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EFFECT OF EXERCISE ON THE INSULIN/IGF-1 SYSTEM IN CANCER PATIENTS

IMPLICATIONS FOR CARDIOMETABOLIC HEALTH AND QUALITY OF LIFE IN BREAST CANCER SURVIVORS AND OLDER ADULTS

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ABSTRACT

Breast Cancer (BC) is the most common female cancer worldwide, and the growing number of adults aged ≥ 65 is expected to increase the population of elderly Breast Cancer Survivors (BCS). However, the effect of tailored aerobic exercise and Mediterranean diet (MD) on overall Quality of Life (QoL) considering fatigue, cardiometabolic health, glucose homeostasis, and the tumor growth regulation, remain unclear. Moreover, the role of reserve of oxygen uptake ($\dot{V}O_2R$) and fatigability as determinant of free-living energy expenditure in older adults is still unknown. Since cancer history is associated with higher fatigability and poor physical functioning, especially in older adults, this Thesis aimed to: (i) investigated the effect of a Lifestyle Intervention (LI), combining supervised aerobic exercise and MD guidance, on a) QoL including cancer-related fatigue (study 1); b) free-living glycemic profile (study 2); and c) Insulin-Like Growth Factor 1 (IGF-1) modulation (study 3) along with cardiometabolic health (e.g., maximum oxygen uptake [$\dot{V}O_{2\mathrm{max}}$], % of fat mass, and caloric intake) in BCS within the MoviS clinical trial; and (ii) to examine $\dot{V}O_2R$ and perceived fatigability as determinants of Activity Energy Expenditure (AEE) in older adults from the ENGAGE project (study 4). Four different studies were conducted: studies 1-3 recruited and randomized BCS into Control (GC) and Intervention (IG) groups, while study 4 followed a cross-sectional design. Study 1 showed that LI improved QoL and cardiometabolic health (e.g., physical functioning and $\dot{V}O_{2max}$) in the short-term. However, benefits returned to the baseline in the long-term, emphasize the need for follow-up education to sustain healthy habits. MD guidance was insufficient to achieve optimal adherence, and age and Tamoxifen influencing QoL changes. Study 2 suggested that the LI improved free-living glycemic control (e.g., insulin level) more in the IG than in the CG. CG showed a worsening trend in time spent above glucose range (> 180 mg/dl). These effects were related to food intake between active and non-active days. Study 3 showed the baseline IGF-1 role in assessing LI efficacy: $\dot{V}O_{2max}$ improvements were associated with an increase in IGF-1 in participants with low baseline levels and with a decrease in those with high levels. Study 4 found $\dot{V}O_2R$ significantly correlated with fatigability and AEE, and $\dot{V}O_2R$, age and % of fat mass were predictors of AEE. Four functional profiles were detected confirming that fatigability was significantly different for women compared to men. Although fatigability was not a predictor of AEE, it was significantly associated to $\dot{V}O_2R$. This Thesis showed the complex interplay between self-reported outcomes (e.g., QoL and fatigability) and physiological

component (e.g., $\dot{V}O_{2max}$ and IGF-1) in two different cohorts. Integrating these measures is essential to understand QoL and cardiometabolic health in order to prevent BC incidence favoring healthy ageing.

PREMISE

Breast Cancer (BC) is the most prevalent cancer among women worldwide, representing one of the chronic diseases with the highest psychological and socio-economic impact. Considering the top five cancer cases and deaths in 2022 (Bray et al., 2024), female BC accounted for 23.8% of 9.7 million new cases and 15.4% of 4.3 million deaths worldwide. However, significant advances in the treatment have been achieved, and BC survival rates have increased in the last few decades due to earlier diagnosis and more effective therapies reaching survival rates of 90% at five years and 80% at ten years (Touraine et al., 2025; Soto-Ruiz et al., 2025). In addition, current demographic data show an increase in the number of older adults aged 65 and above. From 2019 to 2050, this population is estimated to grow by around 90 million (Corselli-Nordblad and Strandell, 2020), representing a further challenge for the healthcare system. As a result, the number of elderly women with a previous history of BC is expected to increase. This high survival rate highlights the need to assess well-being domains, which include both mental (e.g., depression or anxiety) and physical (e.g., cardiometabolic parameters) health to achieve overall Quality of Life (QoL) in both BC survivors (BCS) and older adults (Champion et al., 2014).

Considering the QoL of BCS, recent studies have highlighted that one of the most experienced side-effects of BC drug treatments (e.g., Tamoxifen or Aromatase Inhibitors) is "cancer-related fatigue". Cancer-related fatigue tends to worsen with age (Fitch et al., 2023) and may be exacerbated by typical age-related changes, such as the physiological decline in specific cardiorespiratory and metabolic parameters [e.g., maximum oxygen uptake ($\dot{V}O_{2max}$), reserve of oxygen uptake ($\dot{V}O_2R$), and resting metabolic rate (RMR)]. These changes may influence the behavioural component, such as regular physical activity (PA) and a balanced diet (e.g., Mediterranean diet [MD]), thereby promoting sedentary behaviour that reduces daily activity energy expenditure (AEE) (Thong et al., 2025). In addition, the subjective nature of perceived fatigue as a domain of overall QoL, assessed using questionnaires with general questions (e.g., "Did you need to rest?"), makes it difficult to identify the fatigue level related to specific daily tasks (Gresham et al., 2018) and its association with cardiometabolic health. For this reason, it may be interesting to focus attention on a recent construct, i.e., fatigue perceived in relation to an activity with specific duration and intensity. This construct, namely "fatigability", offers a potentially less biased and more objective approach to measuring the degree to which fatigue limits an individual physically (Glynn et al., 2015). This could be especially important in studies on both BCS and older adults, who - in an effort to reduce perceived fatigue - may modify their level of exertion (e.g., shortening task duration) to maintain a tolerable effort during daily activities (Glynn et al., 2015). Given the differences between younger and older BCS in their specific and overall QoL domains (Champion et al., 2014; Gresham et al., 2018), a history of cancer seems to be associated with higher fatigability and poorer physical functioning. This effect is significantly greater in older adults with a previous cancer diagnosis (Gresham et al., 2018).

It is unknown how Lifestyle Interventions (LI) - including both tailored aerobic exercise and MD guidance - may influence overall QoL (including fatigue as a general domain), cardiometabolic health (e.g., $\dot{V}O_{2max}$, MD adherence, and glycemic homeostasis), and the regulation of key factors for tumor growth (e.g., IGF-1) in BCS in both short- and long-term, as well as it is unknown how $\dot{V}O_2R$ and fatigability (defined as perceived fatigue in relation to an activity with specific durations and intensities) may be determinants of AEE in older adults.

This Thesis is composed of four original research articles that investigated the aforementioned issues as follows.

The first three studies (Study 1, 2, and 3) were carried out at the University of Urbino (Department of Biomolecular Sciences) and investigated the effects of a LI (characterized by a 12-week supervised aerobic exercise training program and MD guidance) on QoL and Cardiometabolic Health in BCS participating in the MoviS clinical trial (ClinicalTrials.gov - NCT04818359). The last study (Study 4) was conducted during a six-month research period abroad at the University of Southern Denmark (Centre for Active and Healthy Ageing [CAHA], located in the Department of Sports Science and Clinical Biomechanics in Odense) and investigated the relationship between $\dot{V}O_2R$ and perceived fatigability as determinants of daily AEE in older adults enrolled in the Energetics in Old Age (ENGAGE) study (ClinicalTrials.gov - NCT04821713). Specifically, Study 1 - *"Short- and long-term effects of a 12-week supervised aerobic exercise training program and mediterranean diet on quality of life and cardiometabolic health in breast cancer survivors: Results from the MOVIS randomized controlled trial"* - aimed to evaluate the effect of the Movis LI on (i) QoL and (ii) on cardiometabolic health in BCS; Study 2 - "*Effect of a 12-week lifestyle intervention program on* *free-living glycemic profile and cardiometabolic health in breast cancer survivors: the Sweet Movis study"* - aimed to evaluate the effects of Movis LI on non-fasting glycemic control metrics in BCS; Study 3 - *"Lifestyle intervention based on aerobic exercise and Mediterranean diet modulates IGF-1 and its binding proteins in breast cancer survivors: results from a randomized controlled trial"* - aimed to evaluate the effect of the Movis LI on modulation of IGF-1 levels, along with anthropometric, body composition, metabolic, and cardiorespiratory parameters; Study 4 - "*The role of $\dot{V}O_2$ reserve and perceived fatigability on free-living activity energy expenditure in older adults"* - aimed to (i) examine possible correlations between *$\dot{V}O_2R$,* AEE, and perceived physical fatigability, (ii) examine whether *$\dot{V}O_2R$* and perceived physical fatigability can predict AEE, and (iii) identify distinct profiles of older adults characterized by specific levels of *$\dot{V}O_2R$*, AEE, and perceived physical fatigability, considering sex differences.

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STUDY 1

Original Article

Short- and Long-Term Effects of a 12-Week Supervised Aerobic Exercise Training Program and Mediterranean diet on Quality of Life and Cardiometabolic Health in Breast Cancer Survivors: Results from the MOVIS Randomized Controlled Trial

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Abstract

The quality of life (QoL) of Breast Cancer survivors (BCS) can be negatively affected by the side effects of oncologic treatments, unbalanced diet and sedentary behaviour. Lifestyle Intervention (LI), which provides physical activity (PA) and Mediterranean diet (MD) guidance, represents an effective non-pharmacological strategy to improve QoL in BCS. The "Movement and Health Beyond Care (MoviS)" is a randomized controlled trial (RCT) which aimed to evaluate the effect of a LI characterized by a 12-week supervised aerobic exercise training program and MD guidance on i) QoL and ii) on cardiometabolic health in BCS women.

One hundred and nine (n=109) sedentary BCS women (age 51.70±8.00 years [mean±SD]) were randomized into a control group (CG, n=56) and an intervention group (IG, n=53). All patients received recommendations on PA and MD at the baseline (T0). IG participants also underwent a 3-time-a-week supervised aerobic training program for 12-week with progressive increases in exercise intensity (40-70% of heart rate reserve) and duration (20-60 minutes). QoL was evaluated using the European Organisation for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30 (EORTCQLQ-C30) version 3.0. Cardiometabolic health was assessed by measuring the cardiorespiratory fitness level ($\dot{V}O_{2max}$ [$mL \cdot min^{-1} \cdot kg^{-1}$]) and the MD adherence (Mediet questionnaire). QoL assessments were done at T0, after the intervention period (T1), in the short- (6 months, T2), and the long-term (12 and 24 months, T3 and T4 respectively), while cardiometabolic health was assessed until T3 (T4 will be done between november and december 2025 as programmed in the Movis Trial). Linear Mixed Models (LMMs) were used to analyse all outcomes and to assess changes between groups over time. All models were adjusted for age and type to endocrine therapy. Statistical significance was set at 0.05 for all analyses.

LMMs show that physical functioning scale, social functioning scale, and QLQ-C30 Summary score increased significantly at T1 in the IG compared to the CG. The same trend was found for cardiorespiratory fitness level: $\dot{V}O_{2max}$ increased significantly at T1 in the IG compared to CG. In addition, LMMs show that financial difficulties were barely significantly lower at T1 compared to T0 in both groups, $\dot{V}O_{2max}$ (at T2) and physical functioning (at T3) were barely significantly higher compared to T0 in both CG and IG. The MD adherence was significantly higher at T1 and at T3 compared to T0 in both groups. Emotional functioning decreased significantly in IG compared to CG at T4, the same trend (but only barely significant) was found in the cognitive functioning scale. Finally, the effects of age and endocrine therapy were found: for each age increase equal to 1-year participants experienced significantly less nausea and vomiting, and diarrhoea symptoms; BCS women who were treated with Tamoxifen experienced significantly more insomnia and appetite loss symptoms compared to BCS with no endocrine therapy.

After the LI (T1), significant improvements were seen in physical functioning, social functioning, $\dot{V}O_{2max}$, and in QLQ-C30 Summary score only in the IG. This highlights the value of exercise specialist supervision for short term enhancements in QoL and cardiorespiratory

fitness level. After 6 (T2)- and 12-month (T3), physical functioning and $\dot{V}O_{2max}$ were barely significantly higher in both groups compared to T0 showing an IG trend in maintaining the beneficial effects reached during the LI, while the CG exhibited slightly improved physical functioning and fitness level, but not to the same extent as the IG in the short term. The MD adherence increased significantly after the LI, at T1 and at T3 compared to T0 in both groups. However, neither of the two groups achieved the minimum score (equal to 8 points) at any time evaluated, indicating that diet guidance may not be sufficient to reach MD adherence. Emotional and cognitive functioning decreased at T4 in the IG suggesting a need for focus on mental health in the long-term. Age and type of endocrine therapy were confirmed as factors which can influence some symptoms such as Nausea and Vomiting, Diarrhoea, Insomnia and Appetite loss in BCS.

Key words: aerobic exercise, breast cancer survivors, quality of life, cardiometabolic health

Introduction

Breast Cancer (BC) is the most prevalent female cancer in the world, representing one of the chronic diseases with the highest psychological and socio-economic impact. Considering the top five cancer cases and deaths distribution in 2022, female BC represented 23.8% of 9.7 million new cases and 15.4% of 4.3 million deaths worldwide (Bray et al., 2024). However, advances in the treatment have been achieved, and BC survival rates have increased in the last few decades due to earlier diagnosis and more effective therapies reaching survival rates equal to 90% at five years and 80% at ten years (Touraine et al., 2025, Soto-Ruiz et al., 2025). In Italy, 55.700 new cases of female BC were diagnosed in 2022, and 125.500 deaths in 2021 were registered (Associazione Italiana Oncologia Medica [AIOM], 2023). A five-year survival rate was equal to 88% (*I numeri del cancro in Italia*, 2022). Hence, the number of long-term BCS, which refers to people who have survived the disease for at least five years since diagnosis, is increasing (Ferlay et al., 2024). This high rate of survival highlights the need to address the Quality of Life (QoL) of BCS, as a central aspect of their general well-being (Champion et al., 2014, Palesh et al., 2018, Rosenberg et al., 2022, Grusdat et al., 2022, Yarosh et al., 2025).

QoL may be influenced by not only BC diagnosis, but also by treatment (e.g., chemotherapy or radiotherapy). In fact, most patients with BC reported treatment side effects which impact their overall health, including both mental and physical disorders (e.g, anxiety, depression, constipation, and appetite loss), which contribute to poor long-term compliance and prognosis (Soto-Ruiz et al., 2025, Mokhtari-Hessari et al., 2020).

Moreover, adjuvant treatments (e.g., Tamoxifen or Aromatase Inhibitors) for BC negatively impact cardiorespiratory fitness (CRF) level, a key predictor of cardiovascular risk (Kotte et al., 2025, Peel et al., 2014). After treatment, many BCS are discharged without receiving follow-up or any support beyond routine controls that are used to detect recurrences (Soto-Ruiz et al., 2025, Koch et al., 2013, Cardoso et al., 2019).

This lack of specialized support leaves many BCS on their own, facing a range of symptoms that can persist for years. These issues include cancer-related fatigue, insomnia, pain, lymphedema, cardiotoxicity, premature menopause, infertility, and sexual health problems (Soto-Ruiz et al., 2025, Koch et al., 2013, Cardoso et al., 2019).

In addition, lifestyle may represent another point which can influence BCS health. For example, an unbalanced diet may contribute to worsening physical functioning and exacerbating specific symptoms (e.g., constipation, diarrhoea, and appetite loss) affecting the overall QoL. In contrast, the Mediterranean diet (MD) could improve BC symptoms related to QoL via anti-inflammatory effects, antioxidant properties, and hormone-receptor interactions (Chen et al., 2023, Castro-Espin et al., 2022). Specifically, MD is a predominant plant-based dietary pattern, characterized by the high intake of olive oil, fruits, vegetables, non-refined cereals, legumes, nuts, and low-to-moderate intake of dairy products, fish and poultry, moderate intake of alcohol, and low intake of red meat and sweets (Chen et al., 2023). Another important aspect to consider is sedentary behaviour: Soto-Ruiz and colleagues reported that BCS who engaged in moderate physical activity (PA) (i.e., 150 to 300 minutes per week) showed significantly improved QoL in both physical and psychological well-being domains. In line with the importance of PA for QoL, the Italian DianaWeb cohort of women with BC (n=781) reported, during the first wave of the COVID-19 pandemic, a marked reduction in PA and an increase in sedentary behavior; notably, higher QoL levels, assessed using the EORTC QLQ-C30 global health status/QoL scale, were associated with lower odds of being sedentary (Natalucci et al., 2021).

Hence, Lifestyle Intervention (LI), which provides PA and MD guidance, represents an effective non-pharmacological strategy to improve QoL in women with BC diagnoses or survivors. The most used Patient-Reported Outcome measure (PROM) to evaluate QoL in patient with cancer was the *"European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30" (EORTC QLQ-C30)"*: a multidimensional QOL questionnaire composed of 30 items organized in 15 different scales (Aaronson et al., 1993, Osoba et al., 1998). The EORTC QLQ-C30 provides a valuable wealth of information about the QoL of patients with cancer, which can be used to tailor LI intervention and improve both mental and physical health. Recently, some researchers proposed a "QLQ-C30 Summary score" to obtain a unique value, which provides a more interpretable QoL changes over time, and represents a good prognostic factor (Hinz et al., 2012, Giesinger et al., 2016, Husson et al., 2020, Gundy et al., 2012). Husson and colleagues reported that QLQ-C30 Summary score was associated significantly with all-cause mortality (hazard ratio [HR], 0.77; 99% confidence interval [CI], 0.71-0.82), and had a stronger association with all-cause mortality than the global QoL scale (HR, 0.82; 99% CI, 0.77-0.86) or the physical functioning scale (HR, 0.81; 95% CI, 0.77-0.85) in patients with cancer (e.g., prostate cancer and colorectal cancer). The QLQ-C30 summary score appears to have more prognostic value than the global QoL, physical functioning, or any other scale within the EORTC QLQ-C30 (Husson et al., 2020).

Given the significant impacts of physical, mental, cognitive, and emotional sequelae on many BCS, it is essential to improve our understanding of how these sequelae affect individuals' QOL in the long-term along with changes in cardiometabolic health.

The "Movement and Health Beyond Care (MoviS)" is a randomized controlled trial (RCT) which aimed to evaluate the Short- and Long-Term effect of a LI characterized by a 12-week supervised aerobic exercise training program and MD guidance on i) QoL and ii) on cardiometabolic health in BCS.

Materials and Methods

Study design and participants

As previously described in Natalucci V. et al. 2023, the Movis Trial is a randomized controlled trial (RCT-ClinicalTrials.gov NCT04818359) conducted at the department of biomolecular sciences of the University of Urbino (Italy) in collaboration with the "Santa Maria della Misericordia" Hospital of Urbino (Italy). The main aims of the Movis Trial were to evaluate the effect of a 12-week supervised aerobic exercise training program on i) QoL (primary outcome) and on ii) cardiometabolic health parameters, including PA level and MD adherence, (secondary outcome) of BCS. Ethical approval was granted by the Human Research Ethics Committee of the University of Urbino (protocol N 21 of July 10, 2019). Written informed consent was obtained from all participants. In this study, 109 BCS women were enrolled (age 51.70±8.00 years [mean±SD]. Eligibility criteria were: histologically confirmed stage 0-III BC with no evidence of recurrent or progressive disease at recruitment; within 12 months after surgery and ≥6 months after radiotherapy and/or chemotherapy; with or without ongoing hormone therapy; age 30-70 years; non-physically active for at least 6 months (i.e., not engaged in at least 60 min/week of structured exercise during the previous 6 months). Participants also had to be at increased risk of recurrence, defined by at least one of the following conditions: body mass index (BMI) ≥ 25 kg/m^2; testosterone ≥ 0.4 ng/mL; serum insulin ≥ 25 µU/mL (170 pmol/L); or metabolic syndrome. Exclusion criteria included pneumological, cardiological, neurological, or orthopedic comorbidities, and mental illnesses that would preclude exercise practice. Recruitment was conducted between January 2020 and September 2023 at the Santa Maria della Misericordia Hospital of Urbino (Italy). General and medical characteristics of all participants were collected by the clinicians, while the cardio-metabolic health parameters (including PA level, and MD adherence) were assessed by the research staff at each time point.

Lifestyle intervention

As reported in Figure 1, this study involved two parallel groups (1:1 randomization ratio with the control arm). Randomization lists were generated using an Excel spreadsheet and the randomized block permutation method (n 4) was used to ensure balance between control group (CG) and intervention group (IG), and stratified according to the anthracycline treatment. Both groups received the same LI guidance on PA and MD according to the American College of Sport Medicine (ACSM) guidelines for cancer survivors (Riebe D. et al., 2018), to the World Cancer Research Fund (WCRF, 2018) recommendations, and to the nutritional and exercise guidelines for BC patients approved by the Italian Ministry of Health in 2017 and 2019. Only the IG underwent a 3-time-a-week aerobic training program for 12-week with progressive increases in exercise intensity (40-70% of heart rate reserve) and duration (20-60 minutes). Each aerobic exercise session performed by the IG was supervised by an exercise specialist: two times per week the training sessions were performed on site at the University of Urbino (each participant could walk or run on a treadmill or use a stationary bike) and one time per week the training session was performed in autonomy (indoors and outdoors, according to the participants' possibilities and preferences) followed the volume prescribed in the previous session by the exercise specialist. In addition, each IGs' participant was instructed to use a heart rate monitor (HR300, Kalenji) to comply with both exercise intensity and duration during all exercise training sessions.

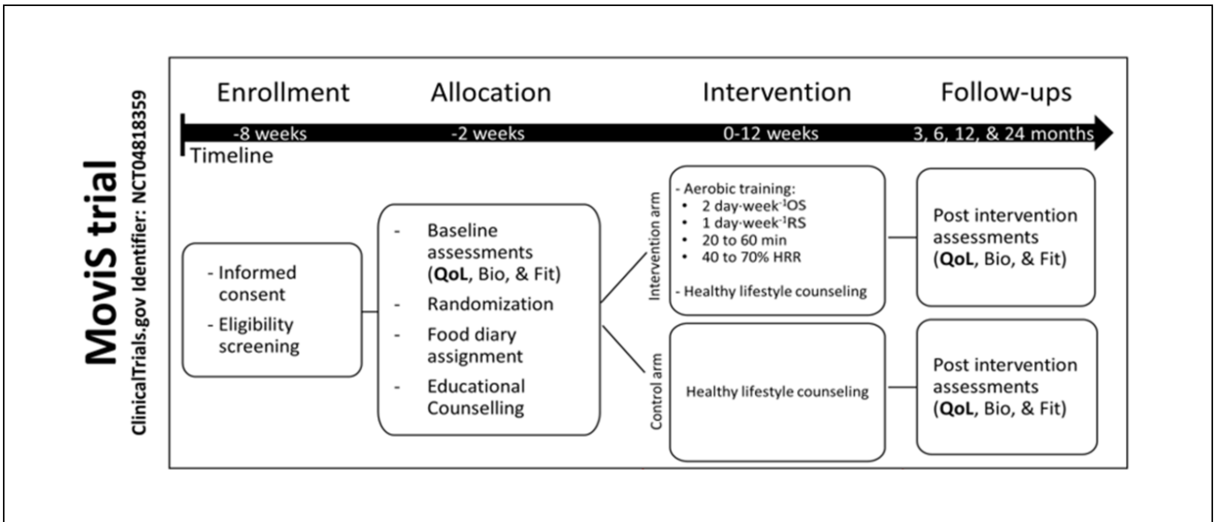

Figure 1. Experimental design of the Movis Trial. **Abbreviations:** QoL, Quality of Life; Bio, metabolic and cancer-related biomarkers; Fit, cardiorespiratory fitness; HRR, Heart Rate Reserve; RS, remotely supervised; OS, on-site supervised.

Quality of Life assessment

QoL was assessed using the "European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30" (EORTC QLQ-C30) version 3.0 (Aaronson et al., 1993)**.** The assessments were done at the baseline (T0), after the intervention period (T1), ad interim in the short term (6 months, T2), and in the long-term (12 and 24 months, T3 and T4 respectively). The EORTC QLQ-C30 is a cancer-specific questionnaire with 30 items organized into 15 scales. Specifically, it is composed of five functional scales (physical, role, cognitive, emotional, and social), nine symptom scales (fatigue, nausea and vomiting, pain), with six individual items (dyspnea, insomnia, appetite loss, constipation, diarrhoea, and financial difficulties), and a global health status/QoL scale. The questionnaire has a 1-week time frame, and all questions are answered on a 4-point Likert-type scale, except for the 2 items of the QoL scale that use a 7-point Likert-type scale (Aaronson et al., 1993). All scores were calculated using the EORTC QLQ-C30 scoring manual (3rd Edition) and were linearly transformed into a 0 to 100 score (Scoring Manual). For functional scales, higher scores indicate better functioning status; for symptoms, higher scores indicate a higher level of symptom burden (Aaronson et al., 1993). In addition, the QLQ-C30 Summary score (ranging from 0 to 100) was calculated according to a validated methodology (Husson et al., 2020): before calculating the mean score, symptoms scales were reversed to obtain uniform scores (e.g., 100 - fatigue) compared to the functional scales (higher scores indicate lower symptom burden). The QLQ-C30 Summary score was calculated as the mean of 13 scale and item scores (excluding global health status/QoL and financial difficulties), with a higher score indicating a better QoL (Hinz et al., 2012, Giesinger et al., 2016, Gundy et al., 2012). QLQ-C30 Summary score was only calculated when all of the required 13 scale and item scores were available (Husson et al., 2020).

