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# Analysis of the COVID-19 Lockdown Impact on Biological Parameters and Physical Performance in Football Players

**Giulia My [1], Santo Marsigliante [1], Okba Selmi [2] and Antonella Muscella [1,*]**

[1] Department of Biological and Environmental Science and Technologies, University of Salento, 73100 Lecce, Italy; giulia.my@unisalento.it (G.M.); santo.marsigliante@unisalento.it (S.M.)
[2] High Institute of Sports and Physical Education of Kef, University of Jendouba, El Kef 7100, Tunisia; okbaselmii@yahoo.fr
* Correspondence: antonella.muscella@unisalento.it

**Abstract:** The COVID-19 pandemic has caused significant changes in global sustainability, but specifically, this study analyses the impact of lockdown on health and behavior in the game of football. The 2020/2021 Italian football competitive season (indicated as "post-COVID"), taking place following an obliged lockdown and longer than the normal summery season break, was characterized by very short recovery times and was compared to the 2018–2019 "pre-COVID" season, which had a regular course. The comparisons were about anthropometric and hormonal responses, muscle damage, and the physical performance of players in the major league (Serie A), and were made considering two extreme points of the competitive seasons: before the preparatory period (T0) and at the end of the season (T1). Turning to the results, it is significant to note the following: (1) body fat percentage was lower at the start (T0) of the post-COVID season than at the start of the pre-COVID season. During both seasons, serum CK and LDH increased in T1 and were significantly higher in both T0 and T1 of the post-COVID season. (2) Cortisol and testosterone concentrations increased in both seasons from T0 to T1; however, in the post-COVID season, concentrations of both were higher than in the previous season. The testosterone to cortisol ratio increased at the end of the pre-COVID season, whilst strongly decreasing at T1 of the post-COVID season. (3) Blood lactate concentrations significantly decreased during the pre-COVID season but remained unchanged during the post-COVID season. We may conclude that the enforced suspension period and the consequent rapid resumption of all activities influenced the physical and physiological state of professional footballers.

**Keywords:** COVID-19 lockdown; Serie A; cortisol; football; testosterone; CK; LDH; physical performance

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## 1. Introduction

The coronavirus disease 2019 (COVID-19) has spread worldwide. To limit the spread of the COVID-19 virus, large restrictive measures were introduced during the first lockdown, which revolutionized many aspects of the daily life of all individuals around the world, causing social distancing and home isolation, with substantial psychological, social and economic consequences [1–3]. These measures also had repercussions on sports and competitive practices [4].

In addition, to the harmful cardiac and respiratory effects caused by the virus [5], long periods of inactivity, the cancellation of sports competitions, and competitive and non-competitive sports practice, and the implementation of the quarantine imposed by the health authority, can have further negative consequences on an individual's well-being, affecting their psychosocial health [6,7] and athletic performance [8], professional football players included [9,10].

With the suspension of championships, athletes have been confined and forced to train at home; some sports researchers have suggested that home training restrictions and the absence of an organized team and competitions would induce some detectives effects that could negatively impact athletes' ability to perform specific actions for the soccer match, especially at high intensity [11–13], and that home confinement could increase the risk of injury when an athlete returns to compete [11,14].

This exceptional state may have had several consequences in clinical practice, due to scarce recovery that could affect athletic performance and increase the risk of sports injuries [15–17]. Seventy-two hour post-match recovery periods may not be sufficient to fully restore homeostatic balance in footballers [18,19]. When the championship resumed, professional footballers were busy playing all of the matches that were not played during the months of lockdown, in just two months.

In a previous study, we investigated the changes caused by the COVID-19 lockdown, on anthropometric and body composition parameters, hormonal status, and performance in a professional soccer team [9]. Despite individual variability in many parameters, significant changes in hemoglobin, hematocrit, ferritin, iron, vitamin D, cortisol and testosterone occurred during the COVID-19 season. From a medical point of view, since all the observed changes were within the normal ranges and were therefore typical modulations of homeostatic concentrations, none of those parameters were considered clinically relevant to the players' health or training conditions. However, the amount of training done while staying at home during the COVID-19 period is not enough for professional soccer players to maintain their jumping power during matches [9]. Continuing with the aforementioned study, the objective here was to investigate possible variations in the anthropometric and hormonal responses, muscle damage and physical performance of high-level footballers in the following season (i.e., the 2020/2021 season, post-COVID), characterized by a summer recovery period much shorter than usual. To our knowledge, no study has investigated these parameters following a forced lockdown and longer than the normal annual seasonal break.

