ORIGINAL RESEARCH

The effect of docosahexaenoic acid (DHA) supplementation on total antioxidant capacity (TAC), superoxide dismutase (SOD), and interleukin-6 levels in underweight pregnant women

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ABSTRACT

Objective: Underweight pregnant women face oxidative stress and inflammation, increasing their risk of intrauterine growth restriction (IUGR) and preterm birth. This study investigates the effects of DHA supplementation on Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), and Interleukin-6 (IL-6) in underweight pregnant women, along with the correlation between DHA and these markers.

Materials and Methods: This experimental pre-test/post-test study focused on underweight pregnant women in the Made District, Surabaya, Indonesia. Eligible participants were in their second or third trimester, had a BMI below 18.5, and were taking DHA regularly. Exclusion criteria included early pregnancy (gestational age < 14 weeks), BMI above 18.5, irregular DHA intake, and withdrawal from the study. The study ran from July to December 2023, using non-probability sampling to select participants. Blood samples were collected before and after two months of DHA supplementation.

Results: Following the intervention, TAC levels demonstrated a noteworthy increase ($p < 0.05$). SOD levels also exhibited a significant difference ($p < 0.05$), and IL-6 levels showed a significant change ($p < 0.05$). A strong and positive correlation ($r = 0.718$) was observed between the increased TAC and SOD levels. DHA influenced both TAC and IL-6, with a significant relationship between TAC and IL-6 ($p < 0.01$). Furthermore, elevated SOD levels were associated with a decrease in IL-6 levels ($p < 0.01$). The correlation coefficient value of 0.718 between changes in SOD and TAC indicated a robust positive correlation.

Conclusion: The findings suggest that DHA supplementation in underweight pregnant women positively affects oxidative stress and inflammation markers, improving TAC, SOD, and IL-6 levels.

Highlights:

1. Underweight pregnant women, face an imbalance in energy and protein intake.
2. TAC, SOD, and IL6 with administration of DHA to pregnant women with chronic energy deficiency in the third trimester provide benefits.

INTRODUCTION

Around 3–4% of women in the United Kingdom commence pregnancy with a low body weight. However, globally, the prevalence of malnutrition predominantly affects low- and middle-income nations. For instance, up to 30% of women of childbearing age in many Sub-Saharan African countries are underweight. Various factors such as body image perceptions, genetics, socioeconomic status, and cultural influences contribute to BMI variations, resulting in a diverse population of underweight women. Nevertheless, extensive cohort studies have revealed that underweight pregnant women are more likely to be younger, unmarried, pursuing education, and unemployed. Moreover, there is evidence indicating a higher prevalence of smoking among underweight women.[1]

During the initial evaluation, it is imperative to rule out treatable factors contributing to low BMI before attributing it solely to idiopathic causes. BMI before pregnancy determines neonatal and maternal outcomes. Pregnant women with underweight have risks in pregnancy and childbirth.[2] Several studies in underweight pregnant women found the risk of preterm birth and low birth weight.[3,4] In underweight pregnancies, especially in the second and third trimesters, there is an increase in oxygen pressure, ROS production, and excessive oxidative stress.[5,6] This process of inflammation will trigger a series of effects that will result in IUGR, preterm birth, and stillbirth.[7] Meanwhile, docosahexaenoic acid (DHA) helped to reduce the production of reactive oxygen species (ROS) in astrocyte cells.[7] Several studies have stated DHA supplementation is important during pregnancy.

However, there are no certain indicators of DHA's influence in pregnancy. This study will be the first study that tries to examine the potential benefits of DHA supplementation for underweight pregnant women by finding the relationship between underweight pregnant women and the incidence of oxidative stress through the TAC and SOD pathways and the inflammatory mechanisms through the IL-6 pathway in underweight pregnant women. We hope that this study will contribute to the development of science, give a better understanding for the clinician regarding DHA supplementation in pregnancy, and thus lessen the complications in pregnancy.

