Introduction

In our previous study we documented strain dependent insulin binding to erythrocytes (5). It was found profound decrease of insulin binding in the SHR/N-cp obese rats as well as in their lean siblings relative to the normotensive rats of Wistar strain. In the other paper (3) we show alleviating effect of terguride in glucose intolerance which is done genetically in the Koletsky obese rats and in their lean siblings. Terguride is potent to alleviate hyperinsulinemia which is based genetically in obese Koletsky rats (4).

Terguride increases specific insulin binding to erythrocytes in both substrains of Koletsky rats except obese females (4). The mentioned data suggest possible causal relationship between glucose intolerance, hyperinsulinemia, and specific insulin binding to erythrocytes. Obese females represents the exception, i.e., in this type of rats terguride induced alleviation of genetically based hyperinsulinemia and glucose intolerance is not accompanied by the changes in specific insulin binding to erythrocytes. It is substantiated looking for a possible drug induced changes in specific insulin binding in the other tissues, i.e., in adipose, muscle and liver tissue. Moreover, such experiments verified a possible different drug sensitivity of insulin independent tissue (see erythrocytes) in comparison with drug sensitivity of insulin dependent tissues (see liver, muscle, adipose tissue) (7).

Nevertheless, there remains the other possibility, i.e., it cannot be a priori excluded that terguride is potent to influence the postreceptor events. There is a reason for monitoring of terguride influence on GLUT-4 glucose transporter. It is well-founded because Friedman et al. (1) found in the obese Koletsky rats decrease of GLUT-4. They did not mentioned sex of the animals.

Summary: Experiments were performed in the genetically hypertensive obese rats of Koletsky type (SHR/N-cp) and in their lean siblings of both sexes. Insulin binding to erythrocytes and to adipose tissue, lever tissue and muscle tissue was monitored in the control animals and in the animals under the long lasting terguride treatment. In control animals insulin binding shows substrain and tissue dependence being elevated in lean rats except insulin binding to erythrocytes where inverse is true. Terguride increases percentage of specific insulin binding to erythrocytes in all groups except obese females, terguride increases percentage of specific binding to adipose tissue except lean females, the mentioned drug remained without effect in muscle tissue in all group except lean females where drug induced elevation was detected. The effect of terguride in liver tissue was monitored only in males of both substrains, elevation was found only in lean. GLUT-4 was analyzed only in muscle tissue. The effect of terguride was found in obese females, i.e., in the group which shows reduced GLUT-4 relative to lean females.

Key words: SHR/N-cp of Koletsky type; Insulin binding to erythrocytes; Adipose tissue; Muscle tissue and liver tissue; Muscle GLUT-4; Long lasting terguride treatment
Methods

Animals

Experiments were performed in obese and lean genetically hypertensive rats of Koletsky type (6) of both sexes at the age of 3-4 months. Lean Koletsky rats represent dominant non-obese homozygotes and heterozygotes whereas the obese siblings are recessive homozygotes. The abnormal animals were obtained by Koletsky (6) when mating spontaneously hypertensive female rat (Okamoto-Kaki strain) with a normotensive Sprague-Dawley male rat. The genetically obese animals appeared after several generations of selective breeding of hypertensive oil-springs of the original cross. The blood pressure (measured by a indirect method) attained in lean genetically hypertensive males 24.6±2.22 KPa (n=15), 17.60±1.32 KPa (n=8) in females (2). The obese genetically hypertensive rats show comparable blood pressure (6).

After weaning at the age of 30 days the animals were kept in groups of four and supplied with water and ST pelleted diet ad libitum. During the experiments the animals were kept in group of two in PVC boxes (humidity: 55±10%, room temperature: 22±1°C, natural lighting). Body weight, water and pellet intake was controlled daily (except Saturdays and Sundays). Obtained differences between lean and obese Koletsky rats are comparable with the data published in our previous paper (3).

Plasma insulin

Plasma insulin was estimated by radioimmunooassay.

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 125I-insulin (3 ng/ml) at 4°C 3 hours. Results were corrected for nonspecific binding. The details of the method are published previously (5).