The formula was as follows:

$(13)^{-1}$ Σ [physical functioning, role functioning, cognitive functioning, emotional functioning, cognitive functioning, (100 - fatigue), (100 - Pain), (100 - nausea and vomiting), (100 - dyspnoea), (100 - insomnia), (100 - appetite Loss), (100 - constipation), (100 - diarrhoea)]

Cardiometabolic health assessment

Cardiorespiratory fitness level

As described by Natalucci and Ferri Marini (Natalucci, Ferri Marini et al., 2023), $\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1}) was assessed, at each time point (T0, T1, T2, and T3), using an individualized submaximal incremental walking test performed on a treadmill (Riebe et al., 2018, Jones et al., 2008). The test comprehended multiple 3-min stages with incremental exercise intensities individualized according to the predicted $\dot{V}O_{2max}$ of each participant. The walking speed, which was individually chosen for each participant, was kept constant throughout the test. The treadmill grade was modified at each stage to induce an exercise intensity of the first stage at about 30% of the predicted oxygen uptake ($\dot{V}O_2$) reserve ($\dot{V}O_2R$), with about 10% $\dot{V}O_2R$ increase in exercise intensity for each stage. Exercise intensity was increased until participants reached 70% of Heart Rate Reserve (HRR) (Jones et al., 2008). The Heart Rate (HR) or $\dot{V}O_2$ values corresponding to the desired percentages of the reserve values (%$\dot{V}O_2R$ or %HRR) will be calculated as follows: (maximal value - resting value) x desired percentage + resting value. Resting $\dot{V}O_2$ was assumed to be 3.5 $mL \cdot min^{-1} \cdot kg^{-1}$ (Riebe D. et al., 2018), $\dot{V}O_{2max}$ was predicted by means of a non-exercise model (Ferri Marini et al., 2021), and $\dot{V}O_2$ was converted to treadmill speed and grade using the ACSM's walking equation (Riebe et al., 2018). Resting HR was measured after sitting for 10 min, using a heart rate monitor (HR300, Kalenji), while maximal HR (HRmax) was predicted as proposed by Gellish et al. (Gellish et al., 2007). Participants' HR was recorded at each stage and was used to create individual submaximal HR-$\dot{V}O_2$ relationship which was extrapolated to the predicted HRmax to estimate $\dot{V}O_{2max}$ (Jones et al., 2008, Riebe et al., 2018). $\dot{V}O_{2max}$ assessment at T4 time point will be done between November and December 2025 (as programmed in the Movis Trial).

Mediterranean diet adherence

Changes in dietary habits were assessed using the Mediet questionnaire: a 14-item MD adherence screener which was filled out by the participants at each time point (T0, T1, T2 and T3). Mediet score higher levels (8-9 or >10 points) indicate adherence to the MD (Natalucci, Ferri Marini et al., 2023, Gianfredi et al., 2020, Villarini et al., 2015). MD assessment at T4 time point will be done between November and December 2025 (as programmed in the Movis Trial).

Statistical analysis

Linear Mixed Models (LMMs) were used to analyse EORTC QLQ-C30 scales, QLQ-C30 Summary score, $\dot{V}O_{2max}$, and MD adherence to assess changes between groups over time. Each score (e.g., fatigue) was considered as a dependent variable, time, group, and time x group interaction were considered as fixed effects, and participants (i.e., MOVIS ID) were considered as random effects. All LMMs were adjusted for age (as continuous variable) and type of endocrine therapy (none=1, tamoxifen=2 or aromatase inhibitors=3). Model terms were tested with the Satterthwaite test Method. A compound symmetry covariance structure was assumed in all models. The CG was considered as a reference group; T0 was considered as reference time (time was considered a continuous variable). Analyses were conducted following the intention-to-treat principle, using all variables data without missing data imputation. All analyses were conducted using JASP software (v. 0.95.2.0).

Results

Subject characteristics

One hundred and nine (n=109) BCS (age 51.70±8.00 years [mean±SD]) were enrolled (baseline clinical characteristics are shown in Table 1). At baseline, no differences were found in demographic and basal clinical data between the two groups (CG, n=56; IG, n=53). All participants met the inclusion criteria. At 24-month post-intervention the following adverse events were observed: 5 (5.45%) relapses (1 between T0 and T1, 3 between T3 and T4, 1 after T4, and 11 (11.99%) dropouts (6 at T2, 3 at T1, and 2 at T3) due to work commitments, little time available and living in a different city to participate in all assessments over time. No cardiovascular incidents were observed over time. For these reasons, as reported in Table 2, some observations in each model were removed due to missing values (all data were analyzed considering the last time available for each participant with relapses, drop out or absence during follow-up assessments). For the statistical analyses 108 participants were considered (because of 1 relapse between T0 and T1) for QoL assessments, 101 for MD adherence and 94 for cardiorespiratory fitness level.

Global effect

Considering the global effect of LI (Table 3), the results showed that no main effect of time was observed in all EORTC QLQ-C30 scales excluding physical functioning (F=2.57, p=0.037) and financial difficulties (F=3.04, p=0.017), fatigue was barely significant (F=2.34, p=0.055). Moreover, the main effect of time was observed in $\dot{V}O_{2max}$ (F=8.22, p < 0.001) and in Mediet score (F=7.36, p < 0.001). No main effects of group were found in all outcomes considered (all *p-values* were > 0.05). Time x group interactions were found in QLQ-C30 Summary score (F=2.60, p = 0.036), emotional functioning (F=2.57, p=0.038), cognitive functioning (F=2.53, p=0.040), and in $\dot{V}O_{2max}$ (F=2.875, p=0.037). Fatigue and diarrhoea were barely significant (F=2.04, p=0.087; F=2.24, p=0.061). In addition, age was significant in two symptom scales: nausea and vomiting (F=6.20, p=0.014) and diarrhoea (F=4.53, p=0.036), while in the constipation scale it was barely significant (F=3.90, p=0.051). Endocrine therapy was significant in other two symptom scales: insomnia (F=3.23, p=0.043) and appetite loss (F=4.03, p=0.020), while in the financial difficulties scale it was barely significant (F=2.52, p=0.085).

Table 1. *Baseline characteristics of Control group (CG) and Intervention group (IG).*

	CG (n = 56) ***Means ± SD***	***IG (n = 53)*** ***Means ± SD***	***p-value***
Age	51.96 ± 8.11	51.41 ± 7.95	0.722
Stage at diagnosis	n(%)	n(%)	
0	9(16.07)	8(15.09)	
I	35(62.50)	31(58.49)	0.748
II	11(19.64)	11(20.75)	
III	1(1.79)	3(5.66)	
Menopausal status			
Premenopausal	23(41.07)	21 (39.62)	0.878
Postmenopausal	33(58.93)	32 (60.38)	
Surgery type			
Mastectomy	8(14.29)	6(11.32)	
Quadrantectomy	48(85.71)	47(88.68)	0.644
Lumpectomy	0(0)	0(0)	
Treatment in addition to surgery			
Only radiation	23(41.07)	19(35.85)	
Only chemotherapy	7(12.50)	4(7.55)	0.501
Radiation and chemotherapy	14(25.00)	20(37.74)	
None	12(21.43)	10(18.78)	
Current endocrine therapy			
None	17(30.36)	19(35.85)	
Tamoxifen	10(17.86)	8(15.09)	0.813
Aromatase Inhibitor	29(51.79)	26(49.06)	

Differences in frequency distributions were compared by chi-squared test.

Table 2. *Sample sizes of the LMMs.*

	n	**Observations (means ± SD)**	**Observations removed for missing values (means ± SD)**
EORTC QLQ-C30 and QLQ–C30 Summary score	108	457.13 ± 4.86	36.50 ± 1.03
	n	**Observations**	**Observations removed for missing values**
$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	94	354	22
Mediet score	101	364	40

Abbreviations: EORTC QLQ-C30, European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30; $\dot{V}O_{2max}$, maximum oxygen uptake.

Table 3. *ANOVA Summary*

Terms	**Age**	**Endocrine Therapy**	**Time**	**Group**	**Time x Group**
Global Health status/QoL	0.481	0.597	0.724	0.847	0.482
Functional Scales					
Physical Functioning	0.151	0.352	**0.037***	0.470	0.109
Role Functioning	0.123	0.710	0.604	0.867	0.091
Emotional Functioning	0.786	0.207	0.851	0.971	**0.038***
Cognitive Functioning	0.999	0.409	0.929	0.987	**0.040***
Social Functioning	0.170	0.382	0.235	0.655	0.182
Symptom Scales					
Fatigue	0.642	0.673	0.055	0.526	0.087
Nausea and Vomiting	**0.014***	0.908	0.156	0.300	0.916
Pain	0.740	0.607	0.711	0.625	0.875
Dyspnea	0.493	0.644	0.167	0.193	0.435
Insomnia	0.654	**0.043* (2)**	0.186	0.422	0.815
Appetite Loss	0.417	**0.020*(2)**	0.436	0.520	0.781
Constipation	0.051	0.993	0.414	0.850	0.443
Diarrhoea	**0.036***	0.289	0.536	0.178	0.061
Financial Difficulties	0.687	0.085	**0.017***	0.386	0.710
Summary Score	0.547	0.481	0.116	0.710	**0.036***
$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	0.547	0.600	**< 0.001*****	0.301	**0.037***
Mediet score	0.678	0.360	**< 0.001*****	0.270	0.909

Abbreviations: EORTC QLQ-C30, European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30; $\dot{V}O_{2max}$, maximum oxygen uptake. Endocrine Therapy (2) = Tamoxifen. *p<0.05; **p<0.01; ***p<0.001.

Fixed Effects Estimates

As reported in Table 4 the effect of time shows that physical functioning was barely significantly higher at T3 compared to T0 (β=2.314,P=0.055) in both groups, financial difficulties were barely significantly lower at T1 compared to T0 (β= - 3.133, p=0.050) in both groups, and $\dot{V}O_{2max}$ was barely significantly higher at T2 compared to T0 (β=1.071, p=0.061) in both groups. The Mediet score was significantly higher at T1 (β=0.679, p=0.010) and at T3 (β=0.622, p=0.023) compared to T0 in both groups.

Time x group interaction was found in the physical functioning scale (β=3.620, p=0.024), in the social functioning scale (β=6.809, p=0.033), and in the QLQ-C30 Summary score (β=2.948, P=0.048). These three scores increased significantly at T1 in the IG compared to the CG, however they tend to baseline values at T2, T3, and T4 with no differences between groups. In addition, time x group interaction was found in the emotional functioning scale at T4: this score decreased significantly in IG compared to CG (β= - 8.210, p=0.045). The same trend (but only barely significant) was found in the cognitive functioning scale: the IG decreased it compared to CG at T4 (β= - 6.648, p=0.054). Time x group interaction was found also in cardiorespiratory fitness level (β=2.104, p=0.007): $\dot{V}O_{2max}$ increased significantly only at T1 in the IG compared to the CG. The effects of age and endocrine therapy were reported in detail in Table 4 caption. Specifically, for each age increase equal to 1-year participants experienced significantly less nausea and vomiting (β= - 0.149, p=0.014), and diarrhoea symptoms (β= - 0.228, p=0.036). In addition, participants who were treated with Tamoxifen experienced significantly more insomnia (β=12.35, p=0.026) and appetite loss symptoms (β=4.852, p=0.009) compared to BCS with no endocrine therapy.

Estimated Marginal Means

In Table 5 all Estimated Marginal Means (EMM) were reported with standard errors, 95% confidence interval, and *p-value* to compare means changes over time (from T0 to T4) and between groups differences (CG vs IG).

Table 4. *Fixed Effects Estimates.*

Time					
Terms	**β**	**SE**	**df**	**t**	***p-value***
Physical Functioning (T3)	2.314	1.203	349.3	1.924	0.055
Financial Difficulties (T1)	-3.133	1.594	346.4	-1.695	0.050
Summary score (T4)	2.541	1.220	362.3	2.082	**0.038***
$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1}) (T2)	1.071	0.569	259.6	1.882	0.061
Mediet score (T1)	0.679	0.262	262.8	2.59	**0.010***
Mediet score (T3)	0.622	0.271	268.4	2.29	**0.023***
Time x group interaction					
Terms	**β**	**SE**	**df**	**t**	***p*-value**
Global Health status/QoL	1.633	3.41	342.9	0.48	0.633
Functional Scales					
Physical Functioning	3.620	1.59	337.8	2.264	**0.024***
Role Functioning	2.969	3.421	336.3	0.868	0.386
Emotional Functioning	-8.210	4.076	348.4	-2.014	**0.045*** †
Cognitive Functioning	-6.648	3.433	345.7	-1.937	0.054†
Social Functioning	6.809	3.179	349.0	2.142	**0.033***
Symptom Scales					
Fatigue	-5.594	3.549	342.8	-1.576	0.116
Nausea and Vomiting	1.064	1.469	342.5	0.725	0.469
Pain	1.854	3.931	343.7	0.474	0.636
Dyspnea	-6.079	3.384	339.1	-1.796	0.073
Insomnia	-4.069	5.001	345.1	-0.814	0.416
Appetite Loss	-0.79	0.246	349.4	-0.352	0.725
Constipation	-5.904	4.036	345.4	-1.463	0.144
Diarrhoea	3.541	2.363	333.5	1.498	0.135
Financial Difficulties	-0.066	2.235	342.5	-0.030	0.976
Summary Score	2.948	1.48	336.5	1.985	**0.048***
$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1})	2.104	0.779	254.1	2.702	**0.007****
Mediet score	0.152	0.372	261.3	0.409	0.683

Abbreviations: β, estimates; SE, standard error; df, degrees of freedom; t, t-value; p, p-value; $\dot{V}O_{2max}$, maximum oxygen uptake. *p<0.05; **p<0.01; ***p<0.001.

All *p-value* reported in the "time x group interaction" section represented group (IG) x time (T1) interaction; †*p-value* represented group (IG) x time (T4) interaction. Other interactions (IG x T2 and IG x T3) were not significant (p > 0.05). Significant *p-values* were in bold. The CG was considered as a reference group; T0 was considered as reference time. Effect of age: a) Nausea and Vomiting, β= - 0.149, SE=0.060, df=100.9, t= -2.491, p=0.014 and b) Diarrhoea, β= - 0.228, SE=0.107, df=94.38, t = -2.129, p=0.036. Effect of Tamoxifen: a) Insomnia, β= 12.35, SE= 5.493, df=120.2, t=2.249, p=0.26 and b) Appetite loss, β=4.852, SE=1.817, df=110.2, t=2.671, p=0.009.

Table 5. *Estimated Marginal Means.*

	Control group (CG) *β (SE)* *[95% CI]*					Intervention group (IG) *β (SE)* *[95% CI]*					Time x group
Terms	**T0**	**T1**	**T2**	**T3**	**T4**	**T0**	**T1**	**T2**	**T3**	**T4**	
Global Health status/ QoL	71.63(2.3) [67.01, 76.25]	72.26(2.4) [67.48, 77.05]	73.83(2.5) [68.87, 78.79]	74.32(2.5) [69.32, 79.31]	76.57(2.7) [71.14, 82.01]	73.84(2.4) [69.12, 78.55]	76.10(2.4) [71.36, 80.85]	73.37(2.4) [68.54, 78.20]	73.46(2.4) [68.62, 78.31]	74.33(2.6) [69.10, 79.57]	0.633
Functional Scales											
Physical	89.64(1.5) [86.69, 92.59]	89.66(1.5) [86.66, 92.67]	90.67(1.5) [87.61, 93.74]	91.95(1.5) [88.87, 95.03]	91.95(1.6) [88.70, 95.19]	90.02(1.5) [87.01, 93.04]	93.67(1.5) [90.65, 96.69]	92.52(1.5) [89.47, 95.57]	92.65(1.5) [89.60, 95.70]	91.77(1.6) [88.56, 94.98]	**0.024***
Role	89.22(2.3) [84.67, 93.76]	87.51(2.4) [82.79, 92.22]	91.32(2.5) [86.43, 96.22]	91.84(2.5) [86.91, 96.77]	93.18(2.7) [87.81, 98.55]	91.35(2.3) [86.67, 96.03]	92.61(2.3) [87.94, 97.29]	92.72(2.4) [87.96, 97.48]	87.64(2.4) [82.86, 92.41]	90.85(2.6) [85.68, 96.02]	0.386
Emotion-al	76.00(2.5) [71.01, 80.98]	75.10(2.6) [69.94, 80.26]	78.69(2.7) [73.35, 84.04]	78.12(2.7) [72.74, 83.51]	82.14(2.9) [76.30, 87.99]	77.58(2.6) [72.48, 82.67]	81.20(2.6) [76.08, 86.33]	77.13(2.6) [71.92, 82.34]	79.15(2.6) [73.92, 84.37]	75.51(2.8) [69.88, 81.15]	**0.045*** †
Cogniti-ve	78.49(2.2) [74.15, 82.84]	77.91(2.2) [73.42, 82.40]	80.99(2.3) [76.36, 85.63]	80.42(2.3) [75.75, 85.09]	83.26(2.5) [78.21, 88.30]	80.49(2.2) [76.05, 84.93]	83.17(2.2) [78.71, 87.63]	78.87(2.3) [74.34, 83.41]	80.12(2.3) [75.58, 84.67]	78.61(2.4) [73.73, 83.49]	0.054 †

Social	94.18(1.8) [90.64, 97.72]	92.61(1.9) [88.88, 96.33]	95.72(1.9) [91.81, 99.62]	94.32(2.0) [90.38, 98.26]	98.37(2.2) [93.97, 102.78]	92.73(1.8) [89.12, 96.35]	97.97(1.8) [94.32, 101.62]	96.98(1.9) [93.24, 100.73]	95.18(1.9) [91.41, 98.94]	96.06(2.1) [91.88, 100.24]	**0.033***
Symptom Scales											
Fatigue	24.63(2.6) [19.50, 29.77]	23.91(2.7) [18.61, 29.20]	19.11(2.7) [13.64, 24.58]	19.12(2.8) [13.62, 24.63]	18.59(3.0) [12.66, 24.53]	22.28(2.6) [17.02, 27.53]	15.95(2.6) [10.68, 21.22]	18.07(2.7) [12.71, 23.42]	21.37(2.7) [16.00, 26.73]	18.26(2.9) [12.45, 24.06]	0.116
Nausea and Vomiting	3.09(0.91) [1.309, 4.890]	0.78(0.95) [-1.07, 2.65]	2.31(0.99) [0.36, 4.255]	2.22(0.99) [0.26, 4.18]	2.17(1.10) [0.02, 4.33]	1.47(0.92) [-0.35, 3.29]	0.23(0.93) [-1.60, 2.06]	0.95(0.95) [-0.92, 2.82]	1.82(0.96) [-0.05, 3.71]	1.32(1.05) [-0.74, 3.38]	0.469
Pain	18.67(2.9) [12.98, 24.36]	15.74(3.0) [9.83, 21.64]	13.90(3.0) [7.85, 19.96]	15.85(3.1) [9.75, 21.94]	15.99(3.3) [7.85, 19.96]	17.94(2.9) [12.12, 23.76]	16.88(2.9) [11.04, 22.72]	17.17(3.0) [11.23, 23.10]	18.63(3.0) [12.68, 24.57]	17.66(3.2) [11.29, 24.02]	0.636
Dyspnea	8.78(2.20) [4.47, 13.10]	9.21(2.28) [4.73, 13.68]	5.78(2.37) [1.13, 10.43]	9.03(2.39) [4.34, 13.72]	7.98(2.62) [2.84, 13.11]	7.82(2.24) [3.43, 12.22]	2.17(2.25) [-2.25, 6.59]	3.45(2.30) [-1.05, 7.96]	7.28(2.31) [2.74, 11.80]	4.96(2.51) [0.03, 9.89]	0.073
Insomnia	20.31(3.5) [13.37, 27.24]	18.04(3.6) [10.88, 25.19]	21.69(3.7) [14.29, 29.10]	21.32(3.8) [13.87, 28.77]	21.29(4.1) [13.21, 29.37]	24.73(3.6) [17.66, 31.80]	18.39(3.6) [11.29, 25.49]	23.10(3.6) [15.88, 30.33]	27.45(3.6) [20.20, 34.69]	24.75(3.9) [16.95, 32.55]	0.416
Appetite Loss	3.76(1.32) [1.16, 6.36]	4.53(1.38) [1.82, 7.24]	4.18(1.44) [1.34, 7.02]	2.59(1.46) [-0.26, 5.45]	2.02(1.62) [-1.15, 5.20]	3.24(1.34) [0.60, 5.88]	3.22(1.35) [0.55, 5.88]	2.10(1.39) [-0.62, 4.83]	3.53(1.40) [0.79, 6.27]	0.91(1.54) [-2.11, 3.94]	0.725

Constipa-tion	9.69(2.88) [4.04, 15.35]	12.85(2.9) [7.02, 18.68]	11.77(3.0) [5.74, 17.81]	8.60(3.09) [2.53, 14.67]	7.99(3.35) [1.42, 14.56]	13.54(2.9) [7.77, 19.31]	10.79(2.9) [5.00, 16.59]	10.26(3.0) [4.37, 16.15]	12.17(3.01) [6.26, 18.08]	7.19(3.24) [0.84, 13.54]	0.144
Diar-rhoea	4.72(1.56) [1.65, 7.78]	2.23(1.62) [-0.94, 5.41]	7.11(1.68) [3.81, 10.42]	3.68(1.69) [0.36, 7.01]	3.64(1.85) [0.00, 7.27]	1.41(1.59) [-1.70, 4.53]	2.47(1.60) [-0.67, 5.61]	1.09(1.63) [-2.11, 4.29]	3.98(1.64) [0.77, 7.20]	1.18(1.78) [-2.30, 4.67]	0.135
Financial Difficulties	3.64(1.25) [1.18, 6.10]	0.51(1.31) [-2.06, 3.09]	1.30(1.38) [-1.40, 4.01]	2.75(1.39) [0.02, 5.48]	0.69(1.55) [-2.35, 3.75]	5.66(1.27) [3.16, 8.16]	2.46(1.28) [-0.06, 4.98]	1.33(1.32) [-1.26, 3.92]	2.10(1.33) [-0.50, 4.70]	2.29(1.47) [-0.60, 5.19]	0.976
Summa-ry Score	87.12(1.2) [84.67,89.57]	87.13(1.2) [84.61,89.65]	88.37(1.3) [85.80,90.94]	88.60(1.3) [86.02,91.19]	89.66(1.4) [86.91,92.41]	87.58(1.2) [85.06,90.10]	90.54(1.2) [88.03,93.05]	89.21(1.3) [86.67,91.75]	87.42(1.3) [84.88,89.96]	88.94(1.4) [86.21,91.66]	**0.048***
$\dot{V}O_{2max}$	30.26(0.8) [28.62, 31.90]	30.80(0.8) [29.15, 32.44]	31.34(0.8) [29.68, 32.99]	30.93(0.8) [29.29, 32.57]	--	30.37(0.8) [28.71, 32.02]	33.00(0.8) [31.36, 34.64]	32.81(0.8) [31.15, 34.47]	31.49(0.8) [29.84, 33.13]	--	**0.007****
Mediet score	6.80(0.27) [6.26, 7.34]	7.48(0.27) [6.93, 8.02]	7.32(0.29) [6.73, 7.90]	7.42(0.28) [6.86, 7.98]	--	7.01(0.27) [6.46, 7.56]	7.84(0.28) [7.28, 8.39]	7.63(0.28) [7.06, 8.20]	7.90(0.27) [7.35, 8.45]	--	0.683

Abbreviations: β, estimates; SE, standard error; 95% CI, 95% confidence interval; $\dot{V}O_{2max}$, maximum oxygen uptake. All *p-value* represented group (IG) x time (T1) interaction; †*p-value* represented group (IG) x time (T4) interaction. Other interactions (IG x T2 and IG x T3) were not significant (p>0.05). Significant *p-values* were in bold. The CG was considered as a reference group; T0 was considered as reference time. *p<0.05; **p<0.01; ***p<0.001.

According to LMMs results, the plots show the mean changes over time for CG and IG for $\dot{V}O_{2max}$ and MD adherence (Figures 1A and 1B), and for physical functioning, social functioning, QLQ-C30 Summary Score, fatigue scale, emotional functioning (Figures 2A, 2B, 2C, 2D, and 2E). Specifically, mean and 95% confidence interval (95% CI) are reported in each figure.

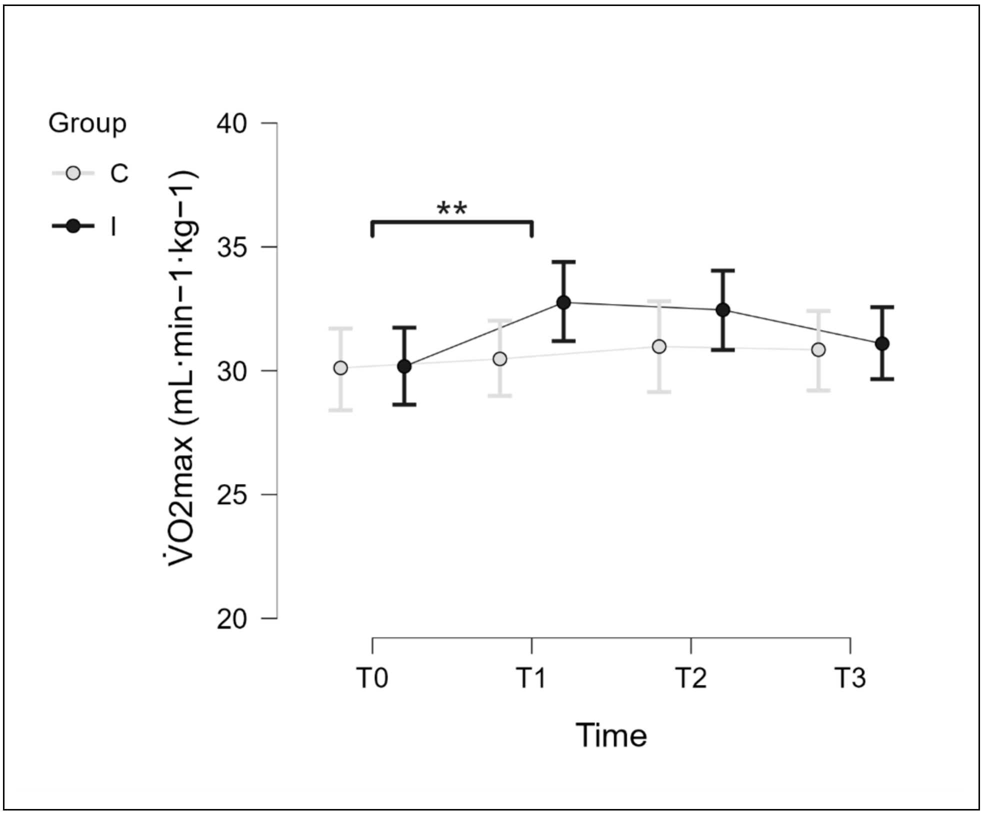

Figure 1A. Cardiorespiratory fitness level across time. **Abbreviations:** C, Control group; I, intervention group, $\dot{V}O_{2max}$, maximum oxygen uptake. Time (T1) x group (I) interaction was represented. β=2.104; SE=0.779; df=254.1; t=2.702; **p=0.007.

