspring of IRI rats (F1 generation) were overweight ($P < .05$) and had an increased pancreatic insulin concentration ($P < .02$). In conclusion, perinatal hyperinsulinism seems to predispose to the later development of syndrome X–like changes in female rats, possibly due to impaired organization of hypothalamic regulators of body weight and metabolism.

BESIDES A VARIETY of other factors, mediobasal hypothalamic nuclei are well known to be involved in the regulation of metabolism and body weight (reviewed in Bray et al[1]). In animals, destruction of the ventromedial hypothalamic nucleus (VMN) leads to hyperinsulinemia, obesity, and an increased diabetes susceptibility.[2,3]

The metabolic syndrome,[4] or syndrome X,[5] represents a complex of disturbances of carbohydrate and fat metabolism characterized by obesity, dyslipoproteinemia, hyperinsulinemia, and impaired glucose tolerance, which were already described to coincide in the 1960s.[6,7] In clinical studies, syndrome X was found to be associated with hypertension and an elevated risk for cardiovascular disease.[8,9] During recent years, the role of hyperinsulinemia and insulin resistance in the complex pathogenesis of these alterations, especially for initiation of hypertension, has been variously discussed.[9-11] In females, syndrome X–like disturbances are often found associated with impaired reproductive function and altered androgen levels, as in the polycystic ovary syndrome (PCO).[12,13]

In clinical studies, overweight and diabetogenic disturbances were found to occur frequently in the offspring of women with diabetes during pregnancy,[14,15] but the pathophysiological mechanisms responsible for these alterations remain unknown. In previous animal investigations, it was shown that an elevated insulin concentration during critical periods of development, as occurs perinatally in the offspring of gestationally diabetic mothers, could lead to permanent malorganization of the VMN, accompanied by diabetogenic disturbances in later life.[16]

For further characterization of a possible impact of early alterations in insulin levels on the later development of metabolic alterations, as well as reproductive function, and a possible association with hypothalamic disorganization, an alternative animal model for early hyperinsulinism was used in the following experiment.

MATERIALS AND METHODS

Animal Model

The investigations were performed in the offspring, bred in our institute, of Wistar rats of an outbred colony strain (Shoe: Wist/2 (Ico)). Virgin female rats were time-mated at the age of 3 months. Under standard laboratory conditions, they were singly housed during pregnancy. On the first day of life, newborn pups were randomly distributed among the mothers. A total of 10 newborn female rats received subcutaneous injections of a long-acting zinc insulin (Berlin-Chemie, Berlin, Germany; Lot No. 081288) from days 8 to 11 of life (IRI group). On days 8 and 9, 0.3 IU was injected, and on days 10 and 11 of life, only 0.1 IU was given. In 10 control (CO) females, an appropriate volume of NaCl was injected. No mortality occurred in either of the two groups. The mean litter size in the CO group was 10.7 ± 1.5 (n = 5), and in the IRI group, 9.8 ± 3.7 (n = 5). All animals were reared under standard conditions with a 12-hour inverse light-dark cycle (lights on from 5:00 PM to 5:00 AM). After weaning on day 21 of life, rats were housed in groups of three to four per Plexiglas cage, except in the period for measurement of food intake on days 110 to 115 of life. They had free access to a standard pellet diet (Altromin, Lage, Germany), and tap water was provided ad libitum. All procedures were approved by the local Animal Care and Use Committee (G 0297/92).

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Functional, Endocrine, and Metabolic Parameters

Body weight was recorded throughout life in all animals. On day 210 of life, relative body weight was evaluated by dividing the body weight by the body length excluding tail length. Additionally, the Lee obesity index[17] was calculated by dividing the cube root of body weight by body length. From days 110 to 115 of life, daily mean food intake was measured by housing the animals individually in feeding cages.

At age 5 months, glucose tolerance tests were performed. After an overnight fast (16 hours), glucose (1.5 g/kg body weight) was injected intraperitoneally using a 20% glucose solution. Before and 15, 30, and 90 minutes after glucose loading, blood samples for determination of glucose (glucosoxidase [GOD]-peroxidase method) were taken by puncture of the retroorbital plexus. Using these values, the area under the curve of glucose (AUCG) during the glucose tolerance test was calculated according to Cederholm and Wibell.[18]

At age 7 months, the mean systolic blood pressure was recorded noninvasively using a modified Riva-Rocci tail-cuff method (TSE, Bad Homburg, Germany) according to Bunag and Teräväinen.[19] Animals were adapted to the measurement conditions on 5 successive days for 10 minutes each. Thereafter, measurements were performed on 5 more successive days during the dark period, and the mean value was calculated for each animal.