## 2. Materials and Methods

### *2.1. Experimental Design*

This prospective cohort study involved an Italian Series A professional soccer team from Southern Italy. The study was conducted during the 2018–2019 and 2020–2021 sports seasons, which are, respectively, the seasons before and after the lockdown due to the COVID-19 pandemic. The two seasons analyzed were interspersed with the 2019–2020 season, which was not considered in this study, which suffered an interruption in performance from 9 March 2020 to 3 May 2020, then end on 2 August 2020 (Figure 1).

All players were evaluated twice during the study: T0 and T1, which correspond to the beginning of the preparatory period (week 0) and the end of the sports season. For the pre-COVID season, T0 coincides with mid-July, while for the post-COVID season, it coincides with the second half of August. T1, on the other hand, coincides for both seasons with the end of May.

### *2.2. Participants*

Eighteen players (aged 24–33 years) from an Italian Serie A football team, participating in both the 2018–2019 and 2020–2021 seasons, were recruited. However, players who suffered injuries and who attended less than 85% of training sessions and scheduled matches were excluded from the study; furthermore, players who tested positive for COVID-19 and goalkeepers were not considered, due to their specific role in the team. In total, 14 players were eligible for inclusion in this study.

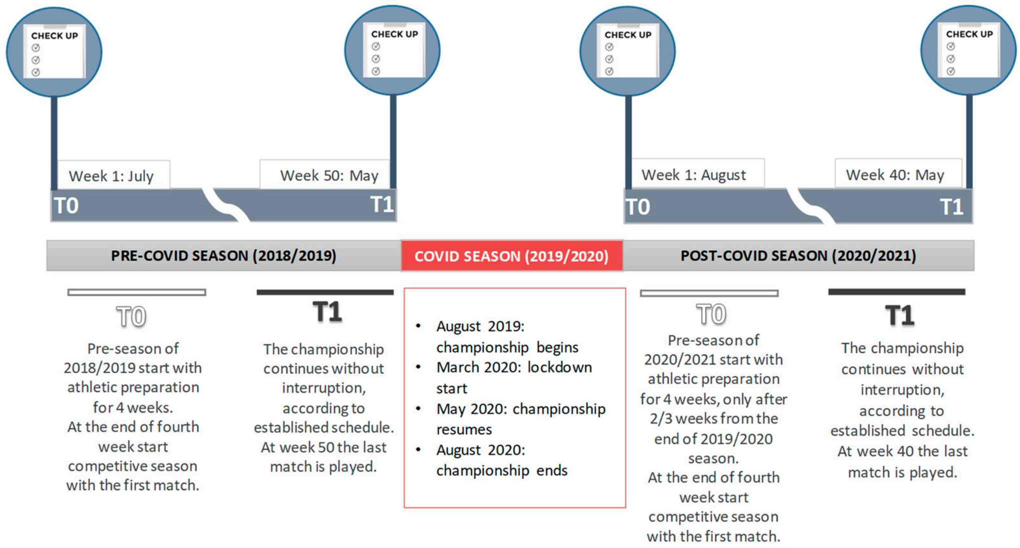


**Figure 1.** A timeline of the 2018–2019 and 2020–2021 Serie A seasons surrounding the impact of the COVID-19 pandemic lockdown.

