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EFFECT OF SUSTANON ON SOME IMMUNOLOGICAL AND HORMONAL PARAMETERS IN RATS

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ABSTRACT

Sustnanon is one of androgenic anabolic steroids, which is in use especially among young people and adolescent, and such drug has many long-term negative side effects; therefore, it has become a major health problem. This study was conducted to examine the effect of intramuscular injection of androgenic anabolic steroide (sustanon) on some hormonal and immunological parameters in albino rats. Three doses of sustanon (0.05, 0.1, 0.2) mg/Kg/day, respectively were injected for six weeks. The hormonal changes included the significant decrease ($P < 0.05$) in the mean levels of adiponectin for both males and females treatment groups compared with control groups, while there was a significant increase ($P > 0.05$) in the mean levels of FSH hormone of males group, compared with the healthy male control group, In contrast, there was asignificant decrease ($P < 0.05$) in the mean levels of FSH hormone in females treated groups, compared with the control groups. Furthermore, there were significant decrease ($P < 0.05$) in the mean levels of LH hormone of both males and females groups, compared with the control groups; a significant decrease ($P < 0.05$) in the mean levels of testosterone hormone of males group, compared with the control group, while there was a significant increase ($P > 0.05$) in the mean levels of this hormone of females group, compared with the control group. Immunological changes included a significant decrease ($P < 0.05$) in the mean levels of IL-1β for males treated group compared with the control group, while there was a significant increase ($P > 0.05$) in the mean levels of this cytokine for females treated group, compared with the control group. Meantime, there was a significant increase ($P > 0.05$) in the mean levels of IL-6 and C-reactive protein of the males and females groups, compared with the control groups.

Key words: Sustanon, Testosterone, Adiponectin, IL-1β, IL-6.

INTRODUCTION

Sustanon is one of AAS, which includes many practical therapeutics treatments. Medically, it is used to treat several cases such as osteoporosis, hypogonadisms in male and infertility[1]. Sustanon characterized by a especially unique and differentiate pharmacological

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structure and properties compared to the other AAS drugs. It is composed of an oily mixture of four different testosterone ester compound, which provides a permanent release of testosterone into the blood serum for length level from 3-4 weeks[2]. Furthermore, these drugs were given in order to race horse and dogs, who wish to enhanced physical presentation[3,4]. It can increase muscle mass and strength, body building. Sustanon acts as fast steroid, in addition to these benefits, there are many side effect produced by using sustanon. Because it can convert to estrogen; thus, increase growth of breast tissues and this state called gynecomastia[5]. Many earlier studies also recorded adverse effects from the use of these drugs including, cardiovascular disorder (mainly amplification of the left ventricle), that leads to sudden death, acute hepatitis with jaundice, testicular dysfunction resulting to infertility, hypertension, behavioral disorders[6,7]. Adiponectin, the circulating peptide hormone secreted by adipocytes, has an important role in insulin resistance, glucose and lipid metabolism and cardiovascular morbidity and mortality[8]. Furthermore, circulating adiponectin concentrations are higher in women than men, independent of the fact that women usually have more overall adiposity than men[9]. The relationship between testosterone and adiponectin is subject to controversy. Testosterone has been shown to decrease adiponectin levels in rodents[10]. In addition, hypogonadal men have higher adiponectin levels, which are reduced by testosterone replacement therapy[11]. The negative feedback of elevated concentrations of testosterone, DHT or estrogens to the hypothalamus and pituitary leads to a suppression of endogenous production of gonadotropic hormones and endogenous steroids associated with morphological effects on endocrine systems (testis), psyche and sexual behavior[12]. Meanwhile, disturbances in sex hormones has been associated with metabolic and cardiovascular risk factors including diabetes, hypertension, dyslipidemia and obesity. While these hormone in physiological concentration protect the cardiovascular system[13]. Clark and Harrold[14] investigated the influence of steroids on male sexual behavior suppression; on the other hand, AAS suppress the hypothalamic–pituitary–gonadal axis (HPG). As a result, the exogenous intake of AAS will decrease the endogenous production of testosterone and gonadotropins (luteinising hormone-LH and follicle-stimulating hormone – FSH). These side effects are gender-specific[15,16].

