Evaluation of insulin resistance in obese castrated New Zealand white rabbits

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SUMMARY

Insulin resistance (IR) is observed in obesity and in the type 2 diabetes mellitus in humans. Although rabbits are used as experimental animal models for IR, the intravenous insulin tolerance test (IVITT), a specific test for evidencing peripheral insulin sensitivity, is not frequently used in this species. This study was conducted to evaluate insulin resistance in obese New Zealand white rabbits by IVITT. A total of 26 rabbits were randomly divided into 3 groups: castrated and obese animals (group CO, n = 7), castrated animals treated with antioxidants (vitamin E and d-limonene, Immunoprotect) (group CIm, n = 7) and not castrated healthy controls (group NC, n = 12). After weighing and determination of the BMI (body mass index), the blood glucose concentrations were measured prior to (0 min) and 5, 10, 15, 25, 30 and 45 min after intravenous injection of human insulin (0.1 U/kg) and the following kinetic parameters were calculated: the index of insulin sensitivity (Kivitt), the glucose disappearance rate (Kg glucose), the glucose half-life time (t½ glucose), the glucose permanence rate (AUCglucose), the minimal glucose concentration (Cmin) and its corresponding time (Tmin). Whereas the Kg glucose and the Kivitt significantly decreased in castrated and obese rabbits (group CO) compared to healthy controls, the other kinetic parameters markedly and significantly increased except for the AUCglucose. When castrated rabbits were supplemented with antioxidants (group CIm), the values of the various parameters were closely related to those calculated in controls except for the Tmin which has remained significantly more elevated. The BMI negatively correlated with the Kg glucose and Kivitt and positively with the other parameters. These results demonstrate that the insulin resistance evaluating throughout the kinetic analysis of the IVITT in rabbits occurs during castration-induced obesity and that antioxidant (vitamin E, d-limonene) supplementation may alleviate this deregulation of glucose metabolism.

Keywords: Obesity, castration, insulin resistance, rabbits, intravenous insulin tolerance test (IVITT), kinetic parameters, glycaemia.

RÉSUMÉ

Evaluation de l’insulino-résistance chez les lapins de Nouvelle Zélande castrés et obèses

Une insulino-résistance est observée lors d’obésité et dans le diabète sucré de type 2 chez l’homme. Bien que le lapin soit couramment utilisé comme modèle expérimental d’insulino-résistance, le test de tolérance à l’insuline injectée par voie intraveineuse (IV-TTI), un test spécifique pour mettre en évidence la sensibilité tissulaire à l’insuline, n’est pas fréquemment mis en œuvre dans cette espèce. L’objectif de cette étude a donc été d’évaluer une situation d’insulino-résistance chez des lapins blancs de Nouvelle Zélande rendus obèses en employant le IV-TTI. Pour cela, 26 lapins ont été répartis aléatoirement en 3 groupes : animaux castrés et obèses (groupe CO, n = 7), animaux castrés et supplémentés en anti-oxydants (vitamine E et d-limonène, Immunoprotect) (Groupe CIm, n = 7) et animaux contrôles (groupe NC, n = 12). Après pesée et détermination de l’IMC (indice de masse corporelle), la glycémie a été mesurée avant (0 min.) et 5, 10, 15, 20, 25, 30 et 45 minutes après l’injection intraveineuse d’insuline humaine (0,1 U/kg) et les paramètres cinétiques suivants ont été calculés : index de sensibilité à l’insuline (Kivitt), le taux de disparition du glucose (Kg glucose), le temps de demi-vie du glucose (t½ glucose), le taux de persistance sanguine du glucose (AUCglucose), la concentration minimale de glucose (Cmin) et le temps nécessaire pour l’atteindre (Tmin). Alors que le Kg glucose et le Kivitt ont diminué significativement chez les lapins castrés et obèses (groupe CO) par rapport aux contrôles, les autres paramètres cinétiques ont augmenté de façon intense et significative excepté l’AUCglucose. Lorsque les lapins castrés ont reçu des anti-oxydants, les valeurs des différents paramètres sont apparues proches de celles calculées chez les contrôles excepté le Tmin qui est resté significativement plus élevé. L’IMC a été négativement corrélé avec Kg glucose ou le Kivitt et positivement avec les autres paramètres. Ces résultats montrent qu’une situation d’insulino-résistance évaluée par l’analyse cinétique de l’IV-TTI chez le lapin survient lors d’obésité induite par castration et que l’apport d’anti-oxydants peut partiellement atténuer cette dérégulation du métabolisme glucidique.