At 7 months of age, blood was taken by puncture of the retroorbital plexus under light ether anesthesia, and the basal blood glucose level was measured (GOD-peroxidase method). Within the same samples, immunoreactive plasma insulin, plasma testosterone, androstenedione, and dehydroepiandrosterone (DHEA) were determined as described later. Additionally, the insulin to glucose ratio was calculated as a measure of insulin resistance.[20]

Cyclicity, Fertility, Pregnancy, and Development of F1-Offspring

The cyclicity of the females was tested by taking vaginal smears daily between 9:00 and 9:30 AM for 12 days at age 3 months. The percentage of estrus days per total number of test days was evaluated.[21] Fertility was examined at 6 months of age by mating each female for 10 days with two normal, nontreated males of proven fertility.[21] The day of conception was confirmed by the presence of sperm in vaginal smears, designated as day 0 of pregnancy. During pregnancy, glucose tolerance was determined on day 20 of gestation as already described.

In F1-offspring, body weight was recorded until day 15 of life. On day 10 of life, some offspring of both experimental groups were killed by rapid decapitation, and trunk blood was collected for determination of blood glucose (GOD-peroxidase method). The remaining animals were decapitated on day 15 of life, and trunk blood was taken for analysis of blood glucose and immunoreactive plasma insulin. Pancreatic insulin concentrations were determined additionally, as described later.

Histologic Measurements

On day 210 of life, IRI and CO females were rapidly decapitated. The brains were quickly removed and immediately processed by fixation in 4% Formalin solution. After fixation, brains were embedded in paraffin. According to Paxinos and Watson,[22] 5-μm thick successive coronal sections were made within plane 27. Sections were Nissl-stained (Nissl+) with cresyl violet, and cover slips were applied. Slides were coded so that analysis could be performed without knowledge of the subject's treatment. Using a light microscope (Zeiss, Jena, Germany) with a stereological test grid (quadratic measurement field with 100 test points), the numerical neuronal density and volume density of neuronal nuclei, neuronal cytoplasm, and whole neurons within the VMN and the lateral hypothalamic area (LHA) were assessed at magnification 1,000×.[23] In both hemispheres of the brain, five (VMN) or six (LHA) microscopic fields of measurement were placed to cover the whole extent of the nuclei. Thereby, every fifth successive section was evaluated in each brain. According to Bereiter and Jeanrenaud[24] only neurons with a distinct nucleolus and soma appearance were included.

Hormone Analysis

For determination of immunoreactive plasma insulin, a double-antibody radioimmunoassay was performed (BioChem ImmunoSystems, Freiburg, Germany). Rat insulin (Novo Nordisk, Copenhagen, Denmark) with a biological potency of 21.3 IU/mg was used as a standard preparation. The intraassay coefficient of variation was 4.5% to 7.4% in a concentration range of 9.1 to 94.2 mIU/L (n = 10). The interassay coefficient of variation was 11.9% to 16.1% between 7.8 and 51.0 mIU/L (n = 22 to 31).

For steroid assays, plasma samples were extracted with ether. After freezing, the ether phase was decanted and evaporated in small tubes. Plasma testosterone was estimated by radioimmunoassay using [1,2,6, 7-^{3}H]testosterone (Du Pont NEN, Bad Homburg, Germany) as a tracer and a rabbit-derived antiserum against testosterone-3-(carboxymethyl)-oxim–bovine serum albumin (BSA) conjugate.[25] Plasma extracts were dissolved in antiserum (1:80,000) containing 10,000 dpm tracer and 0.5% gelatine, mixed for 20 minutes on ice, and incubated overnight (16 hours) at 4°C. After centrifugation (2,000 × *g* for 10 minutes at 4°C), the supernatants were decanted, mixed with a scintillation cocktail for 1 hour at 4°C, and measured by a scintillation counter. No or only very low cross-reactivity (<.01%) with other steroids was detected, except for 5α-dihydrotestosterone. Intraassay variability was 6.6% at 20.4 nmol/L (n = 36).