EXPERIMENTAL

Materials and methods

Preparation of sustanon (250 mg) concentrations

Sustanon ampoules (manufactured by N.V. Organon Oss Inc. Holland) have been obtained from the local pharmacy in Hilla-Iraq. Each ampoule contains 1 mL of oily solution of sustanon. According to the manufacturer, this 1 mL of sustanon consists of four

testosterone ester compounds, which include testosterone propionate, testosterone ephenyl-prpropionate, testosterone isocaproate and testosterone decanoate[17].

Laboratory animals

Forty eight albino rats (*Rattus rattus*) were divided into two main groups. The first group of males (24 rats) were divided into four subgroups (6 replication for each), the first, second and third treatment subgroups were injected sustanon (0.05, 0.1, 0.2) mg/Kg/day, respectively for six weeks, while the fourth subgroup was considered as control group, which was injected by physiological normal saline (0.9% NaCl). The second major group of females (24 animals) has primed with the same drug and doses, and same periods, their weight ranged between 200-250 g.

Blood sampling

Through the course of the study, the animals were anesthesized by using chloroform and sacrificed. About 5 mL of blood was collected directly by heart puncture with sterile syringes, and kept to stand into sterile plastic tubes for 30 min at room temperature to allow clotting. The clotted blood was centrifuged at 3000 rpm for 10 min, and serum was collected for the study (Adiponectin (ADP), Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Testosterone (T), Intrlukin-1B (IL-1B), Interlukin-6 (IL-6), and C-Reactive protein (C.R.P.).

Hormonal assay

ELISA Protocol

The levels of Adiponectin, Testosterone, FSH, LH hormones were estimated by ELISA kit of rat, according to the procedures provided by Elebsience company, China.

Immunological assay

ELISA Protocol

The levels of IL-1β, IL-6 and C-Reactive proteins, were estimated by ELISA kit of rat, according to the procedures provided by Boster company, China.

Statistical analysis

The results of the study were analyzed statistically using SPSS software (Genstat) version (1995). This analysis gives the arithmetic mean and standard error (Mean ± S.E.)

comparison between the averages in different dosage intervals using less ICH difference between middle L.S.D. (Least Significant Differences), and under level probability 0.05.

RESULTS AND DISCUSSION

1-Adiporectin levels

The results of this study showed that changes in the mean of adiponectin in experimental animals, there was a significant decrease ($P < 0.05$) in male and female groups, which were treated with sustanon in different concentrations (0.05, 0.1, 0.2) mg/kg of body weight in males groups mean ± S.E., respectively (8.11 ± 0.55, 6.66 ± 0.33, 0.26.09 ± 0.11), compared with control groups (16.43 ± 1.46), and in females groups mean ± S.E (6.48 ± 0.80, 6.53 ± 1.41, 6.60 ± 0.92) compared with control groups (11.00 ± 0.68) .There was a significant difference between treatment groups primed by (0.05 and 0.2) mg/Kg, but there was no significant difference between treatments groups (0.1 and 0.2) mg/Kg, compared within other groups (L.S.D. (0.05) = 2.19) (Fig. 1).

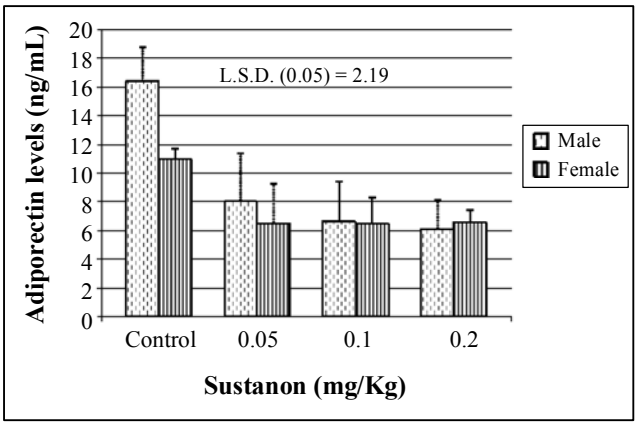

Fig. 1: Adiponectin levels in rats injected by different concentrations of sustanon

