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Salivary adiponectin levels are associated with training intensity but not with bone mass or reproductive function in elite Rhythmic Gymnasts☆

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ABSTRACT

Elite Rhythmic Gymnasts (RGs) constitute a unique metabolic model and they are prone to developing Anorexia Athletica. The aim of the present study was to evaluate the effect of training intensity on salivary adiponectin levels and assess a possible role of salivary adiponectin levels as a predictive factor of reproductive dysfunction and bone mass acquisition in elite RGs. The study included 80 elite female RGs participating in the World Rhythmic Gymnastics Championship tournament held in Montpellier, France on September 2011. Anthropometric values were assessed, training data and menstrual pattern were recorded, bone mass was measured with Broadband ultrasound attenuation (dB/Mhz) and baseline salivary adiponectin levels were determined. The athletes were classified as intensely and very intensely trained, considering the mean training intensity (40.84 h/week). Moreover, considering their reproductive status, they were divided into RG's with normal menstruation, primary amenorrhea and oligomenorrhea. All comparisons were adjusted to age, BMI and body fat percentage differences. Very intensely trained RGs showed higher salivary adiponectin levels ($p = 0.05$). Moreover, salivary adiponectin levels showed significant correlation with training intensity ($r = 0.409$, $p = 0.003$). On the other hand, no association of salivary adiponectin levels was documented with either reproductive function or bone mass acquisition. The results of the present study suggest that, in elite RGs, salivary adiponectin levels are associated with the intensity of training, possibly reflecting the deterioration of energy balance rather than the training stress. On the other hand, a predictive role of salivary adiponectin levels in reproductive dysfunction or bone mass acquisition could not be supported.

1. Introduction

Eating disorders (EDs) encountered in elite athletes have raised great concern and scientific interest and comprise a distinct entity known as anorexia athletica (AA) [24]. Although AA resembles anorexia nervosa (AN), it fails to meet the criteria of EDs and thus it has been suggested to be classified as an eating disorder not otherwise specified (EDNOS) [26].

Elite Rhythmic Gymnasts (RGs) constitute a unique metabolic model prone to develop AA, as the sport specific character favors

Abbreviations: EDs, eating disorders; AA, anorexia athletica; AN, anorexia nervosa; EDNOS, eating disorders not otherwise specified; RGs, Rhythmic Gymnasts; IR, insulin resistance.

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lean physique, leading the athletes to adopt strict dietary restrictions, low caloric intake and chronic negative energy balance (inadequate energy consumption failing to meet energy demands). The inadequate energy consumption characterizing EDs represents a powerful stimulus to the endocrine system, leading to detrimental effects, predominantly involving the reproductive system (primary or secondary amenorrhea, chronic anovulation) and, subsequently, bone metabolism and bone mass (osteopenia and osteoporosis) [22].

During the last decades, the accrual of a large body of evidence has highlighted the emerging role of adipose tissue as an active endocrine organ producing and secreting a number of bioactive molecules (known as adipokines) [6]. Among the numerous adipokines, adiponectin is secreted exclusively from adipose tissue, detected in high levels in serum and involved in metabolism, insulin resistance (IR), inflammatory process and reproductive function [6,12]. Moreover, adiponectin also has an impact on bone metabolism and high adiponectin levels have been shown to increase both osteoclastic and osteoblastic activity [13,14].

Thus, it would be challenging to study the adaptations and variations of adiponectin levels in specific models of energy homeostasis, such as athletes of the highest competitive level. However, a major problem in studying elite athletes on the field of competition lies on the difficulty to obtain blood samples. Thus, the determination of salivary hormone levels provides a convenient, non-invasive and stress-free alternative to blood analysis. In this direction, we [15] and others [25] have reported the determination of adiponectin in saliva and the significant association of salivary with serum adiponectin levels.

Recently, we reported higher levels of salivary adiponectin levels in elite RGs compared to age-matched nonathletic controls [23]. The aim of the present study was to evaluate the effect of training intensity on salivary adiponectin levels, assess a possible role of salivary adiponectin levels as a predictive factor of reproductive dysfunction and bone mass acquisition and further investigate the hypothesis that adiponectin acts as a starvation hormone, signaling low energy availability.