MATERIALS AND METHODS

Our study employed an experimental design incorporating a cohort study framework with a quantitative methodology. This approach allowed us to assess the impact of the treatment administered and substantiate the hypotheses formulated. The primary objective of this study was to investigate the influence of DHA supplementation on TAC, SOD, and IL-6 levels in underweight pregnant women. This assessment was conducted over a specified timeframe of 2 months within a predetermined district.

The study population consisted of underweight pregnant women in Made District, Surabaya, East Java, Indonesia. Inclusion criteria encompassed pregnant women registered in Made District, with a gestational age exceeding 14 weeks (trimesters 2-3), and a BMI below 18.5. Exclusion criteria encompassed a gestational age below 14 weeks, a BMI above 18.5, irregular consumption of DHA supplements, and withdrawal from the study. The study was held from July 2023 to December 2023. Sampling utilized non-probability techniques, combining quota and purposive sampling. The experimental group comprised 21 underweight pregnant women receiving DHA supplementation. TAC, SOD, and IL-6 levels were assessed before and after treatment to evaluate the potential impact of DHA supplementation on reducing TAC, SOD, and IL-6 levels in underweight pregnant women.

The research variables included the dependent variable: TAC, SOD, and IL-6; the independent variable: DHA supplementation; and the control variable: gestational age in the second and third trimester, diagnosis of underweight was done with BMI measurement, no other comorbidities (kidney, heart, diabetes mellitus, and chronic hypertension). TAC was measured using the ELISA method, SOD was measured using the ELISA method with the SOD kit, and IL-6 was measured using the quantitative fluorescent immunoassay method.

SPSS 25 was used in the analysis of the data collected in this study. Normality testing was conducted using either the Kolmogorov-Smirnov test or the Shapiro-Wilk test. For pre-posttest comparisons, the paired t-test will be employed if the data follows a normal distribution. Conversely, if the data does not exhibit normality, the Wilcoxon test was utilized. To assess the correlation among the three variables (TAC, SOD, and IL-6), the researcher employed the Pearson correlation test if the data adheres to a normal distribution. In cases where the data is not normally distributed, the Spearman correlation test was used. These statistical methods were chosen to ensure appropriate analyses were conducted based on the normality or non-normality of the data distribution.

This study has received authorization from the National Unity and Politics Agency (Badan Kesatuan Bangsa dan Politik) under approval number 070/7145/209/2023. All protocols related to research subjects were executed following the ethical guidelines outlined in the approval granted by the Health Research Ethics Committee of the Faculty of Medicine at Ciputra University (Komisi Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Ciputra), with approval number 089/EC/KEPK-FKUC/X/2023. Before their involvement in this research, all participants have given their written consent.

RESULTS AND DISCUSSION

In this study, an evaluation of various characteristics was conducted. The respondents' ages fell within the reproductive age range of 16 to 36 years, and their gestational age ranged from the second to the third trimesters. All participants were classified as underweight, with a BMI ranging from a minimum of 17.33 to a maximum of 18.40. Systolic blood pressure readings ranged from a minimum of 90.0 to a maximum of 128, while diastolic blood pressure ranged from a minimum of 59 to a maximum of 80 (Table 1). Upon conducting normality tests, it was observed that the data for age, gestational age, mid-upper arm circumference, and systolic blood pressure followed a normal distribution. On the other hand, BMI and diastolic blood pressure exhibited a non-normal distribution. These findings provide valuable insights into the demographic and physiological characteristics of the study participants and inform the choice of appropriate statistical tests based on the normality or non-normality of the data.

Table 1. Research characteristics

No	Characteristics	Mean $\pm$ SD
1	Age	27.30 $\pm$ 4.45
2	Gestational Age	19.96 $\pm$ 10.01
3	Weight	41.96 $\pm$ 3.18
4	Height	152.04 $\pm$ 3.35
5	BMI	18.10 $\pm$ 0.28
6	Mid Upper Arm Circumference (LILA)	24.52 $\pm$ 1.67
7	Systolic BP	105.04 $\pm$ 10.32
8	Diastolic BP	67.21 $\pm$ 7.48

Table 2. Total Anti-Oxidant Analysis (T-test)

No	Characteristics	Mean $\pm$ SD	P Value
1	TAC Pre test	0.37 $\pm$ 0.13	<0.001
2	TAC Post-test	0.55 $\pm$ 0.09	