## *2.3. Training Program*

Figure 2 shows the training program followed by the team during the two seasons, which was the same in both. The training scheme for the first two weeks of athletic preparation of the post-COVID season (2020–2021) is also shown, where a day of rest and a day of unloading work in the pool were scheduled at the weekend. This point was a novelty and became necessary, as the players did not have an effective rest time between the end of the previous season and the start of the new season.

| PERIOD | WEEKS | Weekly volume mean of training + matches (min) | Weekly matches/ week (n) | Training sessions/ week (n) | Training distribution (%) *Aerobic* | *Anaerobic* | *Other* | Training week activities description |
|---|---|---|---|---|---|---|---|---|
| **Pre-Covid season (2018/2019)** | 50 | 571 (min 526–max 616) | 1-2 | 5 | 35 | 20 | 45 | ~3 high-intensity technical tactical sessions including simulated soccer matches + 2 or 3 low-intensity technical-tactical sessions + 1 or 2 speed training sessions + 1 or 2 strength training sessions in the gym. |
| ***Pre-season 2020/2021 - Athletic preparation (first 14 days)*** | *2* | *45 (min 35–max 65)* | *0* | *5* | *45* | *10* | *45* | *1 daily session of individual free running (10-15'); 2 sessions of core stability; 2 sessions of isotonic work in the gym; 1 low intensity Technical-tactical sessions; 1 daily session of relaxation and stretching; 1 unloading work session in the pool at the weekend (20').* |
| **Post-Covid season (2020/2021)** | 38 | 457 (min 420–max 493) | 1-2 | 5 | 35 | 20 | 45 | ~3 high-intensity technical tactical sessions including simulated soccer matches + 2 or 3 low-intensity technical-tactical sessions + 1 or 2 speed training sessions + 1 or 2 strength training sessions in the gym. |

**Figure 2.** Weekly training schedule, divided into pre-COVID season (2018–2019), post-COVID season (2020–2021), and period of athletic preparation for the 2020–2021 season.

As already described in ref. [9], all players, after a standardized warm-up of 5–15 min, performed the same number and the same duration of training sessions.

When only one game was played a week (Sunday), after rest on Monday, training was held on Tuesday, Wednesday, Thursday, Friday, and Saturday. When two weekly games were played (e.g., Wednesday and Sunday), the practices were performed on Monday, Tuesday, Thursday, and Friday.

During training, the soccer players achieved average heart rate (HR) values of 145 beats/min, which is approximately 86–96% of the maximum heart rate. For the athletic preparation period, the training protocol included sessions on Mondays, Thursdays, Thursdays, Fridays, and Saturdays. For the athletic training period, the training protocol included sessions on Monday, Thursday, Thursday, Friday, and Saturday. On Saturdays, after training, the training session was also scheduled for the unloading session in the pool. Wednesday was instead set as a day off.

### *2.4. Anthropometric Evaluation*

The anthropometric assessments always were carried out in the early morning, before each workout, as previously published [9,20].

### *2.5. Blood Parameters*

An expert phlebotomist took samples in the morning (between 08:00 and 09:00) after an overnight fast from a supine antecubital vein. Whole blood samples were collected in vacutainer tubes, using an anticoagulant, and were centrifuged (3000 r·min1 for 10 min) within 20 min of sampling, and the serum was separated from the other compounds for all further determinations.

The IMMULITE 2000 Immunoassay System (Medical Systems) analyzed serum testosterone and cortisol, as previously described [21]. Serum testosterone and cortisol reference ranges were 10–75 ng/dL and 7–25 g/dL, respectively.

Serum creatine kinase (CK) and Lactate Dehydrogenase (LDH) concentrations were determined using an optimized UV at 37 °C in the BT 3000PLUS (Biotecnica Instruments, Rome, Italy), in which specific reagents were used to analyze the concentrations of each enzyme. Both CK and LDH were expressed as U/L.

### *2.6. Physical Performances*

To evaluate the speed at which the athlete reaches OBLA (Onset of Blood Lactate Accumulation), the Mognoni test was performed. The test was carried out on the soccer field, where an elliptical path is traced with cones every 50 m. Each athlete must cover 1350 m in 6 min with a speed corresponding to 13.5 km/h. An acoustic signal emitted at regular intervals allows the athlete to adjust the speed in correspondence with the signals placed every 50 m. Each football player must cover 1.350 m in 6′, maintaining a constant speed of 13.5 km/h. To facilitate the correct execution, placed pins were set at regular intervals of 50 m, making sure that the athletes can follow a call that informed them when they had to pass by each one [22]. Immediately after the test, the lactate concentration in capillary blood taken from the earlobe was measured with a portable lactate analyzer (Lactate Plus; Nova Biomedical, Waltham, MA, USA): The best level of aerobic fitness corresponded with a lower lactate value [22]. From the concentration of lactate in mmol/L at the time of arrival, the value of the anaerobic threshold (in km/h) is obtained by applying the Mognoni formula [23].

