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Influence of Physical Activity on Self-Esteem and Anxiety during Pregnancy: A Longitudinal Prospective Study

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Abstract: (1) Background: Women's pregnancy increases the odds of developing anxiety, depression, and low self-esteem. Physical activity can reduce the severity of maternal mental disorders and postpartum symptoms. The aim was to evaluate self-esteem, anxiety, and physical activity patterns among healthy pregnant women during three gestational trimesters (GTs). (2) Methods: A longitudinal prospective study involving 248 Portuguese pregnant women, monitored for a year during all GTs, was conducted. Self-reported questionnaires were used to collect personal and obstetric data, and Rosenberg's Self-Esteem Scale, Zung Anxiety Scale, and a Pregnancy Physical Activity Questionnaire were applied. (3) Results: A total of 152 women presented valid follow-up data considered for analysis. All test scores do not present normality ($p < 0.001$). Over the gestational trimesters, the Zung anxiety scores did not show a significant change between GTs. However, the self-esteem values reduced significantly from 1st to 2nd GT: (1st GT = 43.94 (±9.94) [95%CI: 42.37 to 45.53]; 2nd GT = 27.27 (±9.64) [95%CI: 25.74 to 28.81]; 3rd GT = 25.71 (±10.52) [95%CI: 24.04 to 27.39]; $p < 0.001$), remaining stable in the last GT. Moderate to vigorous (2nd GT = 73.43 (±88.69) [95%CI: 59.34 to 87.54]; 3rd GT = 65.05 (±82.65) [95%CI: 51.92 to 78.19]; $p = 0.008$), vigorous (1st GT = 0.45 (±3.95) [95%CI: 0.83 to 2.08]; 2nd GT = 1.63 (±3.52) [95%CI: 1.07 to 2.19]; 3rd GT = 0.81 (±2.16) [95%CI: 0.46 to 1.15]; $p = 0.0475$), and light (2nd GT = 101.68 (±48.77) [95%CI: 93.93 to 109.44]; 3rd GT = 98.42 (±49.05) [95%CI: 90.62 to 106.22]; $p < 0.001$) physical activity levels were significantly reduced throughout the GTs, but the time spent in sedentary activities did not change. (4) Conclusions: In this follow-up study, it was possible to identify a critical point in the 2nd GT, when the drastic reduction in physical activity levels coincided with periods of lower self-esteem scores. However, anxiety levels do not seem to change over GTs.

Citation: Santos, P.C.; Bernardo, D.; Abdalla, P.P.; Leirós-Rodríguez, R.; da Silva, L.S.L.; Mesquita, C.; Mota, J.; Machado, D. Influence of Physical Activity on Self-Esteem and Anxiety during Pregnancy: A Longitudinal Prospective Study. *Women* **2024**, *4*, 340–350. https://doi.org/10.3390/women4040026

Academic Editor: Philip D. Chilibeck

Received: 1 June 2024
Revised: 11 September 2024
Accepted: 18 September 2024
Published: 24 September 2024

Keywords: self-concept; behavior and behavior mechanisms; gravidity; emotions; depressive disorder; exercise

1. Introduction

Pregnancy and childbirth can be overwhelming and challenging for mothers [1]. In fact, some authors consider pregnancy a time of increased vulnerability for developing anxiety and depression [2,3]. Antenatal anxiety is described as nervousness and fears about the baby's health, the mother's health and appearance, the experience with the healthcare system, and social and financial issues [4]. The relationship between self-esteem, depression, and anxiety is well established in a variety of populations, including pregnant women [5]. During pregnancy, women undergo significant hormonal fluctuations that are crucial for sustaining the pregnancy but can also impact mood and emotional stability. These changes involve not only imbalances in sex steroid hormones but also alterations in cortisol reactivity, both of which have been associated with mood swings, heightened anxiety, and an increased risk of depressive symptoms [6]. In addition, they can be affected by the biopsychosocial environment of altered social dynamics, such as stress from the anticipation of childbirth and potential shifts in relationship dynamics with partners, family, and friends [7]. Body image modification and body weight gain during pregnancy can lead to negative body image and diminished self-esteem, especially during the third gestational trimester (GT) [7,8].

