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Effects of castration-induced visceral obesity and antioxidant treatment on lipid profile and insulin sensitivity in New Zealand white rabbits

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ABSTRACT

Molecular mechanisms, responsible for the impaired insulin-sensitivity state due to the obesity are not fully understood in both humans and animals. The purpose of this study was to investigate the effects of castration-induced visceral obesity and the influence of two antioxidants on constituents of blood lipid profile and insulin sensitivity in New Zealand white rabbits. Twenty-six clinically healthy male New Zealand white rabbits were used in the experiment and were divided into 3 groups: first group (CI, $n = 7$) – castrated-obese and treated with antioxidants "Immunoprotect" for 2 months; second group (CO, $n = 7$) – castrated-obese; third group (NC, $n = 12$) – control group (non-castrated, non-obese). At the end of the follow-up period of 2 months after castration an intravenous glucose tolerance test (IVGTT) was performed after a 12-h fasting period as the blood samples for determination of glucose and insulin and their kinetic parameters were obtained at 5 and 0 min before and at 5, 10, 30, 60 and 120 min after the infusion of the glucose. The constituents of lipid profile, triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL-C) were also assessed in the overnight fasting blood samples. The body weight (BW), body mass index (BMI), amount of the visceral fat (VF) and VF/BW ratio were both measured and calculated before the IVGTT and at the end of the experimental period. All measured markers of obesity (BW, BMI, VF, VF/BW) were significantly higher in both groups of castrated rabbits than in the control group. Apart HDL-C, the plasma concentrations of all constituents of lipid profile (TG, TC, HDL-C) were the highest in CO group. There were generally no differences between CI and NC groups for the same traits. After glucose injection blood glucose concentrations and glucose and insulin kinetic parameters were considerably higher (except of glucose elimination rate) in CO rabbits than in NC ones. Castrated rabbits treated with "Immunoprotect" showed lower fasting plasma insulin and improved glucose kinetics dynamics than CO rabbits, but commensurable values of glucose and insulin kinetics parameters than NC group. The results of the current study clearly indicated that castration-induced visceral obesity affected negatively the lipid profile and insulin sensitivity and/or responsiveness. Treatment with antioxidant supplementation, consisted of d-limonene and vitamin E, improved blood lipid profile, fatty liver, glucose homeostasis and insulin sensitivity in obese rabbits. In addition, based on our results we may suggest that castrated male New Zealand white rabbits might be considered as an appropriate animal model to study various metabolic abnormalities related to visceral obesity, such as dyslipidemia and impaired insulin sensitivity.

1. Introduction

Many features of lipid metabolism in rabbits are similar to humans (so-called LDL mammals), but differ from the most widely used experimental animals – rats and mice, which are predominantly HDL animals (Fan et al., 2001; Kawai et al., 2006). Like humans, rabbits have higher concentrations of cholesterol ester transfer proteins and higher levels of apoB-containing LDL particles compared to rats and mice (Ichikawa et al., 2004; Kawai et al., 2006; Zhao et al., 2007). Moreover, analogously to humans, but different from mice, rabbits can develop cholesterol-diet-induced atherosclerotic lesions (Zhao et al., 2007).

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Despite the increasing number of studies in the last years the molecular mechanisms responsible for the insulin-resistant state due to the obesity are not fully understood in both humans and animals. It has been demonstrated that obesity and insulin resistance, the main features of metabolic syndrome, are closely associated with a state of low-grade inflammation and low expression of peroxisome proliferator activated receptor-γ (PPAR-γ) in adipose tissues (Schwartz and Kahn, 1999; Odegaard et al., 2007). In Humans, visceral or central obesity (due to increased intra-abdominal fat accumulation) is considered as the main predisposing factor for the development of dyslipidemia and insulin resistance (IR), which may progress into metabolic syndrome and type 2 diabetes mellitus (DMT2) (Kawai et al., 2006; Zhao et al., 2007; Hays et al., 2008). Recently, a model of diet-induced central obesity in male Japanese white rabbit has been developed which was accompanied by impairment of glucose metabolism and IR (Zhao et al., 2007). Usually, the typical features of IR are found too late, when β-cell function has been already affected. Therefore, rabbits could be considered as a good animal model to study the early changes of peripheral insulin sensitivity and blood lipid profile in obesity and metabolic syndrome.

In the last years, it has been found that the obesity is accompanied by the development of oxidative stress characterized by increased plasma and tissue concentration of reactive oxygen species (ROS) and free radicals (Anderson et al., 2009). Thus, the oxidative stress, along with other etiological factors, such as intra-myocellular lipid accumulation, increased concentrations of free fatty acids, pro-inflammatory cytokines and adipokines, is proposed to be an important factor for insulin resistance (Keaney et al., 2003; Ceriello and Motz, 2004; Furukawa et al., 2004). However, it is uncertain whether the use of antioxidants in obese individuals leads to improvement of insulin sensitivity and/or other metabolic parameters associated with obesity.

Therefore, the main purpose of the current study was to investigate the effects of castration-induced visceral obesity and the influence of two antioxidants on constituents of blood lipid profile and insulin sensitivity and/or responsiveness in New Zealand white (NZW) rabbits.