### *2.7. Statistical Analysis*

Results were analyzed by GraphPad PRISM 5 software. The descriptive statistics are presented in the form of mean and standard deviation. First, all variables were checked for the normality of distribution by the Kolmogorov–Smirnov test. A mixed ANOVA tested the interaction between time (T0 vs. T1) and group (2018–2019 vs. 2020–2021 seasons). Where indicated in the plots, post-hoc tests (Bonferroni/Dunn) were also performed. The results were considered significant at the 95% confidence level ($p < 0.05$). All data obtained from the study were expressed as mean ± standard deviation.

## 3. Results

### *3.1. Anthropometric Characteristics of Football Players*

The anthropometric parameters of the players, (weight, body mass index, lean body mass (FFM) and body fat percentage (BFP), measured at the beginning (T0) and the end (T1) of the two seasons analyzed, are shown in Table 1.

**Table 1.** Anthropometric characteristics of football players during pre-COVID season (2018–2019) and post-COVID season (2020–2021).

| Parameters | Pre-COVID | Post-COVID | Statistical Analysis | | |
|---|---|---|---|---|---|
| | | | **T0** | | |
| WEIGHT (kg) | 85.6 ± 3.6 | 84.0 ± 3.4 | $p = 0.18$ | F = 1.152 | η = 0.46 |
| BMI (kg/m$^2$) | 24.7 ± 0.8 | 24.3 ± 0.7 | $p = 0.16$ | F = 1.343 | η = 0.58 |
| BFP (%) | 8.9 ± 1.0 | 8.0 ± 0.8 | $p = 0.002$ | F = 1.333 | η = 1.56 |
| FFM (kg) | 78.2 ± 3.0 | 79.5 ± 3.7 | $p = 0.16$ | F = 1.586 | η = 0.39 |
| | | | **T1** | | |
| WEIGHT (kg) | 84.0 ± 3.0 | 82.6 ± 2.9 | $p = 0.11$ | F = 1.053 | η = 1.40 |
| BMI (kg/m$^2$) | 24.3 ± 0.6 | 23.5 ± 0.8 | $p = 0.0004$ | F = 2.155 | η = 1.06 |
| BFP (%) | 7.6 ± 0.7 | 6.7 ± 0.6 | $p < 0.0001$ | F = 1.553 | η = 1.86 |
| FFM (kg) | 79.7 ± 3.0 | 78.6 ± 2.9 | $p = 0.22$ | F = 1.100 | η = 1.23 |

BMI, body mass index; BFP%, percentage of body fat; FFM, fat-free mass.

Mixed ANOVA (time*group) revealed some statistical differences ($p < 0.05$) between the same time of the two different seasons: the percentage of body fat (BFP) was lower at the beginning (T0) of the post-COVID season ($p = 0.002$; F = 1.333; η = 1.56) compared to the beginning of the pre-COVID season. Body mass index ($p = 0.004$; F = 2.155; η = 1.06) and percentage of body fat ($p < 0.001$; F = 1.553; η = 1.86)) were significantly lower at the end (T1) of the post-COVID season rather than at the same time of the pre-COVID season.

So, we can summarize that, among the anthropometric parameters, the ones that actually changed are the BMI in T1 between the two competitive seasons and the BFP both in T0 and in T1 between the two competitive seasons.

### *3.2. Muscle Damage Markers of Football Players*

To check muscle damage and neuromuscular fatigue in football players, creatine kinase (CK) and lactate dehydrogenase (LDH) levels have been used [18]. During both seasons, serum CK and LDH increased in T1 (end of the seasons, $p < 0.0001$, for both by Anova). Furthermore, CK and LDH were significantly higher in both T0 and T1 of the post-COVID season (Figure 3). CK values in T0 of the post-COVID season were very similar to CK values in T1 of the pre-COVID season ($p < 0.20$). In regard to the LDH values in the pre-COVID season, these were lower than the values found in T0 ($p < 0.05$); conversely, serum LDH increased during the post-COVID season with T1 values much higher than T1 of the pre-COVID season ($p < 0.001$).