Mots clés : Obésité, castration, insulino-résistance, lapins, test de tolérance à l’insuline par voie intraveineuse (IV-TTI), paramètres cinétiques, glycémie.

Introduction

The sensitivity of target tissue to metabolic action of insulin is one of the most important factors for the maintenance of systemic glucose homeostasis. Impaired insulin sensitivity, named insulin resistance (IR) is the main abnormality in obesity, metabolic syndrome and type 2 diabetes mellitus (DMT2) [2, 13, 22, 26]. Insulin resistance is defined as a reduced capacity for insulin to stimulate the uptake and intracellular metabolism of glucose in the target cells [5, 14, 16, 20]. Usually, IR leads to a pre-diabetic condition termed impaired glucose tolerance whose main feature is hyper-insulinemia [5, 16, 24, 26].

In contrast to rodents, rabbits exhibited similar lipid profile and metabolism as humans (low density lipoprotein mammals) [17, 18, 26]. Like humans but unlike mice, rabbits are susceptible to the diet induced formation of human atherosclerosis like lesions ranging from fatty streaks to fibrous plaques [9,
19, 26] and this is the reason why rabbits are increasingly used as appropriate animal model to study mechanisms of insulin resistance. Different methods such as the euglycemic clamp technique (“gold standard” for IR), the intravenous glucose tolerance test (IVGTT), the minimal model analysis, the homeostasis model assessment (HOMA-IR) and the quantitative check index (QUICKI) have been developed to evaluate IR. However, most of these tests are complex and laborious, or require special software and/or determination of plasma insulin and thus they are not suitable for the routine evaluation of insulin resistance. The intravenous insulin tolerance test (IVITT) is a direct measure of tissue insulin sensitivity as it evidences an eventual hypoglycaemic effect of exogenously injected insulin [11]. In humans, the IVITT is a simple, quick and reproducible method for assessing insulin sensitivity and has been validated against the euglycemic clamp technique and the HOMA-IR [3, 7]. Recently the IVITT has been validated in dogs and in cats [1, 15, 23]. In rabbits, IR is predominantly assessed by the IVGTT, although it is not strictly specific for IR and it also partially evaluates the β-cell function. Therefore the present study was conducted to evaluate insulin resistance in obese New Zealand white rabbits by the IVITT.

Materials and Methods

ANIMALS

The experimental procedure was approved by the Commission of Ethics at the Faculty of Veterinary Medicine of Trakia University. Twenty six clinically healthy male New Zealand white rabbits (provided by the Agricultural Institute, Stara Zagora) were used in the experiment. At the beginning of the experiment, they were 2 to 2.5 months old. During the experimental period, the recommendations of caring and treatment of rabbits reared as experimental animals were observed. The animals were housed in individual metal cages (80 x 60 x 40 cm) in a temperature-controlled room (20-22°C) and the light/dark regime corresponded to the circadian cycle. The rabbits were fed with a basal diet for adult rabbits (provided by the Agricultural Institute, Stara Zagora) which the composition was: dry matter: 888 g/kg; crude protein: 183 g/kg; fat: 35 g/kg; metabolisable energy: 2556.7 kcal/kg; crude fibres: 12 g/kg and mineral substances: 245.1 mg/kg. The animals had free access to food and water.

During the experimental period the rabbits were determined to be healthy on the basis of routine physical examination and daily monitoring of their behaviour, food and water intake and consistency of their faeces. Erythrocytes and leucocytes number and haemoglobin concentration were determined as well.