For determination of plasma androstenedione, a radioimmunoassay was performed using a rabbit-derived antibody against androstenedione-3-(carboxymethyl)-oxim-BSA and [1,2,6, 7-^{3}H]androstenedione (Du Pont NEN) as a tracer.[25] Samples were incubated with antiserum (1:80,000) for 20 minutes at 4°C, followed by addition of 15,000 dpm tracer and a further incubation overnight (16 hours) at 4°C. Dextran-coated charcoal was added, and the samples were mixed for 10 minutes on ice followed by centrifugation at 2,000 × *g* for 10 minutes. The supernatants were decanted, mixed with scintillation cocktail for 1 hour at 4°C, and measured by scintillation counter. Cross-reactivity with DHEA was 1.5%, testosterone 0.26%, dihydrotestosterone 0.14%, androsterone 2.1%, cortisol 0.004%, pregnenolone less than 0.01%, and progesterone 0.25%. The intraassay coefficient of variation was 5.4% at 3.7 nmol/L (n = 19), and interassay variation was 10.0% at 9.5 nmol/L (n = 30).

Plasma DHEA concentrations were estimated by radioimmunoassay using a rabbit-derived antibody against DHEA-17 hemisuccinate-BSA conjugate and [1,2,6,7-^{3}H]DHEA (Du Pont NEN) as a tracer.[25] Samples were dissolved in anti-DHEA (1:6,000). After mixing, 15,000 dpm tracer was added and the samples were incubated overnight (16 hours) at 4°C. After addition of 200 μL dextran-coated charcoal, the samples were mixed for 10 minutes in an ice bath and centrifuged at 2,000 × *g* for 10 minutes at 4°C, and the supernatants were mixed with 4 mL toluene and scintillation cocktail and measured in a scintillation counter. Cross-reactivity with 3β-hydroxy-5α-androstan-17-one was 25%, 4-androstene-3β-17β-diol 2.5%, 3α-hydroxy-5α-androsten-17-one (androsterone) 25%, 4-androstenedione less than 0.003%, and pregnenolone less than 0.05%. The intraassay variation was 4.8% at 1.5 nmol/L (n = 11), and interassay variation 11.3% at 1.5 nmol/L (n = 11).

All assays were analyzed using the RIA-Calc program (Pharmacia, Uppsala, Sweden).

Extraction of Pancreatic Insulin in F1-Offspring

The pancreas was carefully removed, weighed, and stored at −70°C until further processing. Pancreatic insulin was extracted using a

modification of the method of Ziegler et al.[26] The tissue was cut into small fragments and disintegrated two times at 24,000 rpm (Ultra-Turrax; IKA, Stauffen, Germany) for 60 seconds in 10 mL extraction solution (1 mol/L phosphoric acid and 70% ethanol, distilled water). After sonication by an ultrasonic homogenizer (Sonopuls HD 200; Bandelin, Berlin, Germany) on ice two times for 60 seconds, the samples were incubated after every sonication for 2 hours at 4°C. The extract was centrifuged for 10 minutes at 2,500 × *g*, and the supernatant was removed; 50 µL supernatant was diluted with assay buffer at 1:20, divided into aliquots, and stored at −70°C until assay. For determination of pancreatic insulin, a double-antibody radioimmunoassay was performed as already described.

Statistical Analysis

Data are expressed as the means ± SEM. ANOVA followed by unpaired Student's *t* test (with or without Welch correction) was used for determination of significant differences between the groups. Spearman's rank correlation test was used to analyze relations between two variables. Statistical evaluations were performed using the SPSS for Windows statistical package (SPSS Software, Munich, Germany).

RESULTS

Body Weight

From day 10 of life onward, IRI rats displayed significantly elevated body weight as compared with CO animals ($P < .01$ and $P < .001$, respectively; Fig 1). Body weight remained elevated throughout adult life in IRI females, resulting in significantly increased absolute (CO *v* IRI, 268 ± 7.5 *v* 307 ± 8.6 g, $P < .005$) and relative (CO *v* IRI, 12.0 ± 0.3 *v* 13.2 ± 0.2 g/cm, $P < .005$) body weight on day 210 of life. Moreover, the Lee obesity index was found to be slightly but significantly elevated in IRI rats (CO *v* IRI, 0.290 ± 0.002 *v* 0.300 ± 0.003, $P < .01$).

The increase of body weight was not associated with significantly higher daily mean food intake in IRI rats, as measured from days 110 to 115 of life (CO *v* IRI, 16.0 ± 0.9 *v* 16.4 ± 0.6 g/d, NS).

