ORIGINAL ARTICLE

DEVELOPMENT OF LIPID AND GLYCIDE ABNORMALITIES IN GENETICALLY HYPERTENSIVE OBESE KOLETSKY RATS AND IN THEIR LEAN SIBLINGS

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Summary: Experiments were performed in the genetically hypertensive Koletsky rats and in their lean siblings at the age of two and three months. In the study of development of glycide and lipid abnormalities animal represents control for itself.At the age of two months Koletsky obese rats show relative to their lean controls elevation of plasma triglycerides (males +184%, females +152%) and insulin (males +169%, females +201%). During one month plasma triglycerides elevated in lean males +9%, in lean females 0%, but in obese males +21%, in obese females +139%.Considering insulinemia similar results were obtained. Thus during one month insulin elevates in lean males +19%, in lean females +23%, but in obese males +80%, in obese females +144%. During one month glucose intolerance is elevated as well only in obese rats. Total plasma cholesterol during period of one month shows no changes in both substrains of rats.Similar picture can be found in basal glycemia.In all groups of rats no changes were registered except one, i.e., obese females show decrease.Considering the substrain differences in basal glycemia then at age of one as well as two months obese of both sexes show elevation. As to the body weight at the age of two as well as three months there is increase in obese rats. The changes of body weight during one month are expressively higher in obese rats.

Key words: *Development of glycide and lipid abnormalities; Koletsky obese and lean SHR rats; Insulinemia; Glucose tolerance; Triglycerides; Cholesterol; Basal glycemia*

Introduction

Developmental changes of abnormalities in genetically hypertensive obese Koletsky rats were originally described by Koletsky (8). He analyzed the body weight from two to ten months of age in the obese rats and in the their lean siblings. The author monitored development of plasma triglycerides from two to twelve months. In the period from two months to four months triglycerides elevate from 1.98 mmol/l to 4.67 mmol/l. Total plasma cholesterol in this same period elevated from 2.49 mmol/l to 3.74 mmol/l. The author did analyzed sexual differences.

At recent time one group of authors turn their attention to developmental aspect of metabolic abnormalities in the rats which can be viewed as a potential model of type II diabetes. Ionescu et al. (7) studied blood glucose tolerance in genetically obese (fa/fa) rats at 6- to,7-wk-and 13- to 14-wk-old lean and obese (fa/fa) rats. They found that glucose intolerance became more pronounced with the duration of the syndrom. Moreover, the 6- to 7- wk obese rats showed normal and even higher beta-cell responsiveness to intravenous or oral glucose. In contrast, the 13- to 14-wk obese rats presented a decreased beta-cell responsiveness to such stimuli. Thus the beta-cell function of obese rats worsens with time.

The mentioned authors came to the conclusion that inasmuch as 13- to 14-wk-old obese fa/fa rats have insulin resistance, elevated basal glycemia, and abnormal glucose tolerance, they can be viewed as a potential model of type II diabetes.

Data acumulated in our paper (3) when we monitored plasma triglycerides, basal glucose and glucose tolerance in genetically hypertensive obese Koletsky rats and data presented 1989 (6) when we demonstrated hyperinsulinemia in the mentioned Koletsky obese rats suggest that this type of rats can be judged as a potential genetically based animal model of diabetes II.

DeFronzo et al. (1) when summarizing our recent knowledge in the ethiology of non-insulin dependent diabetes mellitus (NIDDM) came to the conclusion that at the earliest stages of development of NIDDM there is elevated secretion of insulin.

Thus our monitoring of development of glycide and lipid abnormalities in obese Koletsky rats and in their siblings is well founded.

Material and methods

Animals

Experiments were performed in obese and lean genetically hypertensive rats of Koletsky type (8) of both sexes at the age of two months (54 - 59 days) and three months (92 - 95 days). The animal represents the control for itself. Lean Koletsky rats represent dominant non-obese homozygotes and heterozygotes whereas their obese siblings are recessive homozygotes. The abnormal animals were obtained by Koletsky (8) when mating spontaneously hypertensive female (Okamoto-Aoki strain) with a normotensive Sprague-Dawley male rat. The genetically obese animals appeared after several generation of selective inbreeding of hypertensive off-springs of the original cross.

After weaning at the age of 30 days the animals were kept in groups of four and supplied with water and ST-1 pelleted diet ad libitum.

Plasma insulin

Plasma insulin was estimated by radioimmunoassay.

Insulin binding to erythrocytes

Plasma was separated from approximately 3 ml of heparinized blood drawn by cardiac puncture. Erythrocytes were obtained in the presence of constant amount of l25I-insulin (33pM)at l5°C 3 hours.Results were corrected for nonspecific binding. The details of the method were published previously (6).

Plasma lipids

Blood sampled to heparinized capillaries from retrobulbar plexus under light ether anaesthesia was centrifugated and the serum was stored in plastic tubes at -20°C. Total plasma cholesterol and plasma triglycerides were estimated enzymatically by Hitachi analyzer.