The rabbits were randomly divided into 3 groups: The animals of the first group (n = 7) were castrated and treated with “Imunoprotect” for 2 months (Group CIm), whereas castrated obese rabbits (n = 7) constituted the second group (Group CO) and not castrated animals (n = 12) were used as controls (Group NC). The castration of the rabbits was performed under general anaesthesia after sedation with Atropine sulphate (“Vetprom”, Bulgaria, 0.02 mg/kg, SC) following 10 min after with Xylazine (“Alfasan”, Holland, 2 mg in toto, IM) and induction within 10 minutes by Ketamine (“Alfasan”, Holland, 20 mg/kg). The rabbits were lying on their backs as hairs in the scrotal area were depilated and the skin was disinfected. The castration was performed in closed manner. The scrotal wounds remained open. “Imunoprotect” (as gelatinous capsules, synthesized and provided by Pharmaray, Sofia, Bulgaria) is a nutritional supplement which consists of two powerful antioxidants: the vitamin E (10 mg) and the extract from citrus fruits peel (90 mg), whose main ingredient is the d-limonene. The rabbits from the group CIm received 2 capsules / day per os after proper fixation of the animals before the morning feeding for 2 months.

EXPERIMENTAL PROCEDURES

To avoid stress reaction during the injection of insulin and blood samples collection during the intravenous insulin tolerance test (IVITT), all rabbits were familiar with daily handling.

All rabbits were weighed before performing the IVITT and the body weight (BW) and body mass index (BMI) were determined, as markers of adiposity. The BMI was calculated using a model adapted from cats [1]: BMI = body mass (kg) / body length (m) x height (m), where the body length was measured as the distance between the shoulder joint and tuber ischium, and the height as the distance between the shoulder joint and the end of the paw at the lateral position of the rabbit.

The IVITT was performed as has been described previously [1, 21, 23]. Briefly, the following protocol was adopted: after an overnight fasting period of 12 hours a bolus of regular human insulin (0.1 U/kg) (Actrapid®, 40 IU/ml, Novo Nordisk, Denmark) was administered through an ear vein. Blood samples were collected through the opposite auricular vein before (0 min), and 5, 10, 15, 25, 30 and 45 minutes after the insulin injection. After the last sampling all rabbits received a 20% glucose solution (1 mL/kg body weight) independently of the hypoglycaemia occurrence. The glucose concentration was measured immediately after blood collection with a glucose meter (Home Diagnostics, Inc., USA) based on the glucose oxidase method [25] using one drop of whole blood.

The following kinetic parameters of glucose during the IVITT were determined as markers for the insulin sensitivity: the rate of glucose disappearance for IVITT (Kglucose-%/min); the plasma half-life of glucose (t½ glucose- min), the area under the curve glucose concentration / time (AUCglucose 0–45 min, mmol/l.min), the minimal glucose concentration (Cmin, mmol/l) and the time to reach Cmin (Tmin , min). The Kglucose represents the decline in percent of the plasma glucose concentration and was calculated as described by DUJEJÀ et al. [7] and GELONEZ et al. [11] according to the formula: Kglucose (%/min) = (0.693/t½ glucose ) x 100, were t½ glucose is the plasma half-life of glucose decay and was calculated from the slope of the blood glucose concentration during the period from 5 to 15 minutes after insulin administration in which the decline of blood

glucose concentration during the chosen period was linear [7, 11], using the least square analysis. The AUC_{glucose} 0–45 min was calculated by the trapezoidal rule. The values of C_{min} and T_{min} were directly calculated from glucose test results. Low values of K_{glucose} and high values of AUC_{glucose} 0–45 min, C_{min}, T_{min} and t½ glucose reflect variable degrees of insulin resistance [11].

The insulin sensitivity was also expressed by an index, the Kivitt index, calculated as described by Pechereau et al. [23]. This index represents changes in blood glucose concentration after insulin injection relatively to the initial glucose concentration (C0), and was calculated using the following formula: K_{Kivitt} = C_0 – C_{min}/C_0 where C_0 was the initial glucose concentration and C_{min} the minimal glucose concentration during the test. The K_{Kivitt} values were inversely proportional to the insulin resistance.

STATISTICAL ANALYSIS

The statistical processing of data was performed by ANOVA (Statistica for Windows, StatSoft Ins., USA, 1993). All data were presented as mean values ± standard error of the mean (mean ± SEM). The statistical significance of differences before and after insulin injection and between groups were determined by the LSD test of the PostHot procedure of ANOVA and were considered significant if P < 0.05 (Statistica for Windows, StatSoft Ins., USA, 1993). Correlations between glucose kinetic parameters, index of insulin sensitivity and BMI were also determined using the Pearson test.