Plasma lipids

Blood sampled by cardiac puncture (in light ether anesthesia at 07.00 after 14 h starvation) was centrifugated and the serum was stored in plastic tubes at -20°C. Total plasma triglycerides were estimated enzymatically (Ouchterlony, Lachema). Glucose tolerance is expressed as area under the glucose tolerance curve.

Isolation of epidymal fat plaque membranes

Frozen fat tissue was homogenized by Ultraturax in buffer consisting of 50 mM Tris-HCl, 0.25 M sucrose, 1 mM phenylmethylsulfonyl fluoride and 1 mM benazmidoxy, pH = 7.4. The homogenate was filtered through four layers gauze and centrifuged at 4°C, 20 min at 3000 x g. Obtained supernatant was further centrifugated at 200 000g for 15 min. Resulted pellet represents a crude plasma membrane prepa- ration. The pellet was resuspended in 50 mM Tris-HCl, 1 mM EDTA, pH = 7.6 and immediately used for binding assay. Protein content was determined using the Lowry method.

Isolation of muscle plasma membranes

Frozen m. Q was homogenized in liquid nitrogen. Obtained powder was further homogenized in glass-tenlon homogenizer in the buffer consisting of 0.25 M sucrose, 80 mM Hepes, 1 mM EDTA and 1 mM PMSF, pH = 7.5. Centrifugation and the following steps are the same as above described for the fat tissue plasma membranes.

Isolation of liver plasma membranes

Frozen liver tissue is homogenized in five fold amount of buffer consisting of 0.1 M sucrose and 0.025 M Tris-HCl, pH = 7.6. Centrifugation and the following steps are the same as described above.

Determination of insulin specific binding

Insulin binding to isolated plasma membranes was performed in 1.5 ml Eppendorf tubes. The binding assay mixture contained 50 pg of plasma membrane proteins, 100 ng of labeled iodinated insulin (0.2 nM final concentration) and a binding buffer in final volume of 0.5 ml. For fat tissue plasma membranes the buffer consists of 50 mM Hepes, 1 mM CaCl2, 0.1% hovine serum albumine nonlabeled insulin (FBS), pH=7.6 buffer was used. The binding buffer for muscle plasma membranes consisted of 150 mM NaCl, 50 mM Hepes, 0.1% BSA, 100 pM PMSF and 1 pM PEPstatin, pH 7.4. For liver membranes 50 mM Tris-HCl, 0.2% BSA, pH 7.4 binding buffer was used. Nonspecific insulin binding was determined under the same conditions as above in the presence of 1 (for fat and liver) or 10 (for muscle) nM of nonlabeled insulin. Specific binding was determined by subtracting nonspecific binding from total binding in absence and the presence of nonlabeled insulin.

Determination of GLUT-4 in muscle

1. Isolation of total plasma membrane fraction from frozen muscle

Frozen muscle tissue (musculus quadriceps) was stirred with a spatula in liquid nitrogen to obtain a homogeneous powder. The powder was transferred into ice cold buffer (10 mM HEPES, 250 mM sucrose, 20 mM EDTA, 1 mM PMSF and 1 mM leupentin, pH 7.5) in glass homogenizer with a teflon pestle. After homogenization the homogenate was centrifugated in cooled centrifuge (4°C) 15 min at 2000 x g. Obtained sediment containing nuclei and cellular debris was discarded and the supernatant underwent a high speed centrifugation at 150 000 x g for 90 min. Resulted pellet was designed as total plasma membrane preparation. The pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4 and the aliquot of the suspension was used for determination of glucose concentration.