Glucose tolerance

Blood sampled to heparinized capillaries (from retrobulbar plexus under light ether anaesthesia) before glucose loading (basal glycemia) as well as 30,60,l20 and l80 min after glucose loading. Glucose (3g/kg b.w., 30% solution) was applied intragastrically after l4h starvation. Glycemia was estimated enzymatically (Oxochrom glucose, Lachema). Glucose tolerance is expressed as a sum of glycemia obtained 30,60,l20 and l80 min after glucose loading („area under the glucose tolerance curve").

Statistic

The data were analyzed by nonparametric tests, i.e., Mann-Whitney two sample (non-matched) test and by Wilcoxon test for matched pairs (10).

Results

Considering the developmental change in plasma triglycerides (Table l) elevation was found in the obese of both sexes. Taking into account the substrain differences at the age of two and/or tree months obese of both sexes show profound increase. Sex dependence of triglycerides was found only at the age of three month and only in obese rats, in females triglycerides being increased.

Table l: Plasma triglycerides

Group	n	58 days	92 days	P
SHR-M	7	1.20±0.30	1.31±0.36	n.s.
SHR-F	8	1.20±0.26	1.21±0.28	n.s.
SHR-O-M	l2	3.41±0.36D	4.14±0.69D	0.0l
SHR-O-F	8	3.02±0.54D	7.22±2.38d	0.01

Means ± SD. Plasma triglycerides in mmol/l. Abbreviations: SHR:lean genetically hypertemsive rats of Koletsky type, SHR-O: obese genetically hyperttensive rats of Koletsky type,M: males, F:females. Small letters: inter-sex statistical significance, capital: inter-substrain statistical significance (obese versus lean) a = P<0.l0, b = 0.05, c = 0.02, d = P.0.0l. 58 or 92 days: age of animals when measurement was performed.

Total plasma cholesterol (Table 2) was not influenced by the age. Substrain differences were found in females at the age of two months, in males at the age of two months, in obese cholesterol being increased. Sex dependence was expressed in both substrains and at both ages, in females cholesterol being increased.

Table 2: Total plasma cholesterol

Group	n	58 days	92 days	P
SHR-M	7	2.55±0.26	2.40±0.26	n.s.
SHR-F	8	3.43±0.35d	3.49±0.53d	n.s.
SHR-O-M	12	2.90±0.34A	2.77±0.28D	n.s.
SHR-O-F	8	3.99±0.51dC	3.89±0.42d	n.s..

Table 2. Means ± SD. Total plasma cholesterol. Abbreviations are the same as in Table l.

Insulinemia (Table 3) was age dependent in the obese of both sexes, at three months being increased. Obese rats show profound increase at the age of two as well as three months. Sex dependence is apparent only in lean rats, insulin being increased in males.

Table 3: Insulinemia

Group	n	58 days	92 days	P
SHR-M	7	143±38	170±32	n.s.
SHR-F	8	96±26c	118±23d	n.s.
SHR-O-M	l2	384±126D	689±293D	0.01
SHR-O-F	8	289±69D	705±325D	0.01

Table 3. Means ± SD. Plasma insulin. Abbreviations are the same as in Table l.

„Area under the glucose tolerance curve“ (Table 4) shows age dependence only in obese rats of both sexes, at three months being increased. Sex dependence in lean rats is expressed only at the age of two months, being increased in females, and in obese rats is expressed at the age of three months being increased in males. Substrain dependence was found only at the age of three months, elevation is apparent in the obese rats of both sexes.

Table 4: Glucose tolerance

Group	n	58 days	92 days	P
SHR-M	7	561±34	577±43	n.s.
SHR-F	8	620±41^{d}	622±106	n.s.
SHR-O-M	12	543±36	1045±249^{D}	0.01
SHR-O-F	8	602±61	783±99^{dD}	0.01

Table 4. Means ±SD. Glucose tolerance. Abbreviations are the same as in Table l.

Basal glycemia (Table 5) shows age dependence only in obese females, being lower at the age of three months.

Sex dependence was found only in obese rats at the age of two months where glycemia is increased in females. Substrain dependence was found in both sexes and at the age of two as well as three months, basal glycemia being elevated in obese of both sexes.

Table 5: Basal glycemia

Group	n	58 days	92 days	P
SHR-M	7	82±11	74±11	n.s.
SHR-F	8	86±6	78±12	n.s.
SHR-O-M	12	100±15^{D}	96±16^{D}	n.s.
SHR-O-F	8	129±17^{dD}	106±11^{D}	0.01

Table 5. Means ±SD. Basal glycemia. Abbreviations are the same as in Table l.