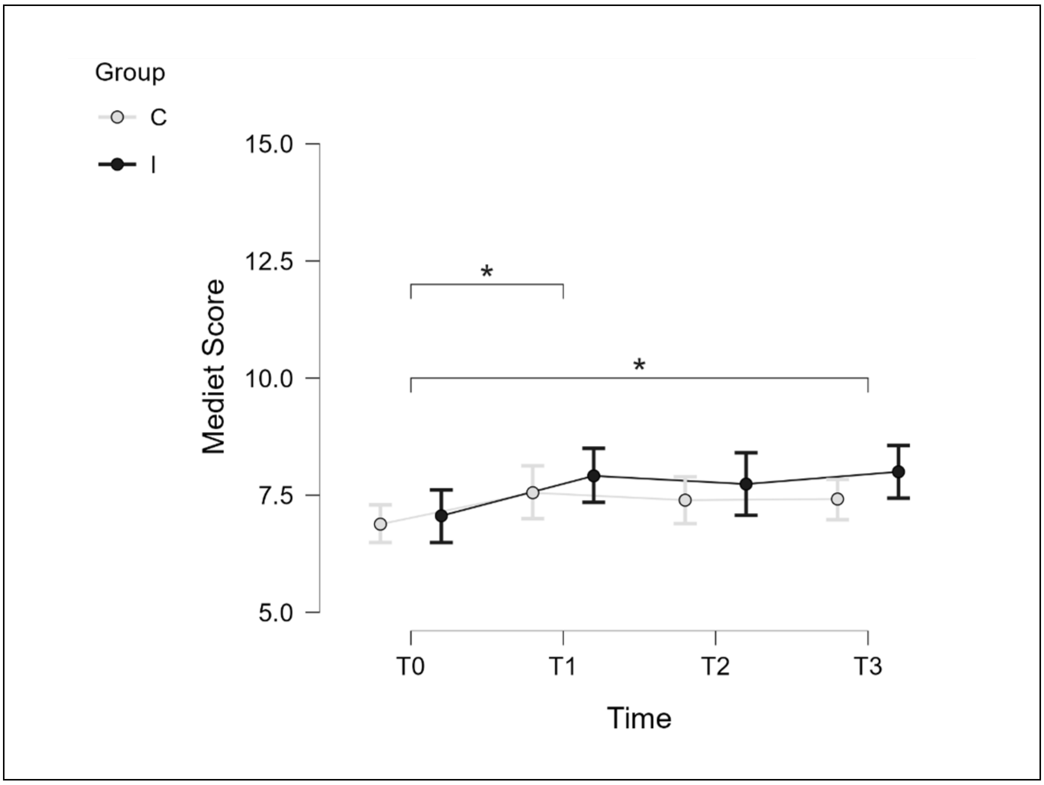

Figure 1B. Mediterranean diet adherence across time**. Abbreviations:** C, Control group; I, intervention group. The effect of time was represented. T1, β=0.679; SE=0.262; df=262.8; t=2.59; p=0.010*; T3, β=0.622; SE=0.271; df=268.4; t=2.29; *p=0.023*.

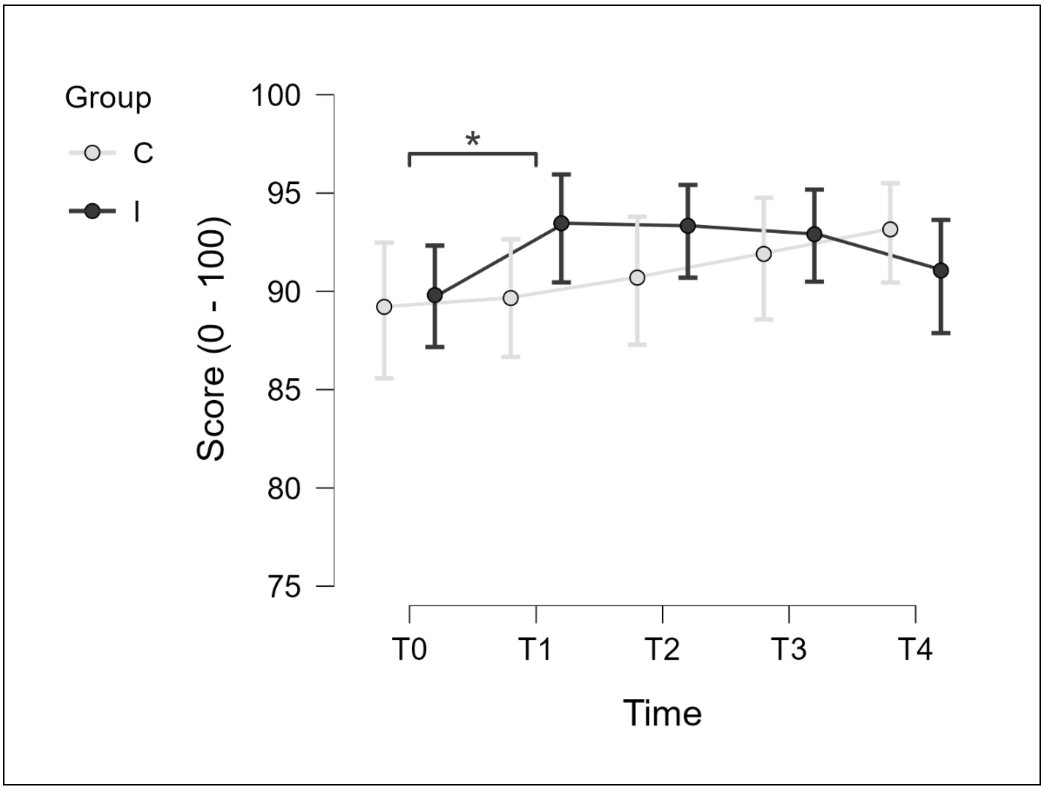

Figure 2A. Physical functioning across time. **Abbreviations:** C, Control group; I, intervention group. Time (T1) x group (I) interaction was represented. β=3.620; SE=1.59; df=337.8; t=2.264; *p=0.024.

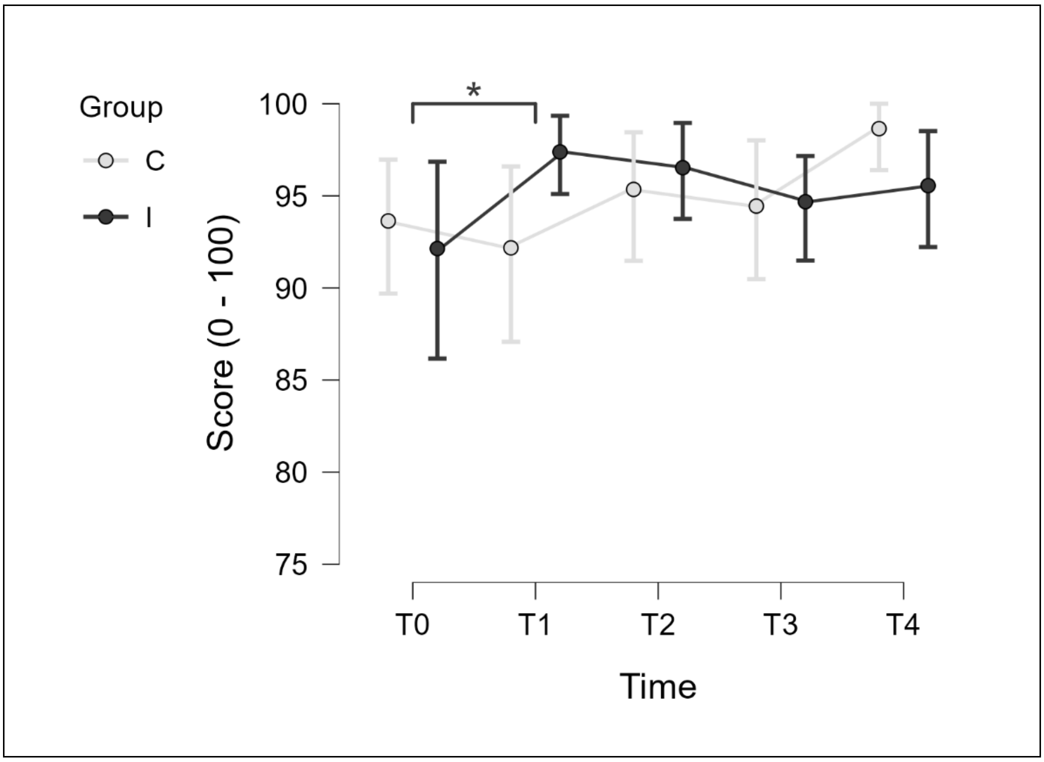

Figure 2B. Social functioning across time. **Abbreviations:** C, Control group; I, intervention group. Time (T1) x group (I) interaction was represented. β=6.809; SE=3.179; df=349.0; t=2.142; *p=0.033.

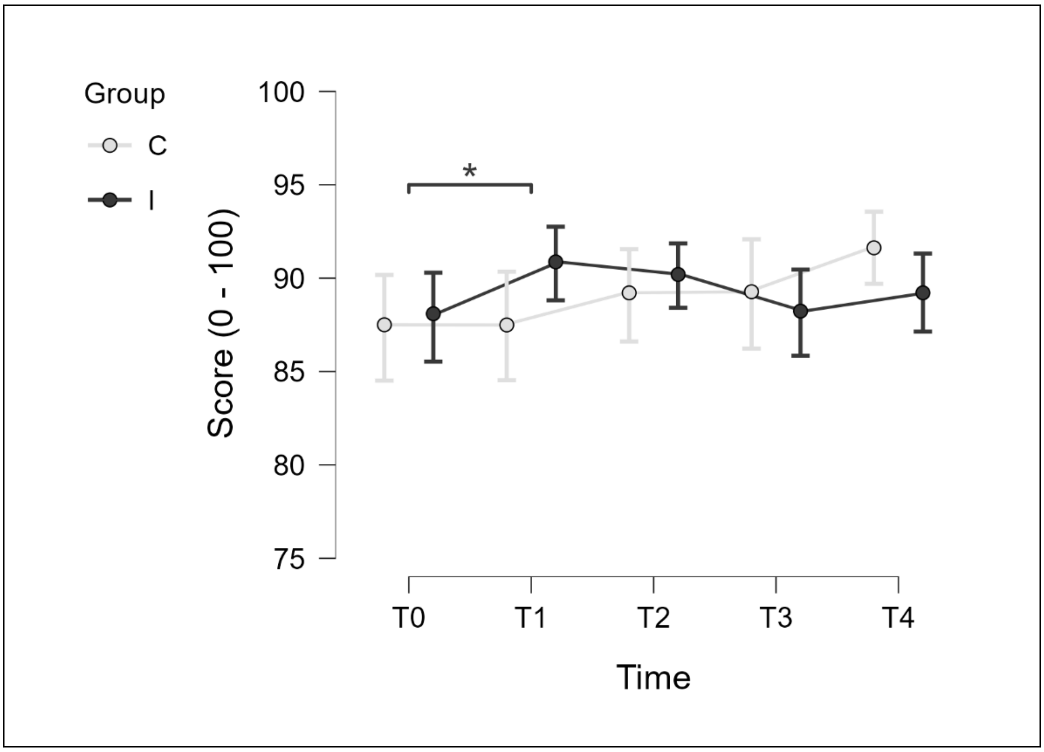

Figure 2C. Summary Score across time. **Abbreviations:** C, Control group; I, intervention group. Time (T1) x group (I) interaction was represented. β=2.948; SE=1.48; df=336.5; t=1.985; *p=0.048.

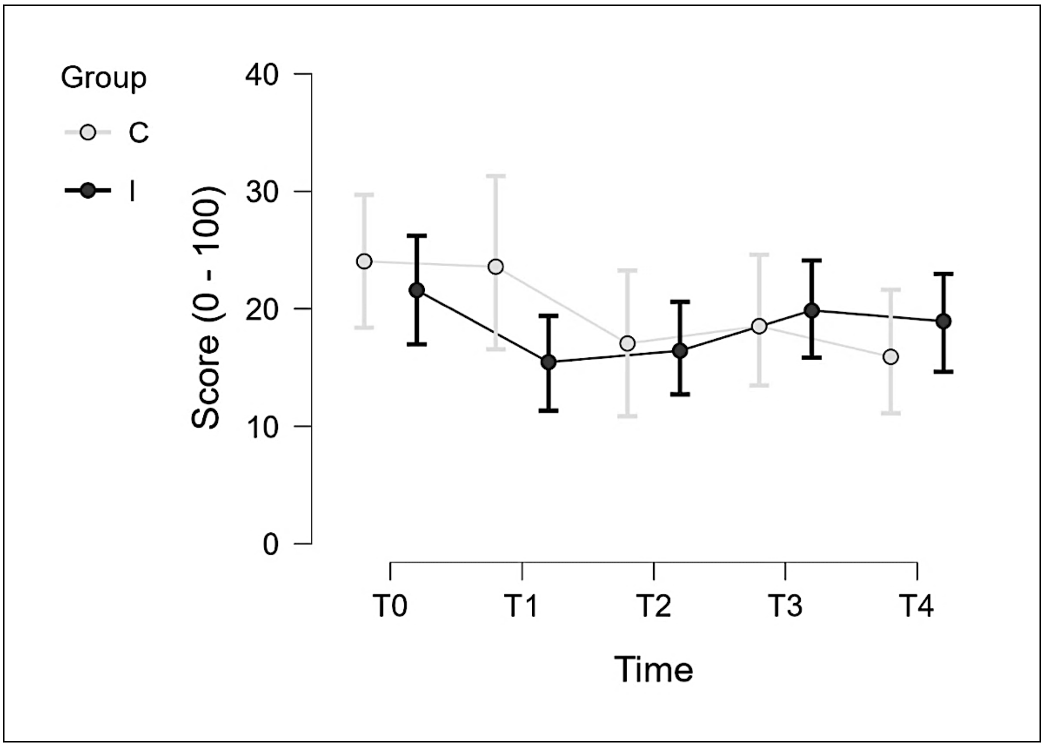

Figure 2D. Fatigue across time. **Abbreviations:** C, Control group; I, intervention group.

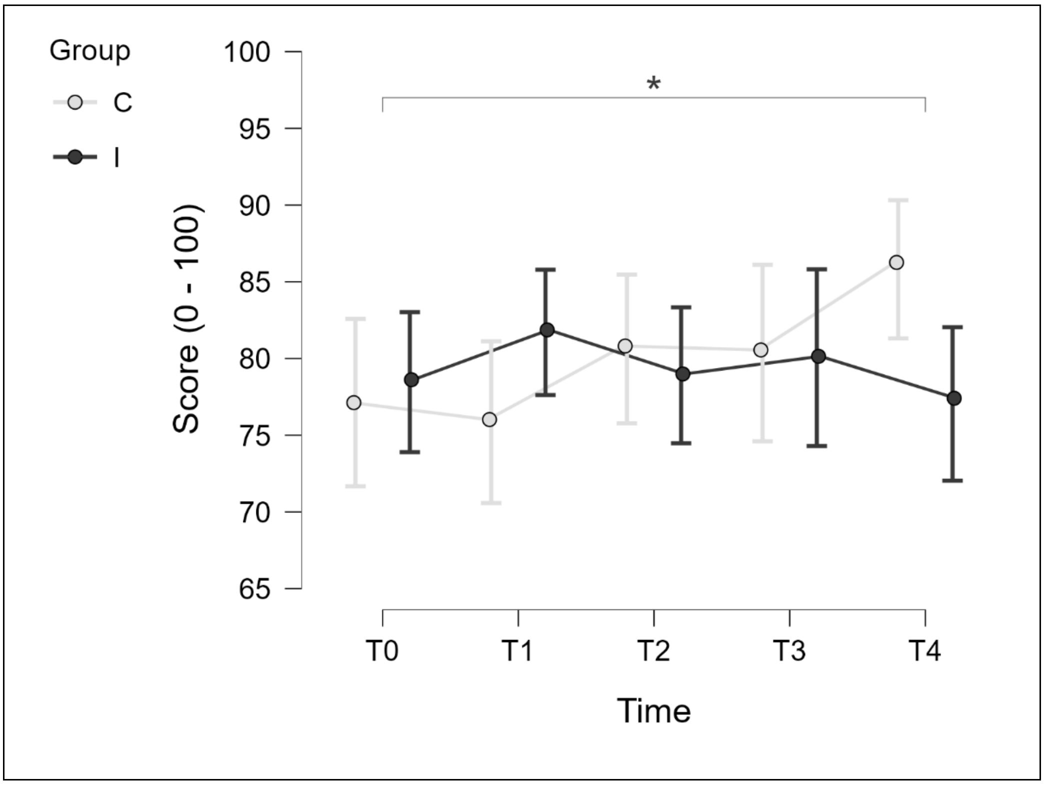

Figure 2E. Emotional functioning across time. **Abbreviations:** C, Control group; I, intervention group. Time (T4) x group (C) was represented. β= -8.210; SE=4.076; df=348.4; t= -2.014; *p=0.045.

Discussion

Considering the importance of QoL and cardiometabolic health assessment in the long-term period in patients with previous cancer diagnosis, this study evaluated the effect of a LI characterized by supervised aerobic exercise and MD guidance on specific functional and symptoms scales, QLQ-C30 Summary score, and cardiometabolic health in BCS.

The global effect (Table 3) of LI was observed in QLQ-C30 Summary score and $\dot{V}O_{2max}$, highlighting that IG improved significantly compared to the CG due to the intervention received. Despite time x group interaction being barely significant on the fatigue scale, only the IG showed a reduction after the intervention, achieving the minimal clinically important difference (MCID) decrease of 5 points (Touraine et al., 2025, Osoba et al., 1998). Fixed effect estimates (Table 4) confirmed that LI improved QLQ-C30 Summary score and $\dot{V}O_{2max}$ only at T1 and showed that an improvement was reached also in physical functioning and social functioning after the LI, but not in the other time points evaluated. Probably, this could be the reason why the increment in these two specific outcomes was not caught by the global effect as a strong difference. This highlights the value of supervised exercise programs for short-term enhancements in QoL and cardiorespiratory fitness level.

In addition, both Table 4 and 5 showed that IG tends to maintain the improvements reached in physical functioning during the intervention until T3 (barely significant trend); however, this outcome tends to decrease in the long-term period (T4). The same trend was observed for cardiorespiratory fitness: $\dot{V}O_{2max}$ increased significantly at T1 only in the IG due to aerobic exercise training, and was barely significantly higher at T2 compared to T0, showing an IG trend in maintaining the beneficial effects reached during the LI. However, it tends to decrease at T3 with no statistical differences compared to T0. The CG exhibited slightly improved physical functioning and cardiorespiratory fitness at T3 and T2, respectively, but not to the same extent as the IG after the LI, showing that only the PA guidelines were not sufficient to achieve significant improvement both in the short- and long-term. Interestingly, the Global Health status/HRQoL did not change over time in both groups compared to QLQ-C30 Summary score. This results was in line with previous studies (Hinz et al., 2012, Gundy et al., 2012, Giesinger et al., 2016, and Husson et al., 2020) which explain that the Summary score could be a good indicator of overall QoL because of the average of both functional and symptoms scale (13 scales, in total) was calculated compared to the only two items included in the Global Health status/HRQoL scale, which may not be particularly well suited, if was considered alone, for detecting changes between patient groups and/or changes over time. However, various methodologies were presented in literature for Summary score calculation (e.g., Hinz et al., 2012 and Nordin et al., 2001), in this study it was calculated according to a validated methodology (Husson et al., 2020). Summary score seems to be better than Global Health status/HRQoL scale only (Giesinger et al., 2016).

The MD adherence increased significantly at T1 and at T3 compared to T0 in both groups. However, neither of the two groups achieved the minimum score (equal to 8 points) in any of the times evaluated, indicating that diet guidance alone was not sufficient to reach MD adherence. Emotional and cognitive functioning decreased at T4 in the IG suggesting a need for focus on mental health in the long-term. The emotional and cognitive side may have been influenced by various personal factors; consequently, it is not possible to directly associate them with the intervention that occurred 2 years earlier. However, long-term monitoring of these two aspects could be interesting to emphasize the possible long-term needs of BCS. In addition, his study confirmed the importance of age and type of endocrine therapy as factors which can influence symptoms such as nausea and vomiting, diarrhoea, insomnia and appetite loss in BCS. In the end, financial difficulties decreased at T1 in both groups, pointing out another interesting factor: the impact of LI on overall QoL as an outcome, which can be affected by various elements, including economic status.

Strengths and limitations

The main strength of this work was the importance of timeliness in assessments: the long-term follow-up until 24 months. In addition, both QoL (considering all 15 items and the QLQ-C30 Summary score) and cardiometabolic health (up to 12 months) were included. Furthermore, the long-term follow-up data will provide important information to optimize a personalized lifestyle intervention characterized by PA and MD diet guidance.

Some interesting interactions were found, including the type of endocrine therapy in the analyses: Tamoxifen had a significant influence on two important symptoms, such as Insomnia and Appetite loss. However, this study had some limitations. The first is the small sample size; it would be interesting to evaluate the same results in larger and prospective trials with patients enrolled from multiple centers. The second is that cardiometabolic health was reported until 12 months (and not until 24 months), making a direct comparison between QoL, diet, and cardiorespiratory fitness level difficult even at T4. In the end, all outcomes were analysed using LMMs; multiple testing may have increased type I error.

Conclusions

This study confirmed the significant impact of LI, particularly in the short-term. The take-home message of this work in the scientific community is: emphasizes the importance of not only conducting follow-ups for individuals with a history of BC to prevent relapses, but also providing educational meetings and programs that promote PA and adherence to the MD. These efforts aim to improve QoL (encompassing mental, physical, and socio-economic well-being) as well as cardiometabolic health both in the short term and the long term.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STUDY 2

Original Article

Effect of a 12-week lifestyle intervention program on free-living glycemic profile and cardiometabolic health in breast cancer survivors: the Sweet Movis study

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Abstract

Endocrine therapies and sedentary behavior increase the risk of type 2 diabetes (T2D) in breast cancer survivors (BCS). Lifestyle Intervention (LI), combining physical activity (PA) and Mediterranean diet (MD), may be an effective adjunctive treatment for BCS, but the impact of

PA and specifically of tailored aerobic exercise on glucose homeostasis remains inconclusive. This study aimed to evaluate the effects of a LI characterized by a 12-week supervised aerobic exercise training program plus MD guidance on non-fasting metrics of glycemic control in BCS participating in the MoviS clinical trial (clinicaltrials.gov identifier: NCT04818359). Twenty-seven sedentary, non-diabetic BCS women (age 52.3±8.6 years) were randomized to control group (CNT, n=12) or intervention group (INT, n=15). All participants received PA and MD recommendations before trial initiation; INT also completed supervised aerobic training 3-times-a-week for 12 weeks with progressive intensity (40-70% of heart-rate reserve) and duration (20-60 minutes). Cardiometabolic health (body weight, BMI, waist circumference, % of fat mass, $\dot{V}O_{2max}$, glucose, insulin, HOMA-IR, triglycerides, total/LDL/HDL cholesterol) and PA (MET-min/week) were assessed at baseline (T0) and post-intervention (T1). Daily glucose level and food intake (Kilocalories [Kcal] and macronutrients) were assessed in the first and last 14 days (12±1.8 days) of study intervention using Continuous glucose monitors (CGMs) and food diaries, respectively. Variability indices (e.g., MAGE, MODD, time in range [TIR]) were computed with EasyGV software (v.9.0.R2). Repeated-measures GLMs tested time (T0 vs T1), group (CNT vs INT), and interactions. Linear mixed models (LMMs) assessed day-to-day intake and CGM outcomes (α=0.05; SPSS v17).

At T1, significant time effects occurred for fat mass (CNT: -2.2%; INT: -3.8%, p=0.015), triglycerides (CNT: -4.2%; INT: -15.9%, p=0.020), insulin (CNT: -14.1%; INT: -15.6%, p=0.006), HOMA-IR index (CNT: -13.7%; INT: -16.3%, p=0.009), and Met-min/week (CNT: +90.8 %; INT: +64.4 %, p=0.005). The main effect of the group was found for fiber intake: INT had higher fiber intake compared to CNT (p=0.011). Significant time x group interaction was found in $\dot{V}O_{2max}$, showing a higher increase in INT group (CNT: +5.3%; INT: +16.4%, p=0.020). Among CGM metrics, the inter-day glycemic variability index MODD increased in CNT at T1 (CNT: +12.5%; INT: -3.6%, p=0.017). Unexpectedly, TIR within 70-140 and 70-160 mg/dL decreased at T1 in both groups (p=0.036 and p=0.038). Mean energy and macronutrient intake did not change at T1; however, day-to-day variation was observed for Kcal (F=5.478, p=0.02), carbohydrates (F =4.261, p=0.039), and oligosaccharides (F=9.061, p=0.003). Accordingly, TIR (range 70-120 mg/dL) also varied by day (F=4.036, p=0.045). Notably, oligosaccharide intake was higher on exercise days, and exercise days were associated with more favorable CGM indices than non-exercise days. Despite cardiometabolic improvements after LI, CGM-derived variability indices did not improve: inter-day variability (MODD) worsened in CNT, and TIR declined in both

groups. Daily-level analyses revealed higher oligosaccharide intake and better glycemic profiles on exercise days, whereas non-exercise days were characterized by poorer CGM metrics, especially in CNT. These findings support combining CGM with dietary logs to characterize day-type patterns (exercise vs non-exercise) and to refine the design and delivery of LI in BCS. Further studies are needed to determine whether structured training, targeted dietary strategies (e.g., carbohydrate quality/timing), and exercise timing can improve TIR and glycemic variability beyond fasting indices, and to clarify implications for T2D prevention.

Key words: aerobic exercise, breast cancer survivors, glycemic homeostasis, endocrine therapies.