2. Materials and methods

2.1. Experimental animals

The experimental procedure was approved by the Commission of Ethics at the Faculty of Veterinary Medicine of Trakia University, Stara Zagora, and during the entire study period the recommendations for caring and treatment of rabbits reared as experimental animals were followed.

Twenty-six clinically healthy male NZW rabbits were used in the experiment. Rabbits were provided by the Agricultural Institute in Stara Zagora, Bulgaria. At the beginning of the experiment they were 2–2.5 months old. The animals were housed in individual metal cages (80 × 60 × 40 cm) in a temperature-controlled room (20–22 °C). The light/dark regime corresponded to the circadian cycle.

The rabbits were given free access to food and water. They were fed a commercially available standard chow diet for adult rabbits, given as dry pellets. The composition of chow diet (% dry matter) was: dry matter: 88.86; crude protein: 16.08; fat: 3.43; crude fiber: 13; neutral detergent fiber: 34.44; acid detergent fiber: 18.29; acid detergent lignin: 4.68; ash: 5.05 and digestibility energy: 10.47 MJ. The chemical composition of diet ingredients expressed as g/MJ was: dry matter: 84.9; crude protein: 15.39; crude fat: 3.28; crude fiber: 12.40; neutral detergent fiber: 32.90; acid detergent fiber: 17.47; acid detergent lignin: 4.47 and ash: 4.82. During the experimental period of 2 months the rabbits were determined to be healthy on the basis of results of routine physical examination and daily monitoring of their behavior, food and water intake and consistency of their faeces. Erythrocyte and leukocyte numbers and hemoglobin concentration were determined twice: before and 2 months after the castration.

The rabbits were divided into 3 groups: (i) First group (CI, $n = 7$) – castrated-obese and treated with Imunoprotect for 2 months; (ii) Second group (CO, $n = 7$) – castrated-obese; and (iii) Third group (NC, $n = 12$) – control group (non-castrated non-obese).

The castration of the rabbits was performed under general anesthesia. The premedication included atropine (Atropini sulphas; "Vetprom", Radomir, Bulgaria), administered subcutaneously (0.02 mg/kg) and 10 min afterwards followed by intramuscular injection (i.m.) of Xylazine (Alfasan, Woerden, The Netherlands; 2 mg/kg). The anesthesia was accomplished 10 min later by i.m. injection of Ketamine (Alfasan; 20 mg/kg). For surgery the rabbits were laid on their backs, the fur in the scrotal area was depilated and the skin was disinfected. The scrotal wounds after castration remained open.

"Immunoprotect" is a nutritional supplement in oil state. It consists of two components: – vitamin E (10 mg equivalent to 15 IU) (Bieri and McKenna, 1981) and organic extract from citrus fruits peel (90 mg), which contains in high proportion limonene (Michaelakis et al., 2009). "Immunoprotect" was produced and gifted by Pharmaray, Sofia, Bulgaria in the form of gelatinous pearls. The rabbits from the first group received two pearls *per os* daily before the morning feeding for 2 months (Penchev Georgiev et al., 2009).

2.2. Experimental procedures

To investigate the obesity-induced insulin resistance, an intravenous glucose tolerance test (IVGTT) was performed 2 months after the castration. All rabbits were familiarized with daily handling in order to avoid the stress reaction during the injection of glucose and blood sampling during IVGTT.

The changes in blood glucose and plasma insulin concentrations were evaluated and kinetic parameters of glucose and insulin, such as rate constant of glucose elimination from the blood ($K_{el\ glucose}$, min^{-1}), plasma half-life of glucose ($T_{1/2\ glucose}$, min), area under the concentration curve of glucose ($AUC_{glucose\ \ 0 \rightarrow 120\ min}$, mmol/l min), area under the concentration curve of insulin ($AUC_{insulin\ \ 0 \rightarrow 120\ min}$, μU ml/min) and $AUC_{insulin\ \ 0 \rightarrow 120\ min}/AUC_{glucose\ 0 \rightarrow 120\ min}$ ratio were determined as measures of insulin resistance in IVGTT tests.

The IVGTT was performed as previously described (Liu et al., 2005; Zhao et al. 2007). Briefly, the following protocol was adopted: food was removed from 12 h overnight and a bolus of 40% glucose (0.6 g/kg) was injected through the ear vein and a blood sample was collected at 5 and 0 min before and at 5, 10, 30, 60 and 120 min after glucose injection. Blood samples for the determination of the concentration of glucose, insulin and of lipid parameters: triglycerides (TG), total cholesterol (TC), HDL-cholesterol and LDL-cholesterol, were collected from the jugular veins. The concentration of the lipid parameters was determined only once in the overnight fasting samples drawn before glucose infusion.

Heparin was used as an anticoagulant. The blood samples were centrifuged immediately after the collection at 800*g* for 15 min. The plasma was stored in plastic tubes at −20 °C until assayed. Glucose concentration was measured in whole blood.

On the day before carrying out the IVGTT the rabbits from the three groups were weighed and the body weight (BW) and body mass index (BMI) were determined as a marker of obesity. BMI