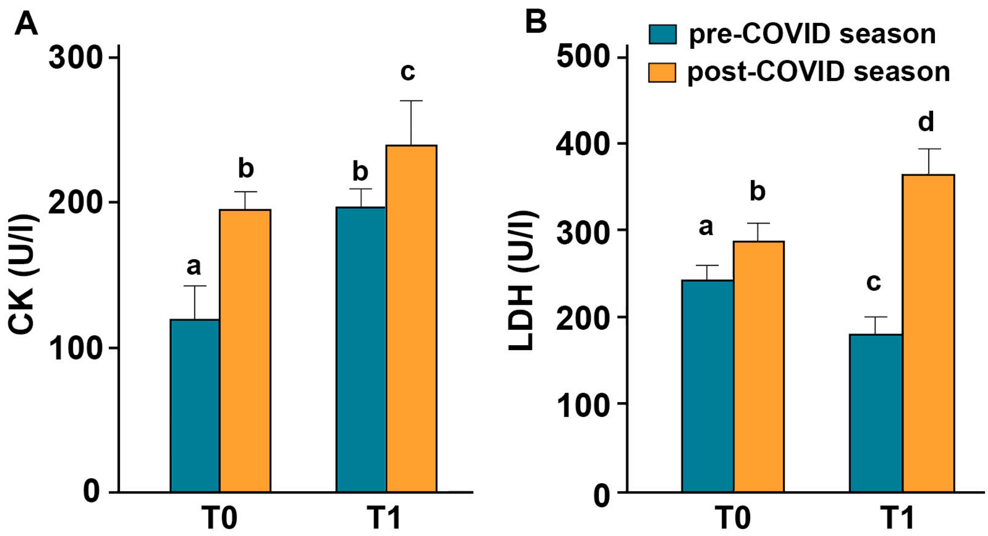


**Figure 3.** Serum CK (**A**) and LDH (**B**) concentration in football players during the 2018–2019 (pre-COVID) and 2020-2021 (post-COVID) seasons. Data are expressed as mean ± SD. Values with the same letters are not significantly different according to the Bonferroni/Dunn post-hoc test.

## *3.3. Indicators of Training Stress of Football Players*

Cortisol (C), Testosterone (T), and T/C ratio measured at the beginning (T0) and end (T1) of both pre-COVID season and post-COVID seasons are shown in Figure 4.