Introduction

Breast Cancer (BC) is the most prevalent cancer among women worldwide, representing one of the chronic diseases with the highest psychological and socio-economic impact. Considering the top five cancer cases and deaths in 2022 (Bray et al., 2024), female BC accounted for 23.8% of 9.7 million new cases and 15.4% of 4.3 million deaths worldwide.

At diagnosis, impaired fasting glucose or previously undiagnosed type-2 diabetes (T2D) are common among women with BC. Approximately 10-20% of postmenopausal women with BC have coexisting T2D, irrespective of stage or receptor status (Eketunde, 2020). Poor glycemic control in those with T2D is associated with higher risks of BC recurrence and mortality (Lu et al., 2014; Stocks et al., 2009). The relationship between BC and T2D has therefore emerged as a distinct area of investigation, with growing interest in the bidirectional mechanisms that may exacerbate both diseases (Thomas et al., 2024; Lu et al., 2014; Natalicchio et al., 2024). Endocrine therapy is the cornerstone of ER-positive (ER+) BC care; 5-year survival for early-stage disease exceeds 90%, and many patients live for decades after treatment (Thomas et al., 2024). Since the 1980s, however, tamoxifen (TMX) and aromatase inhibitors (AIs) have been linked to adverse metabolic effects and an elevated risk of T2D (Thomas et al., 2024). A 2022 meta-analysis reported 30% higher T2D risk versus no endocrine therapy and 19% versus cancer-free controls, with TMX showing the strongest association (Ye et al., 2022). TMX and AIs have also been associated with adverse lipid changes (Thomas et al., 2024) (Montanari et al., 2008) (Francini et al., 2006) and with metabolic-associated fatty liver disease (MAFLD) (Hong et al., 2017).

Beyond drug effects, treatment-related weight gain and menopause-related estrogen loss can shift adipose distribution and promote insulin resistance, potentially increasing both T2D risk and BC recurrence (Harborg et al., 2023; Francini et al., 2006; Thomas et al., 2024). In this context, sedentary behaviour and unbalanced diet can further disrupt glucose and lipid homeostasis. Conversely, lifestyle interventions (LIs) that combine structured physical activity (PA) with dietary counselling represent a practical adjunct to standard care (Campbell et al., 2019; Hojman et al., 2018) and may improve insulin resistance and lower T2D incidence (Jospe et al., 2024; Davies et al., 2011; Rein et al., 2022). Within this framework, supervised and tailored aerobic exercise training programs, and adherence to the Mediterranean diet (MD), which is rich in vegetables, fruits, and whole grains, help mitigate treatment-related gains in fat mass and losses in lean mass during and after therapy (Kudiarasu et al., 2023; Lake et al 2022; Li 2023; Raji et al., 2022). Importantly, reductions in body weight or fat mass are often accompanied by improvements in glucose homeostasis, providing a plausible mechanism by which LIs may help prevent T2D in BC and BC survivors (BCS) (Kudiarasu et al., 2023; D'Alonzo et al., 2021; MakariJudson et al., 2022; Rouque et al., 2017).

Despite these benefits, improvements in glycemic control are not consistently observed in BCS after LIs, particularly in the absence of exercise-induced weight loss (Kang et al., 2017; Campbell et al., 2012; Ligibel et al., 2008). Consistent with this, a recent meta-analysis (Han and Kim, 2021) reported high heterogeneity ($I^2 \approx 90\%$) in the effects of exercise on fasting insulin and HOMA index among women with BC (Dittus et al., 2018; Shaikh et al., 2020).

In this regard, Viskochil and colleagues recently evaluated oral glucose tolerance test (OGTT) responses before and after supervised aerobic exercise training in BCS, enabling assessment of both fasting indices of diabetes risk and post-glucose-ingestion insulin dynamics (Viskochil et al., 2020). Notably, despite no change in fasting insulin or glucose, insulin concentration at 120 minutes post-glucose load declined significantly after training. This focus is clinically relevant because postprandial insulin accounts for ~50-80% of daily insulin exposure, and high postprandial glycemic responses (PPGRs) are hallmarks of prediabetes and predict T2D, cardiovascular disease, and all-cause mortality independent of fasting glucose and glycated hemoglobin (HbA1c). Moreover, exercise training tends to reduce postprandial insulin levels more than fasting insulin levels, largely via potent insulin-sensitising effects on skeletal muscle (Jenkins et al., 2011 Egan and Zierath 2013). In line with this emphasis on postprandial physiology, an increasing number of studies have used continuous glucose monitor (CGM) devices in non-diabetic populations, demonstrating their utility for detailed characterisation of PPGRs and individualised metabolic phenotypes, with the potential to help prevent metabolic deterioration in people with obesity and metabolic syndrome (Wu et al., 2025).

In this study, we aimed to evaluate CGM-derived indices of glycemic variability in 27 BCS participants in the MoviS trial (ClinicalTrials.gov: NCT04818359), an open-label RCT of a multicomponent LI that included aerobic exercise and MD counselling. Participants wore CGM devices for up to 14 days at baseline (T0) and after the intervention (T1) and completed daily logs documenting exercise timing and intensity, meal and snack timing, and sleep-wake patterns. CGM metrics of glycemic variability, dietary intake (energy and macronutrients), and anthropometric, cardiometabolic, and routine laboratory measures were collected at T0 and T1. We hypothesised that the LI would improve both fasting measures and CGM-derived indices of glycemic control in BCS, and that changes in CGM metrics would correlate with diet quality and physical activity patterns.

Materials and Methods

Study design and participants

As previously described in detail in Natalucci, Ferri Marini and colleagues (Natalucci, Ferri Marin et al., 2023) the Movis study was a randomized controlled trial (ClinicalTrials.gov: NCT04818359) conducted at the department of biomolecular sciences of the University of Urbino Carlo Bo (Italy) and at the "Santa Maria della Misericordia" Hospital of Urbino (Italy). The primary outcome of the Movis Trial is the effect of a 12-week supervised aerobic exercise training program on quality of life (QoL) of BCS women. Ethical approval was granted by the Human Research Ethics Committee of the University of Urbino (protocol N 21 of July 10, 2019). The written informed consent was obtained from all participants.

The ancillary study "Sweet Movis" involved a subgroup of twenty-seven (age 52.3±8.6 years) BCS women without diabetes. BCS were eligible if they had histologically confirmed BC (stage 0-III) with no evidence of recurrent or progressive disease at recruitment; were within 12 months after surgery and at least 6 months after radiotherapy and/or chemotherapy, with or without current hormone therapy use; were aged between 30 and 70 years; non-physically active for at least 6 months (i.e., not engaged in at least 60 min/week of structured exercise during the previous 6 months); and were at risk of recurrence due to at least one of the following conditions: body mass index (BMI) ≥ 25 kg/m²; testosterone ≥ 0.4 ng/mL; serum insulin ≥ 25 µU/mL (170 pmol/L); or with metabolic syndrome. Women were excluded if they had pneumological, cardiological, neurological, or orthopedic comorbidities; or mental illnesses that prevented exercise performance. Recruitment was conducted between September 2022 and September 2023 at the Santa Maria della Misericordia Hospital of Urbino (Italy).

General and medical characteristics of all participants were collected by the clinicians, while the Cardiometabolic parameters, PA level, glycemic variability indices, and food intake were assessed by the research staff at each time point (pre- and post-intervention). Baseline characteristics of participants are reported in Table 1.

Table 1. Baseline characteristics of participants.

	Control arm (n=12)		Intervention arm (n=15)		*p-value*
Age (mean ± SD)	52.75±9.16		51.87±8.40		0.796
	n	%	n	%	
Stage at diagnosis					
0	0	0	1	7	0.667
I	6	55	10	66	
II	4	36	3	20	
III	1	9	1	7	
Menopausal status					
Premenopausal	2	18	1	7	0.364
Postmenopausal	9	82	14	93	
Surgery Type					
Mastectomy	2	17	2	13	0.809
Quadrantectomy	10	83	13	87	
Treatment in addition to surgery					
None	4	33	5	34	0.868
Only radiation	1	9	2	13	
Only chemotherapy	4	33	6	40	
Radiation and chemotherapy	3	25	2	13	
Current endocrine therapy					
None	4	33	5	33	0.945
Tamoxifen	3	25	3	20	
Aromatase Inhibitor	5	42	7	47	

Differences in frequency distributions were compared by chi-squared test. For age an independent sample T-test was used.

Lifestyle Intervention

The Sweet Movis study involved two parallel groups (1:1 randomization ratio with the control arm). Randomization lists were generated using an Excel spreadsheet and the randomized block permutation method (n 4) was used to ensure balance between the control group (CNT, n = 12) and the intervention group (INT, n=15). Both groups received the same LI guidance on PA and MD according to the American College of Sports Medicine (ACSM) guidelines for cancer survivors (Liguori et al., 2021), to the World Cancer Research Fund (WCRF, 2018) recommendations, and to the nutritional and exercise guidelines for BC patients approved by the Italian Ministry of Health in 2017 and 2019.

Only the INT underwent a 3-times-a-week aerobic training program for 12-weeks with progressive increases in exercise intensity (40-70% of heart rate reserve) and duration (20-60 minutes). Each aerobic exercise session performed by the INT was supervised by an exercise specialist. Two times per week the training sessions were performed on site at the gym located in the University research centre for physical activity at the University of Urbino. During the on-site training sessions, each participant could walk or run on a treadmill or use a stationary bike. One time per week the training session was performed in autonomy (indoors and outdoors, according to the participants' possibilities and preferences) following the volume prescribed in the previous session by the exercise specialist. In addition, each INTs' participant was instructed to use a heart rate monitor (HR300, Kalenji) to comply with exercise intensity during all exercise training sessions.

Cardiometabolic Health

Anthropometric and body composition parameters were collected at T0 and T1 as follows: height was measured using a fixed stadiometer, and body weight was measured using an electronic scale. Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters. Waist circumference was estimated by identifying the midpoint between the iliac crest and the lower edge of the last palpable rib. Bioimpedance analysis was performed to detect the percentage of fat mass (DC430MA DC430, Tanita Europe).

As described in detail by Natalucci, Ferri Marini and colleagues**,** $\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1}) was assessed using a submaximal cardiorespiratory fitness test performed using a treadmill at each time point (T0 and T1). A personalized test for each participant was created following the ACSM guidelines (Natalucci, Ferri Marini et al., 2023).

Fasting blood glucose, fasting insulin, triglycerides, total, LDL, and HDL cholesterol were determined by colorimetric assays using Beckman Coulter AU Analyzers, and the HOMA-IR index was calculated as follows: [(fasting glucose in mg/dL x fasting insulin in µU/ml) / 405].

Non-fasting metrics of glycemic variability and food diaries

Glycemic variability and food intake were assessed in both groups in the first and last 14 days (12±1.8 days) of the study intervention using the CGMs (FreeStyle Libre 2, Abbott) and a food diary, respectively. Glycemic variability indices (i.e., Coefficient of Variation [CV], Standard Deviation [SD], Mean Amplitude of Glycemic Excursions (MAGE), Mean Of Daily Differences (MODD), Time Below Rage [TBR, < 54 and < 70 mg/dL], Time In Range [TIR, from 70 to 140 and to 160 mg/dL], Time Above Range [TAR, > 140, > 160, > 180 mg/dL]) were calculated with the EasyGV software (v.9.0.R2). Food diaries were used to report main daily food intake (breakfast, lunch and dinner), snacks, and PA eventually performed in autonomy. WinFood software (v. 3.36.2) was used to report each meal and to calculate the daily average of total caloric and macronutrient (protein, lipid, carbohydrates, starch, oligosaccharides and total fibers) intakes.

Physical activity level

The Physical activity (PA) level was assessed using the International Physical Activity Questionnaire-Short Form (IPAQ-SF) at T0 and at T1 (Craig et al., 2003; Lee et al., 2011). This questionnaire assessed PA over the last seven days and asked about the frequency and duration of sitting, walking, moderate-intensity, and vigorous-intensity activities. PA intensity was associated with the metabolic equivalent of task (MET). As reported in the IPAQ-SF guidelines for data processing and analysis, MET equal to 4 or 8 was considered for moderate or vigorous intensity, respectively; MET equal to 3.3 was considered for walking (e.g., Moderate MET-minutes/week = 4.0 x moderate-intensity activity minutes x moderate days). Total PA level was calculated as the sum of walking, moderate and vigorous intensity activities according to the IPAQ-SF guidelines. The IPAQ-SF final score (calculated by converting questionnaire data into MET minutes per week [MET-min/week]) was used as a general indicator of low activity (MET < 600), moderate activity (MET ≥ 600), and high activity (MET ≥ 3000).

Statistical analysis

All non-fasting glycemic variability indices were calculated with the EasyGV software (v.9.0.R2). Differences between groups (between-subjects effects) and differences between pre- and post- intervention (within-subjects effects) for all variables were analyzed using repeated measures general linear mixed models (RM-GLMs) with statistical significance equal to 0.05.

To deeply analyse the day-to-day dietary behaviour, linear mixed models (LMMs) were used to evaluate daily energy and macronutrient intakes, and glycemic variability to assess changes between groups over time. Each outcome (e.g., oligosaccharides) was considered as a dependent variable, time, group, and time x group interaction were considered as fixed effects, and participants (i.e., MOVIS ID) were considered as random effects. All LMMs were adjusted for age and BMI, while PA practice (yes [EXE day] or not [non-EXE day]) and monitored days (to consider day-to-day variability) were used as covariates. Model terms were tested with the Satterthwaite test Method. A compound symmetry covariance structure was assumed in all models. Analyses were conducted following the intention-to-treat principle, using all variables data without missing data imputation. To control for type I errors because of multiple testing, the analyses were adjusted using the Bonferroni correction (SPSS software v.17).

Results

Table 2 summarizes the effects of LI on cardiometabolic health variables. Both CNT and INT showed reductions in fat mass (CNT: -2.2%; INT: -3.8%, p=0.015) and increases in physical activity levels (MET-min/week, CNT: +90.8 %; INT: +64.4 %, p=0.005) at the end of the study (T1). A significant time x group interaction was observed for $\dot{V}O_{2max}$, with a greater improvement in INT compared to CNT (CNT: +5.3%; INT: +16.4%, p=0.020).

Regarding fasting metrics of glucose control and metabolic markers (Table 2), glucose and the cholesterol level did not change after the LI program. In contrast, triglycerides (CNT: - 4.2% INT: -15.9%, p=0.020), insulin (CNT: -14.1%; INT: -15.6%, p=0.006) and HOMA-IR index (CNT: - 13.7%; INT: -16.3%, p=0.009) improved in both groups at T1.

Table 3 presents the absolute and relative energy and macronutrient intakes recorded in the food diaries during the first and last 14 days of the LI. No significant changes over time nor differences between the CNT and INT groups were observed.

Glycemic variability indices and times spent in different glucose ranges, derived from CGM data collected during the first and last 14 days of the LI, are reported in Table 4. Compared with the INT, the CNT showed a worsening glycemic variability indices, evidenced by a borderline increase in glucose SD (CNT: +5.0%; INT: +0.7%, p=0.078) and a significant rise in MODD index (CNT: +12.5%; INT: -3.6%, p=0.017), a widely used measure of day-to-day glycemic variability. TIR declined in both groups at T1 for both 70-120 mg/dL and 70-140 mg/dL ranges, with a larger reduction in CNT (p=0.036 and p=0.038, respectively). Consistently, there was a trend toward a time x group interaction for time spent above 180 mg/dL, reflecting a slight increase in CNT (CNT: from 0.30% to 1.11%) and a decrease in INT (from 1.12% to 0.75%, p=0.064). These results were unexpected given the relative stability of dietary habits recorded at T0 and T1 and concurrent improvements in metabolic markers, particularly insulin level and HOMA-index, observed in both CNT and INT groups.

Table 2. Cardiometabolic health, fasting metrics of glucose control, and metabolic markers.

Anthropometric, body composition parameters, and PA level									
	Control Group Mean ± SD		**%Δ**	**Intervention Group Mean ± SD**		**%Δ**	**Time *p*-value (η^2_p)**	**Group *p*-value (η^2_p)**	**Time x Group *p*-value (η^2_p)**
	T0	**T1**		**T0**	**T1**				
Body weight (kg)	67.82±16.72	67.97±18.13	0.1	65.06±10.66	64.90±11.06	-0.3	0.933 (0.0001)	0.598 (0.011)	0.649 (0.008)
BMI (kg/m^2)	25.90±5.92	25.88±6.62	0.4	25.21± 4.31	24.86±4.52	-1.5	0.776 (0.003)	0.689 (0.007)	0.197 (0.068)
Waist circumference (cm)	80.83±13.52	82.00±14.62	1.4	81.03±13.41	82.20±11.81	2.0	0.247 (0.053)	0.971 (0.0001)	0.986 (0.0001)
Fat mass (%)	32.72±7.96	31.89±7.75	-2.2	31.26±6.20	30.15±6.67	-3.8	**0.015* (0.213)**	0.562 (0.014)	0.717 (0.005)
$\dot{V}O_{2max}$ (mL·min^{-1}·kg^{-1})	28.02±5.56	29.40±5.79	5.3	29.82±4.61	34.32±4.03	16.4	**<0.001*** (0.467)**	0.077 (0.120)	**0.020* (0.283)**
IPAQ SCORE (MET·min/week)	538.75±477.54	1488.66±2265.23	90.8	244.86±245.04	908.7±507.72	64.4	**0.005* (0.280)**	0.197 (0.066)	0.586 (0.012)

Table 2. Cardiometabolic health, fasting metrics of glucose control, and metabolic markers *(continued).*

Fasting metrics of glucose control and metabolic markers									
	Control Group Mean ± SD		**%Δ**	**Intervention Group Mean ± SD**		**%Δ**	**Time *p*-value (η^2_p)**	**Group *p*-value (η^2_p)**	**Time x Group *p*-value (η^2_p)**
	T0	**T1**		**T0**	**T1**				
Fasting blood glucose (mg/dl)	93.00±7.34	93.66±8.00	0.8	94.46±12.17	92.93±12.78	-1.5	0.707 (0.006)	0.927 (0.0001)	0.344 (0.036)
Insulin (µU/ml)	6.57±4.09	5.00±2.47	-14.1	7.01±4.55	5.80±3.72	-15.6	**0.006** (0.272)**	0.677 (0.007)	0.709 (0.006)
HOMA-IR index	1.52±0.95	1.16±0.58	-13.7	1.68±1.14	1.36±0.91	-16.3	**0.009** (0.253)**	0.609 (0.011)	0.840 (0.002)
Triglyceri-des (mg/dl)	87.33±33.68	79.50±28.03	-4.2	108.06±41.64	84.33±25.83	-15.9	**0.020* (0.198)**	0.261 (0.050)	0.223 (0.059)
Total cholesterol (mg/dl)	206.91±37.99	200.00±43.89	-2.9	203.93±26.70	195.40±23.28	-3.8	0.095 (0.107)	0.755 (0.004)	0.857 (0.001)
LDL cholesterol (mg/dl)	124.66±33.08	122.83±43.38	-1.4	126.93±20.08	123.98±17.98	-2.0	0.502 (0.018)	0.886 (0.001)	0.850 (0.001)
HDL cholesterol (mg/dl)	64.00±12.94	66.50±10.36	5.1	59.20±10.16	57.73±13.83	-1.7	0.802 (0.003)	0.115 (0.096)	0.339 (0.037)

Abbreviations: T0, baseline; T1, after intervention period; %Δ, percentage change over time; η^2_p, effect size; PA, physical activity; BMI, body mass index; *$\dot{V}O_{2max}$*, maximum oxygen uptake; IPAQ, International physical activity questionnaire; MET, Metabolic equivalent of task; HOMA-IR, Homeostasis Model Assessment-Insulin Resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein. *p<0.05; **p<0.01; ***p<0.001.

Table 3. Food diary data.

Caloric and macronutrient intakes									
	Control Group Mean ± SD		**%Δ**	**Intervention Group Mean ± SD**		**%Δ**	**Time *p*-value (η^2_p)**	**Group *p*-value (η^2_p)**	**Time x Group *p*-value (η^2_p)**
	T0	**T1**		**T0**	**T1**				
Caloric intake (Kcal)	1490.60±353.78	1388.25±365.60	-6.5	1605.59±333.90	1553.80±314.73	-1.9	0.098 (0.106)	0.269 (0.049)	0.578 (0.013)
Protein (g)	60.05±10.28	54.92±10.55	-7.1	65.17±20.13	65.80±19.01	-3.5	0.359 (0.034)	0.179 (0.071)	0.244 (0.054)
Lipid (g)	64.23±13.92	61.67±16.73	-4.2	67.22±18.68	64.35±17.85	-2.3	0.209 (0.062)	0.655 (0.008)	0.943 (0.0001)
Carbohydrate (g)	170.67±62.81	158.82±59.60	-1.1	191.10±34.35	183.50±30.16	-2.4	0.148 (0.082)	0.199 (0.065)	0.747 (0.004)
Starch (g)	84.94±34.99	86.18±26.70	7.9	95.45±22.29	100.09±23.63	8.5	0.594 (0.012)	0.181 (0.071)	0.758 (0.004)
Oligosaccharides (g)	50.65±24.79	44.75±24.96	-10.9	57.54±15.78	54.38±14.96	-6.5	0.169 (0.074)	0.256 (0.051)	0.672 (0.007)
Total fiber (g)	13.48±5.55	12.94±4.97	-0.8	18.01±5.92	19.07±5.51	10.7	0.769 (0.004)	**0.011* (0.231)**	0.375 (0.032)

Abbreviations: g, grams; T0, baseline; T1, after intervention period; %Δ, percentage change over time; η^2_p, effect size. *p<0.05; **p<0.01; ***p<0.001.

Table 4. Non-fasting metrics of glycemic variability (CGMs data).

Glycemic variability indices									
	Control Group Mean ± SD		**%Δ**	**Intervention Group Mean ± SD**		**%Δ**	**Time *p*-value (η^2_p)**	**Group *p*-value (η^2_p)**	**Time x Group *p*-value (η^2_p)**
	T0	**T1**		**T0**	**T1**				
CV glucose levels (mg/dL)	0.16±0.03	0.17±0.03	8.9	0.17±0.04	0.17±0.04	-1.1	0.516 (0.017)	0.832 (0.002)	0.106 (0.101)
SD glucose levels (mg/dL)	0.93±0.16	1.02±0.25	5.0	1.01±0.35	1.00±0.30	0.7	0.2000 (0.065)	0.795 (0.003)	0.078 (0.119)
MAGE (mg/dL)	2.61±0.59	2.80±0.91	6.1	2.67±0.95	2.69±0.79	4.1	0.325 (0.0039)	0.937 (0.0001)	0.397 (0.029)
MODD (mg/dL)	0.77±0.10	0.87±0.18	12.5	0.88±0.34	0.83±0.27	-3.6	0.474 (0.021)	0.708 (0.006)	**0.017* (0.209)**

Table 4. Non-fasting metrics of glycemic variability (CGMs data) *(continued).*

TBR (%), TIR (%), and TAR (%)							
	Control Group Mean ± SD		**Intervention Group Mean ± SD**		**Time *p*-value (η^2_p)**	**Group *p*-value (η^2_p)**	**Time x Group *p*-value (η^2_p)**
	T0	**T1**	**T0**	**T1**			
% time <54 mg/dL	0.05± 0.16	0.04± 0.12	0.08± 0.20	0.16± 0.35	0.575 (0.013)	0.224 (0.0059)	0.518 (0.017)
% time <70 mg/dL	0.94± 0.97	1.46± 2.75	1.19± 1.72	1.10± 1.43	0.643 (0.009)	0.912 (0.0001)	0.522 (0.017)
% time 70 to 120 mg/dL	83.49±7.31	74.95±18.77	80.71±14.03	78.95±15.69	**0.036* (0.164)**	0.907 (0.001)	0.157 (0.078)
% time 70 to 140 mg/dL	94.65±2.83	89.82±9.50	92.04±8.54	91.43±8.70	**0.038* (0.160)**	0.861 (0.001)	0.102 (0.103)
% time > 140 mg/dL	4.41±3.05	8.71±10.10	6.77±8.80	7.47±8.47	0.071 (0.125)	0.847 (0.002)	0.185 (0.069)
% time > 160 mg/dL	1.20±1.10	3.25±4.83	2.64±5.38	2.50±3.66	0.173 (0.0073)	0.185 (0.002)	0.122 (0.093)
% time > 180 mg/dL	0.30±0.40	1.11±1.73	1.12±2.87	0.75±1.48	0.480 (0.020)	0.734 (0.005)	0.064 (0.130)

Abbreviations: T0, baseline; T1, after intervention period; %Δ, percentage change over time; η^2_p, effect size; CV, coefficient of variation; SD, standard deviation; MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; TIB, time below range; TIR, time in range; TAR, time above range. *p<0.05; **p<0.01; ***p<0.001.

To deeply analyse the day-to-day dietary behaviour, we fit linear mixed-effects models (LMMs) to daily energy and macronutrient intakes. In line with the summary GLM analyses, LMMs showed that the mean of daily energy and macronutrient intakes did not differ between T0 and T1 in either group (all p > 0.05). However, there was a day-to-day variation in caloric intake (F=5.478, p=0.02), carbohydrates (F=4.261, p=0.039) and oligosaccharides (F=9.061, p=0.003) (Figure 1). Accordingly, TIR (70-120 mg/dL) also varied from day-to-day (F=4.036, p=0.45), supporting that eating behavioural changes contributed to glycemic variability.