2. Electrophoresis of plasma membranes and immuno- blotting of GLUT-4.

Fresh total membrane preparation was solubilized with the Laemmli sample buffer (14) at 37°C for 30 min. The solubi- lized membranes were separated on a 12% polyacryla- mide gel electrophoresis using a Bio-Rad Mini-Protein gel apparatus. Protein were then electrotransferred from the gel to nitrocellulose membrane (HYBOND, Amersham, USA) at 100 V for 1 hour using a Bio-Rad Mini Trans-blot appar- tus. The membranes were blocked with 5% nonfat dry milk in 50 mM Tris-HCl, 2 mM CaCl2, 80 mM NaCl, pH 8 for 1 hour at room temperature. After blocking the membranes were incubated overnight at 4°C with rabbit antil GLUT-4 an- tibody (Charles River Pharmaceutics, USA) diluted 1:500 with blocking solution. The solution of the antibody was dis- carded and the membranes were washed 4 x 10 min with TERN buffer (50 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, pH 7.4) containing 0.05% Tween 20 followed by 3 x 10 min wash PBS. At the end of final wash the membranes were in- cubated with horseradish peroxidase conjugated secondary antibody linked to horsecarpside phosvitin (anti-rabbit IgG, PIERCE, USA) in PBS with 0.05% Tween 20 for 1 hour at room temperature. Finally the membranes were washed with PBS-Tween (5 x 10 min) and desorbed water (3 x 5 min). The protein bands containing GLUT-4 were visualized by exposing the membranes to en- hanced chemiluminesence reagent according to manufac- turer’s instructions (Amersham, USA). Autoradiography was carried out using Hyperfilm ECL (Amersham, USA). The specific band intensities were quantitated by optical den- sitometry using Kodak DS DC40 camera and ID Image Analysis Software (Eastman Kodak, USA). The results are expressed in arbitrary units of signal intensity.

Terguride treatment

The drug was applied i.p. in two daily doses (70.00 and 14.00) for 21 days (when lipemia was investigated) or for 11 days only (when glucose tolerance was monitored). Terguride maleate was administered at a dose of 0.1 mg/kg. 0.1 mg of terguride maleate was dissolved in 1 ml of water pre injecti- one. Solution of drug was applied at the dose 1 ml/kg b.w.

Statistics

The data were analyzed by Student t-test to determine ter- guride effect in individual groups and individual parameters.

Results

Insulin binding to erythrocytes (Table 1)

In control animals obese males as well as females show lower insulin binding than lean males and females. Terguride elevates insulin binding to erythrocytes in obese males as well as females and in lean males.

Insulin binding to adipose tissue (Table 2)

In control animals obese males as well as females show lower insulin binding than lean males and females. Terguride increases insulin binding to erythrocytes in obese males as well as females and in lean males.

Insulin binding to muscle tissue (Table 3)

In control animals obese males show lower insulin bind- ing to muscle tissue than lean males. In lean animals there is apparent sex dependence in the level of insulin binding to muscle tissue, i.e., being higher in males. Terguride increases insulin binding to muscle tissue only in lean females.

Table 1: Insulin binding to erythrocytes

| Group | Control | Terguride | P     |
|-------|---------|-----------|-------|
| SHR-M | 7 0.19±0.05 | 0.79±0.10 | n.s.  |
| SHR-F | 0.79±0.02 | 0.94±0.02 (6) | 0.05 |
| SHR-GM | 0.59±0.13 | 0.65±0.10 | n.s. |
| SHR-GF | 0.59±0.13 | 0.53±0.19 (5) | n.s. |

Table 2: Means ± SD. Specific insulin binding to erythrocytes

| Abbreviations: SHR - lean genetically hypertensive Koletsky rats, SHRO - obese genetically hypertensive Koletsky rats, M - males, F - females, number in brackets = number of animals per group. Statistical evaluation: differences between control and terguride treated animals is evaluated at the right side, differences between control male and female of lean as well as obese rats is expressed by small let- ters, differences between lean and obese rats is expressed by capital: a or A- P<0.10, b or B- P<0.05, c or C- P<0.02, d or D- P<0.01. |

Table 3: Means ± SD. Insulin binding to tissue muscles.

| Abbreviations are the same as in Table 1. |
Isolation of epididymal fat placenta membranes
Frozen fat tissue was homogenized by Ultraturrax in buffer consisting of 50 mM Tris-HCl, 0.25 M sucrose, 1 mM phenylmethylsulfonyl fluoride and 1 mM benzamidine, pH 7.4. The homogenate were filtered through four layers gauze and centrifuged at 4°C, 20 min at 3000 x g. Obtained supernatant was further centrifuged at 200 000 xg for 15 min. Resulted pellet represents a crude plasma membrane preparation. The pellet was resuspended in 50 mM Tris-HCl, 1 mM EDTA, pH 7.6 and immediately used for binding assay. Protein content was determined using the Lowry method.