All of these physical, hormonal, and emotional changes heighten the predisposition to comorbidities like anxiety and depression during pregnancy. Maternal anxiety and depression during pregnancy not only contributes to adverse delivery outcomes, such as preterm labor, increased pain, prolonged labor, obstetric complications, and a higher likelihood of operative delivery [9], but also serve as significant risk factors for the newborn's well-being, with prenatal anxiety and depression negatively impacting both the clinical aspects of labor and, indirectly, the Apgar score [10].

It is recommended that all clinicians screen for prenatal mental health at least once during pregnancy [11]. However, screening alone does not improve perinatal outcomes. Public health systems should ensure early, consistent, and efficient screening through adequate assessment, detection, intervention, and monitoring tools for women with identified perinatal mental disorders [12,13], promoting well-being of both mothers and babies in the long term. Therefore, knowledge of specific risk factors could help create a screening tool aimed at more vulnerable [14] women.

Standard treatments for anxiety include pharmacological and psychological intervention, but long-term drug use might also have neurodevelopmental effects on prenatally exposed offspring [14]. One potentially promising method for reducing the odds, severity, and symptoms of maternal anxiety is physical activity (PA) [15]. PA plays a key role in health, and during pregnancy its practice has minimal risk [16] and positively impacts the health of both the mother and the child [16,17]. Current international guidelines recommend that women without contraindications to exercise should be physically active throughout pregnancy [16,18,19]. Pregnant women should accumulate at least 150 min of moderate-intensity PA each week to achieve clinically meaningful health benefits and reductions in pregnancy complications [18]. These guidelines were endorsed by the World Health Organisation, reaffirming that more physical activity is better for optimal health outcomes while also providing a new recommendation on reducing sedentary behaviours. These guidelines stress the importance of regular aerobic and muscle-strengthening activities [20].

Women's pregnancy is associated with increased odds of developing anxiety, depression, and low self-esteem, making maternal mental health a significant concern [2]. At the same time, sociodemographic, obstetric, and psychological factors are associated with the development of these mental health disorders [20].

In parallel, the benefits of physical activity include the prevention and improvement of mental health [21,22]. In fact, PA has been shown to reduce the severity of maternal mental disorders and postpartum symptoms [23], but the longitudinal effect of PA during pregnancy remains unclear. Thus, knowing the longitudinal effect of PA during pregnancy is important for clinical practice and public health policies aiming to promote physical activity and mental health during pregnancy.

In response to the increasing recognition of maternal mental health as a critical component of quality perinatal care, we developed this study with two major aims: firstly, to evaluate self-esteem, anxiety, and PA patterns among healthy pregnant women in three GTs; secondly, to compare PA levels with self-esteem and anxiety among pregnant women. We hypothesize that there is a relationship between PA levels and self-esteem among healthy pregnant women across the three GTs.

2. Results

A total of 248 women were initially invited to participate in the study. However, only 152 completed the questionnaires throughout the different gestational trimesters, resulting in a follow-up loss of 38.7% (96 participants). Losses during the follow-up were primarily due to incomplete questionnaire responses.

Physical activity, self-esteem, anxiety, body mass, and height variables did not present normality, and non-parametric tests were used.

Regarding the baseline characteristics of the sample (Table 1), it was observed that the participants had an average age of 29 (5.2) years, with a pre-gestational body mass index of 24.3 kg/m^2, classified as normal. Additionally, it was noted that most participants spent the majority of their time engaging in light activities. Concerning anxiety and self-esteem levels, it was found that at baseline, women exhibited 43.9 (9.9) and 35.5 (5.5), respectively.

Table 1. Baseline (1st gestational trimester) characteristics of the sample (n = 152).

Variables	Mean	SD	95%CI		Normality K–S Statistic
			LL	UL	
Age (years)	29.9	5.2	29.2	30.8	0.086 *
Anthropometry					
Weight (kg)	74.2	7.6	57.4	90.9	0.383 *
Pre-pregnancy weight (kg)	63.4	9.8	61.1	65.7	0.101
Height (m)	1.61	0.05	1.60	1.62	0.095
Pre-pregnancy body mass index (kg/m^2)	24.3	3.7	23.3	25.2	0.135 *
Physical activity levels					
Sedentary (MET-h.wk-1)	54.8	35.0	49.2	60.4	0.122 *
Light (MET-h.wk-1)	118.5	53.1	110.0	127.0	0.081 *
Moderate (MET-h.wk-1)	92.5	97.7	76.9	108.2	0.173 *
Vigorous (MET-h.wk-1)	1.5	4.0	0.8	2.1	0.407 *
Self-esteem (score)	43.9	9.9	42.4	45.5	0.081 *
Anxiety (score)	35.3	5.5	34.4	36.2	0.171 *

SD = standard deviation; CI = confidence interval; LL = lower limit; UL = upper limit; * Kolmogorov–Smirnov (K–S) not normally distributed = $p < 0.05$.