Metabolic Parameters

IRI rats showed basal hyperinsulinemia in adult life ($P < .02$) and a significantly elevated insulin to glucose ratio on day 210 of life ($P < .05$). This was accompanied by elevated mean systolic blood pressure in these rats ($P < .025$; Fig 2). Basal plasma insulin was significantly correlated with absolute and relative body weight ($r = .45$, $P < .05$ and $r = .45$, $P < .05$, respectively). Furthermore, the insulin to glucose ratio displayed significant positive correlations with absolute body weight ($r = .52$, $P < .02$), relative body weight ($r = .45$, $P < .05$), and mean systolic blood pressure ($r = .45$, $P < .05$).

Glucose tolerance tests on day 160 of life revealed an impaired glucose tolerance in the IRI group, showing significantly higher blood glucose before as well as 15 minutes after glucose loading ($P < .05$ and $P < .005$, respectively; Table 1). Impaired glucose tolerance in IRI females was confirmed by a significantly elevated AUCG, as compared with controls (CO *v* IRI, 6.2 ± 0.2 *v* 7.4 ± 0.4 mmol/L · h, $P < .05$).

Reproductive Function

In both groups of rats, regular 3- to 4-day cycles were observed, without significant differences in the frequency of estrus days between the groups (CO *v* IRI, 35.0% (42 of 120 days) *v* 38.3% (46 of 120 days), NS). Neither testosterone (CO *v* IRI, 1.1 ± 0.1 *v* 1.1 ± 0.1 nmol/L) nor androstenedione (CO *v* IRI, 2.8 ± 0.1 *v* 2.8 ± 0.3 nmol/L) levels were changed at the age of 7 months in IRI females compared with controls. Only DHEA showed a weak but nonsignificant elevation in the IRI group (CO *v* IRI, 3.4 ± 0.3 *v* 4.2 ± 0.5 nmol/L, NS). Fertility was not found to be significantly decreased in IRI females compared with CO (CO *v* IRI, 60% (six of 10) *v* 30% (three of 10), NS).

Morphometric Analysis of Hypothalamic Structures

Analysis of morphometric parameters within the mediobasal hypothalamus of 210-day-old rats showed a significant reduc-

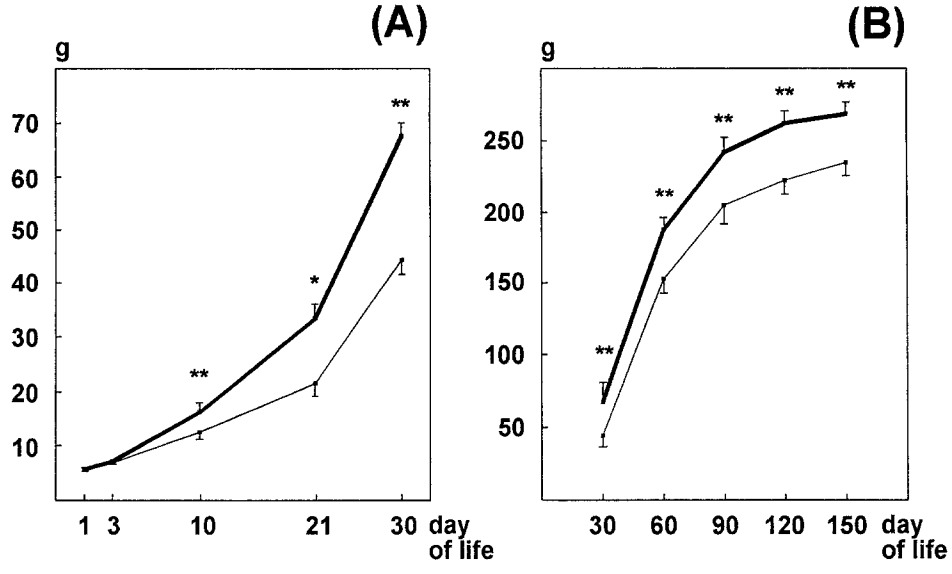

Fig 1. Body weight from days 1 to 30 of life (A) and days 30 to 150 of life (B) in IRI female rats (bold line, n = 10) *v* CO females (thin line, n = 10). Data are the mean ± SEM. *$P < .01$, **$P < .001$ *v* CO.

