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Metabolic effects of 20-OH-Ecdysone in ovariectomized rats

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ABSTRACT

Postmenopausal women develop often obesity which may be prevented by 20-OH-Ecdysone (Ecd). This was investigated in ovariectomized (ovx) rats. They were orally treated with 3 doses of Ecd (18, 56 or 116 mg/day/animal). Positive controls received 159 μg estradiol (E2). Quantitative computer tomography at the level of the abdomen and the metaphysis of the tibia allowed estimation of surface, fat depots and muscles. The highest dose of Ecd resulted in serum concentrations of 0.4×10^{-6} M. Serum E2 concentrations in the positive controls were 73.3 ± 24.41 pg/ml. E2 but not Ecd stimulated uterine weights. Under Ecd ovx animals gained less fat but had more muscle mass. Serum TSH, T4 and T3 levels remained unaffected while E2 treatment increases T4 but decreases T3 levels. Ecd at the lowest dose lowered serum LDL and did not result in increased serum triglycerides, an effect seen in the E2 treated rats. At the Ecd highest dose serum HDL was higher than in the controls.

In conclusion Ecd has beneficial effects on fat and muscle tissue and may be able to prevent the metabolic syndrome and sarcopenia by a non-estrogenic mechanism.

1. Introduction

Chronic obesity reaches epidemic dimensions and may result in the metabolic syndrome of which the end stage is type II diabetes, hypertension and arteriosclerosis with all consequent following diseases such as heart attacks and strokes [1–4]. According to WHO 1 billion people are overweight in the world. Thereof 300 million people are morbidly obese. For several reasons particularly aged people are prone to put on weight [5–7] and therefore the metabolic syndrome develops frequently in the postmenopause [8,9]. In addition aged persons often develop a loss of skeletal muscle (sarcopenia) which decreases their mobility [10,11]. It is long known that ovariectomized (ovx) rats develop obesity [6,12] and that hormone replacement therapy (HRT) of postmenopausal women or estrogen treatment of ovx rats influence the development of the metabolic syndrome positively particularly in conjunction with increased bodily exercise and food restriction [6,7]. In the past few years however, HRT was less frequently practiced because of a number of adverse side effects such as a slight increase in the incidence of mammary cancer and of arteriosclerosis which resulted in increased cases of heart attacks and strokes [13]. Hence, other means to prevent the metabolic syndrome and sarcopenia are currently investigated.

Ecdysteroids particularly 20-OH-Ecdysone (β-Ecdysone = Ecd) are known to be produced by arthropods to initiate metamorphosis, the so-called molting process [14] and a number of plants produce ecdysteroids which protect them from herbivory [15–17].

In mammals including the human Ecd is known to stimulate muscular growth [15–17]. We observed recently that the administration of Ecd has antiosteoporotic effects in ovx rats [18]. The ovx induced increase of body weight is due to fat accumulation and can be prevented by E2 treatment which has antilipotropic/lipolytic effects and increases mobility and thereby energy expenditure [5,6]. In our previous experiments we observed that the Ecd treatment did not result in lower body weight in comparison to the ovx control rats. This promoted us to speculate that more muscles were formed on the account of less fat accumulation. Therefore we determined in the present study the amount of fat depots by quantitative computer tomography (qCT). Utilizing this qCT method we have recently described a method to quantify intraabdominal fat depots as well as small fat depots in the lower hind leg which we called paratibial fat depot [19]. These fat depots are sensitively regulated by estrogens and increase dramatically in size following ovx [19–22]. The qCT plane of the lower hind leg details primarily 3 compartments: the surface of bones, of fat depots and muscles. In the present contribution we propose therefore that quantification of fat and of bone surfaces allows calculation of lower hind leg muscle surface and this is also determined in the present study. If in Ecd treated ovx rats fat accumulation is prevented and muscle accumu-

Abbreviations: Ecd, 20-OH-Ecdysone; ovx, ovariectomized; E2, estradiol; HRT, hormone replacement therapy; qCT, quantitative computer tomography; OECD, Organisation of Economic Cooperation and Development; sf, soy-free; TSH, thyroid stimulating hormone; T4, thyroxin; T3, triiodothyronine; SEM, standard errors of means; L4/L5, lumbar vertebral body 4/5; RXR, retinoid x-receptor.

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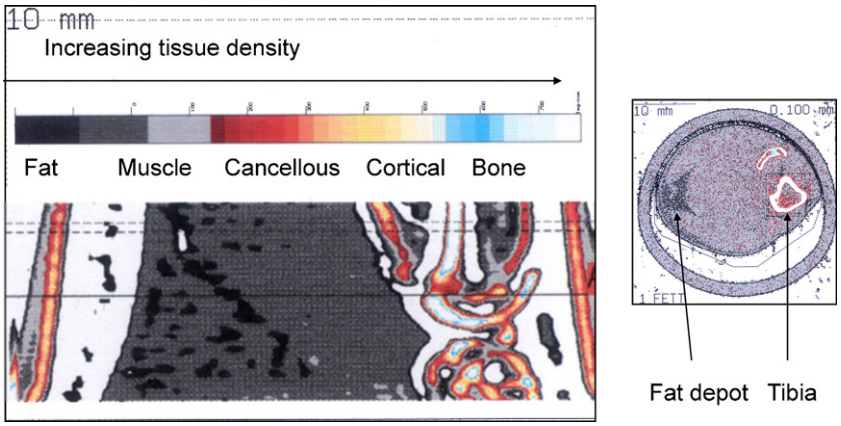

Fig. 1. Scout view and qCT slice of tibial fat depot.

lation increased serum leptin levels may also be affected by Ecd. Leptin is a product of adipocytes and is therefore high in ovx obese and low in intact and E2 treated ovx animals [6,23]. In addition serum cholesterol, LDL and HDL as well as triglycerides, TSH, T3 and T4 levels were measured as additional metabolic parameters.