The comparison of results in the three gestational trimesters is shown in Figure 1.

Regarding vigorous physical activity, there was a difference among the three GTs (1st GT = 0.45 (±3.95) [95%CI: 0.83 to 2.08]; 2nd GT = 1.63 (±3.52) [95%CI: 1.07 to 2.19]; 3rd GT = 0.81 (±2.16) [95%CI: 0.46 to 1.15]; p = 0.0475), but no interaction between the GTs. Anxiety scores were not modified throughout the three GTs.

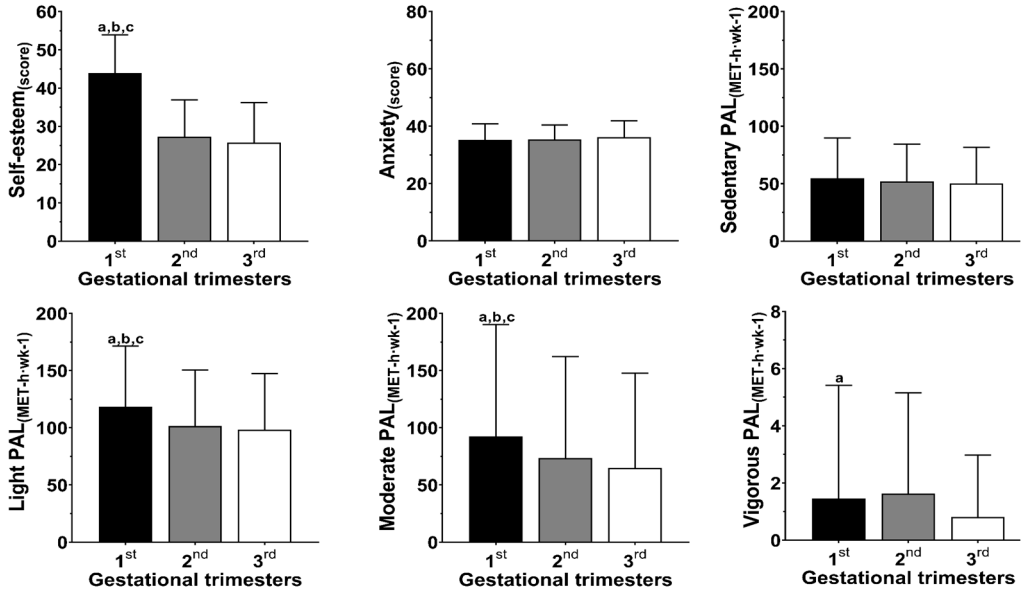

Figure 1. Comparison of three gestational trimesters' self-esteem, anxiety scores, and physical activity level: a = significant difference alongside gestational trimester ($p < 0.05$); b = significant difference compared to 2nd gestational trimester ($p < 0.05$); c = significant difference compared to 3rd gestational trimester ($p < 0.05$).

In comparison to the 1st GT, self-esteem was significantly reduced in the 2nd and 3rd GTs (1st GT = 43.94 (±9.94) [95%CI: 42.37 to 45.53]; 2nd GT = 27.27 (±9.64) [95%CI: 25.74 to 28.81]; 3rd GT = 25.71 (±10.52) [95%CI: 24.04 to 27.39]; $p < 0.0015$) (Figure 1). Similarly, light (2nd GT = 101.68 (48.77) [95%CI: 93.93 to 109.44]; 3rd GT = 98.42 (±49.05) [95%CI: 90.62 to 106.22]; $p < 0.001$) and moderate (2nd GT = 73.43 (±88.69) [95%CI: 59.34 to 87.54]; 3rd GT = 65.05 (±82.65) [95%CI: 51.92 to 78.19]; $p = 0.008$) PA levels experienced significant reductions throughout the 2nd and 3rd GTs ($p < 0.05$). Regarding vigorous PA, there was a difference among the three GTs ($p < 0.05$), but no interaction between the GTs. Anxiety scores were not modified throughout the three GTs.