2. Subjects and methods

2.1. Subjects

The study was conducted during the 2011 World Rhythmic Gymnastics Championship tournament held in Montpellier, France on September 2011 and included 80 elite RGs from 25 countries. The study protocol was approved by the Ethics Review Committee of Nimes, France (Commission de Protection des Personnes, Sud Mediterranee III) and permission for the clinical trials was granted by the French Medicine and Health Care Products Regulatory Agency (Agence Française de Securite Sanitaire des Produits de Santé). Moreover, the study protocol was authorized by the International Gymnastics Federation (FIG).

The athletes participated voluntarily in all parts of the study and informed consent was obtained from athletes, their coaches and parents, in accordance with article 7 of the medical organization of F.I.G. competitions. Before the study (sampling procedure and questionnaire completion), all athletes were familiarized with the sampling techniques and informed about the protocol and the objectives of the study.

2.2. Methods

The study protocol included noninvasive clinical and laboratory investigations as well as the completion of a questionnaire. Standing height was measured with a stadiometre to the nearest 0.1 cm and recorded as the mean of two consecutive measurements. Body composition and body weight were evaluated using a portable apparatus (Tanita, BC-418MA, TANITA UK LTD), calculating body fat and total body water, via Bioelectrical Impedance Analysis. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m) (kg/m^2). Furthermore, the athletes completed a questionnaire including training history data (age of training onset and usual training intensity).

2.2.1. Questionnaires

The participants completed a series of questionnaires designed to assess the general medical and menstrual history, with questions about the age of menarche and the pattern of menses, including the menstrual cycle duration and the absence or irregularity of menstruation. Primary amenorrhea was defined as absence of menstruation above 15 years of age, secondary amenorrhea was defined as absence of menstruation for 3 months in the post-menarche period and in the absence of pregnancy and oligomenorrhea was defined the presence of menstrual interval of more than 35 days, with four to nine periods in the past year. The use of oral contraceptive pills (OCP) was also recorded and OCP users were excluded from the study. The latter might explain the absence of RGs reporting secondary amenorrhea in the studied population. It should also be noted that 3 (three) athletes failed to complete the questionnaire regarding the menstrual status.

In addition, detailed information about the training history was collected, including data on the age of training onset and training intensity (hours per week).

2.2.2. Quantitative ultrasound measurement

Quantitative ultrasound (QUS) measurements were made with the Osteospace densitometer (Medilink, Mauguio, France). Osteospace measures broadband ultrasound attenuation (BUA in dB/MHz). The variables are automatically computed after the ultrasonic wave has transversed the calcaneus. The dominant heel was measured in all subjects. The precision (reproducibility) error for BUA is 1.72% [19].

2.2.3. Assays

Saliva samples were collected using Salivettes (Sarstedt Co. Ltd., Nümbrecht, Germany). Saliva samples from athletes were obtained during the tournament in the morning (0800–1000 h) after an overnight fasting, before brushing their teeth.

After centrifugation (3000 rpm for 15 min) at room temperature, saliva samples were stored at −20 °C until the assay. Salivary adiponectin levels were measured using commercially available enzyme immunoassay kits for serum determinations with minor modifications, as we reported in a recent study [15].

Salivary adiponectin concentrations were measured by the quantitative sandwich enzyme immunoassay technique (R&D Systems, United Kingdom) with dynamic range: 3.9–125.0 ng/ml. The method's sensitivity was 0.246 ng/ml and the intra- and interassay CVs were 2.5% and 5.8%, respectively. Salivary adiponectin determinations were carried out at the Laboratory of Reproductive Endocrinology of Patras Medical School.

2.2.4. Statistical analysis

All statistical procedures were performed using SPSS 19.0 for Windows (IBM SPSS Statistics, IBM software). Test for normality was done using Kolmogorov–Smirnov test. Variables normally distributed are presented as mean ± S.D, while skewed variables are presented as median and interquartile range (25th and 75th).

Comparison of mean values between RGs subgroups was performed using the independent t-test or one-way ANOVA when data distribution was normal and Mann–Whitney or Kruskall–Wallis test when the continuous variables were nonnormally distributed.