If Ecd exerts similar beneficial effects as E2 but without the adverse effects of estrogens an estrogenic action of the ecdysteroid in the uterus must be excluded. The Organisation of Economic Cooperation and Development (OECD) recommends testing of estrogenicity by the so-called *uterotropy-assay* which utilizes the growth promoting effects of estrogens in uteri of ovx rats [24,25]. Therefore, in the present study we investigated the effects of 3 orally administered doses of Ecd given over a period of 3 months on the above mentioned parameters and on uterine weights and compared them with those in ovx and E2 treated ovx rats. In essence we hope that Ecd may be used as a means to prevent obesity, arteriosclerosis and sarcopenia.

2. Materials and methods

Female Sprague Dawley rats ($n = 60$) were used for the present experiments. Allowance to perform these experiments was obtained from the Bezirksregierung Braunschweig (permission No. Az.G 82.06). The test substance was 20-OH-Ecdysone (=β-Ecdysone = Ecd) provided by Changzhou Dahua Imp. and Export (Group) Corp. Ltd. Changzhou, Jiangsu, China, 97.2% purity. For control purposes estradiol-17beta (E2)-benzoate (Order no. E-9000, Sigma–Aldrich, St. Louis, MO, USA) was tested.

Three months old Sprague Dawley rats (Winkelmann, Borken, Germany) weighing 250 ± 10 g were adjusted to our animal facilities (6 animals/cage; 12 animals/group; light phase 06.00 a.m. to 06.00 p.m., relative humidity 55%) and were kept on soy-free, pelleted food (sf food) (V 1355 R-Z, 10 mm, poor phytoestrogens, ssniff, Borken, Germany) in which isocaloric protein supplementation was secured by added potato proteins. After 1 week of adjustment animals were anaesthetized with isoflurane (Forene, Abbott-AG, Baar, Switzerland), weighed and subjected to quantitative computer tomography (qCT with the XCT Research SA, Stratec Medizintechnik, Pforzheim, Germany) for the determination of fat depots in the abdomen of the levels of L4/L5 and of a small fat depot in the lower hind leg which we named paratibial fat depot. The scanner was positioned at the level of L4/L5 and 3 tomographic slices were taken. For evaluation of the fat depot in the lower hind leg the scanner was positioned at the epiphysis of tibia and a coronal computed radiograph (Fig. 1) was carried out. The scout view was used to position the scanner at the site of measurement and 3 tomographic slices at a distance of 3.75 or 4.25 or 15 mm distal of the reference line were used for the determination of fat depots.

In these planes the amount of fat and bone tissue was calculated and all values below 40 mg/cm^3 were considered to be fat, between 40 and 99 mg/cm^3 muscle and values between 280 and 400 mg/cm^3 cancellous bone. The qCT plane of the tibia at the level of the upper metaphysis is shown in Fig. 1. In this qCT plane the densities of the different tissues are shown in different grey tones. Computer assisted perimetry of the surface of the fat depot allows calculation of fat surface in mm^2. Similarly the surface of the tibia and fibula can be calculated. The total surface of the CT plane minus surface of fat and bone tissue gives an estimate of the muscle surface in this qCT plane. It is known that ovx animals continue to grow and to develop obesity while intact or E2 treated animals grow less and remain slim [19]. As a consequence the absolute values of muscle tissue surface in the qCT plane are misleading and therefore these surfaces are given in absolute values/100 g body weight while changes in fat tissue are given in % of the pre-treatment values.

After this initial CT-scan rats were ovariectomized (ovx) under the same anaesthesia. After ovx control animals were maintained on soy-free potato protein enriched food, the treated ovx animals were placed on Ecd and E2 containing food for 3 months ($n = 12$/group). On the basis of the twice weekly measurement of food consumption per cage divided by the number of animals per cage the food intake of each rat could be estimated. The type of food, Ecd content of the food as well as average food intake, final body weights (BWs) and the estimated amount of daily uptake of the test substances are detailed in Table 1. After the treatment period

Table 1
Food intake and calculated daily uptake of the test substances.

	Dose	Food intake in ovx rats g/animal/day	Substance intake mg/animal/day	Final body weight in g
ovx, sf	V 1355 ssniff	17.32	–	324.7 ± 21.47
ovx + E2	10 mg/kg food	15.92	0.159	274.5 ± 23.69*
ovx + Ecdysone	1 g/kg food	18.02	18.02	324.4 ± 30.46
ovx + Ecdysone	3 g/kg food	18.86	56.58	332.0 ± 21.09
ovx + Ecdysone	6 g/kg food	19.3	115.8	344.5 ± 10.86

* $P < 0.05$ vs ovx, sf.