Only self-esteem, from the 1st to the 2nd GT, showed a large effect size (ES) of 1.7 (Figure 2). This large ES means that the participants' self-esteem was clinically impaired throughout the GT. The other variables (anxiety and physical activity levels) and GTs showed a small (−0.4 to 0.2) to null (0) ES, meaning that although some variables had statistical difference, the clinical effect was low.

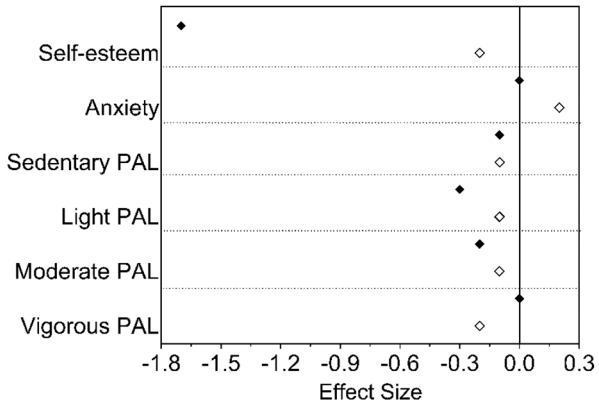

Figure 2. Effect size along gestational trimesters for self-esteem, anxiety, and physical activity levels (PALs). Note: Black diamonds mean the effect size between 1st and 2nd gestational trimesters, whereas the white diamond means the effect size between 2nd and 3rd gestational trimesters.

3. Discussion

The findings of this study shed light on the complex relationship between anxiety, self-esteem, and PA levels throughout the GTs. We observed that anxiety and self-esteem may be associated with a gradual reduction in the expected PA levels in healthy pregnant women. This suggests that as pregnancy progresses, factors such as hormonal changes, physical discomfort, and concern for the baby's health may contribute to a decrease in motivation or ability to be physically active. Specifically, fluctuations in hormones such as serotonin, estrogen, and cortisol [24] can lead to mood swings and fatigue. When combined with the physical strain and musculoskeletal issues specific to pregnancy, such as lower back pain [25], these factors can diminish a pregnant woman's inclination to engage in regular exercise. Together, these factors may create barriers that impede the maintenance of an active lifestyle throughout the different stages of pregnancy [26–28].

Moreover, the study revealed that self-esteem scores were lower during the 2nd GT, when there was a significant reduction in PA levels. This critical point highlights the importance of identifying and addressing the potential decline in self-esteem during this period, as it may have implications for the mental well-being of pregnant women [12,20].

Additionally, our findings showed that while the PA level reduces throughout pregnancy, the time spent in sedentary activities remains relatively constant. This suggests that pregnant women may compensate for reduced PA by engaging in sedentary behaviors [29]. Understanding this pattern is crucial for developing targeted interventions that promote an active lifestyle and diminish the negative impact of sedentary behaviors on mental health outcomes.

Studies have shown that PA during pregnancy can positively impact women's mental health outcomes. However, the relationship between PA levels and maternal mental health during pregnancy is complex and multifactorial. In our study, we found that anxiety and self-esteem may be associated with the reduction in expected PA levels during the pregnancy of healthy women. During pregnancy, women experience significant physical and emotional changes, which can affect their inclination to engage in physical activities. This finding is consistent with our previous research that has shown a decline in PA levels during pregnancy [26]. Additionally, a comparison with the existing literature on sedentary behavior during pregnancy reveals a consistent pattern of elevated sedentary time among pregnant women, which is often linked to adverse outcomes. Studies have shown that sedentary behavior tends to increase as pregnancy progresses, with many women spending a significant portion of their day in sedentary activities [27]. This trend is concerning, given the established association between high levels of sedentary behavior and negative health consequences such as excessive gestational weight gain, gestational diabetes, and poor cardiovascular health [27]. Our findings align with these observations, further emphasizing the critical need for interventions that specifically target sedentary behavior during pregnancy to improve overall maternal health.

Anxiety and self-esteem have also been linked to PA levels during pregnancy, previously [30]. Specifically, a positive effect was found of past-pregnancy PA on self-efficacy and postpartum weight retention [30]. Furthermore, a systematic review found that exercise interventions during pregnancy had positive effects on anxiety and self-esteem [31]. When considered together, our findings could suggest that maintaining PA levels during pregnancy may be important for supporting maternal mental health outcomes, particularly for anxiety and self-esteem. This highlights the need for interventions and support programs that promote PA among pregnant women to promote overall well-being.