Isolation of muscle plasma membranes
Frozen muscle tissue was homogenized in liquid nitrogen. Obtained powder was further homogenized in glass-tenlon homogenizer in the buffer consisting of 0.25 M sucrose, 10 mM HEPES, 1 mM EDTA and 1 mM PMSF, pH 7.5. Centrifugation and the following steps are the same as above.

Isolation of liver plasma membranes
Frozen liver tissue is homogenized in five fold amount of buffer consisting of 0.3 M sucrose and 0.025 M Tris-HCl, pH 7.6. Centrifugation and the following steps are the same as described above.

Determination of insulin specific binding
Insulin binding to isolated plasma membranes was performed in 1.5 ml Eppendorf tubes. The binding assay mixture contained 50 μg of plasma membranes proteins, 100 μl of labeled iodinated insulin (0.2 μM final concentration) and a binding buffer in final volume of 0.5 ml. For fat tissue plasma membranes a 100 μl of Tris-HCl, 2 mM EDTA, 1 mM orthophenaldiamine, 1 mM CaCl2, 0.1% bovine serum albumin proteinase free (BSA), pH 7.6 buffer was used. The binding buffer for muscle plasma membranes consisted of 150 mM NaCl, 50 mM HEPES, 0.1% BSA, 100 μM PMSF and 1 mM PEP, pH 7.4. For liver membranes 50 mM Tris-HCL, 0.2% BSA, pH 7.4 binding buffer was used. Nonspecific insulin binding was determined under the same conditions as above in the presence of I (for fat and liver) or I0 (for muscle) μM of nonlabeled insulin. Specific binding was determined as the difference between total and nonspecific binding in absence and the presence of nonlabeled insulin.

Determination of GLUT-4 in muscle
1. Isolation of total plasma membrane fraction from frozen muscle
Frozen muscle tissue (musculus quadriceps) was stirred with a spatula in liquid nitrogen to obtain a homogeneous powder. The powder was transferred into ice cold buffer (10 mM HEPES, 250 mM sucrose, 20 mM EDTA, 1 mM PMSF and 1 mM Leupeptin, pH 7.5) in glass homogenizer with a teflon pestle. After homogenization the homogenate was centrifuged in cooled centrifuge (4°C) 15 min at 2000 x g. Obtained sediment containing nuclei and cellular debris was discarded and the supernatant underwent a high speed centrifugation at 150 000 xg for 90 min. Resulted pellet was designed as total plasma membrane preparation. The pellet was resuspended in 50 mM Tris-HCl buffer, pH 7.4 and the aliquot of the suspension was used for determination of protein concentration.

2. Electrophoresis of plasma membranes and immunoblotting of GLUT-4.
Fresh total membrane preparation was solubilized with the Laemmli sample buffer (1/3 at 37°C for 10 min). The solubilized membranes were separated on a 12% polyacrylamide gel electrophoresis using a Bio-Rad Mini-Protein gel apparatus. Proteins were then electrotransferred from the gel to nitrocellulose membrane (HYBOND, Amersham, USA) at 100 V for 1 hour using a Bio-Rad Mini-Trans Blot apparatus. The membranes were blocked with 5% nonfat dry milk in 50 mM Tris-HCl, 2 mM CaCl2, 80 mM NaCl, pH 8 for 1 h at room temperature. After blocking the membranes were incubated overnight at 4°C with rabbit anti-GLUT-4 antibody (Charles River Pharmaservices, USA) diluted 1:500 with blocking solution. The solution of the antibody was discarded and the membranes were washed 4 x 10 min with TNE buffer (50 mM Tris-HCl, 5 mM EDTA, 150 mM NaCl, pH 7.4) containing 0.05% Tween 20 followed by 3 x 10 min wash PBS. At the end of final wash the membranes were incubated with secondary antibody linked to horseradish peroxidase (anti-rabbit IgG, PIERCE, USA) in PBS with 0.05% Tween 20 for 1 h at room temperature. Finally the membranes were washed with PBS-Tween (5 x 10 min) and deionized water (3 x 5 min). The protein bands containing GLUT-4 were visualized by exposing the membranes to enhanced chemiluminescence reagent according to manufacturer’s instructions (Amersham, USA). Autoradiography was carried out using Hyperfilm ECL (Amersham, USA). The specific band intensities were quantified by optical densitometry using Kodak DS DC40 camera and Image Analysis Software (Eastman Kodak, USA). The results are expressed in arbitrary units of signal intensity.