Results

The body weight (BW) and the BMI in the 2 both castrated groups (groups CIm and CO) were significantly (P < 0.001) higher than in controls (Table I). In the 3 groups a similar response profile of blood glucose concentration to exogenous insulin injection during an IVITT was found (Table I): plasma glucose concentrations significantly declined since 10 minutes after insulin administration compared to the initial values (P < 0.05) for reaching a minimum around 20-25 minutes and then increased until 45 minutes post-injection. Nevertheless, as shown in Table I, the glucose clearance rate in castrated and obese rabbits (group CO) was slower (statistically significant at 15 and 20 minutes) than in control rabbits (group NC). By contrast, no significant difference of glycaemia after insulin injection was evidenced between castrated rabbits orally supplemented with Immunoprotect or not (groups CIm and CO) or between castrated and Immunoprotect-treated rabbits and controls (groups CIm and NC).

The glycaemia kinetic parameters measured during the IVITT were presented in Table II. Whereas the rate of glucose disappearance (K_{glucose}) (P < 0.01) and the K_{Kivitt} index (P < 0.05) were significantly decreased in castrated obese rabbits (group CO) compared to the not castrated controls (group NC), the other kinetic parameters measured during the IVITT (the plasma half-life of glucose (t½ glucose) (P < 0.05), the minimal glucose concentration (C_{min}) (P < 0.01) and the time necessary to obtain it (T_{min}) (P < 0.01) except for the glucose permanence rate given by the AUC_{glucose} were markedly and significantly increased in this group. The area under the curve tended also to be enhanced but the difference with controls was not significant. By contrast, the glucose kinetic parameters were not significantly altered in the group CIm (castrated and Immunoprotect supplemented rabbits) compared to the healthy controls except for the T_{min} which remained significantly elevated (P < 0.05). Moreover, the K_{Kivitt} index (the index of the insulin sensitivity) was significantly higher in the group CIm than in the group CO (P < 0.05) whereas the other parameters determined with the IVITT did not significantly differ between the both 2 groups of castrated rabbits.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>CIm (n = 7)</td>
</tr>
<tr>
<td></td>
<td>4.09 ± 0.11^A</td>
</tr>
<tr>
<td></td>
<td>CO (n = 7)</td>
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<tr>
<td></td>
<td>3.89 ± 0.11^A</td>
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<tr>
<td></td>
<td>NC (n = 12)</td>
</tr>
<tr>
<td></td>
<td>2.89 ± 0.10^B</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>68.6 ± 0.9^A</td>
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<tr>
<td></td>
<td>65.5 ± 1.2^A</td>
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<tr>
<td></td>
<td>60.6 ± 1.3^B</td>
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<tr>
<td>IVITT – glycaemia (mmol/L)</td>
<td></td>
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<tr>
<td>0 min</td>
<td>6.9 ± 0.7^A</td>
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<td></td>
<td>7.0 ± 0.6^A</td>
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<td></td>
<td>6.0 ± 0.7^A</td>
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<tr>
<td>5 min</td>
<td>6.0 ± 0.7^A</td>
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<td></td>
<td>5.9 ± 0.7^A</td>
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<td>5.2 ± 0.7^b</td>
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<td>10 min</td>
<td>5.0 ± 0.7^b</td>
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<td></td>
<td>4.2 ± 0.6^bcAB</td>
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<td></td>
<td>4.7 ± 0.2^bcAB</td>
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<tr>
<td>15 min</td>
<td>3.7 ± 0.7^cAB</td>
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<td></td>
<td>4.6 ± 0.1^bcA</td>
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<td></td>
<td>3.2 ± 0.2^bc</td>
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<tr>
<td>20 min</td>
<td>3.8 ± 0.8^c</td>
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<td></td>
<td>4.1 ± 0.2^c</td>
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<td></td>
<td>3.4 ± 0.3^bc</td>
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<tr>
<td>25 min</td>
<td>4.0 ± 0.8^bc</td>
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<td></td>
<td>4.4 ± 0.2^bc</td>
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<td></td>
<td>4.7 ± 0.3^bc</td>
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<tr>
<td>30 min</td>
<td>4.7 ± 0.9^b</td>
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<td></td>
<td>4.6 ± 0.3^bc</td>
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<tr>
<td>45 min</td>
<td>6.0 ± 0.3^b</td>
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<tr>
<td></td>
<td>4.7 ± 0.3^b</td>
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</table>

BMI: body mass index; IVITT: intravenous insulin tolerance test.