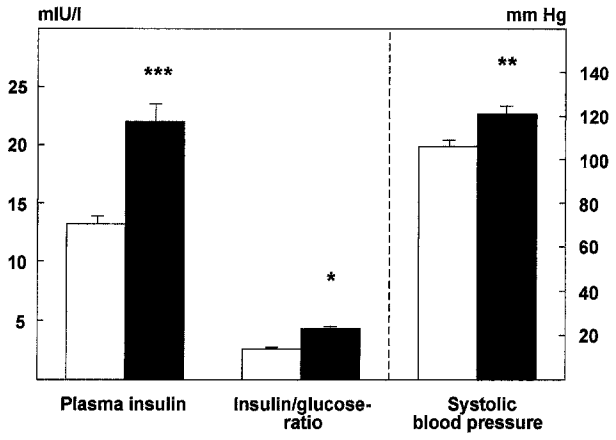

Fig 2. Plasma insulin, insulin to glucose ratio, and mean systolic blood pressure in 7-month-old IRI female rats (■, n = 10) *v* CO females (□, n = 10). Data are the mean ± SEM. *$P < .05$, **$P < .025$, ***$P < .02$ *v* CO.

tion of the mean numerical density of neurons within the VMN of IRI females ($P < .025$). This was accompanied by a significant decrease of the volume density of neurons and neuronal cytoplasm, respectively, in the VMN of IRI females ($P < .05$ and $P < .02$, respectively; Fig 3). In contrast, within the LHA, none of the stereological parameters were significantly different in IRI versus CO rats (Fig 4).

Development of F1-Offspring

A glucose tolerance test during pregnancy revealed a weak but nonsignificant increase of the AUCG in IRI compared with CO females (CO *v* IRI, 5.2 ± 0.4 (n = 6) *v* 5.9 ± 0.2 mmol/L · h (n = 3), NS). The litter size was similar in both groups (CO *v* IRI, 8.5 ± 4.1 (n = 6) *v* 9.3 ± 5.7 (n = 3), NS), as well as the mortality rate in the offspring of CO mothers compared with offspring of IRI mothers until day 15 of life (offspring of CO *v* IRI, 2.0% (one of 51) *v* 3.6% (one of 28), NS). Figure 5 shows the development of body weight in both groups of offspring. Starting from the day of birth, offspring of IRI mothers displayed an elevation in body weight that was significant on days 1, 10, and 15 of life compared with offspring of CO mothers. Blood glucose was similar in both groups of offspring on day 10 of life (CO *v* IRI, 6.0 ± 0.2 (n = 23) *v* 6.0 ± 0.1 mmol/L (n = 13)), as well as day 15 of life (CO *v* IRI, 6.1 ± 0.2 (n = 26) *v* 5.9 ± 0.2 mmol/L (n = 14), NS). Plasma insulin showed no significant difference between the offspring of CO and IRI rats on day 15 of life (CO *v* IRI, 30.8 ± 2.5 (n = 26) *v* 25.4 ± 7.5 mIU/L (n = 14), NS), but the offspring of IRI rats had an increased pancreatic insulin concentration (CO *v* IRI, 9.6 ± 0.8 (n = 12) *v* 12.7 ± 0.8 mIU/L (n = 14), $P < .02$).

Table 1. Glucose Tolerance on Day 160 of Life in IRI Female Rats Compared With CO Females (1.5 g glucose/kg body weight intraperitoneally; mean ± SEM)

Group	0 min	15 min	30 min	90 min
CO (n = 10)	3.6 ± 0.1	9.8 ± 0.4	6.8 ± 0.5	5.3 ± 0.1
IRI (n = 10)	4.4 ± 0.2†	11.3 ± 0.5*	7.7 ± 0.6	5.8 ± 0.3

*$P < .05$ *v* CO.
†$P < .01$ *v* CO.