Numerous physiological and psychological changes occur during pregnancy, including changes in PA levels, anxiety, and self-esteem [32]. Several studies have explored the multifactorial relationship between PA benefits during pregnancy [33,34]. One of these studies found that neighborhood environments conducive to the practice of PA during pregnancy had a positive effect on exercise habits throughout the GTs [35]. Another study reported that self-esteem was negatively affected during the 2nd GT of pregnancy, possibly due to physical changes such as weight gain and body shape changes, that women

experience during this time [36]. Likewise, anxiety levels have been reported to increase during pregnancy due to hormonal changes, life events, and changes in relationships and support systems [37]. According to the *2019 Canadian Guideline for Physical Activity throughout Pregnancy* [18], it is recommended that pregnant women aim to accumulate at least 150 min of moderate-intensity PA each week. This guideline emphasizes the importance of engaging in regular PA to attain meaningful health benefits and reduce the risk of pregnancy complications. The PA level in the baseline (1st GT) was not adequate to WHO recommendations for pregnant or postpartum women (at least 150 min per week of moderate-intensity aerobic physical activity), being only ~93 min/week [38]. Although, it is important to note that pregnant women should consult with their healthcare provider before starting or modifying an exercise routine, especially if they have pre-existing medical conditions or other factors that may require specific considerations [16]. Healthcare professionals can provide personalized recommendations based on individual circumstances and ensure that any exercise program is tailored to the woman's health status and gestational stage. Studies show that there is good adherence to remote exercise programs of moderate intensity that can be performed at home, which may enhance compliance [39]. By following guidelines, pregnant women can optimize their health and well-being while reducing the risk of complications [16,40].

Research has also explored the positive relationship between PA levels and mental health outcomes during pregnancy. A systematic review found that women who engaged in regular moderate-intensity PA had lower levels of anxiety during pregnancy compared to those who did not engage in physical activity [41]. Another study found that increasing PA levels during pregnancy were associated with a reduction in depressive symptoms [42]. Additionally, several studies have reported a positive relationship between PA and self-esteem during pregnancy [21,22,42]. When analyzed together, these findings suggest that anxiety and self-esteem are associated with changes in PA level throughout pregnancy. Engaging in regular PA during pregnancy may be an important strategy for improving mental health outcomes and self-esteem [43]. Future research should explore the long-term consequences of reduced physical activity during pregnancy, particularly its effects on postpartum recovery and maternal mental health, to emphasize the role of sustained physical activity in promoting lasting maternal health benefits. Additionally, it is essential for clinical practice to promote early intervention programs, including health education during pregnancy and the postpartum period, to enhance women's knowledge and empowerment during this stage. Encouraging activities that support physical and emotional well-being, such as exercise programs, is also important.

The authors recognize that our study has several limitations. Firstly, the study relied on self-reported measures, which are subject to recall bias and social desirability effects. Additionally, the study was conducted in a specific region, which may limit the generalizability of the findings to other populations or cultural contexts. In addition, the research instruments used are not designed or specifically validated for use in pregnant women, a population group in which the constructs evaluated could have different conditioning factors than the rest of the population.

Despite these limitations, we also recognize the strengths of this research: one of them is its longitudinal design, allowing us to observe changes over time and capture the dynamic nature of these variables throughout the gestational period, and another strength is the inclusion of a relatively large sample size. We also emphasize that this study contributes to the understanding of the relationship between physical activity, self-esteem, and anxiety during pregnancy, highlighting the need for further research and targeted interventions to support the mental well-being of pregnant women.

As for the implications for practice and research, the findings of our study are important for both practice and future research. In terms of practice, healthcare professionals working with pregnant women should be aware of the potential impact of anxiety, self-esteem, and PA levels on maternal mental well-being. Our results suggest the need for integrated approaches that address pregnancy's psychological and physical aspects. Inter-

ventions should focus on promoting PA engagement, enhancing self-esteem, and managing anxiety symptoms throughout specific exercise programs, while respecting the different moments and sensitivities of the GTs. They can also provide guidance, support, and personalized recommendations to encourage pregnant women to maintain an active lifestyle while addressing their mental health needs. For future research, it should be valuable to explore the specific mechanisms underlying the association between anxiety, self-esteem, and PA during pregnancy. Longitudinal studies with larger and more diverse samples with the participation of pregnant women from other regions and countries could provide a deeper understanding of the complex dynamics between these variables.