The superscripts "^a,b,c" in the same column indicate significant differences (P < 0.05) according to time for a given group.

The superscripts "^A,B,C" in the same row indicate significant differences (P < 0.01) between the 3 groups.

Table I: Variations of body weights and BMI (body mass index) and blood glucose concentrations measuring during the IVITT (intravenous insulin tolerance test) in male New Zealand white rabbits castrated or not and supplemented with Immunoprotect or not (group NC: n = 12, rabbits were not castrated and not supplemented; group CO: n = 7, rabbits were only castrated and were obese; group CIm: n = 7, rabbits were castrated and orally supplemented with Immunoprotect for 2 months).

Moreover, positive significant correlations were found between the body weight, the BMI, the plasma glucose half-life, the glucose permanence rate, the minimal glucose concentration and the corresponding time (r ranged from 0.43 to 0.94, \( P < 0.05 \) to \( P < 0.001 \)) (Table III). On the other hand, all these parameters negatively significantly correlated with the rate of glucose disappearance and with the \( K_{ivitt} \) index (r ranged from -0.40 to -0.67, \( P < 0.05 \) to \( P < 0.01 \)) and a strong positive correlation between \( K_{glucose} \) and \( K_{ivitt} \) was found (r = 0.90, \( P < 0.001 \)).

Taken together the results of IVITT indicated that castration induced obesity was accompanied by marked impairment of insulin sensitivity. However, concomitant administration of antioxidants improved insulin resistance.

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Taken together the results of IVITT indicated that castration induced obesity was accompanied by marked impairment of insulin sensitivity. However, concomitant administration of antioxidants improved insulin resistance.

### Discussion

The current study was undertaken to evaluate insulin resistance in obese New Zealand white rabbits based on IVITT and to test the hypothesis that antioxidants would improve the insulin sensitivity. It is well known that in individuals with insulin resistance the insulin production is strengthened in order to counteract a moderate hyperglycaemia [1, 2]. As the IVITT allows measure of the direct hypoglycaemic effect of exogenous insulin, it is considered as specific for the evaluation of peripheral insulin sensitivity [1, 7, 21, 23]. Only changes in glycaemia after exogenously injected insulin are usually analyzed in rabbits. In the present study, the calculation of the glucose kinetic parameters and the determination of the \( K_{ivitt} \) index dependent from minimal and initial glucose concentrations complete the exploitation of the IVITT. These more precise parameters constitute reliable markers of insulin resistance and better describe the fate of glucose following insulin injection than iterative measures of glycaemia alone. Similar changes in blood glucose concentrations and in the values of \( K_{glucose} \) (rate of glucose disappearance) are reported in cats [1] and dogs [15].