The evaluation of total antioxidant levels before and after DHA supplementation revealed a significant difference in TAC results between the pre and post-test, indicating a substantial increase in TAC levels (p < 0.05) (Table 2). This suggested that DHA supplementation had a notable effect on enhancing total antioxidant levels. Underweight pregnant women, as evidenced by reduced TAC levels, experienced a decline in antioxidant capacity. The inhibitory impact of DHA on NF-kB activation, a key factor in the synthesis of inflammatory cytokines, vascular adhesion molecules, metalloproteinase, and VEGF, contributes to this effect.[8,9] Furthermore, DHA enhances TAC through mechanisms associated with its ability to increase SOD and catalase enzymes. By inhibiting NF-kB and reducing reactive oxygen species (ROS), DHA effectively elevates antioxidant levels in the blood of underweight pregnant women.[10]

Table 3. Superoxide Dismutase Analysis (T-test)

No	Characteristics	Mean $\pm$ SD	P Value
1	SOD Pre test	15.55 $\pm$ 2.54	0.008
2	SOD Post test	17.44 $\pm$ 2.62	

The study identified a significant difference in SOD levels before and after the intervention (p < 0.05), with an observed increase in SOD values in underweight pregnant women receiving DHA (Table 3). This indicates that DHA supplementation has a positive impact on enhancing SOD levels. DHA's ability to inhibit oxidative stress and exert anti-inflammatory effects contributes to the improvement of endothelial function. Additionally, DHA plays a crucial role in eliminating ROS-induced DNA damage and reducing H2O2 formation. Through its supplementation, DHA increases the production of SOD, enhancing its scavenging ability in redox signaling. The nuclear factor E2-related factor 2 (Nrf2) emerges as a key player in fighting oxidative stress, and DHA plays a pivotal role in regulating the expression of genes responsible for increasing SOD levels. This regulation by Nrf2, a critical transcription factor, triggers the cellular antioxidant defense system to effectively combat Reactive Oxygen Species (ROS). The findings underscore the multi-faceted impact of DHA on SOD levels and its role in bolstering the cellular defense mechanisms against oxidative stress.[11,12]

Table 4. IL-6 Analysis (Wilcoxon)

No	Characteristics	Mean $\pm$ SD	P Value
1	IL 6 Pre test	9.83 $\pm$ 17.8	0.005
2	IL 6 Post test	7.28 $\pm$ 11.003	

The IL-6 levels in this study exhibited a significant change (p < 0.05), demonstrating a notable decrease (Table 4). This indicates that DHA supplementation has a discernible effect on IL-6 levels in underweight pregnant women. DHA, known for its anti-

inflammatory role, operates through competitive inhibition against arachidonic acid (ARA) in phospholipid membranes, resulting in a metabolic shift from pro-inflammatory ARA-derived eicosanoids to DHA-derived lipid mediators. The decrease in ARA is directly linked to the increased DHA levels in the blood, as there is competition between DHA and ARA metabolism involving enzymes such as phospholipase A2, COX, and lipoxygenase. Elevated ARA levels are directly proportional to IL-6 production, indicating an increased inflammatory response, particularly in cases of underweight pregnant women. DHA supplementation effectively lowers ARA levels, thereby reducing the production of inflammatory cytokines, including IL-6. These findings highlight the anti-inflammatory potential of DHA in mitigating inflammatory markers such as IL-6 in underweight pregnant women.[13,14]

Table 5. Pearson's correlation test between TAC, SOD and IL-6

No	Characteristics	P Value	Pearson's correlation
1	TAC and SOD	< 0.05	0,718
2	TAC and IL 6	< 0.05	- 0.600
3	SOD and IL 6	< 0.05	- 0.592