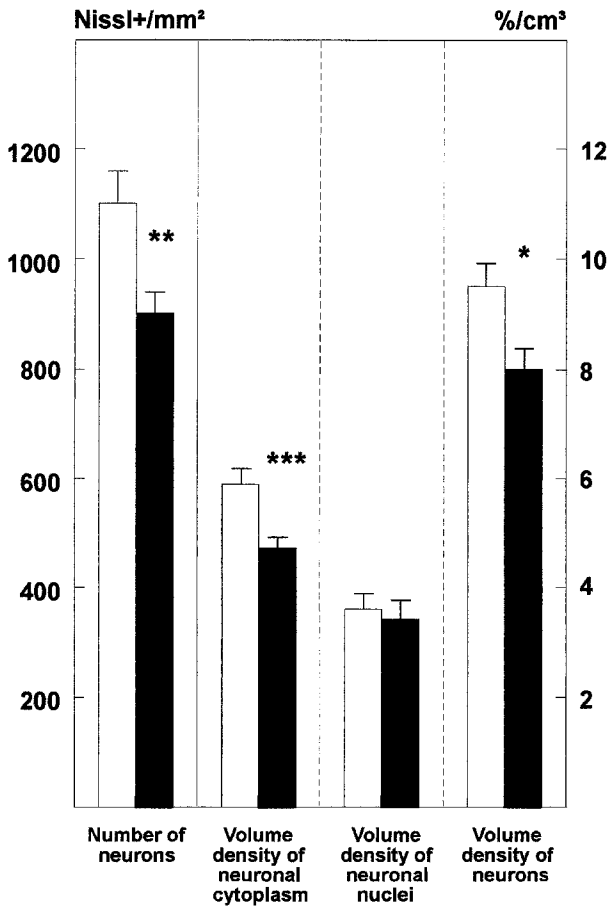

Fig 3. Stereological parameters within the VMN of 210-day-old IRI female rats (■, n = 10) *v* CO females (□, n = 10). Data are the mean ± SEM. *$P < .05$, **$P < .025$, ***$P < .02$ *v* CO.

DISCUSSION

The results of the present study indicate that temporary hyperinsulinism during a critical period of early development could lead to permanent alterations of body weight, glucose metabolism, and blood pressure associated with disorganization of the VMN, which is known to be a regulatory center of body weight and metabolism. Since the discovery of the ob/ob-coded protein leptin, attention has focused again on the hypothalamic structures involved in body weight regulation and their possible pathogenetic role in the development of obesity.[27,28] In this context, although the significance of the hypothalamic-arcuate-paraventricular axis is mainly discussed,[29] one of the most intensively investigated hypothalamic regulation centers of metabolism remains the VMN.[30-32] Whereas the sympathetic VMN is known to inhibit insulin secretion and food intake and to stimulate lipolysis, the antagonistic parasympathetic LHA stimulates insulin output, food intake, and lipogenesis.[1,33,34] Lesions of the VMN result in hyperinsulinemia, obesity, and increased diabetes susceptibility, which were reported to be even more pronounced than after lesions of the paraventricular hypothalamic nucleus.[35]

In rats, the differentiation of these hypothalamic nuclei is known to last until day 15 to 20 of postnatal life.[36,37] The period around day 10 of life seems to represent a particularly critical

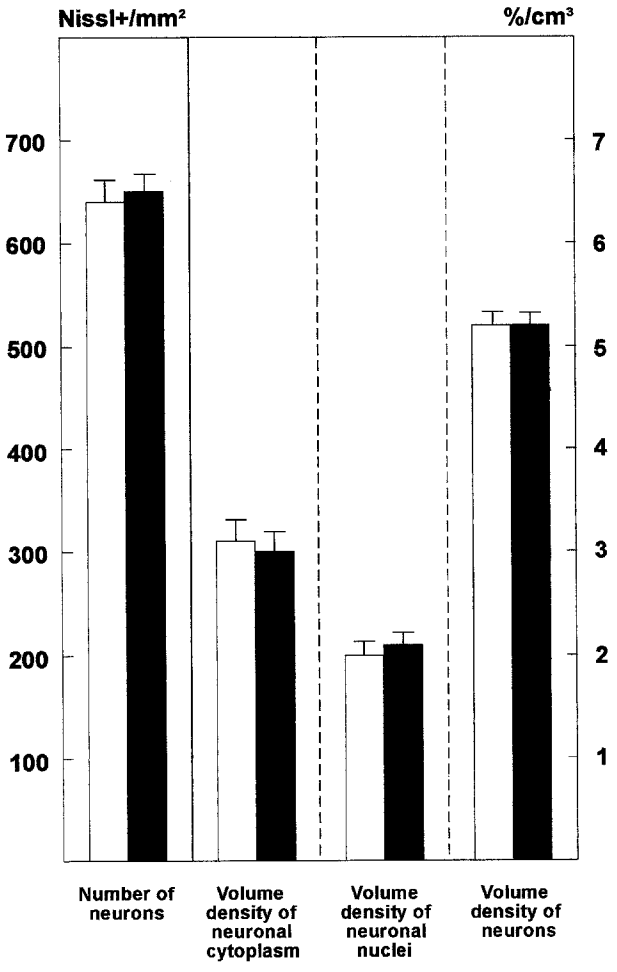

Fig 4. Stereological parameters within the LHA of 210-day-old IRI female rats (■, n = 10) *v* CO females (□, n = 10). Data are the mean ± SEM.