2-FSH levels

Results of study reveled changes in the mean of FSH. There was a significant increase ($P > 0.05$) in males groups, which primed by different concentrations of sustanon (0.05, 0.1, 0.2) mg/kg of body weight, mean ± S.E., respectively (11.93 ± 0.70, 11.00 ± 0.79, 12.08 ± 0.34) compared with control groups (5.83 ± 0.09), and there was a significant decrease ($P < 0.05$) in females treatment groups (12.39 ± 1.05, 11.40 ±1.00, 13.03 ± 0.48) compared with control group (16.39 ± 0.28) and between treatment groups alone. There was a significant difference between treatment groups in females (0.05, 0.1, 0.2) mg/kg of body

weight , while there was no significant differences between treatment groups of males, in contrast within other groups L.S.D. (0.05) = 1.679 (Fig. 2).

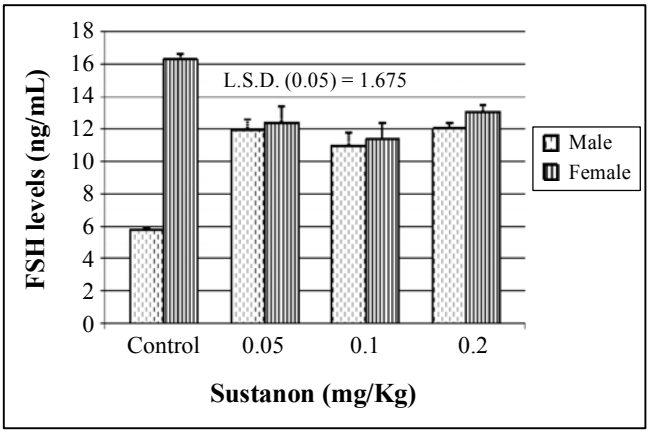

Fig. 2: FSH levels in rats primed by different concentrations of sustanon

3-LH levels

The results showed changes in LH mean of laboratory animals, which pointed out a significant decrease ($P < 0.05$) in males and females treated groups (0.05, 0.1, 0.2) mg/Kg of body weight, mean ± S.E. in male groups, respectively (2.35 ± 0.50, 1.42 ± 0.21, 1.02 ± 0.06) compared with control groups (6.67 ± 0.46), and in females groups mean ± S.E. (2.08 ± 0.45, 1.30 ± 0.25, 0.82 ± 0.30), compared with control groups (3.38 ± 0.09), L.S.D. (0.05) = 0.472, as well as there was a significant difference among treatments for males with females, when compared within other groups (Fig. 3).

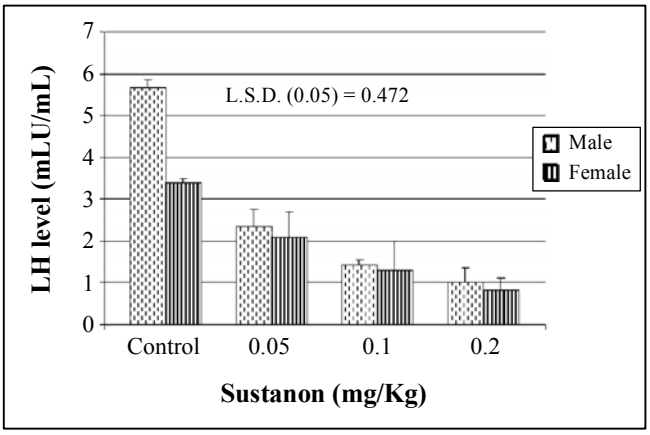

Fig. 3: LH levels in rats injected by different concentrations of sustanon

4-Testosterone levels

Results of this study show changes in testosterone means of rats, which revealed a significant decrease ($P < 0.05$) in males groups treated with sustanon (0.05, 0.1, 0.2) mg/Kg of body weight, mean ± S.E (0.51 ± 0.008,0.67 ± 0.03, 0.73 ± 0.07), compared with control groups (1.12 ± 0.04), as well as, there was a significant difference among treated males (0.05, 0.2) mg/Kg, but there was no significant difference observed between (0.1, 0.2) mg/Kg, when compared within other groups. On the other hand, there was significant increase ($P > 0.05$) in females groups, which treated with sustanon (0.1) mg/Kg of body weight, means (0.60 ± 0.09, 0.70 ± 0.14, 0.66 ± 0.05), compared with control groups (0.58 ± 0.03), (L.S.D. (0.05) = 0.174). In addition, no significant difference was there between females treated groups, when compared within other groups (Fig. 4).