Terguride treatment
The drug was applied i.p. in two daily doses (70.00 and 14.00) for 21 days (when lipemia was investigated) or for 11 days only (when lipolytic tolerance was monitored). Terguride maleate was administered at a dose of 0.1 mg/kg, 0.1 mg of terguride maleate was dissolved in 1 ml of water pro injecti- one. Solution of drug was applied at the dose 1 ml/kg b.w.

Statistics
The data were analyzed by Student t-test to determine terguride effect in individual groups and individual parameters.

Results
Insulin binding to erythrocytes (Table 1)
In control animals obese females show higher insulin binding to erythrocytes than lean females.

When considering terguride effect on the insulin bind- ing to erythrocytes there is increase in all group except obese females.

| Group | Content | Terguride | P |
|-------|---------|-----------|---|
| SHR-M | 2.050 (5) | 0.970 (9) | 0.08 |
| SHR-F | 1.652 (5) | 2.330 (5) | 0.08 |
| SHR-M | 2.542 (5) | 1.05 (5) | 0.03 |
| SHR-F | 2.626 (5) | 3.331 (5) | 0.03 |

Table 1: Means ± SD: Specific insulin binding to erythrocytes
Abbreviations: SHR - lean genetically hypertension Koletsky rats, SHR-O - obese genetically hypertension Koletsky rats, - males, F - females, n= number of animals per group.

Statistical evaluation: differences between control and terguride treated groups are evaluated at the right side, differences between control male and female of lean as well as obese rats is expressed by small let- ters, differences between lean and obese rats is expressed by capital a of A - P<0.01, b or B - P<0.05, c or C - P<0.02, d or D - P<0.01.

Insulin binding to adipose tissue (Table 2)
In control animals obese males as well as females show lower insulin binding than lean males and females.

Terguride elevates insulin binding to erythrocytes in obese males as well as females in lean males.

Table 2: Insulin binding to adipose tissue

| Group | n | Control | Terguride | P |
|-------|---|---------|-----------|---|
| SHR-M | 7 | 4.276 (5) | 3.96 (5) | 0.05 |
| SHR-F | 6 | 4.150 (5) | 4.55 (5) | 0.03 |
| SHR-O-M | 6 | 1.780 (5) | 1.95 (5) | 0.03 |
| SHR-O-F | 6 | 1.765 (5) | 2.190 (5) | 0.02 |

Table 2: Means ± SD: Specific insulin binding to adipose tissue
Abbreviations: n - number of animals per group, the other abbreviati- ons are the same as in Table 1.
Insulin binding to liver tissue (Table 4)

In control animals basa! glycemia shows sexdependence. In lean being lower in sub males and substrate difference being lower in males of obese rats obese. Terguride decreasa glycemia in males of both substrains. At the level of trend (P<0.05) there is elevation in lean females.