In this study, a significant and robust correlation was observed between TAC and SOD, providing insights into the influence of DHA on TAC and SOD levels with a strong positive correlation coefficient ($r = 0.718$) (Table 5, Figure 1). This finding suggests that the administration of DHA affects increasing both TAC and SOD activity. The mechanism underlying this effect can be explained through the inhibition of phosphatidylinositol 3-kinase and protein kinase B (PKB), which modulate oxidative stress activation. This modulation subsequently hinders the induction of the NF-kB pathway, as elucidated in the study conducted by Mahdi Sepidarkish.[15] The correlation observed in this study aligns with existing research, supporting the notion that DHA supplementation may contribute to enhanced antioxidant capacity and SOD activity in underweight pregnant women.[15]

The study indicates a relationship between DHA supplementation and alterations in TAC and IL-6 levels. The association between TAC and IL-6 can be explained by the increase in ROS, which triggers elevated expression of inflammatory cytokines, including IL-6, through the activation of NF-kB and activator protein-1 (AP-1). Subsequently, the infiltration of macrophages into adipose tissue is stimulated, leading to increased production of inflammatory cytokines due to enhanced ROS production. These findings align with research conducted by Martinez, who investigated DHA supplementation for 3 months in

patients with keratoconus. Martinez found that DHA could elevate TAC levels while concurrently decreasing IL-6 levels. This underscores the potential of DHA in addressing states of chronic inflammation and oxidative stress occurring simultaneously. The study provides valuable insights into the multifaceted impact of DHA on antioxidant capacity and the modulation of inflammatory markers, highlighting its potential therapeutic role in mitigating conditions characterized by inflammation and oxidative stress.[16,17]

The relationship between TAC and IL-6 can be further explained by the activation of the NF-kB pathway initiated by ROS. This activation induces inflammatory processes, triggering the production of pro-inflammatory cytokines, including IL-6. These cytokines, in turn, enhance the synthesis of matrix metalloproteinase (MMPs). The increased presence of MMPs contributes to elevated oxidative stress, causing damage to extracellular constituents, cell membranes, nucleic acids, and protein structures, ultimately resulting in tissue protein damage. DHA, as a source of antioxidants, plays a crucial role in inhibiting tissue damage caused by oxidative stress. By acting as a negative regulator, DHA mitigates the impact of pro-inflammatory cytokines like IL-6. This highlights the potential of DHA in modulating the intricate interplay between oxidative stress, inflammation, and tissue damage, underscoring its significance as a protective factor in such processes.[18]

The correlation test between SOD and IL-6 demonstrated a significant and inversely correlated relationship, where an increase in SOD was associated with a decrease in IL-6 levels. This inverse correlation exhibited moderate strength with a negative correlation coefficient ($r = -0.592$). The relationship between SOD and IL-6 in underweight pregnant women can be elucidated by examining how decreased SOD levels may contribute to endothelial damage. Endothelial damage triggers an inflammatory process through inflammatory cells, particularly monocytes, which migrate to the sub-endothelium and bind to endothelial adhesive molecules. These monocytes then differentiate into macrophages, and activated macrophages secrete pro-inflammatory cytokines. IL-6 is induced due to the influence of low SOD expression through inflammation mediated by neutrophils. Activated neutrophils, in turn, bind to the endothelium, migrate to the extracellular space, and release reactive oxygen species (ROS), protease enzymes, and chemokines in significant quantities. DHA, recognized as a potent antioxidant, especially in increasing SOD activity, plays a crucial role in mitigating this process.[4,19,20]. This study aligns with the research of Losano, supporting the proposition that DHA supplementation can reduce IL-6 levels,