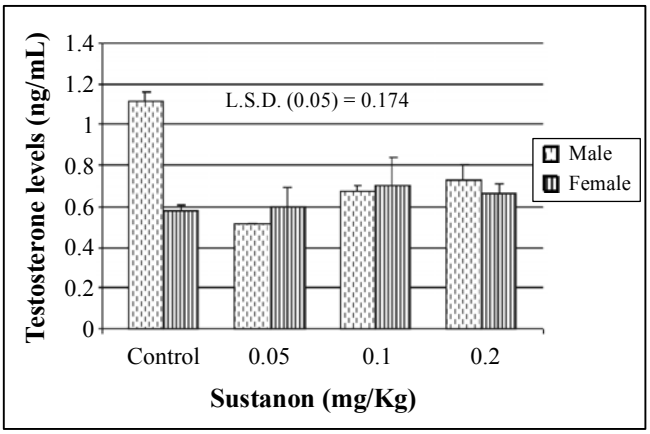

Fig. 4: Testosterone levels in rats injected by different concentrations of sustanon

5-Levels of IL-1β

The results of this study showed changes in IL-1β means in laboratory animals. There was a significant decrease ($P < 0.05$) in males treated groups with sustanon (0.05, 0.1, 0.2) mg/Kg of body weight, (41.50 ± 3.75, 46.13 ± 2.18 ,64.06 ± 0.75), compared with the control groups (75.13 ± 6.53), as well as there was significant increase ($P > 0.05$) in females groups, which was treated by sustanon (0.05,0.1,0.2) mg/Kg of body weight means (84.73 ±1.44, 82.73 ± 6.02, 49.33 ±5.46) compared with control groups (49.33 ± 5.46), L.S.D.(0.05) = 10.433, also showed significant difference among treatment males and females groups (0.05, 0.2) mg/Kg of body weight. Furthermore, no significant difference was observed between treatment males and females groups (0.05, 0.1) mg/Kg of body weight, compared within other groups (Fig. 5).

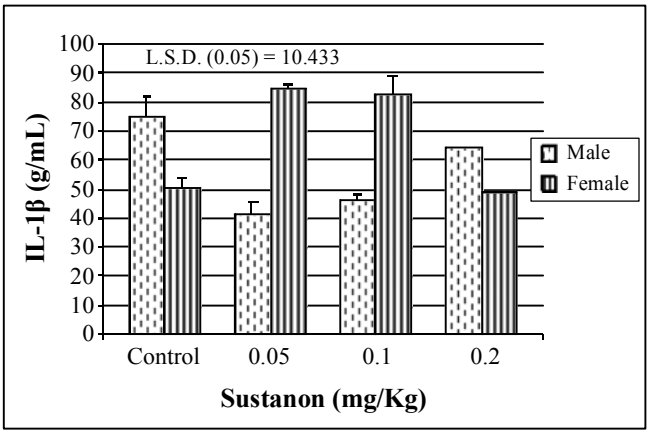

Fig. 5: Levels of IL-1β in rats immunized by different concentrations of sustanon

6-Levels of IL-6

There were changes in the levels means of IL-6 between treatment and control groups. The results showed a significant increase ($P > 0.05$) in males treated groups by sustanon (0.1) mg/Kg of body weight, mean ± S.E. (26.73 ± 7.53), compared with control group (11.16 ± 0.37), as well as there was significant increase ($P > 0.05$) in females groups, which was treated by sustanon (0.2) mg/Kg of body weight (19.93 ± 6.48), compared with the control group (12.03 ± 0.31), L.S.D. (0.05) = 7.854. It also revealed a significant difference among treatment males groups (0.1, 0.2) mg/kg of body weight, compared with the other groups, while no significant difference was observed between treatment females groups, compared with the other groups (Fig. 6).