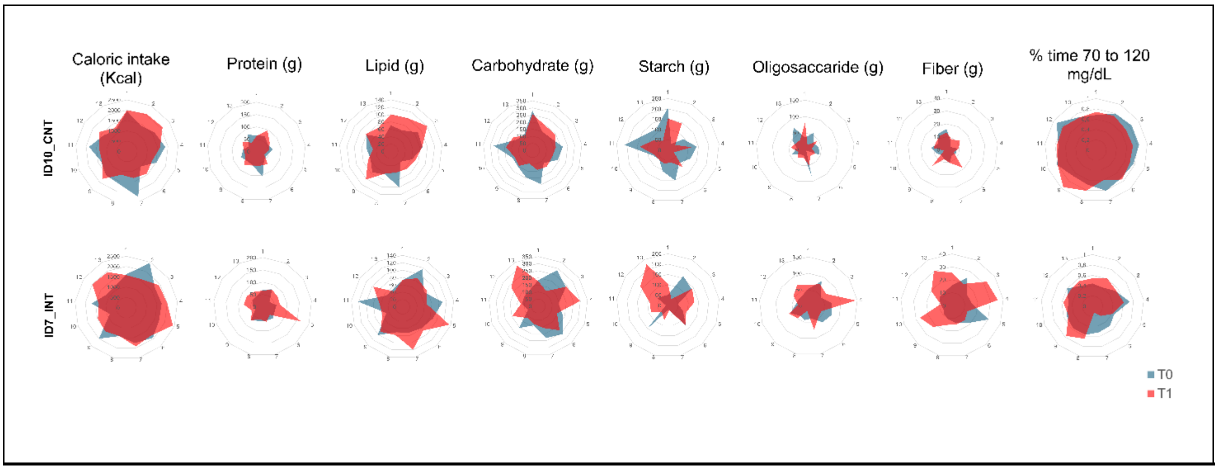

Figure 1. Daily caloric and macronutrient intakes. In this figure, one participant from the control group and one participant from the intervention group were represented as examples.

Substantial evidence suggests that exercise can influence dietary habits (Reily et al., 2023). Because both the CNT and INT groups performed PA on alternate days, we tested whether macronutrient intake differed between exercise (EXE) and non-exercise (Non-EXE) days, and whether CGM metrics varied accordingly (Figure 2). Overall, total energy and macronutrient intakes were similar on EXE and Non-EXE days. The only significant difference was observed for oligosaccharide intake, which was higher on EXE than on Non-Exe days at T1 (F=3.415, p=0.034) (Figure 2A). Notably, the CNT group exhibited a marked difference in oligosaccharide intake between EXE and Non-EXE days at T1, rising from 37.7±24.1 grams on Non-EXE days to 60.5±42.2 grams on EXE days, whereas the INT group showed only a smaller change (from 53.3±24.9 grams to 57.0±27.2 grams). GCM metrics showed different patterns by group when comparing EXE and Non-EXE days (Figure 2B-E). In the CNT group, mean of glucose (F=3.878, p=0.021), percentage of time above 160 mg/dL (F=3.200, p=0.041), and 140 mg/dL (F=3.200, p=0.041), indices of poorer glycemic control, were higher on Non-EXE compared with EXE days, and especially at T1. In contrast, these parameters were more stable in the INT and tended to improve on EXE days at T1 compared to T0. Consistently, time in the optimal glucose range (70-120 mg/dL) showed the opposite pattern (F=3.730, p=0.025), being lower on Non-EXE than EXE days.

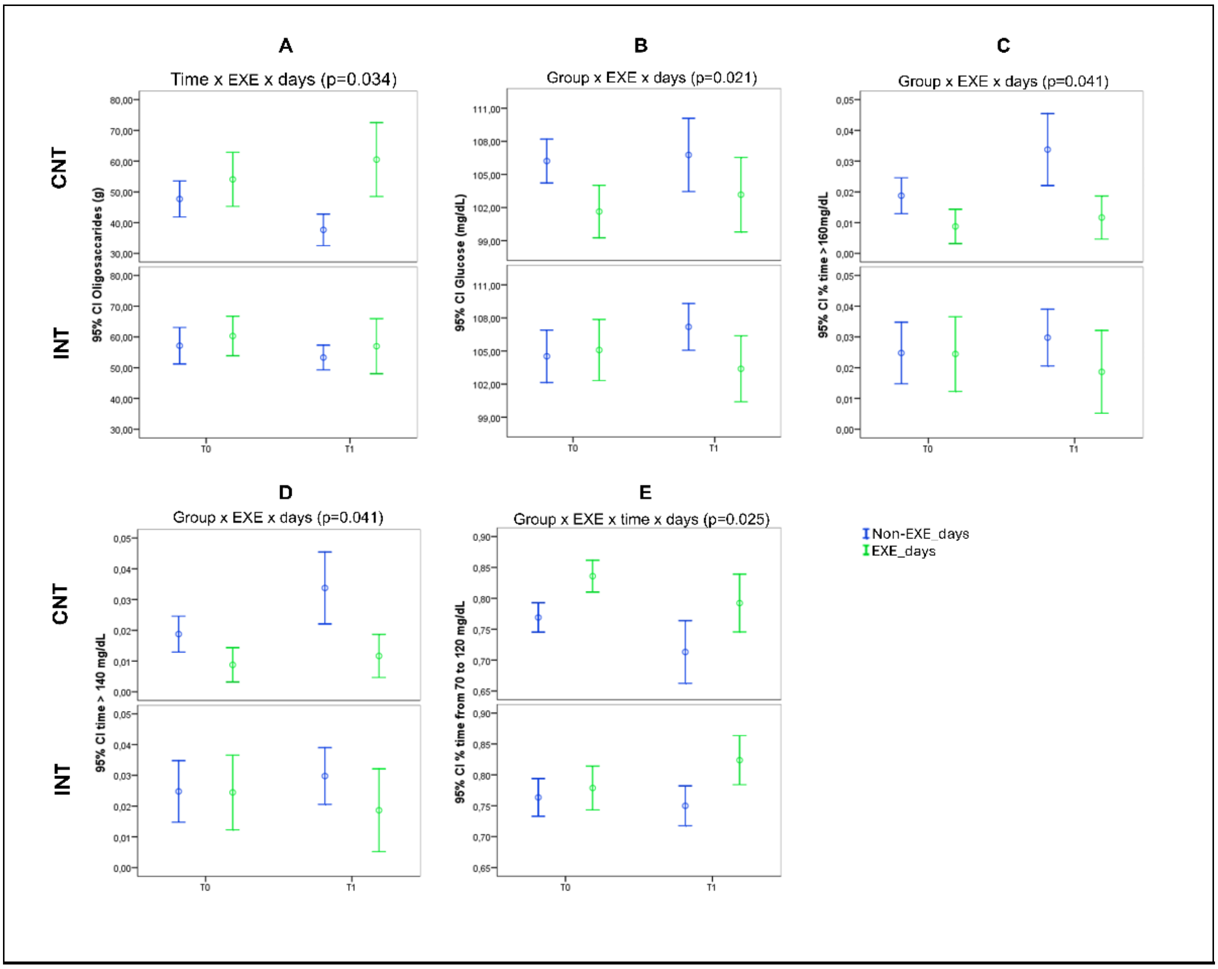

Figure 2. Changes in macronutrient intake between exercise (EXE) and non-exercise (Non-EXE) days, along with CGM data.

Discussion

In this study, a 3-week LI led to significant improvements in anthropometric and metabolic markers in BCS at elevated metabolic risk. These improvements were similar in the CNT and INT groups, despite only the INT completing a structured, supervised aerobic exercise program. As expected, $\dot{V}O_{2max}$ increased only in INT, even though MET-minutes/week recorded from the IPAQ increased in both groups. Given these favorable changes, we anticipated improvements in CGM-derived glycemic indices at T1; instead, we observed higher day-to-day glucose variability (i.e., increased MODD index) in CNT and a reduction in TIR in both groups.

Prior LI studies have focused mainly on fasting metrics of glucose control in BCS (e.g., glucose, insulin and HOMA-IR) and generally report benefits in individuals with poorer baseline metabolic profiles who achieve weight loss (Kang et al., 2017; Campbell et al., 2012; Ligibel et al., 2008). Our findings align with these studies suggesting that the modest decrease in fat mass likely contributed to lower fasting insulin and HOMA-IR in both CNT and INT. The inclusion of relatively metabolically unhealthy BCS subjects may have facilitated these favorable fasting changes (Viskochil et al., 2020). Notably, structured training did not further improve % of fat mass, triglycerides, fasting insulin, or HOMA-IR beyond CNT, despite a ~16% increase in $\dot{V}O_{2max}$ achieved by the INT group.

Although CGM is increasingly used in non-diabetic populations (Sofizadeh et al., 2022), there is still little consensus on how to integrate it effectively into preventive LI programs. To our knowledge, this is the first study to apply CGM to assess glycemic variability in sedentary, non-diabetic BCS at elevated metabolic risk undergoing a 12-week LI. Surprisingly, classic variability indices such as daily mean glucose, SD, CV, MAGE (intra-day), and MODD (inter-day), did not improve at T1 in either group. On the contrary, MODD worsened in CNT, and TIR (70-120 mg/dL and 70-140 mg/dL) decreased in both groups.

One possible explanation, namely changes in dietary habits across the LI, was not supported by our dietary records. Indeed, total energy and macronutrient intakes (proteins, lipid, carbohydrate, starches, oligosaccharide, and fiber) were stable from baseline to T1 in both groups. This suggests that the worsening glycemic indices were not explained by end-of-study changes in average diet. They also indicate that generic Mediterranean diet counseling provided to both groups was insufficient to produce measurable dietary change over this timeframe.

We next tested the hypothesis that dietary habits change on a day-to-day basis, possibly explaining the variation of MODD index and sporadic time spent above optimal glucose levels. Interestingly, we found that daily energy, carbohydrate, and oligosaccharide intake varied across days and coincided with day-to-day variation in time spent in TIR (70-120 mg/dL). Between-day variations in energy and macronutrient intake, together with fluctuations in glucose, prompted us to examine more closely the relationship between exercise and dietary behavior. Because the INT protocol scheduled exercise on alternate days and CNT participants likewise reported physical activity on alternate days in their dietary logs, we conducted additional analyses comparing EXE versus Non-EXE days. Notably, oligosaccharide intake was higher on EXE compared to Non-EXE days, and this difference was particularly evident in the CNT group at T1, which consumed, on average, over 20 grams more oligosaccharides on EXE versus Non-EXE days. CGM indices also differed between EXE and Non-EXE days: mean daily glucose and time spent above 160 and 140 mg/dL were higher during Non-EXE compared to EXE days, particularly in CNT. Accordingly, TIR was higher during EXE compared to Non-EXE days in both groups and CNT showed a deterioration of TIR at T1 compared to T0, particularly in Non-EXE days. Although earlier reports suggested that exercise might worsen dietary habits by increasing energy intake or leading to less healthy food choices (King et al., 2007; King et al., 2012; Melanson et al., 2013), recent free-living work specifically examining everyday eating did not confirm compensatory shifts toward less healthy foods on exercise versus non-exercise days (Reily et al., 2023). Instead, participants tended to consume healthier meals on exercise days, albeit with larger portions at post-exercise meals compared with random meals on non-exercise days. Moreover, a recent meta-analysis underscores exercise’s role in modulating appetite and intake, showing significant reductions in hunger, prospective food consumption, and energy intake (Li et al., 2025). Thus, the increased consumption of oligosaccharides observed in the present study is consistent with a shift versus healthier food in EXE days. Moreover, the increased oligosaccharide consumption might have directly contributed to the better glycemic control observed during the EXE days (An et al., 2022). Taken together, these observations are consistent with prior reports, and they may help explain the higher between-day glucose variability observed in the CNT group at T1, as well as the lower TIR seen in both groups - changes that occurred mainly on Non-EXE days.

Conclusions

In conclusion, our findings suggest that short-duration LI can improve fasting cardiometabolic risk without necessarily translating into better CGM variability metrics. They also underscore the potential value of integrating CGM with simple dietary logging to track adherence and to target high-risk (non-exercise) days with tailored strategies (e.g., optimizing carbohydrate quality and timing and ensuring sufficient activity). Future, longer trials should evaluate whether more intensive or longer structured training, personalized dietary guidance, and attention to exercise timing can improve TIR and glucose variability in BCS. These results highlight the importance of day-to-day behavioral coupling - dietary intake and exercise - rather than relying solely on averaged measures across weeks to track adherence to LI in BCS in real-life settings.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STUDY 3

Original Article

Lifestyle intervention based on aerobic exercise and Mediterranean diet modulates IGF-1 and its binding proteins in breast cancer survivors: results from a randomized controlled trial

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Abstract

Cancer-related biomarkers such as insulin-like growth factor-1 (IGF-1) may help identify high-risk breast cancer survivors (BCS) who could benefit from lifestyle interventions (LIs). However, the effect of LIs on modulation of IGF-1 levels in BCS remains inconclusive.

Fifty inactive BCS were randomized into a control group (CG, n=26) and an intervention group (IG, n=24). Both groups received recommendations on exercise and the Mediterranean diet; the IG additionally followed a supervised 3-month aerobic exercise program (MoviS trial, NCT04818359). Associations between baseline and LI-induced changes (Δ) in IGF-1, IGFBP1 and IGFBP3 levels, along with anthropometric, metabolic, and fitness parameters, were assessed using linear and quadratic models.

Both groups increased physical activity (MET-min/week) and Mediterranean diet adherence (Med diet score) after the LI, while $\dot{V}O_{2max}$ increased only in the IG. Reductions in BMI, fat mass, insulin levels, HOMA-IR index, total and LDL cholesterol were observed in both groups and were associated with increased IGFBP1 and decreased IGFBP3 levels. Mean IGF-1 levels remained unchanged in both groups. Baseline IGFBP1 was inversely correlated with IGF-1, LDL, BMI, fat mass, and insulin, while baseline IGFBP3 was positively correlated with IGF-1, insulin, and HOMA-IR. Baseline IGF-1 levels were negatively correlated with Δ IGF-1: participants with IGF-1 ≤ 94.7 ng/mL showed increases, whereas those with IGF-1 ≥ 173.3 ng/mL exhibited decreases post-intervention. Similar trends were found for IGFBP3 but not for IGFBP1. A three-dimensional quadratic model revealed a U-shaped relationship between baseline IGF-1, Δ IGF-1, and Δ $\dot{V}O_{2max}$: improvements in $\dot{V}O_{2max}$ were associated with IGF-1 increase in participants with low baseline IGF-1 and decrease in those with high levels. Conversely, an inverted U-shaped relationship was found between baseline IGF-1, Δ IGF-1, and Δ fat mass.

These findings underscore the importance of accounting for IGFBP modulation and baseline heterogeneity in IGF-1 levels when evaluating the efficacy of LIs targeting the IGF-1 system in high-risk BCS.

Trial registration: ClinicalTrials.gov NCT04818359.

Keywords: IGF-1, IGF-1 binding proteins, breast cancer survivors, lifestyle intervention, aerobic exercise, Mediterranean diet

Introduction

Breast cancer (BC) is the most commonly diagnosed cancer in women and the second most frequently occurring cancer worldwide in 2022, with 2.3 million new cases (Bray et al., 2024). Although BC diagnoses continue to increase, advances in screening and treatment have significantly reduced the mortality recurrence rate (Bray et al., 2024). As a result, many survivors seek information on how lifestyle factors such as physical activity and diet may influence their prognosis. Evidence has consistently shown that lifestyle interventions (LIs) can reduce symptoms, such as fatigue, and improve the overall quality of life in BC survivors (BCS) (Ballard-Barbash et al., 2012). However, only a limited number of randomized controlled trials (RCTs) have evaluated the effects of LIs on cancer-related biomarkers in BCS. Consequently, the biological mechanisms linking physical activity, diet, and prognosis in BCS remain poorly understood.

One potential mechanism underlying the association between lifestyle and cancer is the insulin-like growth factor-1 (IGF-1) signaling pathway (Baldelli et al., 2024; Pollak et al., 2004). The IGF-1 system comprises a ligand (IGF-1) and six IGF-1 binding proteins (IGFBPs), which regulate IGF-1 bioactivity (Allard & Duan, 2018). IGFBP production is, in turn, regulated by hormones (e.g., growth hormone (GH), insulin, and glucocorticoids) as well as nutritional status. For example, GH stimulates liver IGFBP3 production to extend IGF-1's stability and ensure its physiological function (Blum et al., 1993). Conversely, liver IGFBP1 production increases in response to catabolic conditions (e.g., starvation, hypoxia, and stress) (Kajimura et al., 2006) and is negatively regulated by insulin (Suwanichkul et al., 1994). This mechanism ensures that free, bioactive IGF-1 levels increase only under anabolic conditions.

When free IGF-1 binds to its receptor (IGF-1R), it activates key oncogenic signaling pathways, including MAPK and PI3K/Akt/mTOR, which regulate cell proliferation, survival, and energy metabolism (Pollak et al., 2004). These findings imply that elevated IGF-1 levels or bioactivity can promote cell proliferation and survival, potentially contributing to carcinogenesis (Pollak et al., 2004). Notably, approximately 75% of patients with BC and 87% of those with invasive BC show IGF-1R signaling activation (Denduluri et al., 2015; Ireland et al., 2018). Moreover, increased IGF-1 levels are associated with disease progression, resistance to standard therapies, and increased all-cause mortality in women with estrogen receptor (ER)-positive BC

(Denduluri et al., 2015; Duggan et al., 2013; Yerushalmi et al., 2012). Unsurprisingly, targeting the IGF-1 system is one of the most investigated areas in anticancer drug development (P. Wang et al., 2023).

LIs, including physical activity and diet, have been proposed as non-pharmacological strategies for decreasing IGF-1 levels and bioactivity (Han & Kim, 2021; Hu et al., 2022; Invernizzi et al., 2022; D.-W. Kang et al., 2017; X.-Y. Kang et al., 2020; Meneses-Echávez et al., 2016; Y. Wang et al., 2020; Wei et al., 2017). Indeed, LIs may influence IGF-1 signaling by lowering circulating hormone levels or modulating IGFBP expression. However, the effect of LIs on the modulation of the IGF-1 system in BCS remains inconclusive. For example, three meta-analyses reported IGF-1 reductions (Hu et al., 2022; X.-Y. Kang et al., 2020; Meneses-Echávez et al., 2016), whereas others found no effect on IGF-1 levels (Han & Kim, 2021; D.-W. Kang et al., 2017; Y. Wang et al., 2020) after LIs in BCS. These meta-analyses also reported high heterogeneity (I^2) among studies. Similarly, findings regarding LI-induced changes in serum IGFBP3 and IGFBP1 levels remain inconsistent (Han & Kim, 2021; Hu et al., 2022; Invernizzi et al., 2022; D.-W. Kang et al., 2017; X.-Y. Kang et al., 2020; Meneses-Echávez et al., 2016).

Individual variability in baseline IGF-1 levels may partly explain the discrepancies and inconsistent findings regarding the effect of LIs on the IGF-1 system (Devin et al., 2016). In support of this hypothesis, some studies have found an inverse relationship between baseline IGF-1 levels and LI-induced changes, as observed with exercise training (Nishida et al., 2010; Orsatti et al., 2008; Sillanpää et al., 2010) or fasting-mimicking diets (Wei et al., 2017) in healthy participants. Thus, LIs may exert stronger effects on individuals with relatively high IGF-1 baseline levels (Devin et al., 2016; Y. Wang et al., 2020). Additionally, large population studies have suggested a U-shaped relationship between IGF-1 levels and age-related outcomes, including cardiovascular disease, diabetes, dementia, cancer, and all-cause mortality, where both high and low IGF-1 levels are associated with increased risk (Lin et al., 2023; Mukama et al., 2023; Rahmani et al., 2022; Zhang et al., 2021). However, whether BCS with different baseline IGF-1 levels respond differently to the same LI remains unexplored. In this study, we analyzed IGF-1, IGFBP1, and IGFBP3 levels in 50 BCS participating in the MoviS trial (ClinicalTrials.gov reference number: NCT04818359), an open-label RCT based on multi-component LI that combined aerobic exercise with dietary recommendations. We investigated the association between LI-induced improvements in anthropometric, metabolic, and fitness parameters, and IGF-1 system components. Moreover, using both linear and quadratic modelling, we examined the relationship between baseline IGF-1, IGFBP1, and IGFBP3 levels and the changes induced by LIs.

Materials and Methods

Study design and participants

The MoviS trial was a monocentric trial (protocol: NCT 04818359) conducted at the Santa Maria della Misericordia Hospital (Urbino, Italy) and the Department of Biomolecular Sciences of the University of Urbino Carlo Bo (Italy). As reported elsewhere (Natalucci et al., 2023), ethical approval was granted by the Human Research Ethics Committee of the University of Urbino Carlo Bo (Protocol N 21 of July 10, 2019), and written informed consent was obtained from all participants. Women were eligible for the study if they had histologically confirmed BC (stage 0-III) with no evidence of recurrent or progressive disease at recruitment; were within 12 months after surgery and at least 6 months after radiotherapy and/or chemotherapy, with or without current hormone therapy use; were aged between 30 and 70 years; non-physically active for at least 6 months (i.e., not engaged in at least 60 min/week of structured exercise during the previous 6 months); and were at risk of recurrence due to at least one of the following conditions: body mass index (BMI) ≥ 25 kg/m²; testosterone ≥ 0.4 ng/mL; serum insulin ≥ 25 µU/mL (170 pmol/L); or metabolic syndrome. As reported in the CONSORT flow diagram (Figure 1), 50 participants were included in this study.

Lifestyle Intervention

LIs began after surgery and completion of primary treatments (post-adjuvant chemotherapy or radiotherapy). As previously described (Natalucci et al., 2023), the participants were randomized into either the Control group (CG) (n=26) or Intervention group (IG) (n=24). Both groups attended a one-hour meeting consisting of a 45-minute group session and a 15-minute personalized session, during which an oncology nutritionist and an exercise oncology specialist presented and discussed the latest guidelines on physical activity and the Mediterranean diet (structured counselling session). The recommendations were based on the WCRF 2018 guidelines and the most recent nutritional and exercise guidelines for BC patients approved by the Italian Ministry of Health in 2017 and 2019 (Ministero Della Salute Linee Di Indirizzo Percorsi Nutrizionali Nei Pazienti Oncologici. Available Online: Https://Www.Salute.Gov.It/Imgs/C_17_pubblicazioni_2682_allegato.Pdf,n.d.; Ministero Della Salute Linee Di Indirizzo Sull'attività Fisica per Le Differenti Fasce D'età e Con Riferimento a Situazioni Fisiologiche e Fisiopatologiche e a Sottogruppi Specifici Di Popolazione. Available

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Additionally, all participants had the opportunity to register on the DianaWeb platform (Gianfredi et al., 2020; Villarini et al., 2015), which provides daily nutritional advice aligned with the Mediterranean diet, while only the IG participated in the MoviS training.

The MoviS training consisted of a 3-month supervised aerobic training program with progressively increasing intensity (ranging from 40% to 70% of the Heart Rate Reserve [HRR]) and duration (from 20 to 60 minutes). An exercise specialist supervised two exercise sessions per week (on-site sessions), while participants independently completed one additional session per week (remote session) following the prescribed exercise intensity under remote supervision. Each participant was instructed to use a heart rate monitor (HR300, Kalenji) to verify exercise intensity in each session (on-site and remote sessions). Each participant could walk or run on a treadmill or use a stationary bike during the on-site sessions, while the remote sessions were performed both indoors and outdoors, according to the participants' possibilities and preferences.

Anthropometrics and body composition

Anthropometric and body composition parameters were collected at T0 and T1 as follows: body height was measured using a fixed stadiometer, and body weight was measured using an electronic scale. Body mass index (BMI) was calculated by dividing the body weight in kilograms by the square of the body height in meters. Measurements of bioimpedance to obtain the percentage of fat mass were performed using DC430MA DC430 (Tanita Europe).

Dietary habits, physical activity level and cardiorespiratory fitness

The modified MeDiet questionnaire, consisting of a 14-item adherence screening, was used to assess adherence to the Mediterranean diet (8–9 or >10 points in the 14-item score indicate higher adherence) (Martínez-González et al., 2012). Each participant completed the questionnaire during assessments at T0 and T1. The Physical activity (PA) level was assessed using the International Physical Activity Questionnaire – Short Form (IPAQ-SF) to determine the participants' habitual PA level (Craig et al., 2003; Lee et al., 2011). This questionnaire contains questions on PA during the last seven days and assesses the frequency and duration of sitting, walking, moderate-intensity activities, and vigorous-intensity activities. The IPAQ-SF was completed at T0 and T1. Based on the participants' responses, total PA levels were calculated by converting questionnaire data into the metabolic equivalent of task (MET) minutes per week (MET-min/week), and exercise intensity was associated with MET (MET=8 for vigorous, MET=4 for moderate, MET=3.3 for walking), and total PA level included walking and moderate and vigorous intensity activity according to the IPAQ-SF guidelines (Craig et al., 2003; Lee et al., 2011). The IPAQ-SF score expressed as MET-min/week was used as a general indicator of low activity (MET < 600), moderate activity (MET ≥ 600), and high activity (MET ≥ 3000). Cardiorespiratory fitness ($\dot{V}O_{2max}$ [$mL \cdot min^{-1} \cdot kg^{-1}$]) was assessed using a submaximal cardiorespiratory fitness test performed at T0 and T1. A personalized test for each participant was created according to the American College of Sports Medicine (ACSM) guidelines, as described in detail by Natalucci et al. 2021 (Natalucci et al., 2021).

Metabolic and hormonal analyses

Blood glucose, insulin, triglycerides, HDL, LDL, and total cholesterol concentrations were determined by colorimetric assays using Beckman Coulter AU Analyzers, and the homeostasis model assessment was used to estimate insulin resistance (HOMA-IR index), as detailed in (Natalucci et al., 2021). Serum concentrations of IGF-1 and IGFBP3 were measured using a solid-phase, enzyme-labeled chemiluminescent immunometric assay with the IMMULITE 2000 analyzer (Siemens Healthcare s.r.l., Italy), according to the manufacturer's protocols. The concentration of IGFBP1 was measured using Human Duo-Set enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) according to the manufacturer’s instructions. The sensitivities of the assays were 20 ng/mL (IGF-1), 0.03 ng/mL (IGFBP1), and 0.1 µg/mL (IGFBP-3). The total intra- and inter-assay CVs were respectively <3.9 and <7.7% for IGF-1 and <4.8 and <7.3% for IGFBP3 across the concentrations observed in the study. The IGFBP1 Human Duo-Set ELISA had a coefficient of variation of less than 10% across the standard curve for both intra- and inter-assay precision. The technicians analyzing the serum samples were blinded to the patient allocation.

Statistical analysis

Descriptive statistics were used to summarize the baseline characteristics. Continuous variables are expressed as mean ± standard deviations (SD) or median with interquartile range (IQR), while categorical variables are presented as absolute numbers and percentages. Between-group comparisons were performed using the chi-squared test (χ^2) or Fisher's exact test, as appropriate.