highlighting the potential of DHA in modulating the intricate relationship between SOD, inflammation, and oxidative stress in underweight pregnant women.[21]
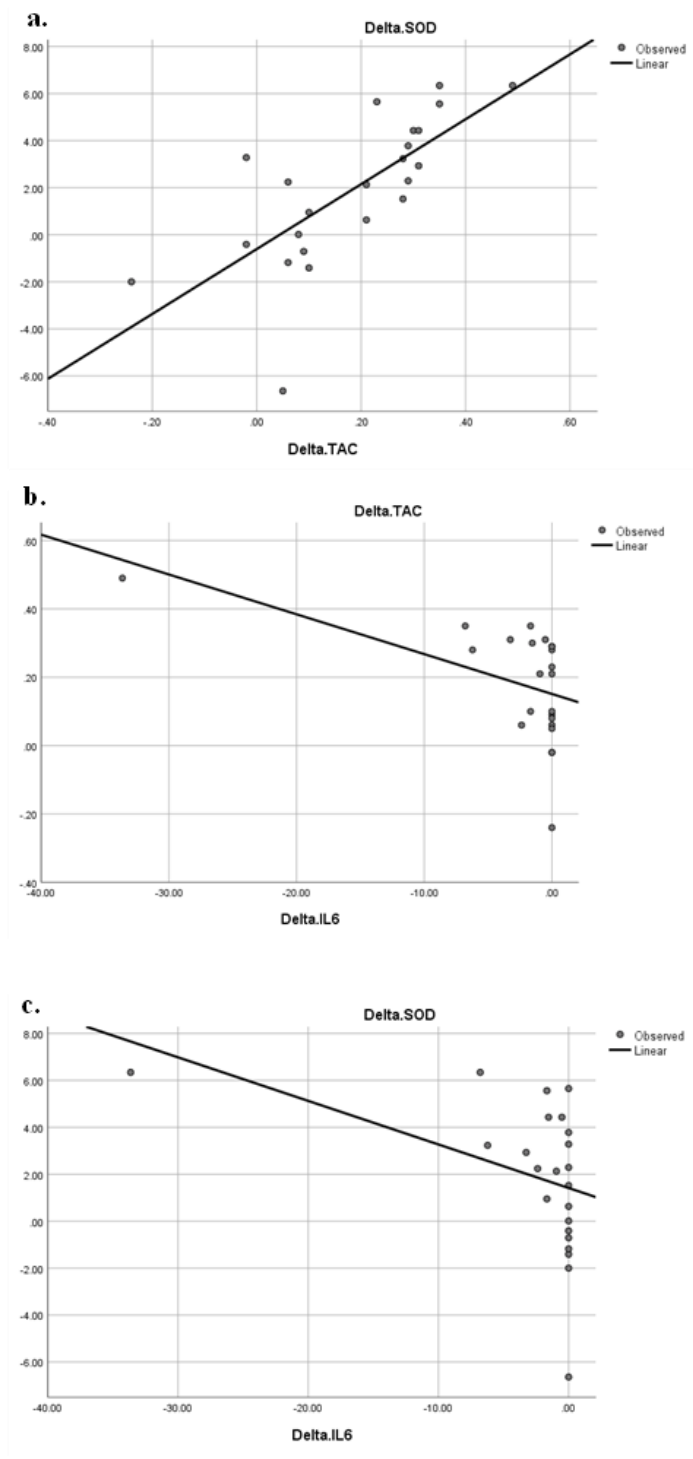

Figure 1. a. Scatter plot of correlation of Delta SOD and Delta TAC; b. Scatter plot of correlation of Delta TAC and Delta IL-6; c. Scatter plot of correlation of Delta SOD and Delta IL-6.

Based on the findings from the three markers in this study, it can be concluded that DHA plays a significant role in underweight pregnant women, particularly in its function against ROS-induced cell damage and death. The production of superoxide radicals (O2-) through the donation of one electron to molecular oxygen (O2) marks the initial step in the formation and dissemination of ROS inside and outside mitochondria. The imbalance between ROS production and enzymatic antioxidant systems increases the susceptibility of tissues and organs to oxidative radical damage. Superoxide radicals can react with nitric oxide (NO) to form peroxynitrite (ONOO-) in a reaction controlled by the production and bioavailability of radicals. Furthermore, in the transition from iron, superoxide radicals and hydrogen peroxide (H2O2) interact with the Haber-Weiss reaction to generate hydroxyl radicals (OH). The high reactivity of peroxynitrite and hydroxyl radicals poses a risk to cell nucleic acids, proteins, or lipids, leading to overall organism damage. Mitigating superoxide radicals can be achieved by increasing mitochondrial superoxide dismutase, subsequently reducing the formation of peroxynitrite and hydroxyl radicals. Hydrogen peroxide can be inhibited by activating glutathione peroxidase (GPX) and catalase (CAT), mechanisms facilitated by the consumption of DHA. This underscores the protective role of DHA against oxidative stress and highlights its potential to preserve cellular integrity in underweight pregnant women.[22]