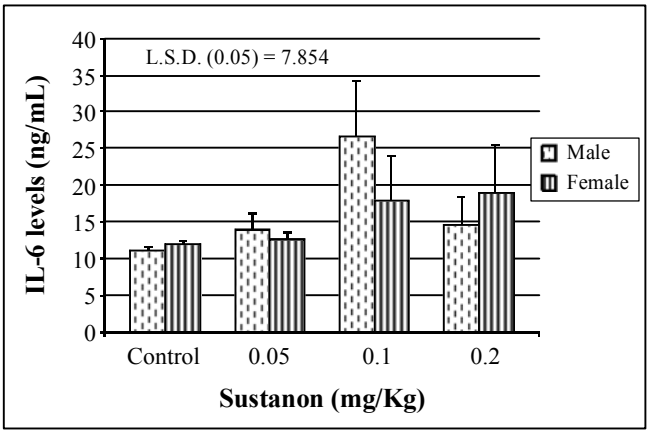

Fig. 6: Levels of IL-6 in rats injected by different concentrations of sustanon

7-C-RP levels

The results revealed that changes in C-RP. means in experimental animals, there was a significant increase ($P > 0.05$) in males and females treated groups by sustanon (0.05, 0.1, 0.2) mg/kg of body weight, means of male, respectively (9.60 ± 0.30, 14.74 ± 0.47, 22.39 ± 0.32), compared with control group (2.38 ± 0.61), while the means of female groups (9.63 ± 0.37, 15.04 ± 0.92, 24.10 ± 2.31), compared with control group (4.21 ± 0.71), L.S.D. (0.05) = 2.416, also showed a significant difference among treated males and females groups (0.05, 0.1, 0.2) mg/kg, when compared within other groups (Fig. 7).

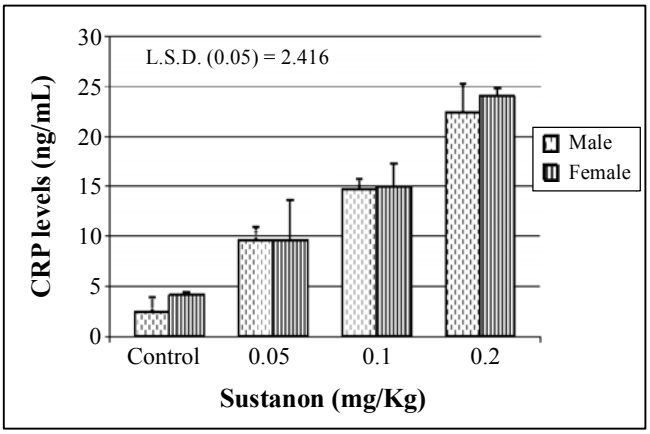

Fig. 7: Levels of C-RP in rats injected by different concentrations of sustanon

Three different doses of sustanon, which have been chosen regarding the doses used by athletes, were administered to the rats. This study revealed that the different doses of sustanon, which was used in this research caused a significant decrease in adiponectin levels in both male and female groups of rats. Use of AAS can cause a decrease in serum adiponectin levels as a negative correlation and this agree with the results obtained by Lanfranco et al.[11] and Page et al.[18] Furthermore, these researchers suggested a direct suppressive effect of exogenous testosterone, which is not associated with the gender. Meantime, Xu et al.[19] revealed that, in rats, T administration selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from the fat tissue. In addition, the study of Tsujimura et al.[20] refer to inverse relation between adiponectin levels and testosterone in rodents and humans. The results revealed that a significant increase in FSH levels of males treated groups than control groups, because the increase in testosterone level in the blood of athletes can cause increase secretion of FSH from hypothalamus-pituitary gland and enhance appearance of male secondary characteristics. On the other hand, FSH levels of females treated groups showed a significant decrease than control groups