Despite glycaemia decreased according a similar pattern in castrated and not castrated rabbits in response to injected insulin, the kinetic analysis has evidenced some alterations of the glucose response in castrated and obese rabbits (group CO) compared to healthy controls: the minimal glucose concentration (Cmin) and the time necessary to obtain Cmin. \( K_{ivitt} \): the index of the insulin sensitivity; NS: Not significant (P > 0.05).
the glucose half-life whereas the rate of glucose disappearance (K_{glucose}) was significantly depressed, suggesting that the blood glucose was distributed to tissues more slowly in this group. In addition, the C_{min}, the T_{min}, the t_{1/2} glucose and the AUC_{glucose} positively correlated together and were negatively associated with the K_{glucose}. The index of the insulin sensitivity, the K_{ivitt} index, corresponding to the relative difference between initial and minimal glycaemia during the IVITT, was also dramatically depressed in castrated and obese rabbits compared to controls. Based on IVITT, PECHEREAU et al. [23] have reported 3 degrees of insulin sensitivity: K_{ivitt} > 0.5 – normal insulin sensitivity; 0.4 < K_{ivitt} < 0.5 - borderline insulin sensitivity and K_{ivitt} < 0.4 – low insulin sensitivity. In rabbits however there is no such classification. Nevertheless, if this criterion was applied in the present study, it appeared that castrated and obese rabbits exhibited low insulin sensitivity while the insulin sensitivity was normal in not castrated rabbits (group NC) or castrated animals orally supplemented with Immunoprotect (group Clm). The strong positive correlation between K_{glucose} and K_{ivitt} recorded in the present study indicates that the calculation of both parameters is a method of choice to evaluate insulin resistance. Consequently, the insulin regulation of glycaemia was impaired in the group of the castrated rabbits. Furthermore, as the values of K_{glucose} and K_{ivitt} negatively correlated with the BMI, the insulin resistance may result from obesity induced by the castration.

The current results confirmed our previous data derived from intravenous glucose tolerance test in the same animals showing that castrated rabbits became insulin resistant as a consequence of visceral obesity (unpublished observation). According to PECHEREAU et al. [23] the evaluation of insulin resistance on the basis of IVITT has some advantages over IVGTT (intravenous glucose tolerance test) and can be used in animals. The IVITT is easier to perform and ensure precise and reproducible results and does not require glucose infusion or determination of plasma insulin concentration. In addition, in humans, the results of IVITT significantly correlates with those of euglycemic clamp test which is thought as the “gold standard” for the evaluation of insulin resistance and HOMA-IR. This implies to carry out additional studies on the basis of IVITT in rabbits in order to define exact criteria able to evaluate insulin resistance and tissue glucose disposal. However there are some limitations for the use of IVITT: the insulin induced hypoglycaemia leads to release of counter-regulatory hormones, such as glucagons, adrenaline, glucocorticoids and somatotropin. Therefore, the glucose elimination rate is not only function to the insulin [1]. But, as the concentration of these hyperglycaemic hormones started to increase after the 15th minute following the insulin injection, the blood glucose concentrations measured within the 15 first minutes of IVITT were not affected by the counter-regulatory hormones and decreased as a linear function of the insulin sensitivity of target tissues [1, 12]. That is the reason why the glucose disappearance rate (K_{glucose}) was calculated in the present study by considering the slope of the glycaemia according to time within the first 15 minutes. Furthermore, a severe hypoglycaemia can occur during the IVITT: in one rabbit from the control group, strong clinical signs of hypoglycaemia including neurological symptoms were observed and justified glucose infusion which has lead to the exclusion of this control to the experiment.

On the other hand, the glucose response to the exogenous insulin did not seem to be significantly altered in the group of castrated rabbits treated with the Immunoprotect (group Clm) although the T_{min} remained significantly elevated compared to the healthy controls. Moreover, the mean K_{ivitt} index was closely related to the value calculated in controls and was significantly higher than in the group of castrated and not treated animals (group CO) whereas the other kinetic parameters have not significantly differed between the 2 groups of castrated rabbits (groups CO and Clm). Again, these results clearly indicate that the K_{ivitt} index can be considered as a very sensitive and reliable marker of insulin resistance during IVITT and confirm previous preliminary results based on the IVGTT suggesting that the Immunoprotect treatment of obese rabbits may improve the insulin sensitivity (unpublished observations). As it was established in the recent years that the occurrence of an oxidative stress coupled with hypertriglyceridemia and lipid accumulation in skeletal muscles contributes to the impairment of insulin actions and to the pathogenesis of insulin resistance [4, 6, 8, 10], the protective effect of the Immunoprotect treatment would be partially due to the powerful antioxidant properties of vitamin E and d-limonene, leading to a rapid utilization and degradation of fatty acids.

As a conclusion, this study shows that, in one hand, the intravenous insulin tolerance test (IVITT) and the inherent calculations of kinetic parameters constitute a simple and reliable method for evaluation of insulin resistance in rabbits and that, on the other hand, the combination of plant derived antioxidants and vitamin E could be used for the improvement of insulin sensitivity.

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References