Body weight (Table 6) shows substrain dependence at the age of two as well as three months, being elevated in obese of both sexes. Sex dependence is apparent in lean as well as in obese rats, body weigth being elevated in males. Age dependent changes in body weight shows sex and substrain dependence. In lean rats lower age dependent increase is in females, in obese rats lower age dependent increase is in males. Age dependent changes are in obese rats twofold (in males) and fourfold (in females) higher than in lean rats.

Table 6: Changes in body weight

Group	n	58 days	92 days	change in %
SHR-M	7	170±12	246±10	44±10
SHR-F	8	140±10^{d}	183±14^{d}	30±6^{d}
SHR-O-M	11	215±10^{D}	416±22^{D}	93±8^{D}
SHR-O-F	8	162±26^{dC}	396±22^{bD}	148±29^{dD}

Table 6. Means ± SD. Changes in body weight.Abbreviations are the same as in Table l.

Insulin specific binding to erythrocytes (Table 7) shows substrain differences in males, being higher in obese rats. Sex dependence is apparent only in obese rats, being higher in males.

Table 7: Specific insulin binding to erythrocytes

Group	n	% of specific binding
SHR-M	7	3.14±0.76
SHR-F	8	2.27±0.94
SHR-O-M	l2	5.71±3.21^{D}
SHR-O-F	8	2.68±0.73^{d}

Table 7. Means and SD. % of specific binding to erythrocytes. Abbreviations are the same as in Table l.

Discussion

We have documented that at the age of two month Koletsky obese rats show relative to their lean controls very profound elevation of triglycerides (males +l84%, females l52%) and insulin (males +169%, females 201%).

Moreover, during one month triglycerides elevated in lean males +9%, in females 0%,but in obese males + 21%, in obese females +l39%.

When studying the insulinemia we obtained similar results. Thus, at the age of two months Koletsky obese rats relative to their lean siblings show very profound elevation of insulinemia (males +l69%, females +201%). During one months insulin elevates in lean males +l9%, in females +23%, but in obese males +80%, in obese females + 144%.

There are opened two serious questions. The first one, why the greatest differences between obese and lean rats at the age of two months were found in plasma triglycerides and plasma insulin. The second one, to what extent it is possible postulate the causal relationship between elevated plasma insulin and plasma triglyceride.

DeFronzo at al. (1) when summarizing recent knowledges in pathogenesis of non-insulin dependent diabetes mellitus (NIDDM) came to the conclussion that at the earliest stages in the natural history of NIDDM, insulin secretion is augmented compared with age-matched and weight-matched controls. In our previous study (3) we documented that in Koletsky obese rats there is a cluster of abnormalities in lipid and glycide metabolism which resembles deviations described by Reaven (9) in the patients suffering from NIDDM. Friedman et al. (2) obtained data in Koletsky obese rats which are in consonace with our findings and they came to the conclusion that this type of rats represents animal model of syndrom X. Thus hyperinsulinemia in our obese Koletsky rats can be considered as an expression of developing NIDDM. It would be desirable to monitore insulinemia in our obese rats in earlier stage of development, i.e., at the age of one month. At the age of one month it is possible to distinquish the obese from their lean sibling by

the morphologic signs (obese show smaller head and barrel-like chest).

Now to the a possible causal relationship between hyperinsulinemia and hypertriglyceridemia in our obese rats. It is generally accepted (11) that insulin increases triglyceride stores. Increased entry of glucose into adipose tissue facilitates fatty acid and glycerophospate synthesis, which, by mass action, drives tryglycerides synthesis. In addition, insulin inhibits the enzyme which catalyzes triglyceride breakdown. Insulin thus increases triglycerides stores by a double effect: driving triglyceride synthesis by facilitating glucose entry and at the same time inhibiting triglyceride breakdown via the lipase.

We have verified the above mentioned statement by the multiple regression analysis of parameters of lipid and glycide metabolism in Koletsky obese and lean rats (4). Regression analysis was performed when plasma triglycerides was used as a dependent variable and plasma insulin, insulin binding to erythrocytes, basal plasma glucose and glucose tolerance data were used as the independent variables.

It was found that insulinemia represents dominant independent variable in all group of rats except obese females where the dominant independent variable was represented by basal plasma glycemia. The obtained data are in consonace with above mentioned statement (11). A short notion to the rats with exceptional independent variable i.e. to the obese females. In our previous paper (5) we documented that in obese females muscle glucose transporter GLUT4 is lower than in lean Koletsky females.

In males this difference was not found. Moreover, only in obese females terguride is potent to increase GLUT4.

In the present series of experiment (Table 7) obese females relative to lean ones show no differences in insulin binding to erythrocytes. On the other had, in our previous paper (5) we documented that in obese females specific binding to adipose tissue is lower than in lean females. It can be judged as an expression of down regulation mechanism. The same can be stated in obese males rats. Hyperinsulinemia is accompanied by reduced insulin binding to adipose tissue (5).

It remains to be solved the reason of different dynamics of insulin binding in the erythrocytes when obese versus lean Koletsky rats are judged.

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