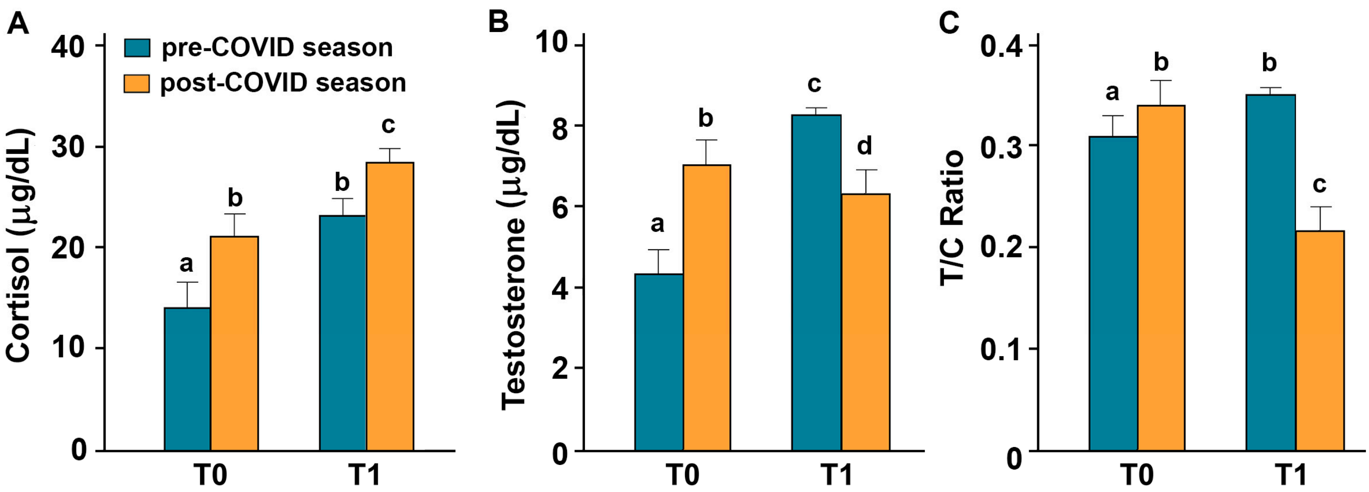


**Figure 4.** Serum cortisol (**A**) and testosterone (**B**) concentration, and T/C ratio (**C**), in football players during 2018–2019 (pre-COVID) and 2020–2021 (post-COVID) seasons. Data are expressed as mean ± SD. Values with the same letters are not significantly different according to the Bonferroni/Dunn post-hoc test.

Serum cortisol and testosterone concentration increased in both seasons from T0 to T1 ($p < 0.0001$ for both by Anova); however, in the post-COVID season, concentrations of both were higher than in the previous season (Figure 4A,B). To be precise, both testosterone and cortisol concentrations at the beginning of the post-COVID season were similar to those in T1 of the pre-COVID season ($p = 0.55$, Figure 4A,B).

Testosterone to cortisol ratio (T/C) increased at the end of the pre-COVID season, while strongly decreasing at T1 of the post-COVID season ($p < 0.0001$, by Anova Figure 4C).

## *3.4. Physical Performances of Football Players*

Blood lactate concentrations significantly decreased during the pre-COVID season ($p < 0.001$) but remained unchanged during the post-COVID season ($p < 0.10$; Figure 5).

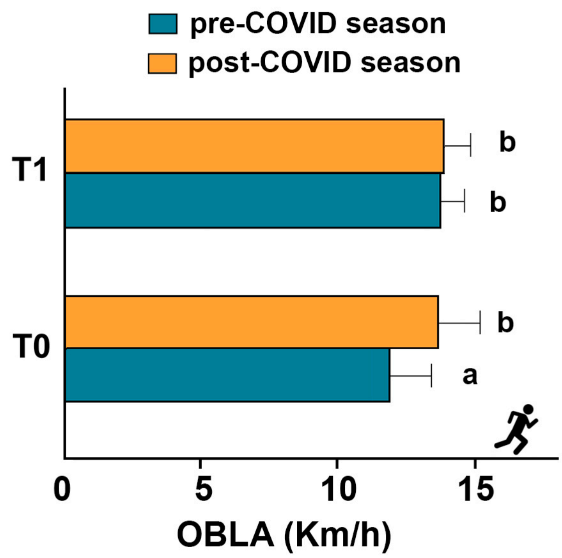


**Figure 5.** Anaerobic threshold values in football players during the 2018–2019 (pre-COVID) and 2020–2021 (post-COVID) seasons. Data are expressed as mean ± SD. Values with the same letters are not significantly different according to the Bonferroni/Dunn post-hoc test.

## 4. Discussion

Prolonged and intense physical activity induces physiological stress, which could cause significant changes in biochemical and hematological parameters [9,24]. Regular monitoring of these parameters in elite athletes during and after a competitive season is helpful for exercise planning and training scheduling and recovery [25]. Stable hematological indices are one of the key determinants of optimal exercise performance, particularly in endurance sports such as soccer [26].

The congested schedules post-pandemic in professional football reduced the amount of time for players to recover between matches and between seasons. Therefore, this non-interventional study aimed to evaluate the effects of a short period of rest between COVID and post-COVID seasons in professional soccer players. To our knowledge, no studies of elite footballers have examined the effects of a long-term suspension period, followed by a rapid recovery of all previously suppressed competitive activities. The two different seasons analyzed were the 2018–2019 season (pre-COVID), which followed a regular course, and the 2020–2021 season (post-COVID) in which professional football players had just returned to play after a very short rest period (2/3 weeks).