(Fig. 2). This result is in agreement with reports get by Gao and Dalton[21], Attardi et al.[22] and Karbalay-Doust and Noorafshan[23]. These researchers pointed out that increase of anabolic androgens reduces gonadotropin release by way of a negative feedback mechanism, and decreases estrogen secretion in rats and mice. The results illustrated that significant decrease in LH levels, whether in males or females treated groups than control groups (Fig. 3). These data may belong to many factors such as, high levels of exogenous testosterone as sustanon caused of suppression sex hormones in the blood of laboratory animals. On the other hand, in males elevation of exogenous testosterone may effect on leydig cells and caused a decreased in LH levels product by testis[24]. Moss et al.[25] referred that use of AAS inhibits both GnRH and gonadotropin secretion, which results in negative feedback on the HPG axis, and this action suppresses the secretion of FSH and LH by the hypothalamus. The present data revealed that a significantly decreased and increased in testosterone hormone levels of rats treated by different concentrations of sustanon in males and females groups, respectively, compared with the control groups (Fig. 4). AAS as sustanon led to suppression of testosterone production by testis through suppressing both GnRH productions by the hypothalamus and LH production by the pituitary gland[24,26], In contrast, Shiono[27] established that enchanting testosterone and its derivatives caused a height of the serum level of testosterone in rats. Meantime, not only testosterone derivatives could increase the level of serum testosterone, but giving other anabolic androgenic steroids, which are used to increase blood testosterone levels for the purposes of increasing strength, lean body mass and sexual performance such as androstenedione and dehydroepi androsterone were found to elevate testosterone levels, which can be seen in females groups treated by sustanon causing an increase of blood T levels[28] and also the levels of testosterone hormone in normal female less than in male. Therefore, the injection of exogenouse testosterone (sustanon) cause arise of T levels in females groups. Fig. 5 revealed a significant decrease in the levels of IL-1β in males treated groups, compared with control groups. In contrast, there was significant increase in females treated groups compared with control groups. The males rats primed by sustanon may cause immunosuppressive, reduction of cytokines production, high level of exogenouse testosterone weaken immune competence in male because testosterone has anti-inflammatory properties, which suggests that there was a connection between the male sex hormone and immune response, while in women, there was higher blood levels of signaling proteins that immune cells pass back and forwards to start inflammation, required for activation of immune-system[29]. These evidence confirm, why IL-1β decreased in males and increased in females, affect immune responsiveness as evidenced by a decrease in antibody secretion[30]. Similarly, Sthoeger[31] have shown that testosterone uptake inhibits the proliferation and differentiation of B lymphocytes. While others suggest that AAS enhance immune function[32]. In contrast, the nature of their effects on the immune system depends on the type and dose of AAS used, as well as timing of administration. Nevertheless, it has been

shown that different AAS can act in either immunosuppressive or immunostimulatory manner[33].

The results of the present study demonstrated that a significant increase in the levels of IL-6 in both males and females treated groups, than control groups (Fig. 6). There was a positive correlation between sustanon (AAS) and IL-6 concentration, because high levels of artificial testosterone were given to laboratory animals stimulate production of IL-6 as a pro-inflammatory cytokine, that leads to stimulate immune response by T cells and macrophages. Ferguson-Smith et al.[34] reported that IL-6 increased, when exposed to infection and after injury or other inflammation as a pro-inflammatory cytokine. In contrast, IL-6 plays a roles in muscles tissues, cytokine produced from muscles. It is considered a first cytokine that arise in blood circulation through exercises and therefore, use of AAS by athletes caused elevatation of its concentrations, which leads to muscle contractions[35]. While, D'Agostino et al.[36] have suggested that testosterone suppresses pro-inflammatory cytokines and may up-regulate anti-inflammatory cytokines. It was revealed a significant elevation in CRP levels of treated groups than control groups (Fig. 7). These results may be due to many causes, one of them is that CRP secreted by hepatocytes in response to any inflammatory events *in vivo*, and rise of CRP may be regarded a first sign of cardiovascular disorder. This event may cause through using testosterone as AASs by athletes[37]. These results explained that elevated of IL-6 in the present study by injection of sustanon leads to elevatation of CRP concentration in the blood stream, which agree with reports of Biasucci et al.[38], who revealed that raised levels of C- reactive protein (CRP) are associated with rise of interleukin (IL-6), because CRP up regulates some pro-inflammatory cytokines as IL-6. Furthermore, CRP stimulates peripheral blood monocytes to produce tissue factor (TF), necessary for the body, developed during exposure to exo- endogenous agents and acts as chemotaxis and procoagulant[39] during injury or inflammation by using exogenouse testosterone. Additionally, us of AAS by athletes caused increase of body mass with decreased HDL, waist circumference and height of CRP, which is a significant correlation between CRP levels and rate of metabolic activity[40].

CONCLUSION

The present study concludes that increasing the doses of sustanon drug may cause clear pathological changes in most of the study parameters.

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