time in development, with an increased vulnerability of neuroendocrine systems to develop permanent malorganization.[21,38]

Morphometric alterations in the VMN of IRI rats were similar to those observed in former experiments in which a temporary, only intrahypothalamically localized hyperinsulinism was induced by stereotactic application of insulin into the mediobasal hypothalamus of rats at the same age (day 8 of life), leading to clear overweight and increased diabetes susceptibility later in life.[39]

The mediobasal hypothalamus shows a high density of insulin receptors, which are known to be present in the rat brain already during fetal life.[40,41] Insulin receptors in the developing central nervous system (CNS) are regulated by peripheral insulin concentrations.[42] Furthermore, insulin is known to act as a modulator of central neuronal development.[43] Therefore, it seems possible that elevated insulin concentrations could influence the development of hypothalamic structures, which are involved in the regulation of body weight and metabolism. Besides the presence of a receptor-mediated uptake of insulin into the CNS (reviewed in Schwartz et al[44]), an increased permeability of the blood-brain barrier is characteristic of the developing brain, particularly the hypothalamus.[45] Therefore, a peripheral increase of insulin concentrations as occurs, eg, in the fetuses of gestationally diabetic mothers[46] also may influence the development of hypothalamic structures.

Interestingly, disorganization of the VMN was also observed in genetic animal models of obesity and diabetogenic disturbances. In ob/ob mice, Bereiter and Jeanrenaud[24] described malorganization of this nucleus, while the LHA was found to be unchanged. Similar results were reported from investigations in the diabetic Chinese hamster.[47] In the db/db mouse, a decreased neuronal density within the VMN was also observed.[48]

Morphometric alterations in the VMN of IRI rats in our study may lead to a disturbed functional activity of this nucleus, as could be reflected by a decreased volume density of neuronal cytoplasm.[49] As a consequence of disturbed VMN organization and possibly successive dysfunction, a relative hyperactivity of the antagonistic LHA could be suggested, as observed in VMN-lesioned rats,[3] leading to hyperinsulinemia, impaired glucose tolerance, and obesity. In further studies on this experimental model, morphometric measurements in the paraventricular and arcuate nucleus should be performed, especially since animal models of endogenous postnatal hyperinsulinism (small litters, offspring of gestationally diabetic mothers) show morphometric alterations within these nuclei,[50,51] as well as genetic models of obesity and associated diabetic disturbances.[47,48]

The development of overweight, probably due to an increase of body fat, in our animal model was confirmed by elevation of the Lee obesity index, which is a good measure to characterize increased body fat, especially in VMN-lesioned rats.[52] Only a small but significant increase of the Lee index was seen in IRI rats. However, a small elevation of the Lee index indicates a relatively large increase in body fat, as shown by other investigators.[52] On the other hand, the validity of the Lee index is still controversial, since it was shown to correlate with body fat in some but not all animal models of obesity.[53,54]

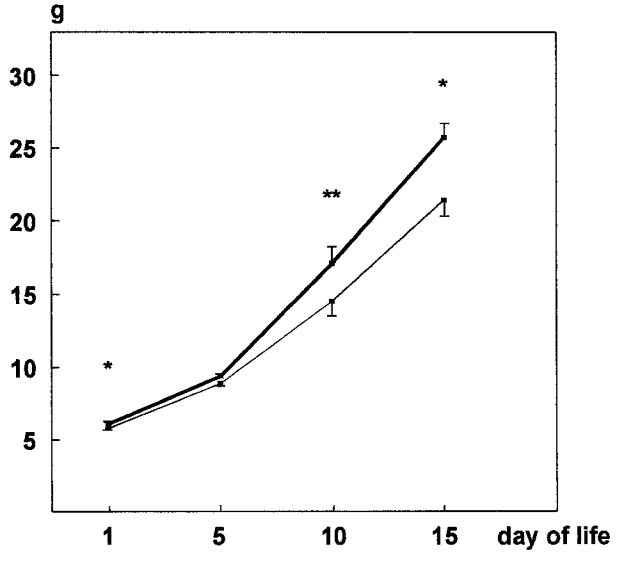

Fig 5. Body weight from days 1 to 15 of life in F1-offspring of IRI female rats (bold line, n = 14) *v* offspring of CO females (thin line, n = 26). Data are the mean ± SEM. *$P < .05$, **$P < .0005$ *v* offspring of CO.