Generalized Linear Models for Repeated Measures

To evaluate the effects of the LI program on IGF-1, IGFBP1, and IGFBP3 levels as well as anthropometric, metabolic, and fitness parameters, a generalized linear model (GLM) for repeated measures was applied. This model accounted for within-subject correlations across time points (T0 vs. T1) while testing for the time effect (T0 vs. T1), group effect (intervention vs. control), and time × group interaction to assess whether responses to the intervention differed between groups. For significant effects, post-hoc tests were conducted to examine pre-to-post changes within each group, with p-values adjusted using the Bonferroni correction to control for multiple comparisons. Effect sizes were calculated using partial eta-squared (η^2) and interpreted as small (η^2=0.01), medium (η^2=0.06), or large (η^2=0.14).

Correlation Analyses

To explore the relationships between IGF-1, IGFBP1, IGFBP3, and metabolic/anthropometric variables, a correlation matrix was computed, and a correlation plot was generated. The pairwise Spearman's rank correlation was used. Additionally, an unsupervised hierarchical clustering approach was applied to the correlation matrix to identify the clusters of highly correlated variables. Specifically, distance metric: Euclidean distance was used to quantify the similarity between variables; linkage method: Ward's minimum variance method was employed to minimize intra-cluster variance. The number of clusters was chosen by inspecting the dendrogram structure and the cophenetic correlation coefficients. Hierarchical clustering and correlation visualization were performed using the corrplot and hclust functions in R (packages: "corrplot", "stats").

Regression Models

Linear regression analysis was conducted to examine whether T0 IGF-1 levels predicted T1 changes in the IGF-1 levels. Moreover, to determine the T0 IGF-1 levels at which no significant T1 changes in IGF-1 were observed, the 95% confidence interval (CI) for the elevation values in the linear regression analysis was calculated. The same approach was applied to IGFBP3 and IGFBP1 variables.

Quadratic Modeling

Given the observed variability in IGF-1 responses, we assessed whether changes in IGF-1 (Δ IGF-1) exhibited a non-linear dependence on T0 IGF-1 and fitness/metabolic adaptations. A complete quadratic regression model was fitted to evaluate the potential U-shaped or inverted U-shaped relationship. The final model was selected using backward stepwise elimination based on the Akaike Information Criterion (AIC). A two-tailed p-value < 0.05 was considered statistically significant for all tests. All statistical analyses were conducted using the R software or SPSS (version 22; IBM Corp., Armonk, NY, USA).

Results

The baseline characteristics of BCS enrolled in this study are shown in Table 1. The average age at T0 was 51.0 years (±6.4) for the CG and 52.5 years (±7.2) for the IG. The BC stage at diagnosis, menopausal status, surgery type, treatment in addition to surgery, and current endocrine therapy were not significantly different between the groups (Table 1).

Effects of LI on body composition, cardiorespiratory fitness, metabolic profile, MeDiet score and physical activity level

There was a significant interaction between group and time for $\dot{V}O_{2max}$, total PA level, and MeDiet scores, which increased more in the IG than in the CG (Table 2). No significant group × time interaction was found for BMI, fat mass, or metabolic variables. However, the main effect of time was a reduction in BMI, fat mass, insulin, HOMA-IR index, and total and LDL cholesterol in both groups at T1. The main effect of group showed that total cholesterol and total PA level were higher in the IG than in the CG.

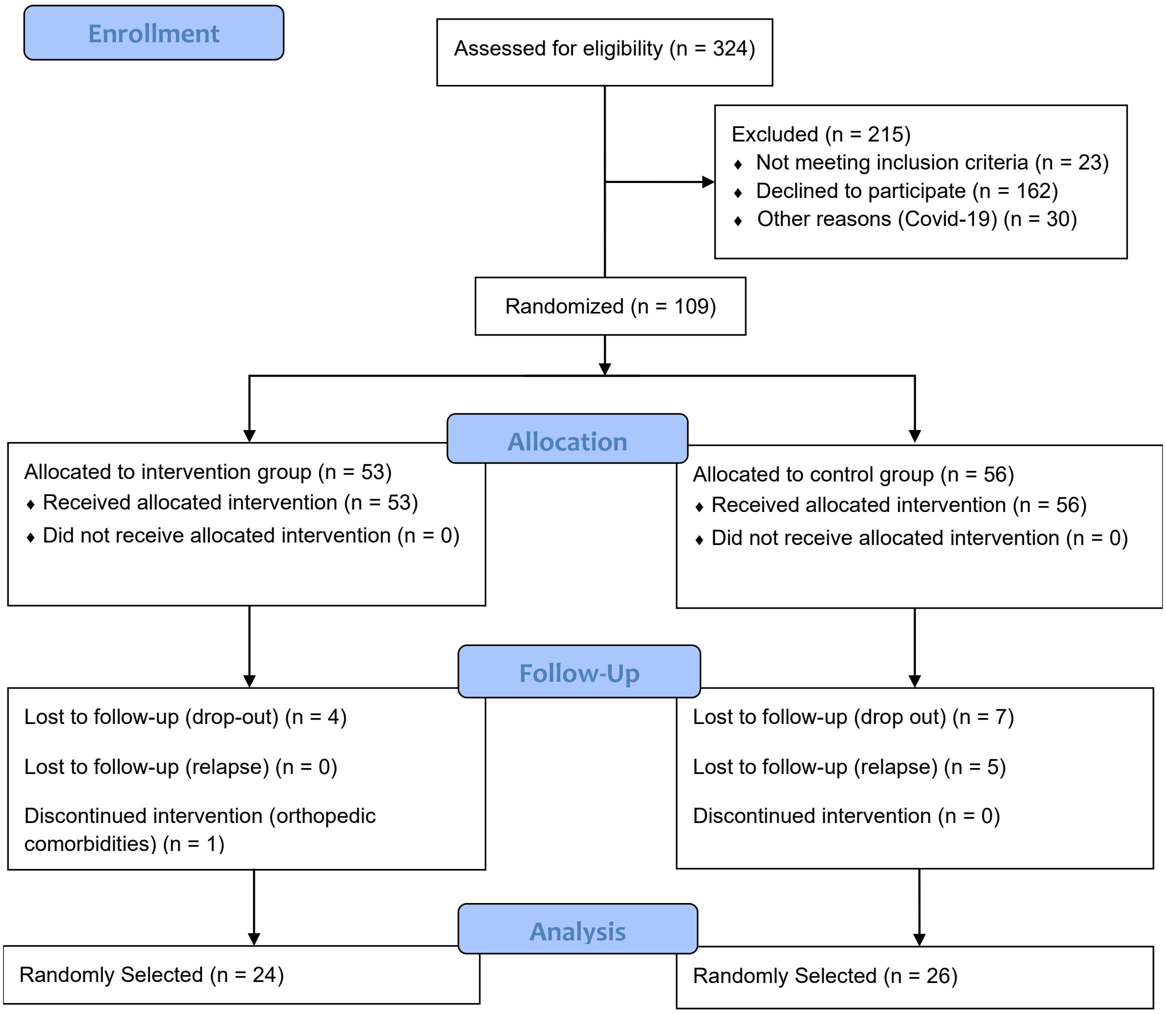

Figure 1. CONSORT flow diagram. Flow diagram of the progress through the phases of a randomized trial of two groups (i.e., enrolment, intervention, allocation, follow-up, and data analysis). CONSORT = Consolidated Standards of Reporting Trials.

Table 1. Baseline characteristics of Control group (CG) and Intervention group (IG).

	Control group (CG; n=26)		Intervention group (IG; n=24)		*p-value*
	n	%	n	%	
Stage at diagnosis					
0	7	27	6	25	
I	15	58	13	54	0.767
II	4	15	4	17	
III	0	0	1	4	
Menopausal status					
Premenopausal	11	42	10	42	0.963
Postmenopausal	15	58	14	58	
Surgery Type					
Mastectomy	4	15	2	8	
Quadrantectomy	22	85	21	88	0.769
Lumpectomy	0	0	1	4	
Treatment in addition to surgery					
Only radiation	9	35	10	42	
Only chemotherapy	2	8	1	4	0.872
Radiation and chemotherapy	9	34	9	37	
None	6	23	4	17	
Current endocrine therapy					
None	10	38	10	42	
Tamoxifen	2	8	3	12	0.786
Aromatase Inhibitor	14	54	11	46	

Differences in frequency distributions were compared by chi-squared test.

Table 2. Comparison between T0 and T1 of anthropometric and body composition, cardiorespiratory fitness, metabolic profile, MeDiet score and PA level.

	Control group (CG; n=26)			Intervention group (IG; n=24)			p (n^2_p)	p (n^2_p)	p (n^2_p)
	T0	**T1**	**Δ%**	**T0**	**T1**	**Δ%**	**Time**	**Group**	**Time × Group**
BMI (Kg/m^2)	24.7±4.7	24.0±4.4	-2.8	26.1±5.2	25.3±5.0	-3.1	**<0.001** (0.351)	0.400 (0.015)	0.665 (0.004)
Fat mass (%)	30.4±6.4	29.3±6.2	-3.6	31.9±7.6	30.1±7.1	-5.6	**<0.001** (0.362)	0.541 (0.008)	0.225 (0.033)
$\dot{V}O_{2max}$ (mL/Kg/min)	31.8±4.1	31.1±4.2	-2.2	31.3±4.9	33.2±4.6	+6.1	0.073 (0.068)	0.711 (0.003)	**0.002** (0.187)
Glucose (mg/dL)	95.2±10.2	94.3±6.6	-0.9	97.1±9.6	97.1±10.3	0.0	0.663 (0.004)	0.484 (0.011)	0.944 (0.000)
Insulin (µU/mL)	6.2±2.5	5.7±2.1	-7.1	7.0±5.21	6.1±4.8	-11.9	**0.018** (0.116)	0.606 (0.006)	0.445 (0.013)
HOMA-IR index	1.5 0.7	1.3 0.5	-10.0	1.7±1.4	1.5±1.3	-12.3	**0.019** (0.114)	0.579 (0.007)	0.591 (0.006)
Triglycerides (mg/dL)	81.6±28.9	75.5±34.3	-7.5	99.5±78.5	93.2±79.8	-6.3	0.112 (0.054)	0.144 (0.046)	0.352 (0.019)
Total Cholesterol(mg/dL)	204.0±31.4	196.9±35.4	-3.5	237.1±44.5	214.8±34.0	-9.4	**<0.001** (0.216)	**0.043** (0.086)	0.072 (0.068)
HDL (mg/dL)	61.0±13.4	62.3±13.7	+2.1	62.9±12.0	64.3±12.9	+2.2	0.062 (0.074)	0.811 (0.001)	0.826 (0.001)
LDL (mg/dL)	122.8±23.9	118.5±26.4	-3.5	145.0±31.4	129.3±25.3	-10.8	**<0.001** (0.216)	0.071 (0.069)	0.059 (0.075)
MeDiet score	7.1±1.3	7.3±2.1	+2.9	6.8±2.2	8.2±2.0	+27.9	**0.003** (0.181)	0.611 (0.006)	**0.033** (0.099)
PA level(MET-min/week)	485.4±325.6	530.4±320.4	+35	510.9±304.6	972.3±321.5	+152.4	**<0.001** (0.376)	**0.005** (0.162)	**<0.001**(0.290)

Abbreviations: T0, baseline; T1, post intervention; n^2p, partial eta squared; Δ%, percentage changes over time; BMI, body mass index; VO_{2max}, maximal oxygen uptake; HOMA, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MeDiet score, adherence to Mediterranean diet; PA level, Physical activity level.

Effects of lifestyle intervention on IGF-1, IGFBP1, and IGFBP3 levels

There was no significant interaction between group and time on IGF-1, IGFBP1, or IGFBP3 levels (Table 3). However, the main effect of time was an increase in IGFBP1 and a reduction in IGFBP3 levels at the end of the study.

Table 3. Comparison between T0 and T1 of IGF-1, IGFBP1 and IGFBP3 levels.

	Control group (CG; n=26)			Intervention group (IG; n=24)			p (η^2_p)	p (η^2_p)	p (η^2_p)
	T0	**T1**	**Δ%**	**T0**	**T1**	**Δ%**	**Time**	**Group**	**Time × Group**
IGF-1 (ng/mL)	154.3± 57.1	152.3± 51.8	-1.3	137.2± 41.6	138.9± 34.0	+1.2	0.847 (0.001)	0.274 (0.026)	0.746 (0.002)
IGFBP1 (ng/mL)	31.1± 23.9	38.0± 28.0	+22.2	28.5± 28.9	43.8± 41.6	+53.7	**0.001** (0.208)	0.850 (0.001)	0.206 (0.036)
IGFBP3 (µg/mL)	6.0±1.2	5.8± 1.1	-4.3	5.8± 0.9	5.3± 0.7	-8.3	**0.001** (0.202)	0.146 (0.045)	0.285 (0.025)

Abbreviations: T0, baseline; T1, after intervention period; %Δ, percentage change over time; IGF-1, Insulin-like Growth Factor-1; IGFBP1, IGF-1 Binding Protein 1; IGFBP3, IGF-1 Binding Protein 3.

Correlation between IGF-1, IGFBP1 and IGFBP3 levels with variables measured at T0 and with changes (Δ) between T0 and T1

The correlations between IGF-1, IGFBP1, and IGFBP3 levels and body composition, $\dot{V}O_{2max}$, and metabolic variables at T0 are shown in Figure 2. IGF-1 levels at T0 were negatively correlated with IGFBP1 (r= -0.33; p=0.046) and positively correlated with IGFBP3 (r=0.40; p=0.002). IGFBP1 levels were negatively correlated with LDL levels (r= -0.40; p=0.007), BMI (r= -0.52; p < 0.0001), fat mass (r= -0.54; p < 0.0001), and insulin levels (r= -0.41; p=0.041). IGFBP3 levels were positively correlated with insulin levels (r=0.33; p=0.008) and HOMA-IR index (r=0.29; p=0.011). Hierarchical cluster analysis showed that the variables could be categorized into three clusters (highlighted boxes in Figure 2). As expected, BMI, fat mass, and metabolic variables shared the same cluster (bottom-right box in Figure 2). IGF-1 and IGFBP3 levels belonged to the same median cluster and were positively correlated. The top-left cluster contained IGFBP1, $\dot{V}O_{2max}$, and HDL levels, which showed a positive correlation.

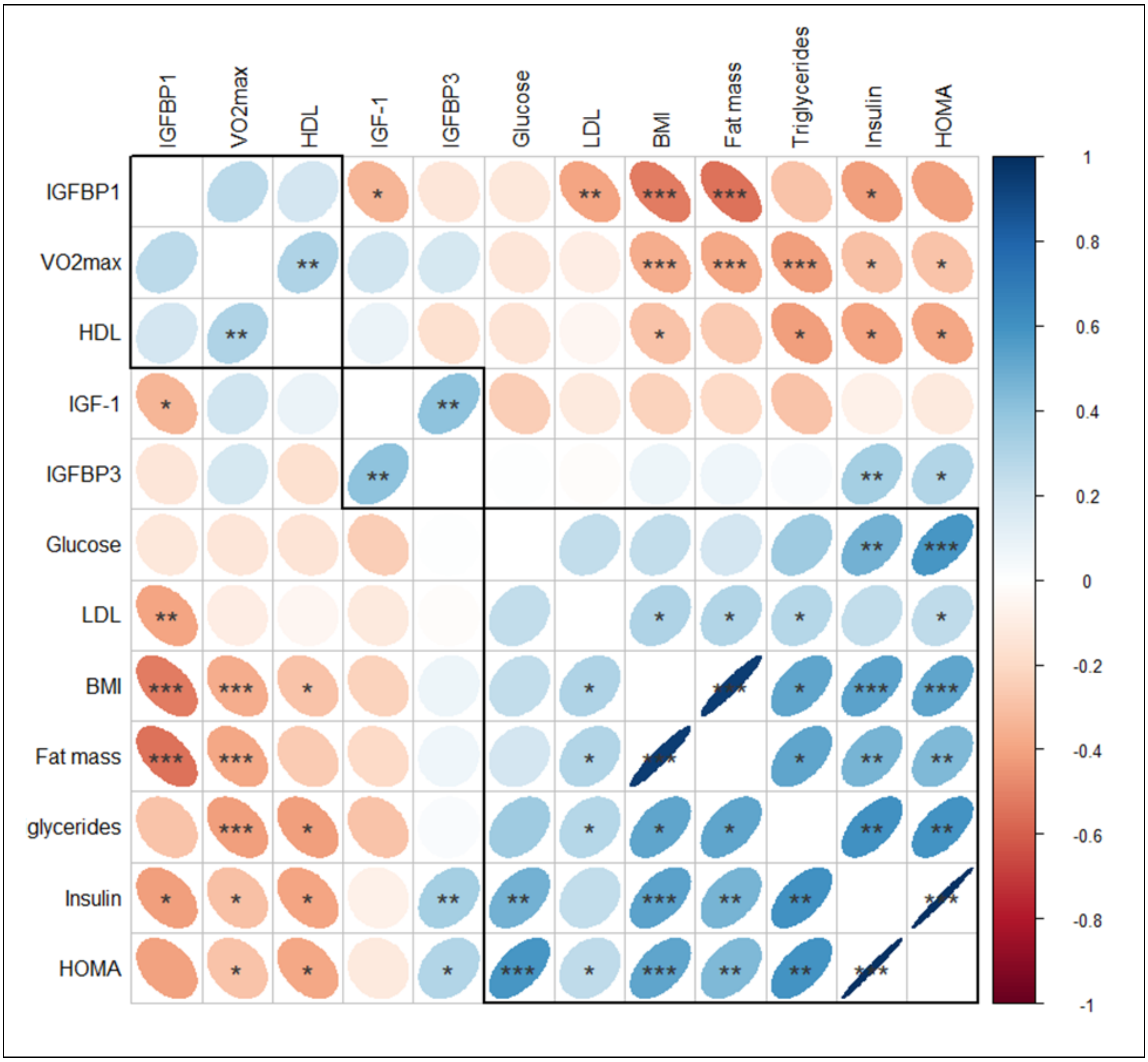

Figure 2. Correlation plot of IGF-1, IGFBP1 and IGFBP3 levels and body composition, $\dot{V}O_{2max}$, and metabolic variables measured at T0. The highlight boxes indicate highly correlated variables. Spearman rank correlation, *p <0.05; **p<0.01; ***p<0.001.

Subsequently, we tested the hypothesis that IGF-1, IGFBP1, and IGFBP3 levels change depending on their T0 values (Figure 3) (Devin et al., 2016; Nishida et al., 2010; Orsatti et al., 2008; Sillanpää et al., 2010; Wei et al., 2017). There was a negative correlation between T0 IGF-1 level and Δ IGF-1 ($r = -0.32$; $p=0.026$). We then calculated the 95% CI for the elevation values in the linear regression to define the T0 IGF-1 values associated with non-significant Δ

IGF-1 (i.e., ordinate equals zero). The CI for IGF-1 ranges from 94.7 ng/mL to 173.3 ng/mL, with a mean of 137.5 ng/mL. This indicated that participants with T0 IGF-1 values within this range exhibited no significant Δ IGF-1 following LI. In contrast, IGF-1 levels increased in participants with T0 values below 94.7 ng/mL and decreased in those with T0 values above 173.3 ng/mL. Similar results were obtained for IGFBP3 (r= -0.42; p=0.002; CI for the elevation values 2.5 µg/mL to 5.4 µg/mL; mean=4.7 µg/mL). IGFBP-1 values showed marked asymmetry in distribution, and no relationship was found between T0 IGFBP1 values and Δ IGFBP1 (r= -0.13; p = 0.366).

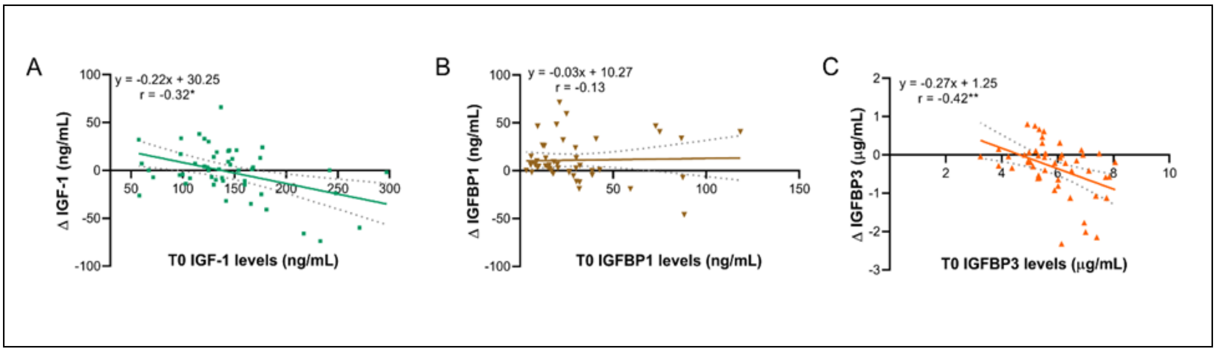

Figure 3. Correlations between: (A) T0 IGF-1 levels and Δ IGF-1; (B) T0 IGFBP1 levels and Δ IGFBP1; (C) T0 IGFBP3 levels and Δ IGFBP3. Spearman rank correlation, *p<0.05; **p<0.01.

The correlation between Δ IGF-1, Δ IGFBP1, Δ IGFBP3, Δ BMI, Δ fat mass, Δ $\dot{V}O_{2max}$, Δ insulin, Δ HOMA-IR index, Δ LDL, Δ PA level, and Δ MeDiet score was also analyzed (Figure 4). Δ fat mass was positively correlated with Δ IGF-1 (r 0.35; p 0.02) and negatively correlated with Δ IGFBP1 (r= -0.33; p=0.02) (Figure 4A); Δ insulin was negatively correlated with Δ IGFBP1 (r= -0.35; p=0.01) and positively correlated with Δ IGFBP3 (r=0.42; p=0.002) (Figure 4B); and, Δ IGFBP1 was negatively correlated with Δ IGF-1 (r= -0.34; p=0.02) and Δ IGFBP3 (r= -0.34; p=0.02) (Figure 4C). Subsequently, we analyzed the three-dimensional quadratic relationship between T0 IGF-1 levels, Δ IGF-1, and Δ $\dot{V}O_{2max}$ (Figure 5A) or Δ fat mass (Figure 5B). This analysis tested the non-linear dependence of Δ IGF-1 versus T0 IGF-1 levels, considering also LI-induced $\dot{V}O_{2max}$ or fat mass variation. Δ $\dot{V}O_{2max}$ was chosen because it mainly depends on PA levels, whereas Δ fat mass mostly depends on variations in dietary habits. The relationship between T0 IGF-1 levels, Δ IGF-1, and Δ $\dot{V}O_{2max}$ followed a U-shaped pattern; participants with T0 IGF-1 levels above the 82nd percentile, corresponding to the upper limit of the 95% CI for the elevation level, exhibited a decrease in IGF-1 levels when $\dot{V}O_{2max}$ improved. Conversely, in patients with T0 IGF-1 levels below the 12th percentile, corresponding to the lower limit of

95% CI, an increase in $\dot{V}O_{2max}$ was associated with an increase in IGF-1 levels. The relationship between T0 IGF-1 levels and Δ IGF-1 and Δ fat mass displayed an inverted U-shaped pattern: participants with T0 IGF-1 levels above the 82nd percentile showed a reduction in IGF-1 levels when fat mass decreased, whereas those with T0 IGF-1 levels below the 12th percentile showed an increase in IGF-1 levels in response to fat mass reduction.

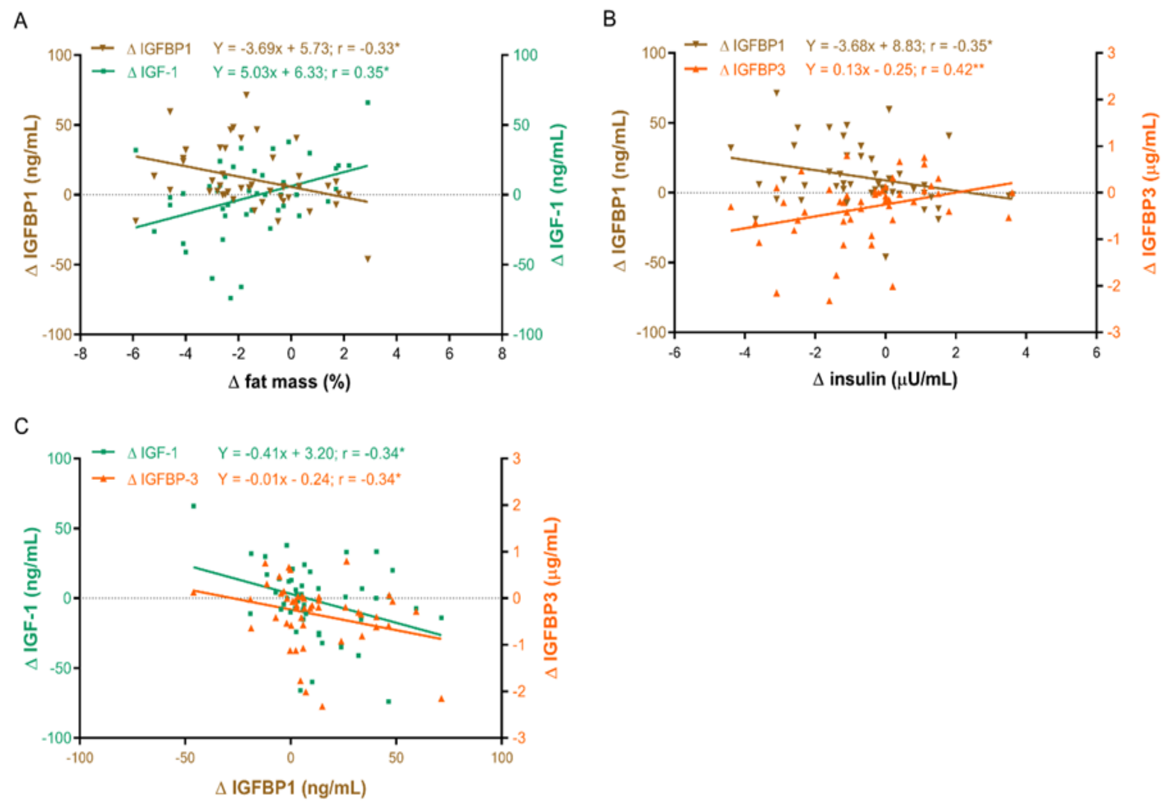

Figure 4. Correlations between: (A) Δ fat mass, IGFBP1 and IGF-1; (B) Δ insulin, IGFBP1 and IGFBP3; (C) Δ IGFBP1, IGF-1 and IGFBP3. Spearman rank correlation, *p<0.05; **p<0.01.