Table 6: Effect of long term terguride treatment on the parameters of glycide metabolism

| Variables | drug | SHR-M | SHR-F |
|-----------|------|-------|-------|
| Basal glycemia (mmol/l) | Co | 4.592(12)±0.67 | 5.058(12)±0.67 |
| | Te | 4.820(12)±0.81 | 5.058(12)±0.67 |
| Insulinemia (mmol/l) | Co | 2438(7)±1.10 | 2834(7)±2.25 |
| | Te | 1798(4)±0.67 | 2648(6)±0.67 |
| Glucol tolerance (mmol/l) | Co | 29.32±2.17 | 29.80±2.17 |
| | Te | 26.62±2.93 | 27.21±2.93 |

Table 6: Means ± SD. Effect of long lasting terguride treatment on parameters of glycide metabolism. Abbreviations: number in brackets - number of animals in group. P<0.05, c- P<0.02, d- P<0.01: significance of terguride effect; significance between controls versus control lean is labeled by capital (A,B,C,D), significance between control males and control females is labeled l,2,3,4 Co - control animals, Te - animals treated by terguride. The other abbreviations are the same as in Table 1.

Discussion

In the discussion we want to pay an attention not only to the effect of terguride in the insulin binding to the differ- ent tissues but it will be valuable to take notice of insulin binding in the control animals as well.

Thus it cannot be omitted substrate differences in the insulin binding to the different tissues. Considering eryth- rocytes obese females show higher insulin binding than the lean females. In the other tissues inverse is true, i.e., in obe- se animals the insulin binding is lower than in lean animals. It is so in both sexes in adipose tissue, in the males in musc- le tissue. Till now in liver tissue we measured insulin bind- ing only in the males. It was again elevated in lean animals.

The decreased insulin binding to adipose, muscle and li- ver tissue in the genetically hypertensive obese Koletsky rats is accompanied by hyperinsulinemia (compare the data in Table 6 and 7). Thus the decrease of insulin binding in adipose, muscle and liver tissue can be judged as an ex- pression of down regulation. It is known (8) that hyperin- sulinemia per se induced the decrease of insulin receptors. There remained open question why it is not so in insulin bind- ing to erythrocytes. To what extent it can be considered as consequence of the functional characteristic of different tissues (on one side erythrocytes as insulin insensitive tis- sues, and on the other side adipose muscle and liver as an insulin sensitive tissues) (7), it remained to be solved.

The above mentioned tissue dependence in the substrate in differences of the level of insulin binding is not expressed in the terguride effect on the insulin binding. It is apparent that terguride shows its effect in both types of tissue, i.e., in the insulin sensitive and in insulin insensitive as well. When the effect of terguride is expressed in the changes of insulin binding, then in every case it is increased. We have never found decrease.

Considering the terguride sex and substrate dependen- ce, then minimal dependence is expressed in insulin bind- ing to erythrocytes and to adipose tissue. In other words, the effect of terguride is expressed in the changes of insulin binding to erythrocytes opposite to the decrease of the effect in the males. Again, the sex of animals is not mentioned. This findings is comparable with our males where we found lower insulin binding in muscle of obese rats relative to lean rats.

Acknowledgement

This paper was supported by Internal Grant Agency of Ministry of Health of the Czech Republic No 3864/3. The authors wish to thank Carl T.Hansen, Animal Genetics Division, National Institute of Health, Bethesda, USA, for providing the genetically hypertensive rats of Koletsky type, and GALENA, Czech Republic for providing terguride ma- terial. The authors also wish to thank the etical commission of Medical Faculty of Charles University, Hradec Kralove, for kind approving with the project of Grant 3864/3.

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Inulin binding to liver tissue (Table 4)
Inulin binding to liver tissue was not yet measured in females rats. In the males we obtained following results.

In control animals lean males show higher inulin binding to liver tissue than obese males.

Table 4: Specific insulin binding to liver tissue

| Group | n | Control | Terguride | P       |
|-------|---|---------|-----------|---------|
| SHR-M  | 5 | 31.6±3.1 | 32.0±2.1  | n.s.    |
| SHR-F  | 3 | 57.6±3.0 | 63.4±3.4  | n.s.    |
| SHR-OM | 5 | 36.4±3.9 | 37.6±4.3  | n.s.    |
| SHR-OF | 5 | 37.2±3.0 | 38.9±4.0  | n.s.    |