The results of this study indicate that the pre-test and post-test outcomes for TAC, SOD, and IL-6 were statistically significant, signifying that the administration of DHA brought about changes in these three variables. The administration of DHA increased TAC and SOD levels, while IL-6 levels decreased. Furthermore, in the examination of the correlation test, a significant relationship or correlation was identified between the change (delta) in TAC with both SOD and IL-6. The relationship between TAC and SOD was positive, indicating they moved in the same direction, while the relationship between TAC, SOD, and IL-6 was negative, suggesting an opposite direction based on the correlation test. These findings underscore the impact of DHA on antioxidant capacity, Superoxide dismutase activity, and IL-6 levels, providing valuable insights into the multifaceted effects of DHA supplementation in underweight pregnant women.

The researchers also conducted a demographic test with the change (delta) in TAC, SOD, and IL-6. Based on the results of the demographic test, there was no significant difference observed for the three variables representing changes in TAC, SOD, and IL-6 (Table 6). This implies that demographics were not a confounding variable in this study. The non-significant findings in the demographic test suggest that, regardless of demographic factors, the administration of DHA can be conducted. The study indicates that demographic variables were not influential in the changes observed in SOD, TAC, and IL-6. Therefore, the alterations in these three research markers were attributed purely to the effects of DHA and were not related to demographic factors. This strengthens the inference that the observed changes are indeed a result of DHA supplementation in underweight pregnant women, providing a more robust understanding of the intervention's impact.

Mammalian cells utilize oxygen as the final electron acceptor in energy metabolism, but this process generates unwanted oxygen-based byproducts collectively known as ROS. At low levels, ROS can function in redox signaling and biological processes, but at high levels, they can lead to nonspecific and potentially harmful reactions. The susceptibility of fatty acids to oxidation is believed to depend on the degree of saturation. DHA, despite its high degree of saturation, is susceptible to oxidation, and the relationship between its structure and susceptibility to ROS oxidation is intricate. Both in vitro and in vivo studies have demonstrated that DHA can induce an antioxidant response at the transcriptional or post-transcriptional level. Understanding the molecular mechanisms behind the protective role of DHA is increasingly important due to its potential in preventing chronic diseases. The transcription factor Nrf2, which regulates cellular redox homeostasis, plays a crucial role in activating genes that enhance antioxidant and detoxification capacity. DHA can activate Nrf2 reversibly, initiating a cellular antioxidant response. DHA can undergo enzymatic and non-enzymatic oxidation, resulting in various oxidation metabolites.

Table 6. Correlation Test of Demographic Relationship with Delta TAC, SOD and IL6

No	Characteristics	Delta TAC	Delta SOD	Delta IL-6
1	Age	0.132	0.445	0.117
2	Gestational Age	0.790	0.604	0.445
3	Mid Upper Arm Circumference (LILA)	0.473	0.755	0.744
4	Systolic BP	0.733	0.337	0.709
5	Diastolic BP	0.450	0.586	0.627
6	BMI	0.428	0.416	0.972

These oxidized DHA products can interact with Keap1, a protein involved in Nrf2 degradation, inhibiting this degradation process. As a result, Nrf2 can carry out its activity in regulating the antioxidant response. Understanding these mechanisms provides valuable insights into the role of DHA in cellular antioxidant responses and its potential implications in preventing chronic diseases.[23]

However, this study had some constraints. It focused solely on one district of Surabaya (Made District, Surabaya, East Java, Indonesia). Our study's strength lies in the selection of our sample, as we meticulously monitored their DHA consumption from the outset of the research to ensure proper adherence.

CONCLUSION

In this study, it was found that the administration of DHA can have an impact or change on TAC, SOD, and IL-6 in pregnant women with chronic energy deficiency. In addition, this study also proves that DHA corrected the oxidative status and reduce inflammation.

DISCLOSURES

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Conflict of interest

No conflicts of interest could influence the results or interpretation of this research

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Author contribution

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting, and approval for publication of this manuscript.

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