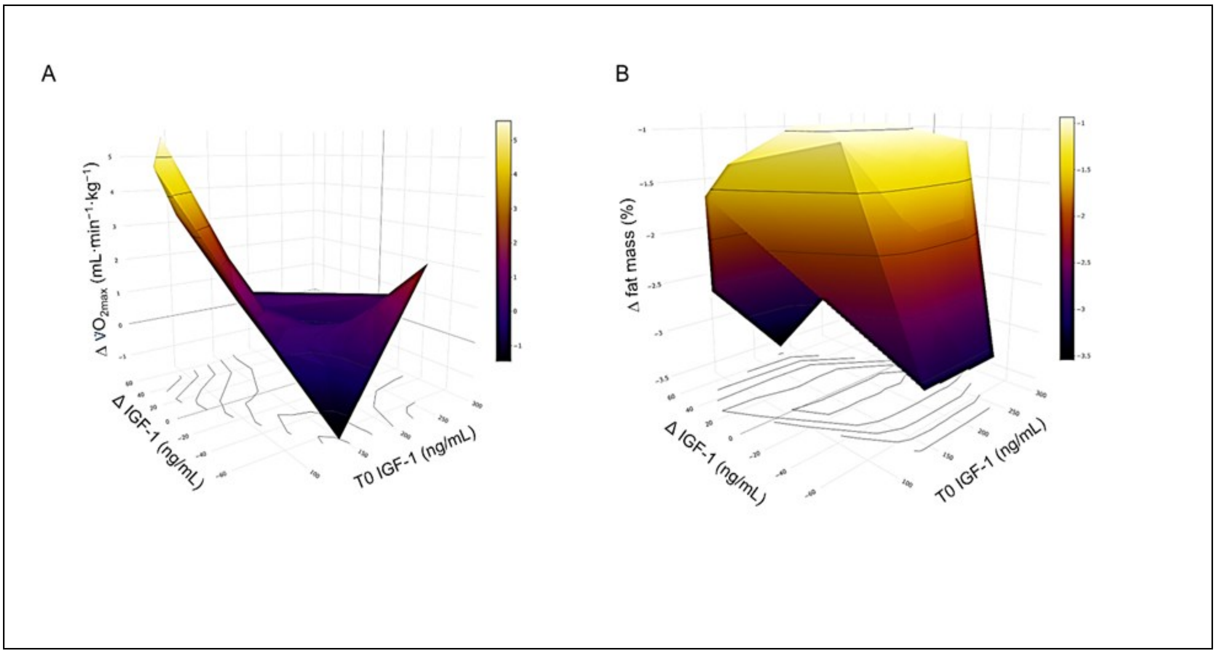

Figure 5. Three-dimensional quadratic relationship between T0 IGF-1 levels, Δ IGF-1, and (A) Δ $\dot{V}O_{2max}$ and (B) Δ fat mass.

Discussion

This study demonstrated that a LI program based on exercise and Mediterranean diet recommendations (MoviS trial, protocol: NCT 04818359) increased IGFBP1 levels, decreased IGFBP3 levels, and modulated IGF-1 levels in BCS, depending on its baseline values. The BCS participants in the MoviS trial showed improvements in anthropometric and body composition values (BMI and fat mass percentage), physical activity level (PA level), adherence to the Mediterranean diet (MeDiet score), and metabolic markers (insulin, HOMA-IR index, total and LDL cholesterol) in both CG and IG. The magnitude of improvement was greater in the IG, particularly for the $\dot{V}O_{2max}$, total PA, and MeDiet scores, likely due to the more structured and supervised aerobic exercise training provided in this group. With regard to the IGF-1 system, although mean serum IGF-1 levels remained stable after LI, significant changes in IGFBPs were observed in both the CG and IG. Specifically, IGFBP1 levels increased, whereas IGFBP3 levels decreased following the 3-month LI program, suggesting that LI modulated IGF-1 bioavailability indirectly through IGFBPs regulation.

Baseline analyses showed that IGFBP1 levels were negatively correlated with IGF-1, LDL, BMI, fat mass, and insulin levels. This is consistent with previous findings indicating that liver IGFBP1 expression and secretion decreases under anabolic conditions, leading to increased IGF-1 bioactivity (Kajimura et al., 2006; Suwanichkul et al., 1994). Thus, the reductions in fat mass and insulin observed at the end of LI may have directly contributed to increased IGFBP1 levels. Notably, IGFBP-1 is unique among IGFBPs in its rapid response to metabolic and hormonal variations (Katz et al., 2002; Rajwani et al., 2012), allowing it to regulate IGF-1 bioactivity in response to fasting and insulin fluctuations. Conversely, IGFBP3 was positively correlated with IGF-1, insulin, and HOMA-IR levels at baseline, and with LI-induced changes in insulin levels. These opposing trends between IGFBP1 and IGFBP3 underscore the complex regulation of IGFBPs in response to lifestyle changes and highlight the importance of measuring IGFBPs when assessing the impact of interventions on the IGF-1 system.

Another key finding of this study was that changes in IGF-1 and IGFBP3 levels were dependent on their baseline levels. Specifically, participants with higher baseline IGF-1 levels (> 173 ng/mL) exhibited a reduction in IGF-1 levels after LI, whereas those with low baseline IGF-1 levels (< 95 ng/mL) showed an increase. A similar pattern was observed for IGFBP3.

The high inter-individual variation in circulating IGF-1 levels, attributable to genetics, age, nutritional status, and health-related factors, complicates the prediction modelling of IGF-1 responses to LI. Furthermore, the absence of a clinically defined threshold for IGF-1 levels further challenges the ability to predict the effects of LI on IGF-1 levels. To address this complexity, we tested for the presence of a non-linear relationship between LI-induced changes in IGF-1 levels and anthropometric, $\dot{V}O_{2max}$ and metabolic variations, accounting for baseline IGF-1 levels.

We demonstrated that a non-linear model better explained the association between baseline IGF-1 levels and changes in $\dot{V}O_{2max}$, fat mass, and IGF-1 levels, revealing a U-shaped relationship. Specifically, the LI-induced improvement in $\dot{V}O_{2max}$ was associated with increased IGF-1 levels in participants with low baseline values (i.e., below the 12th percentile) and with a reduction in those with high baseline values (i.e., above the 82nd percentile). Along this line, an inverted U-shaped relationship emerged between baseline IGF-1 levels and changes in IGF-1 and fat mass, where a reduction in fat mass was associated with an increase in IGF-1 in participants with low baseline values and a decrease in those with high baseline values. In contrast, BCS participants who did not improve $\dot{V}O_{2max}$ or fat mass, as well as those with mid-range baseline IGF-1 levels (between the 12th and 82nd percentiles), exhibited minimal changes in IGF-1 levels.

These findings align with prior research suggesting a U-shaped relationship between IGF-1 levels and disease risks, including cardiovascular disease, diabetes, and overall mortality, in which both low and high IGF-1 levels are associated with adverse health outcomes (Lin et al., 2023; Mukama et al., 2023; Rahmani et al., 2022; Zhang et al., 2021). Similarly, a recent meta-analysis involving 30,876 participants suggested that low-to mid-range IGF-1 levels (120-160 ng/ml) might represent an optimal range indicative of health (Rahmani et al., 2022). Additionally, a large study of 945 older adults in the U.S. found that individuals with stable IGF-1 levels over a 11.3-year follow-up showed lower mortality compared to those with fluctuating IGF-1 levels (Sanders et al., 2018). In this study, we demonstrated that the modulation of systemic IGF-1 levels after the LI program occurred only in participants with low or high basal values, helping to normalize IGF-1 levels to a potentially physiological range. This highlights the importance of considering baseline IGF-1 levels to accurately model and predict the effects of LI on the IGF-1 system.

The findings of this study have several important implications for the design of future RCTs and other studies that assess the effects of LI on the IGF-1 system. First, our results indicate that lifestyle-based interventions markedly affect IGFBP levels, with opposing responses observed for IGFBP1 and IGFBP3. Given that approximately 99% of circulating IGF-1 is bound to IGFBPs (Allard & Duan, 2018), ignoring the effects of LI on IGFBPs may yield misleading conclusions regarding IGF-1 system modulation through diet and exercise.

Second, in high-risk populations such as BCS, IGF-1 should not be viewed solely as a pro-tumoral factor, but also as a potential health marker (Nindl et al., 2011; Nindl & Pierce, 2010). Indeed, while high IGF-1 is causally associated with an increased cancer risk in BCS, low IGF-1 levels might indicate poor general health due to cancer therapies, reduced lean mass, and inadequate nutritional intake (Clemmons, 2007; De Santi et al., 2023; Devin et al., 2016; Fanti & Longo, 2024). This observation emphasizes the need for personalized approaches when designing interventions aimed at modulating the IGF-1 system. In this scenario, monitoring circulating IGF-1 levels might allow us to identify at-risk individuals, namely those with relatively low or high IGF-1 levels and those with high IGF-1 fluctuation, who are most likely to benefit from a LI. Rather than focusing on a global increase or decrease in IGF-1 levels, the modulation of IGF-1 in response to LIs should be viewed as an adaptive response aimed at maintaining homeostasis.

This study had several limitations. First, the BCS participants analyzed were non-physically active and had at least one hormonal risk factor or metabolic syndrome, which may limit the generalizability to other, healthier BCS groups. Second, since both CG and IG participants showed improvements in Mediterranean diet adherence, physical activity level, anthropometric and body composition measurements, and metabolic markers, we could not clearly distinguish the independent effects of the Mediterranean diet and aerobic exercise on the IGF-1 system. The structured counselling session about lifestyle recommendations provided to all MoviS participants may explain the lack of significant differences between the CG and IG. Third, since only IGFBP3 and IGFBP1 levels were measured, we cannot exclude the possibility that other IGFBPs that potentially affect IGF-1 bioactivity may have been modulated by LI. In this regard, further efforts should be directed toward the development of high-throughput assays to directly measure IGF-1 bioactivity (Chen et al., 2003).

Conclusions

In conclusion, our study demonstrates that the IGF-1 system is strongly influenced by LI, particularly through alterations in IGFBP1 and IGFBP3 levels. Furthermore, the apparent stability of circulating IGF-1 levels pre- and post-intervention was due to the divergent modulation observed in BCS participants with relatively low or high baseline IGF-1 levels. Given its affordability, robustness, and consistency, IGF-1 may serve as a valuable biomarker for identifying high-risk individuals, namely those with relatively low or high IGF-1 levels or with high IGF-1 variability over time, for inclusion in LI programs. Future research should focus on exploring the dynamic interactions between IGF-1, its binding proteins, and lifestyle factors, and examine long-term IGF-1 trajectory changes and their implications for health and disease outcomes.

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the Human Research Ethics Committee of the University of Urbino Carlo Bo (Protocol N 21 of July 10, 2019), and written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

GA, DS, MDS, RE and EB conceived the study; VN, MI, CFM, FL and SA supervised exercise intervention and performed fitness evaluations and analyses; AV, MI, EB and RE assessed dietary habits; LV, RS, GB and SB performed blood sample collection and analyses; MF, VC and RE performed clinical parameters evaluation; DS and MBLR performed data collection and statistical analysis; EB and RE supervision and acquisition of funding. GA, DS and MDS drafted the manuscript. All authors revised the article and approved the submitted version.

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Study 4

Original Article

The Role of $\dot{V}O_2$ Reserve and Perceived fatigability on Free-living Activity Energy Expenditure in Older Adults

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Abstract

Reserve of oxygen uptake (*$\dot{V}O_2R$)* and perceived fatigability may be related and represents two important components of physical capacity that could influence the Activity Energy Expenditure (AEE) in free-living conditions in older adults. However, the relationship between physiological factors and subjective measurements is a complex field that needs further research in relation to ageing. This study aims to examine how $\dot{V}O_2R$ and perceived physical fatigability influenced free-living AEE in older adults. Participants (n=77, 43% Female) were from the cross-sectional study of ENerGetics in Old AGE (ENGAGE) baseline cohort (aged 80.23±3.70 years).

The ENGAGE study included 14 days of both laboratory and free-living assessment: anthropometric and body composition parameters, Resting Metabolic Rate (RMR), cardiorespiratory fitness level ($\dot{V}O_{2max}$ [mL·min^{-1}·kg^{-1}]), Total Energy Expenditure (TEE) and AEE, Pittsburgh fatigability Scale [PFS physical score], and Brief Pain Inventory (BPI) questionnaire. $\dot{V}O_2R$ was calculate using the following formula: ($\dot{V}O_{2max}$ - RMR), and AEE was calculated considering the TEE measured by Doubly-Labeled Water (DLW) method and using the following formula by Weir: [AEE = (TEE x 0.9) - RMR]. Correlations were done to examine possible relation between $\dot{V}O_2 R$, AEE, and PFS physical score. A Wilcoxon rank-sum test was

used to verify if participants with a low level of PFS physical score were different in all variables measured in this study compared to participants with a high level of PFS physical score. Multiple Linear Regression Models (MLRs) were used to examine whether *$\dot{V}O_2$ R* and PFS physical score can predict AEE in older adults. A mediation analysis was done to verify whether PFS physical score may be a mediator in relation between *$\dot{V}O_2R$* and AEE. A K-medians Clustering analysis was performed to divide all participants in different clusters using the medians of *$\dot{V}O_2R$* and AEE.

$\dot{V}O_2R$ was significantly correlated with PFS physical score (Pearson's r= - 0.360, p=0.002), and with AEE (Spearman's rho=0.268, p=0.018), but no correlation was found between PFS physical score and AEE (Spearman's rho= - 0.065, p=0.580). Wilcoxon rank-sum test showed that older adults with high level of PFS physical score had low level of both *$\dot{V}O_2R$* (p=0.020) and $\dot{V}O_{2max}$ (p=0.022). In the MLR final model (progressively adjusted for age, sex, body weight or % of fat mass, n chronic conditions and pain) *$\dot{V}O_2R$* ($p < 0.001$), age (p=0.014), and % of fat mass (p=0.069) explained 30.5% of the variance in AEE (adjusted $R^2 = 0.305$, $p < 0.001$). Other variables were excluded by the stepwise procedure at $p \geq 0.10$. Mediation analysis revealed a significantly direct effect between *$\dot{V}O_2R$* and AEE ($p < 0.001$). No indirect effect of PFS physical score was found (p=0.824). The K-medians Clustering analysis divided all 77 participants in 4 different clusters representing different profiles of older adults considering sex differences.

The results shown that *$\dot{V}O_2R$* was significantly negative correlated with PFS physical score, this aspect was confirmed by the Wilcoxon rank-sum test (older adults with high level of *$\dot{V}O_2R$* reported low level of fatigability). Despite *$\dot{V}O_2R$* and AEE was significantly correlated, no differences were found in terms of AEE between participants with low or high level of fatigability perceived. In addition, MLRs revealed that *$\dot{V}O_2R$,* age and % of fat mass were predictors of AEE, while fatigability was excluded in the final model. The mediation analysis confirmed a direct effect of *$\dot{V}O_2R$* on AEE with no mediation of PFS physical score. Interestingly, 4 different clusters were detected, highlighting different older adult profiles, and showed that fatigability was significantly different for women in relation to the cluster where they were allocated.

Key words: older adults, *$\dot{V}O_2R$*, perceived fatigability, activity energy expenditure, free-living conditions

Introduction

In Europe, current demographic data showed an increase in the number of older adults aged 65 and above. From 2019 to 2050, this specific population is estimated to grow by around 90 million (Corselli-Nordblad and Strandell, 2020). Longer life expectancy (the population aged from 77 to 84 and from 85 and above is projected to increase by 56% and 114%, respectively) represents a challenge for the European healthcare system. The problem is that a longer life expectancy does not always correspond to good quality of life and health. In the older population, diseases and comorbidities are being diagnosed and are often accompanied by malnutrition and a sedentary lifestyle (Christensen et al., 2009, Izquierdo et al., 2025). The ratio of healthy life years to overall life expectancy has generally increased: in 2022, a 65-year-old European man was expected to live 8.9 years without activity limitations, 9.2 years if considering a woman with the same age (Eurostat, 2025)[1]. However, in some countries, such as Denmark, Sweden, and Germany, there was a decline in healthy life years expectancy from 2010 to 2022 (Eurostat, 2025)[2]. The concept of healthy ageing was defined as "*the process of developing and maintaining the functional ability that enables well-being in older age"* by the World Health Organization (Healthy ageing and functional ability, WHO-2022). Having *functional ability* skills (e.g., capacity to meet basic needs, having a social active life or build relationships) was extremely related to healthy lifestyle characterized by physical (e.g., physical fitness components) and mental (e.g., cognitive function) health (Healthy ageing and functional ability, WHO-2022). However, both physical and mental health may be influenced by age-related changes, which can influence behavioral factors such as low physical activity (PA) levels and increased sedentary lifestyle. In addition, social environments can affect health directly or through barriers or incentives that affect opportunities and healthy decisions (e.g., eating a balanced diet, engaging in regular PA, and refraining from tobacco use), (Ageing and Health, WHO-2025). This is the reason why WHO proposed the "Global Action Plan" to promote PA levels and maintain physical and mental health supporting healthy ageing and

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[1]Eurostat, 2025. https://ec.europa.eu/eurostat/databrowser/view/tepsr_sp320/default/table?lang=en&category=t_hlth-t_hlth_state

[2]Eurostat, 2025. https://ec.europa.eu/eurostat/statistics-explained/SEPDF/cache/1274.pdf

reducing inactivity-related health issues (Global action plan on physical activity 2018-2030, WHO). One of the key points of age-related changes is the physiological decline in specific cardiorespiratory and metabolic parameters such as maximum oxygen uptake ($\dot{V}O_{2max}$) and Resting Metabolic Rate (RMR) respectively. Scientific evidence reported that reductions in muscle oxygen delivery, due to reduced cardiac output or to its maldistribution, appear to play the dominant role up until late middle age. On the other hand, there is a decline in both skeletal muscle and skeletal muscle oxidative capacity with ageing, due in part to mitochondrial dysfunction (Betik et al., 2008, Kim et al., 2016, Shur et al., 2021). Declines in muscle mass also influence energy balance and consequently RMR more than body fat mass changes. Furthermore, a 12-year longitudinal study (Lührmann et al., 2009) of well-functioning older adults (aged 60-90) (n=516) showed a decline in RMR up to 5% per decade. A recent systematic review reported that RMR in older adults was approximately 25% lower (2.7 $mLO_2 \cdot min^{-1} \cdot kg^{-1}$) than the conventional estimation equal to 3.5 $mLO_2 \cdot min^{-1} \cdot kg^{-1}$ (Leal-Martín et al., 2022). RMR decline reflected both Total Energy Expenditure (TEE) and Activity Energy Expenditure (AEE) in older adults (Manini, 2010). AEE is also associated with reduced mobility impairment and thus preserved physical function (Manini, 2010, Manini et al., 2009). AEE and reduced physical functioning may be related to various factors such as perceived pain, chronic conditions or perceived fatigability (Barbosa et al., 2016, Eldadah, 2010). Fatigability, which defines fatigue in relation to an activity with specific duration and intensity (Glynn et al., 2015), was associated with worse physical performance, lower PA level, mobility decline, and impaired cognition (Gay et al., 2025, Barbosa et al., 2016, Qiao et al., 2022). Perceived fatigability prevalence in older adults (from 20 to 89.5%) is lower among men than in women. These findings suggest different perceptions considering sex differences (Gay et al., 2025). Considering all these aspects, studying specific parameters such as the reserve of oxygen uptake ($\dot{V}O_2R$), defined as the difference between $\dot{V}O_{2max}$ and RMR, may be a good strategy to account for the physiological decline due to ageing and individual physiological characteristics, rather than $\dot{V}O_{2max}$. Similarly, investigating TEE and AEE may be relevant to better understand both behaviour and physical functioning in older adults. In fact, TEE was defined as the sum of RMR, AEE and Diet-Induced Thermogenesis (DIT), while AEE encompasses increased energy expenditure derived from both exercise (e.g., aerobic exercise and/or resistance training), non-exercise activities (e.g., cycling for transportation) and Activities of Daily Living (ADL) (Warren et al., 2010, Hills et al., 2014). For these reasons AEE is a result of multidimensional behaviour which represents the most variable component of TEE. The gold standard method to measure TTE is the Doubly Labeled Water (DLW) method; the most accurate method for calculating AEE in free-living conditions is to subtract the measured or estimated RMR and the estimated DIT from the TEE measured using the DLW method (Hills et al., 2014). Recently, the literature showed an interest in studying the relationship between physiological components and subjective measurements (such as questionnaires) to combine the results and find a better way to explain adaptation of specific intervention in older adults or to explain their behaviour in free-living condition (Glynn et al., 2015, Aftab et al., 2022). Actually, it is unknown whether $\dot{V}O_2R$ and perceived physical fatigability may interact in their contribution to free-living AEE in older adults. The interaction between physiological components and subjective measurements may explain free-living behavior in older adults and may also explain the AEE in relation to ageing, a known factor that influences physical function.

The main aims of this study were to (i) examine possible relation between $\dot{V}O_2R$, AEE, and perceived physical fatigability, (ii) to examine whether $\dot{V}O_2R$ and perceived physical fatigability can predict AEE in older adults, and (iii) to find different profiles of older adults characterized by specific level of $\dot{V}O_2R$, AEE, and perceived physical fatigability considering sex differences. We hypothesized that older adults with high level of $\dot{V}O_2R$ and low perceived physical fatigability had high AEE.

Materials and Methods

Participants

Seventy-seven (43% female, age 80.23±3.70 years [mean±SD]) community-dwelling Danish older adults were recruited (e.g., nationally regulated preventive home visits and newspaper announcement) for this study. All participants received an informational letter, and they are recruited following specific inclusion criteria: (i) age 75 years and above; (ii) intact cognitive function (score > 3 in the Short Form Mini-Mental State Examination) (Haubois et al., 2011); and (iii) passed a medical screening (during which all participants reported number of chronic conditions). Metabolic abnormality (i.e., severe organ or mental disease, currently under chemotherapy treatment, had a drug and/or alcohol addiction, currently bed-ridden) with potential altered metabolic state or if cardiorespiratory fitness level ($\dot{V}O_{2max}$) assessments were inadvisable by the general practitioner were considered as exclusion criteria. All 77 participants signed a written informed consent before the beginning of the study.

Study Design

Participants were from the cross-sectional study of Energetics in Old Age (ENGAGE) baseline cohort which included both laboratory and free-living assessment. The ENGAGE study is part of a larger Horizon 2020 project named PROMISS (Prevention of Malnutrition in Senior Subjects) and was carried out between August 2019 and June 2022. The Ethical approval was received from The Regional Scientific Ethical Committees for Southern Denmark (S- 20170150) and was registered in clinicaltrials.gov (NCT04821713). All participants underwent laboratory assessments within a 14-days period.

During day 1, anthropometric, body composition, and Resting Metabolic Rate (RMR) measurements were performed. Day 2 and 3 were used for the data collection of Doubly-Labeled Water method (DLW) to obtain the Total Energy Expenditure (TTE) in free-living conditions. During day 14, the participants performed a $\dot{V}O_{2max}$ test, and reported a perceived physical fatigability and pain by filling out the Pittsburgh Fatigability Scale (PFS) and the Brief Pain Inventory (BPI) questionnaires, respectively. Collection of the last samples was done 14 days after the first laboratory measurement day for DLW. $\dot{V}O_2R$ was calculate using the following formula: ($\dot{V}O_{2max}$ - RMR). Activity Energy Expenditure (hereafter referred to as "AEE") was calculated considering the TEE measured by DLW and using the following formula by Weir: [AEE = (TEE x 0.9) - RMR] (Weir, 1949).

Anthropometrics and body composition measurements

During day 1, anthropometric and body composition measurements were obtained. Height (cm) was accurately measured using a standard stadiometer (SECA, Germany) up to the nearest 0.1 cm. Waist circumference (cm) was assessed as the minimal abdominal circumference using a flexible, non-elastic tape (ruler width approximately 0.7 cm). During the measurements, participants were in a standing position. The technical error of measurement was 0.48 cm for men and 1.15 cm for women. The bioelectrical impedance analysis (BIA) device (TANITA, model BC-420MA) was used to measure body weight (Kg) and body composition (% of fat mass and % of fat-free mass). An estimated weight of the clothes (0.7 kg) was subtracted from the total body weight measurement of each participant (Skjødt et al., 2025). Body Mass Index (BMI) was calculated by dividing the body weight in kilograms by the square of the height in meters (kg/m^2).

Resting Metabolic Rate

During day 1, RMR ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$) was assessed in the morning after an overnight fast of 12h. Participants were also refraining from exercise, smoking, consumption of coffee, tea, and alcohol, and were resting in a supine position in a quiet room with comfortable temperature (22-24 °C). The collection of expired gas started 30 min after quiet laying to ensure steady state using the gold standard Douglas bag method (Shephard et al., 2017). During $\dot{V}O_2$ measurement all participants have worn a facemask (V2 mask, Hans Rudolph, IL, USA), the mask was connected to a two-way non-rebreathing valve (Innovision A/S) which was connected to two tubes (one for inhaling and one for exhaling). The tube for exhaling was connected to a custom-built three-way valve system (Håkan Eriksson, Karolinska University Hospital, Stockholm, Sweden), which was equipped with a stopwatch (in this way the researcher was enabled to distribute the expired air either into the ambient or into the Douglas bag [130 L, PU-coated fabric; C. Fritze Consulting Svedala, Sweden]). After the steady state period, the expired air was collected and analyzed within a short while to avoid gas diffusion. The gas analyzer (INN00400, Innocor, Denmark) was calibrated with a two-point calibration before the measurements in each Douglas bag (21% O_2 / 0% O_2 and 18 % O_2 / 6% CO_2) and was used to measure the concentration of O_2 and CO_2 in the expired air of all participants.