Some studies report changes in anthropometric characteristics and body composition during periods of soccer training, and their significant impact on player performance [27,28]. However, in both seasons analyzed, there were constant decreases in weight, BMI, and percentage of fat mass, and increases in lean mass; at the beginning of the post-COVID season, body fat percentage was lower than at the beginning of the pre-COVID season because players were still conditioned.

This study's results confirm a significant reduction in the content of adipose tissue in the analyzed period, by other studies that describe fat reduction with a concomitant increase in lean body mass during the training period [29,30]. Indeed, body fat percentage has been shown to correlate with aerobic and anaerobic fitness capacities [31,32], while fat-free-mass strongly relates to movements that involve rapid skeletal–muscle activation [33], pointing to the importance of decreasing BF% and increasing FFM throughout a period of conditioning [34].

Football is an aerobic–anaerobic sport and involves eccentric muscle contractions, resulting in muscle cell injury and dispersion of cell contents, which leads to elevations of plasma muscle enzymes in footballers above normal values throughout the competitive season; therefore, indicative of extensive cell damage and the impaired functional state of muscle tissue [35].

Thus, increased activities of CK and LDH enzymes in plasma, considered biochemical markers of muscle damage, are commonly used in clinical practice [36]. CK levels are generally used to monitor muscle damage in elite soccer players [37–39] and other sports [40,41], but little information is available on long-term prospective studies in football leagues [42]. Our results demonstrate that CK levels were significantly higher in the post-lockdown congested period than in the uncongested season when players played single-game weeks. Elevated CK values have been suggested as a symptom of overreaching or overtraining [43,44]. However, unusually higher CK values have been routinely measured in soccer players (until 1492 U/L) [45,46] and further higher values following COVID [47]. This may be related to the nature of football training and playing, which involves a large amount of weight-bearing activity, which includes eccentric contractions of the leg muscles [48]. Our findings could be explained by considering that players reduced the number of low- and medium-intensity actions they participated in, but maintained the same number of high-intensity actions during periods of congested scheduling [49]. Exercise is known to increase the activity of enzymes involved in glycolysis and glycogenolysis, such as glycogen phosphorylase, phosphofructokinase, and LDH [42,50,51].

The level of the LDH enzyme also increases during muscle damage/non-functional overcoming state, which can lead to reduced physical performance [52,53]. Therefore, an interesting hypothesis to test would be to investigate whether the cumulative fatigue and metabolic demand that occurred after the COVID-19 lockdown could be similar to the fatigue situation experienced after performing very intense exercises [54].

Generally, LDH increases due to the effects of training throughout the season [55] and specifically, this change was observed in post-COVID season. However, the mean value of LDH decreased at the end of the pre-COVID season. The last result of the present study did not support all previous studies, reporting that LDH activity in soccer players remained unchanged for twelve weeks of the season [39].

However, on the other hand, when the activity level of LDH was measured in the midseason of competitive football, a significant decrease in the plasma activity of this enzyme was noted [36].

Hormonal signals play an important role in the repair and recovery mechanisms, with testosterone playing a major role in stimulating protein synthesis, and with cortisol being a major catabolic influence [56].

Thus, monitoring the changes in testosterone and cortisol might provide some insights into training load/stress [57].

Although cortisol values followed an increasing trend from the beginning (T0) to the end (T1) of both seasons, for the post-COVID season, higher values were already obtained at the beginning (T0), where they were almost similar to the end values (T1) of the pre-COVID season. These results agree with previous studies [21,58–60], according to which, the increase in blood cortisol during the season is a physiological adaptation to physical and psychological stress.

In fact, during training, i.e., a long period of aerobic exercise, an increase in cortisol concentration helps to maintain adequate energy intake [61]. Cortisol prevents the re-esterification of fatty acids obtained from the lipolysis caused by catecholamines [62]. A catabolic process activation is an essential tool for adaptation in conditions of high stress [63].

Increased cortisol concentrations have been reported to have an inhibitory effect on testosterone synthesis [60,64], which would interfere with athlete performance and recovery; in fact, testosterone participates in protein synthesis and erythropoiesis [65], and therefore, its decrease could even negatively affect the health of athletes, causing bone low-mineral content and low density and infertility [66].