In future research, objective measures of physical activity, such as accelerometers, could be used to objectively measure PA.

4. Materials and Methods

4.1. Study Design and Sample

This longitudinal prospective study was conducted on a consecutive sample of pregnant women who attended routine prenatal visits in maternal health centers and one hospital in the north of Portugal. The study was approved by the Ethical Review Board of Hospital S. João in Portugal (code: 09988), following the ethical guidelines outlined in the 1975 Helsinki Declaration (rev. 2013). Written informed consent was obtained from all individuals included in the study after a brief explanation of the study objectives and evaluations.

The inclusion criteria were as follows: (a) women aged between 18 and 40 years; (b) with a spontaneous singleton pregnancy; and (c) gestational age between 10th and 13th weeks. They were ineligible if they had any of the following characteristics: (a) diabetes, hypertension, heart disease, thyroid disease, or an uncontrolled chronic disease; (b) persistent bleeding after 12 weeks of pregnancy; (c) severe anemia; (d) pre-eclampsia; (e) a lack of competence in the Portuguese language or a cognitive impairment that precludes comprehension of the questionnaire in order to obtain an answer to said questionnaire; and (f) those who did not complete all the stages or desired to withdraw from the study. Physiotherapists or nurses had identified women with confirmed pregnancies in the first trimester and invited them to participate in the study.

The sample size calculation considered the desired maximum error (ε) and degree of confidence (Zy = 95%), based on the previously known population variability ($\sigma 2$) [44]. To determine the sample size, we considered the highest variability (Self-Rating Anxiety Scale; standard deviation = 12.3) expected for this population [45]. By setting the predetermined maximum desired error ($\varepsilon \leq 2\%$), the ideal sample size for this study was determined to be n = 152 [44].

4.2. Procedure

Women were evaluated in three stages, corresponding to each GT of pregnancy: the first, second and third GTs. After the medical consultation, pregnant women followed at the hospital in the first GT (<12 weeks of gestation) were referred by the physiotherapist or nurse to an office. The principal investigator checked the eligibility and exclusion criteria and gave general information about the study. The principal investigator scheduled the follow-up evaluation during their second GT (12 to 28 weeks) and third GT (>28 weeks). Reassessments were always scheduled from the 1st GT to the others to coincide with routine maternal health consultations. All women had an identification code in the pregnant health bulletin. This identification code contained all the questionnaires given to each woman to allow the pairing of the questionnaires. The same code was on the follow-up table that each healthcare professional monitored. Women who missed the scheduled day were contacted by phone to reschedule the assessment, and in case of a second absence, a home visit was made to ensure reassessment and avoid losses in the follow-up. Pregnant women were monitored for a year during all GTs.

Self-reported questionnaires were used to collect socio demographic data, also including the following:

Rosenberg's Self-Esteem Scale [46]: This unidimensional scale included ten items aimed to globally assess the positive or negative attitude of the individual towards herself. Five items are considered indicators of positive self-attitudes, and the other five are representative of negative self-attitudes. For each item, there are six possible answers, ranging from '6—strongly agree' to '1—strongly disagree'. After subtracting the scores of the negative responses, the sum of the results reflects greater self-esteem, with higher scores indicating higher self-esteem. In this study, we obtained satisfactory internal reliability. The present study used a version with transcultural Portuguese adaptation with adequate internal consistency, with a Cronbach's value of 0.86, and good temporal stability, given that with a two-week interval between assessments, the Pearson correlation coefficient was equal to 0.90 [47].

Zung Self-rating Anxiety Scale (SAS): This scale translated and validated in Portuguese [48] with 20 items that cover a variety of anxiety symptoms, both psychological (e.g., "I feel afraid for no reason at all" and "I feel like I'm falling apart and going to pieces") and somatic (e.g., "My arms and legs shake and tremble" and "I feel my heart beating fast"). Responses were given on a 4-point scale, ranging from 1 (none, or some of the time) to 4 (most, or all of the time). Participants were instructed to base their answers on their experiences over the last week. Items included both negative and positive experiences (e.g., "I fall asleep easily and get a good night's sleep."), with the latter being reverse scored. Raw scale scores for the SAS ranged from 20 to 80. The SAS had satisfactory psychometric properties, including internal consistency (Cronbach's alpha = 0.82) [49], concurrent validity (r = 0.3 with the Taylor Manifest Anxiety Scale) [50], and the capacity to discriminate between clinical and non-clinical samples, and anxiety and other psychiatric disorders [50].