In our animal model, overweight was not associated with a significant elevation of the mean food intake. Nevertheless, assuming that the small difference in food consumption existed throughout life, it can be presumed that it was sufficient to account for the differences in body weight. On the other hand, Kennedy[30] in 1957 and Han and Frohman[55] showed that obesity due to VMN lesions in weanling and adult rats develops independently of food intake, as confirmed by Balkan et al,[2] possibly due to decreased brown adipose tissue thermogenesis.[56] Additionally, it cannot be excluded that housing the animals in group cages in our experiment may have contributed to the results obtained in measurements of feeding behavior (performed in single cages), blood pressure, and/or blood glucose.

IRI rats in our study displayed features like overweight, hyperinsulinemia, impaired glucose tolerance, elevated blood pressure, and signs of insulin resistance that can be interpreted as a predisposition to syndrome X–like abnormalities. In recent years, especially the role of hyperinsulinemia and insulin resistance in the pathogenesis of syndrome X has been discussed.[10,11,57] In genetic animal models, an association between elevated insulin levels and hypertension has been frequently described.[58,59] However, despite the higher insulin and blood pressure levels in IRI animals, no significant correlation between insulin and blood pressure was found in our study. In contrast, the insulin to glucose ratio was positively correlated with blood pressure, possibly indicating that insulin resistance plays a role in the development of these alterations. Furthermore, other metabolic factors, eg, hypertriglyceridemia due to hyperinsulinemia as occurs in VMN-lesioned rats,[60] may have contributed to the elevation of blood pressure in these animals.

In females with PCO, hyperinsulinemia, insulin resistance, obesity, and hypertension are found to be associated with subfertility or infertility, hyperandrogenemia, and an irregular cycle, while elevated insulin levels are frequently discussed as a possible cofactor in the complex pathogenesis of this disease.[12,61] Hyperinsulinemia was also described in animal models of PCO.[62] However, despite the presence of clear functional and metabolic alterations like overweight, hyperinsulinemia, and hypertension, no significant disturbances of reproductive parameters were observed in this study in IRI compared with CO female rats. In conclusion, factors other than those altered in this particular animal model may significantly contribute to the development of PCO-like changes in female rats.

In the offspring of streptozotocin-diabetic female animals, a maternofetal transmission of elevated diabetes susceptibility due to gestational hyperglycemia, and hence fetal and neonatal hyperinsulinism, has been described through successive generations.[16,63] Therefore, in a preliminary study, we investigated body weight changes and glucose and insulin levels in infantile offspring of IRI mothers. Interestingly, F1-offspring of IRI mothers displayed clearly elevated body weight from days 1 to 15 of life, as well as increased pancreatic insulin concentrations. However, no significant differences in blood glucose or insulin levels were observed between the groups. Nevertheless, it must be emphasized that in IRI mother rats, only a tendency for elevated blood glucose during pregnancy was found, possibly due to the relatively low number of pregnancies in the IRI group.

In summary, in female rats, peripheral hyperinsulinism during a critical period of perinatal development leads to complex functional and metabolic disturbances like overweight, impaired glucose tolerance, hyperinsulinemia, and elevated blood pressure in later life, similar to the changes in body weight and metabolism observed during prior experiments in male rats,[39,64,65] which can be interpreted as a predisposition to syndrome X–like changes. No significant alterations of reproductive function or sex steroids were detected. Functional and metabolic alterations were found to be associated with disorganization of a hypothalamic metabolic regulatory center, the VMN, while the antagonistic LHA was unchanged. The infantile offspring of IRI female rats displayed overweight and elevated pancreatic insulin concentrations.

These results point to perinatal hyperinsulinism as a possible risk factor for the later development of syndrome X–like alterations, possibly due to impaired organization of hypothalamic regulators of body weight and metabolism.

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