In our research, levels of serum testosterone increased in pre-COVID season, reflected the anabolic activity increment related to the volume of strength training and physical performance improvements [9]. Instead, athletes suffered a significant decrease in basal T levels during post COVID season; a fact that would indicate that there is an alteration of the HPT [67].

Additionally, testosterone levels at the start of the post-COVID season were significantly higher than those at the end of the previous season as well. This could be caused by the fact that, after the COVID period, football players have trained with the highest weekly volume with less recovery between sessions, which would favor the maintenance of high-cortisol concentrations.

These results agree with several studies reporting a negative relationship between T concentrations and high-training volumes, also in other sports [68–70]. In addition to high volumes of exercise, multiple other factors, such as sleep deprivation, energy restriction, and psychological stress, can all contribute to reductions in serum testosterone [69].

However, although hormone concentrations showed significant changes in this study, they all remained within the reference range of normality [9,21,66,71].

The decrease in testosterone levels during the season affects the testosterone/cortisol ratio during the post-COVID period (it decreases by about 35%). To monitor and evaluate the athlete's physiological responses to prolonged training-induced stress, the T/C ratio is widely used [72,73]. However, some studies have reported that since a 30% decrease in the T/C ratio did not necessarily correspond to worse athlete performance, this variable cannot be used as a means of control [74].

Aerobic fitness was evaluated by the OBLA test [75,76]. The decreased blood lactate concentrations ([La−]), measured at the end of the pre-COVID season, indicate that this period has positively affected the football player's aerobic capacities. Contrastingly, [La−] remained stable from T0 to T1 during the post-COVID season. These findings confirm previous studies reporting aerobic fitness capacity to be improved following the preparation period and the competitive phase of the season [9,23,75,76]. On the other hand, thanks to the effectiveness of the strategies adopted by the technical staff to enhance aerobic capacity even in the post-COVID period, no deterioration in aerobic fitness and player performance was observed.

Some limitations should be considered in this research. Athletes were selected from just one team; thus, data collection might not be representative of the overall football-playing population. In addition, the study involved the same players for both seasons, therefore, it reduced the sample size because new players were not included in the season two data. Finally, only a limited number of parameters and physiological capacities could be assessed. Therefore, we suggest that future studies utilize a wider range of test parameters.

This study was conducted to provide baseline data on professional footballers, comparing the normal trend of a competitive season (2018–2019) with that of the 2020–2021 (post-COVID) football season, in which footballers returned to activity after only 3 weeks of recovery. In conclusion, the parameters considered showed differences between the two seasons, proving that the forced stop period and the consequent rapid resumption of all activities influenced the physical and physiological state of professional footballers.

The study of specific parameters highlights the lack of physiological and neuromuscular recovery due to the poor recovery between the two seasons.

According to our findings, player substitution, and rotation strategies, as demonstrated by other studies [3], with a particular focus on midfielders, should be strongly encouraged in times of match congestion. Thus, the five-substitution rule, a football rulebook, facilitates substitution decisions to improve performance and decrease the likelihood of injury [77].

## 5. Conclusions

Football clubs are under pressure from fans, owners, and sponsors regarding the sustainability of the football system itself, and this pressure also affects the health of the players. The COVID-19 pandemic has significantly changed the dynamics of the world economy and brought new concerns to countries. The changes have positive and negative valences about sustainability and, in general, the COVID-19 pandemic has caused changes associated with health, sustainability, and ethical behavior in the game of football. We have shown here that the period of forced closure and the consequent rapid resumption of all activities has profoundly influenced not only the performance of the sports industry and its contribution to sustainable development, but has also had a significant impact on the physical state and physiology of professional footballers.

**Author Contributions:** Conceptualization, A.M., O.S., G.M. and S.M.; methodology, G.M. and O.S. software, G.M.; validation, A.M. and S.M.; formal analysis, A.M. and S.M.; investigation, G.M. and O.S.; data curation, A.M. and G.M.; writing—original draft preparation, G.M. and A.M.; writing—review and editing, S.M. visualization, A.M., O.S., G.M. and S.M.; supervision A.M. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Data available on request due to restrictions, e.g., privacy or ethics.

**Conflicts of Interest:** The authors declare no conflict of interest.

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