Pregnancy PA Questionnaire (PPAQ) [51]: This questionnaire listed 32 activities, organized into four intensity levels according to the score obtained in each, and measured in metabolic equivalents (METs). One MET corresponded to the metabolic equivalent of energy expended at rest. Sedentary activities corresponded to an energy expenditure < 1.5 METs; light activities between ≥1.5 and <3 METs; moderate activities between ≥3 and <6 METs; and a vigorous energy expenditure ≥6 METs. The energy expenditure on the activity in METs (intensity) was multiplied by the duration of the activity per day, and then we obtained an average measurement of energy spent weekly (MET-h·wk−1) [51]. This questionnaire was validated for the Portuguese population with interclass correlation coefficient values between 0.87 and 0.70 for the different types of activities, and a weak and negative coefficient correlation (r = −0.030) for total activity [52].

4.3. Statistical Analysis

Descriptive data considered measures of mean, standard deviation, and 95% confidence interval (95%CI) from the baseline. Firstly, data normality was tested with the Kolmogorov–Smirnov test. The Friedman test compared the differences in Rosenberg's Self-Esteem Scale (SAS) and PPAQ scores between GTs. The Dunn post-hoc test was used to make multiple pairwise comparisons between groups after detecting a significant difference with the Kruskal–Wallis test. The minimal clinically important difference in Rosenberg's Self-Esteem Scale (SAS) and PPAQ scores between GTs (1st vs. 2nd; 2nd vs. 3rd) was assessed using the effect size (ES). The ES was calculated by dividing the mean difference between the pre-test and post-test scores by the pre-test standard deviation (1st vs. 2nd; 2nd vs. 3rd). The Cohen's d (>0.9: large; <0.9 to >0.5 medium; and <0.5 small for positive ES; <−0.9: large; >−0.9 to <−0.5 medium; and >−0.5 small for negative ES) was used to classify the ES. All analyses were performed using SPSS Statistics, v. 20.0 (IBM, Chicago, IL, USA), with a significance level previously established (α = 5%). The graphs were built with GraphPad Prism v. 9.3 software.

5. Conclusions

This study successfully achieved its two major aims. Self-esteem, anxiety, and PA patterns were evaluated among healthy pregnant women across three GTs, providing valuable insights into the changes and associations between these variables. We observed a significant decrease in self-esteem scores during the 2nd GT, coinciding with a reduction in PA levels. Additionally, this study found that PA levels changed across GTs, with a notable decrease in light to moderate physical activity. These findings highlight the importance of considering the mental well-being of pregnant women and the potential impact of PA on self-esteem and anxiety. However, anxiety levels do not seem to change over GTs.

Interventions that address anxiety, promote self-esteem, and provide tailored strategies to overcome barriers to PA can have a positive impact on the mental well-being of pregnant women, ultimately improving their overall health and quality of life.

Author Contributions: Conceptualization, P.C.S., D.B. and J.M.; methodology, P.C.S. and C.M.; software, P.P.A., D.M. and L.S.L.d.S.; valid formal analysis, D.M., P.P.A., R.L.-R. and L.S.L.d.S.; investigation, P.C.S. and D.B.; data curation, P.C.S.; writing—original draft preparation, D.B., P.C.S. and D.M.; writing—review and editing, P.P.A., R.L.-R., J.M. and C.M.; funding acquisition, P.C.S. and D.B.; Supervision: J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Portuguese Foundation for Science and Technology (FCT); UID/DTP/00617/2020 [Research Center in Physical Activity, Health, and Leisure—CIAFEL] and LA/P/0064/2020 [Laboratory of Interdisciplinary Research—ITR] and FCT: PTDC/DES/116586/2010; SFRH/BD/45375/2008; PROTEC SFRH/BD/50002/2009.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Review Board of Hospital S. João in Portugal (code: 09988).

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

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author/s.

Conflicts of Interest: The authors declare no conflicts of interest.

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