Table 5: GLUT4 in the muscle tissue

| Group | n | Control | Terguride | P       |
|-------|---|---------|-----------|---------|
| SHR-M  | 5 | 31.6±3.1 | 32.0±2.1  | n.s.    |
| SHR-F  | 3 | 57.6±3.0 | 63.4±3.4  | n.s.    |
| SHR-OM | 5 | 36.4±3.9 | 37.6±4.3  | n.s.    |
| SHR-OF | 5 | 37.2±3.0 | 38.9±4.0  | n.s.    |

Table 6: Effect of long lasting terguride treatment on parameters of glycide metabolism

| Variables | Terguride | P       |
|-----------|-----------|---------|
| Basal glycemia (mmol/l) | SHR-M | SHR-F |
| Co | 5.47±0.55(b) | 4.65±0.67(b) |
| Te | 6.82±0.51(b) | 5.05±0.51(b) |
| Insulinemia (ng/ml) | SHR-M | SHR-F |
| Co | 243±37(b) | 205±38(b) |
| Te | 179±49(b) | 189±26(b) |
| Glucose tolerance (mmol/l) | SHR-M | SHR-F |
| Co | 59.3±2.9(b) | 29.8±6.3(b) |

Table 7: Effect of long lasting terguride treatment on parameters of glycide metabolism

| Variables | Terguride | P       |
|-----------|-----------|---------|
| Basal glycemia (mmol/l) | SHR-M | SHR-F |
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| Te | 6.82±0.51(b) | 5.05±0.51(b) |
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Discussion

In the discussion we want to pay an attention not only to the effect of terguride in the insulin binding to the different tissues but it will be valuable to take notice of insulin binding in the control animals as well.

Thus it cannot be omitted substrate differences in the insulin binding to the different tissues. Considering eryth- rocytes obese females show higher insulin binding than the lean females. In the other tissues inverse is true, i.e., in obe- se animals the insulin binding is lower than in lean animals. It is so in both sexes in adipose tissue, in the males in musc- le tissue. Till now in liver tissue we measured insulin bin- ding only in the males. It was again elevated in lean animals.

The decreased insulin binding to adipose, muscle and li- ter tissue in the genetically hypertensive obese Kolsetky rats is accompanied by hyperinsulinemia (compare the data in Table 6 and 7). Thus the decrease of insulin binding in adipose, muscle and liver tissue can be judged as an ex- pression of down regulation. It is known (8) that hyperin- sulinemia per se induced the decrease of insulin receptors.

Considering the terguride sex and substrain depen- dence in the insulin binding to the different tissues. Considering eryth- rocytes obese females show higher insulin binding than the lean females. In the other tissues inverse is true, i.e., in obe- se animals the insulin binding is lower than in lean animals. It is so in both sexes in adipose tissue, in the males in musc- le tissue. Till now in liver tissue we measured insulin bin- ding only in the males. It was again elevated in lean animals.

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The above mentioned tissue dependence in the substra- in dependence being increased in obese of both sexes. The above mentioned tissue dependence in the substra- in dependence being increased in obese of both sexes. The above mentioned tissue dependence in the substra- in dependence being increased in obese of both sexes. The above mentioned tissue dependence in the substra- in dependence being increased in obese of both sexes.

Table 8: Means ± SD of glucose tolerance in control and terguride treated rats

| Group | n | Duration | Control | Terguride | P |
|-------|---|----------|---------|-----------|---|
| SHR-M  | 5 | 31.6±3.1 | 32.0±2.1  | n.s.    |
| SHR-F  | 3 | 57.6±3.0 | 63.4±3.4  | n.s.    |
| SHR-OM | 5 | 36.4±3.9 | 37.6±4.3  | n.s.    |
| SHR-OF | 5 | 37.2±3.0 | 38.9±4.0  | n.s.    |

Acknowledgement

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The space for photo of a representative autoradiogram of GLUT4

Fig. 1: A representative autoradiogram of GLUT4 protein levels in muscle tissue membranes of female rats. Abbreviations: N: nonobese animals, O: obese animals, N+O: nonobese animals treated with ter- guride, O+T: obese animals treated with terguride.
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Submitted May 1998. Accepted June 1998.

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