A gas spirometer pump was used to empty each bag (Rayfield Equipment, Vermont, USA) and the volume of expired air was calculated. The gas spirometer was previously validated by placing it in series with a 120 L Tissot spirometer. In the end, the temperature of the air inside the bags and the barometric pressure in the room was collected (this step is necessary to later convert the volume into the Body Temperature, ambient Pressure, Saturated with water vapor [BTPS] and to Standard Temperature, Pressure, Dry - no water vapor [STPD] units).

Cardiorespiratory fitness level

$\dot{V}O_{2max}$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$), was assessed through an incremental treadmill walking test. A computerized mixing chamber system was used to measure the expired gas (O2CPX, Oxigraf, USA, version 8.02, Innocor, Denmark). Before the assessment the gas and flow analyzers were calibrated with a two-point calibration of known gas concentrations and a 3L syringe, respectively. All participants performed a short warmup before the $\dot{V}O_{2max}$ test to favoring the familiarization to the treadmill (Skjødt et al., 2025). During the test, participants walked with a constant comfortable self-selected walking speed (the protocol for selecting the walking speed is described in detail by Skjødt et al., 2024), while the slope was increased every second minute starting at 0% inclination followed by 5%, 10%, 12% inclinations and continuing with a 2% increase every second minute until exhaustion. Heart Rate (HR) was continuously monitored during the test and Rate of Perceived Exertion (RPE) was reported immediately after completion of the test. $\dot{V}O_{2max}$ was calculated as the three highest consecutive 10-s intervals ($\dot{V}O_2$ data were provided for each 10-s intervals). The $\dot{V}O_{2max}$ level was defined valid if al least two of the following three criteria were reached: (1) HR within 10 beats/min of age-predicted maximal HR (220 - age), (2) respiratory exchange ratio > 1.10, (3) a Borg scale score (RPE) of ≥ 17. Participants were given strong verbal encouragement during the test to achieve their maximum exertion.

Doubly-Labeled Water Method

DLW method is a gold standard method for measuring TTE and it is an isotope-based technique for measuring the total carbon dioxide production (rCO_2) in free-living human (and animals) (Speakman et al., 2021; Schoeller et al., 1996; Schoeller et al., 2008).

The method is based on the observation that the oxygen (O_2) in respiratory carbon dioxide (CO_2) is in complete isotopic equilibrium with the O_2 in body water (H_2O). Labeled O_2 introduced into the body H_2O is eliminated as both H_2O and CO_2, while a simultaneously introduced label of hydrogen, such as deuterium, will be eliminated only as H_2O. The difference in elimination rates of the two isotopes gives a measure of rCO_2 (Speakman et al., 2021). In the DLW short protocol (which was used in this study) participants are required to give a total of six urine and one saliva samples: four urine and one saliva samples before and after drinking an initial dose of DLW ($^2H^{18}O$), and then two urine specimens will be collected after 14 days. During the period between the initial and final urine samplings, participants are free to carry out their normal activities (for this reason the DLW method is an ideal method because it is noninvasive and nonrestrictive). In this study, all participants arrived at the laboratory in a fasting state and provided the baseline urine sample, and then they ingested a pre-weighed dose (according to individual body weight) of $^2H^{18}O$ (100 ml, 0.20 g/Kg total body water of labelled ^{18}O and 0.12 g/Kg total body water of ^2H). Urine samples were also collected after 2, 3 and 4 hours after DLW consumption along with the sample of saliva (between 2- and 3- hours post-ingestion of $^2H^{18}O$. On-site examinations will be performed in the exercise physiology laboratory at the University of Southern Denmark or other suitable facilities (e.g., exercise center in the municipality) by project personnel. After 14 days, a project personnel member will visit the participant's home in the morning to collect the two final urine samples (one hour apart). The DLW method requires specialized equipment and expertise, especially when analyzing the results. Urine samples collected will be frozen immediately after the collection period ends and then sent to a research partner's laboratory in University of Madison, USA. All samples were analyzed by isotope mass spectrometry, and the dilution spaces were determined by the plateau method (Cole and Coward, 1992). rCO_2 was calculated using recently developed equations (Speakman et al., 2021), and TEE was calculated using the Weir equation (respiration quotient=0.86), (Weir, 1949).

Pittsburgh Fatigability Scale

Pittsburgh Fatigability Scale (PFS) is a validated 10-item scale to assess both physical and mental perceived fatigability (Glynn et al., 2015). The concept of fatigability is different from the concept of general fatigue because it defines fatigue in relation to an activity with specific duration and intensity. To reduce or avoid fatigue, older adults may modify their exertion level (e.g., slow down or shorten task duration) to maintain a tolerable effort (engage in self-pacing), for this reason using PFS could give a more objective approach. PFS is arranged in two subscales, physical fatigability (PFS physical) and mental fatigability (PFS mental); the subscales final score for both physical and mental fatigability ranges from 0 to 50, and a higher score represents higher level of fatigability. The established threshold (≥ 15) was used to identify participants with high level of physical fatigability compared to participants with low level of physical fatigability (< 15) (Schrack JA., et al., 2020; Simonsick EM. et al., 2018). All participants filled out the PFS questionnaire during the last day of assessments (day 14), and they reported physical fatigability level (0=no fatigue to 5=extreme fatigue for all questions). Specifically, they imagined they would feel fatigue after a specific activity (e.g., "brisk or fast walk for one hour" or "high-intensity activity for 30 minutes"). In this study, a mental fatigability score was not considered (the established mental fatigability threshold to distinguish high or low level is ≥ 13).

Brief Pain Inventory

Brief Pain Inventory is a self-administered questionnaire used to assess the perceived pain (Poquet and Lin, 2016). It is available in a short (nine items), which was used in this study, and long (17 items) form. The BPI short form is more frequently used in research (Cleeland et al., 1994). The BPI gives two main scores: pain severity (each 4 sub-items is rated from 0, no pain, to 10, pain as bad as you can imagine) and a pain interference score (each 7 sub-items are rated from 0, does not interfere, to 10, completely interferes). However, for this study only the response to the first question (reported at the end of this paragraph) was considered to include this item in the statistical analyses (otherwise, considering pain severity and pain interference, older adults with no pain would not have been taken into account in the statistical analysis because these two scores were available for participants who reported experiencing pain), (Cleeland et al., 1994).

"Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?" (1, Yes or 2, No)

Statistical Analysis

Before all statistical analyses, data of PFS physical score were imputed for 4 participants following the instruction published by Cooper and colleagues in 2018 (Cooper et al., 2018 - supplemental materials). Unfortunately, 2 scores were not imputed due to both fatigability score and the corresponding activity question missing values. At the end of the imputation process, 75 of the 77 total values were considered for PFS physical score.

Shapiro-Wilk test was done to assess normality distribution for *$\dot{V}O_2R$,* PFS physical score and AEE, and parametric or non-parametric correlations (Spearman's rho) were done between *$\dot{V}O_2R$*, AEE, and PFS physical score.

A Wilcoxon rank-sum test (o Mann-Whitney U) was used to verify if participants with a low level of PFS physical score (< 15) were different in all variables measured in this study compared to participants with a high level of PFS physical score (≥ 15).

Multiple linear regression models *(MLRs)* with stepwise-backward entry method were used to examine whether *$\dot{V}O_2R$* and PFS physical score (independent variables) can predict AEE (dependent variable) in older adults. The regression models were adjusted progressively for age, sex, body weight (or % of fat mass), number of chronic conditions and perceived pain (yes or not).

A *mediation analysis* was done to verify whether and to what extent the effect of *$\dot{V}O_2R$* (independent variables) on AEE (dependent variable) may be explained by PFS physical score (mediator). Age, % of fat mass and sex are considered as background confounders.

In addition, a *K-medians Clustering* analysis was performed to divide all participants into different clusters (Neighborhood-Based Clustering). Each cluster was determined using the median of *$\dot{V}O_2R$* ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$) and the median of AEE (Kcal/Kg). In this study we hypothesized that older adults with "high" or "low" level of *$\dot{V}O_2R$* may have "high" or "low" level of AEE respectively. However, considering the behavioural component, may be older adults with "high" level of *$\dot{V}O_2R$* but "low" level of AEE, in contrast with older adults with "low" level of *$\dot{V}O_2R$* but "high" level of AEE. In addition, we were interested to evaluate how physical fatigability was distributed in these clusters considering sex differences (Chi-Squared Tests). *Contingency tables and Chi-Squared tests* were used to analyze the distribution of male and female in each cluster and the possible association between clusters and PFS physical score considering sex differences (clusters were considered as rows, the established threshold for PFS physical score [< 15, low physical fatigability; ≥ 15 high physical fatigability)] was considered as columns, and sex was considered as layers).

All analyses were performed using JASP *(vs. 0.95.0.0)* considering statistical significance equal to 0.05. The imputation was performed using R (*vs. 2025.07.0).*

Results

All variables and participants characteristics were presented as mean and standard deviation (mean±SD) in Table 1.

The Shapiro-Wilk test revealed that $\dot{V}O_2R$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$) and PFS physical scores were normally distributed (p=0.429 and p=0.081), while AEE (Kcal) was not normally distributed (p=0.018).

$\dot{V}O_2R$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$) was significantly correlated with PFS physical score (Pearson's r= - 0.360, p=0.002), and with AEE (Spearman's rho=0.268, p=0.018), but no correlation was found between PFS physical score and AEE (Spearman's rho= - 0.065, p=0.580). All correlations were presented in Table 2.

A Wilcoxon rank-sum test showed no significant differences in all variables between participants with low PFS physical score and high PFS physical score excluding $\dot{V}O_2R$ (p=0.020) and $\dot{V}O_{2max}$ (p=0.022). BMI (p=0.052) and waist (p=0.055) were only barely significant. All data are presented in Table 3.

Table 1. Participants characteristics.

	All (n = 77) ***Means ± SD***
Age	80.23 ± 3.70
Sex (female, %)	33, 43%
Number of chronic diseases	2.53 ± 1.54
Height (cm)	169.45 ± 9.20
Body weight (Kg)	73.32 ± 13.41
BMI ($Kg \cdot m^{-2}$)	25.37 ± 3.16
Waist (cm)	94.58 ± 11.10
FM (%)	30.72 ± 6.57
FFM (%)	69.29 ± 6.58
RMR ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	2.66 ± 0.34
RMR (L/min)	0.19 ± 0.03
RMR (Kcal)	1343.81 ± 238.26
$\dot{V}O_{2max}$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	24.06 ± 5.47
$\dot{V}O_{2max}$ (L/min)	1.77 ± 0.56
$\dot{V}O_2$ R ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	21.50 ± 5.32
$\dot{V}O_2$ R (L/min)	1.59 ± 0.53
TTE (kcal)	2184.74 ± 483.88
AEE (Kcal)	620.53 ± 324.27
PFS_Physical Fatigability Score*	13.28 ± 7.51

Abbreviations: SD=standard deviation, BMI=body mass index, FM=fat mass, FFM=fat free mass, RMR=resting metabolic rate, $\dot{V}O_{2max}$=maximum oxygen uptake, $\dot{V}O_2R$=reserve of oxygen uptake, TTE=total energy expenditure, AEE=energy expenditure of activity, PFS=Pittsburgh Fatigability Scale. * n=75 (2 missing values).

Table 2. Correlations.

			Pearson		Spearman	
			r	*p*	*rho*	*p-value*
Physical Fatigability Score	-	$\dot{V}O_2R$ (L/min)	-0.242	0.037	**-0.252***	**0.029**
Physical Fatigability Score	-	$\dot{V}O_2R$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	-0.360**	0.002	-0.320**	0.005
Physical Fatigability Score	-	TEE (Kcal)	-0.075	0.521	-0.048	0.682
Physical Fatigability Score	-	AEE (Kcal)	-0.065	0.580	-0.022	0.850
Physical Fatigability Score	-	AEE (kcal/Kg)	-0.074	0.528	-0.060	0.610
Physical Fatigability Score	-	$\dot{V}O_2$ max (L/min)	-0.224	0.054	**-0.253***	**0.043**
Physical Fatigability Score	-	$\dot{V}O_2$ max ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	-0.353**	0.002	-0.315***	0.006
$\dot{V}O_2R$ (L/min)	-	TEE (Kcal)	0.707***	< 0.001	**0.714*****	**< 0.001**
$\dot{V}O_2R$ (L/min)	-	AEE (Kcal)	0.503***	< 0.001	**0.448*****	**< 0.001**
$\dot{V}O_2R$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	-	TEE (Kcal)	0.433***	< 0.001	0.400***	< 0.001
$\dot{V}O_2R$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	-	AEE (Kcal)	0.376***	< 0.001	**0.268***	**0.018**
TEE (Kcal)	-	AEE (Kcal)	0.845***	< 0.001	0.788***	< 0.001

Abbreviations: $\dot{V}O_{2max}$=maximum oxygen uptake, $\dot{V}O_2R$=reserve of oxygen uptake, TTE=total energy expenditure, AEE=energy expenditure of activity, PFS=Pittsburgh Fatigability Scale. *p<0.05; **p<0.01; ***p<0.001.

Table. 3 Wilcoxon rank-sum test.

†Physical Fatigability Score (CUT-OFF)	*Means ± SD*	*Means ± SD*	
	< 15† (n = 45)	**≥ 15† (n = 30)**	***p-value*** **(η^2_p)**
Female (%)	19 (57%)	14 (42%)	0.704
Age	80.11 ± 2.956	80.56 ± 4.67	0.991 (0.002)
Number of chronic diseases	2.44 ± 1.42	2.56 ± 1.71	0.982 (0.004)
Height (cm)	170.04 ± 9.19	167.66 ± 8.73	0.267 (0.264)
Body weight (Kg)	71.92 ±12.55	74.25 ± 14.21	0.458 (-0.176)
BMI ($Kg \cdot m^{-2}$)	24.74 ± 3.07	26.19 ± 3.17	0.052 (-0.465)
Waist (cm)	92.41 ± 10.38	97.46 ± 11.81	0.055 (-0.459)
FM (%)	29.81 ± 7.05	32.26 ± 5.78	0.118 (-0.373)
FFM (%)	70.19 ± 7.06	67.75 ± 5.80	0.120 (0.371)
RMR ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	2.68 ± 0.37	2.64 ± 0.29	0.554 (0.140)
RMR (L/min)	0.19 ± 0.03	0.19 ± 0.04	0.739 (-0.079)
RMR (Kcal)	134.15 ± 226.06	1348.22 ± 261.29	0.785 (-0.064)
$\dot{V}O_{2max}$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	25.24 ± 5.04	22.32 ± 5.69	**0.022* (-0.551)**
$\dot{V}O_{2max}$ (L/min)	1.81 ± 0.49	1.69 ± 0.65	0.357 (0.219)
$\dot{V}O_2 R$ ($mLO_2 \cdot min^{-1} \cdot kg^{-1}$)	22.73 ± 4.74	19.68 ± 5.66	**0.020* (-0.317)**
$\dot{V}O_2 R$ (L/min)	1.63 ± 0.46	1.50 ± 0.62	0.113 (-0.218)
TTE (kcal)	2164.08 ± 438.49	2155.36 ± 513.60	0.774 (-0.040)
AEE (Kcal)	615.44 ± 309.68	587.06 ± 319.11	0.758 (-0.043)
AEE (Kcal/Kg)	8.56 ± 3.97	7.89 ± 3.91	0.475 (0.169)

Sex Differences were analysed through Chi-Squared test.

Abbreviations: SD=standard deviation, BMI=body mass index, FM=fat mass, FFM= fat free mass, RMR=resting metabolic rate, $\dot{V}O_{2max}$=maximum oxygen uptake, $\dot{V}O_2R$=reserve of oxygen uptake, TTE=total energy expenditure, AEE=energy expenditure of activity, PFS=Pittsburgh Fatigability Scale. * n=75 (2 missing values). *p<0.05; **p<0.01; ***p<0.001.

As reported in table 4, in the MLR model 1 (progressively adjusted for age, sex, and body weight) *$\dot{V}O_2R$ (*p < 0.001) and age (p=0.024) explained 28.2% of the variance in AEE (adjusted R^2=0.282, p < 0.001). PFS physical score, sex, and body weight were excluded by the stepwise procedure at p ≥ 0.10. As reported in table 5, in the MLR model 2 (progressively adjusted also for % of fat mass [instead of body weight], n chronic conditions and pain) *$\dot{V}O_2R$* (p < 0.001), age (p=0.014), and % of fat mass (p=0.069) explained 30.5% of the variance in AEE (adjusted R^2=0.305, p < 0.001). PFS physical score, sex, n chronic conditions, and pain were excluded by the stepwise procedure at p ≥ 0.10. For all MLRs, absolute value of *$\dot{V}O_2R$* (L/min) was considered because of the adjustments (e.g., body weight or % of fat mass).

Table 4. Multiple Regression Model 1.

MODEL 1					
AEE (Kcal)				***p-value***	***Adjusted R^2***
$\dot{V}O_2R$ (L/min)				***< 0.001***	**0.243**
+ PFS physical score				*0.575*	**0.239**
****MODEL 1** (adjusted for* age, sex, body weight)					
	β (95% CI)	***t***	***SE***	***p-value***	***Adjusted R^2***
AEE (Kcal)				**< 0.001**	**0.282**
$\dot{V}O_2R$ (L/min)	349.83 (224.62, 475.01)	5.56	62.81	**< 0.001**	
Age	20.82 (2.85, 38.78)	2.31	9.01	**0.024**	
PFS physical score, sex and body weight were excluded in the final model.					
PFS physical score	1.05 (-7.811, 9.92)	0.238	4.44	0.813	
Sex (0 = M; 1 = F)	-86.88 (-262.81, 89.05)	-0.98	88.23	0.328	
Body weight	-2.11 (-8.98, 4.74)	-0.61	3.44	0.540	

Abbreviations: ***$\dot{V}O_2R$*=**reserve of oxygen uptake, **PFS=**Pittsburgh Fatigability Scale, F=female, M=male.
*Stepwise-backward entry with adjustments.

Table 5. Multiple Regression Model 2.

*MODEL 2 (*adjusted for % of fat mass, number of chronic conditions and pain)*					
	β (95% CI)	***t***	***SE***	***p-value***	***Adjusted R^2***
AEE (Kcal)				**< 0.001**	**0.305**
$\dot{V}O_2R$ (L/min)	312.24 (182.50, 441.98)	4.80	65.07	**< 0.001**	
Age	22.48 (4.72, 40.25)	2.52	8.91	**0.014**	
Fat mass (%)	-9.07 (-18.88, 0.73)	-1.84	4.92	0.069	
PFS physical score, sex, n chronic conditions and pain were excluded in the final model.					
PFS physical score	0.766 (-8.25, 9.78)	0.17	4.51	0.866	
Sex (0 = M; 1 = F)	-27.97 (-217.63, 161.68)	-0.29	95.03	0.769	
n chronic conditions	-4.10 (-47.16, 38.95)	-0.19	21.57	0.850	
pain (yes or no)	16.20 (-121.89, 154.31)	0.23	69.22	0.816	

Abbreviations: *$\dot{V}O_2R$*=reserve of oxygen uptake, PFS=Pittsburgh Fatigability Scale, F=female, M=male, n=number.
*Stepwise-backward entry with adjustments.

Mediation analysis revealed a significantly direct effect of *$\dot{V}O_2R$* on AEE ($p < 0.001$). No indirect effect of PFS physical score was found (p=0.824). This analysis was in line with the MLRs results, confirming the relationship between *$\dot{V}O_2R$* and AEE without physical fatigability mediation.

The K-medians Clustering analysis divided all 77 participants into 4 different clusters (for 2 participants only the PFS physical scores were not included for missing data). As reported in the boxplots (Figure 1. A, B and C), each cluster was different considering *$\dot{V}O_2R$* and AEE values (medians).

In the cluster number 1, 19 older adults with *$\dot{V}O_2R$*=23.93 [7.21], AEE=12.59 [5.03], and PFS physical scores=9.50 (6.50), (median [Interquartile Range]) were included.

In the cluster number 2, 30 older adults with *$\dot{V}O_2R$*=23.40 [3.77], AEE=7.23 [2.37], and PFS physical scores=11.00 (8.75), (median [Interquartile Range]) were included.

In the cluster number 3, 13 older adults with $\dot{V}O_2R$=16.23 [2.12], AEE=9.39 [1.33], and PFS physical scores=17.50 (11.50), (median [Interquartile Range]) were included.

In the cluster number 4, 15 older adults with $\dot{V}O_2R$=16.75 [2.54], AEE=4.74 [2.43], and PFS physical scores=19.00 [13.0], (median [Interquartile Range]) were included.

Each cluster may represent a "profile" obtained combining physiological measurements and perceived physical fatigability: cluster 1 may represent older adults with high level of $\dot{V}O_2R$ and AEE, and low level of physical fatigability; cluster 4 may represent older adults with low level of $\dot{V}O_2R$ and AEE, and high level of physical fatigability; cluster 3 may represent the "resilient" older adults with low level of $\dot{V}O_2R$, high level of AEE and high level of physical fatigability; cluster 3 represent older adults with high level of $\dot{V}O_2R$, low level of AEE and high level of physical fatigability.

Finally, the contingency table (Table 6) showed the distribution of male and female in each cluster and the *Chi-Squared* test showed a significant association between clusters and PFS physical score only for women (n=33, X^2=13.084; p=0.004), but not for men (n=42, X^2=1.560; p=0.668). These results suggested that perceived PFS physical score was significantly different for women in relation to the cluster where they were allocated.

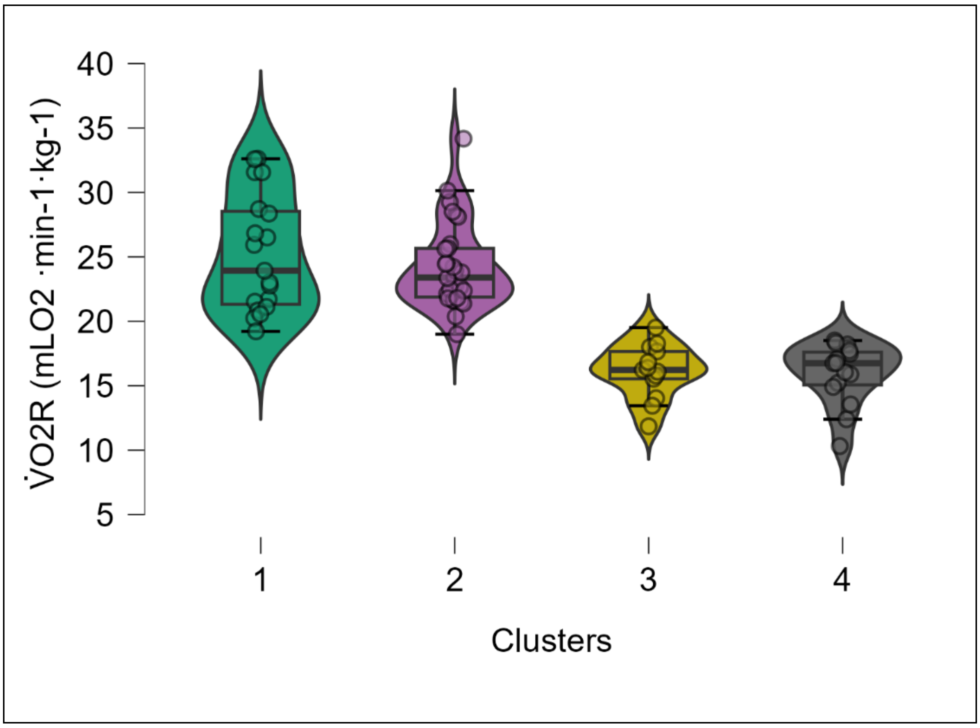

Figure 1A. Clusters for V̇O₂R (median). **Abbreviations:** V̇O₂R, reserve of oxygen uptake.

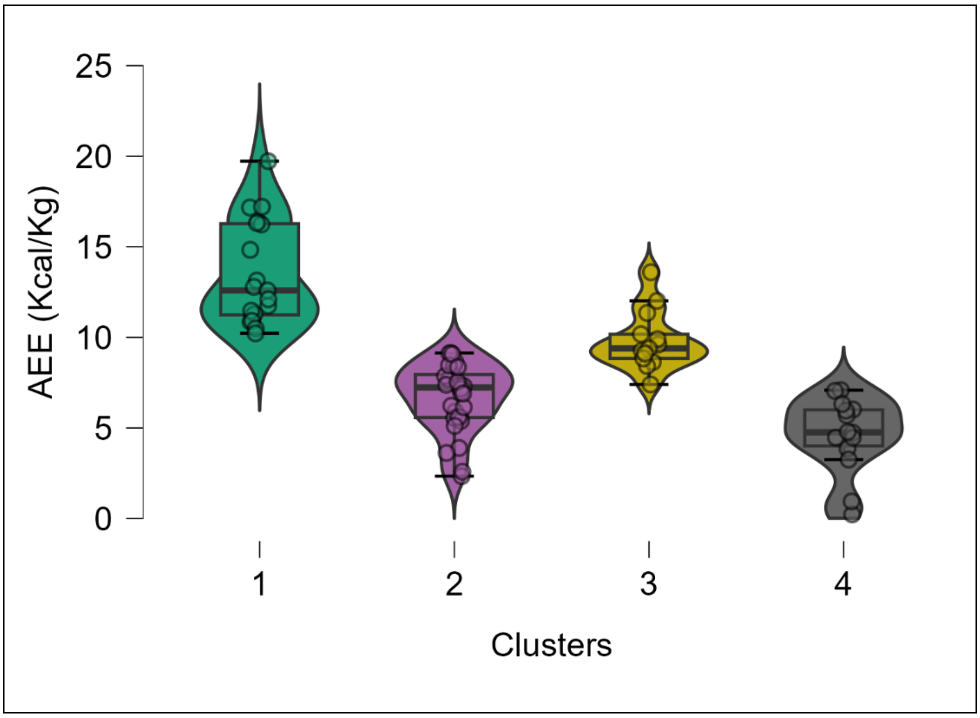

Figure 1B. Clusters for AEE (median). **Abbreviations:** AEE, Activity Energy Expenditure.

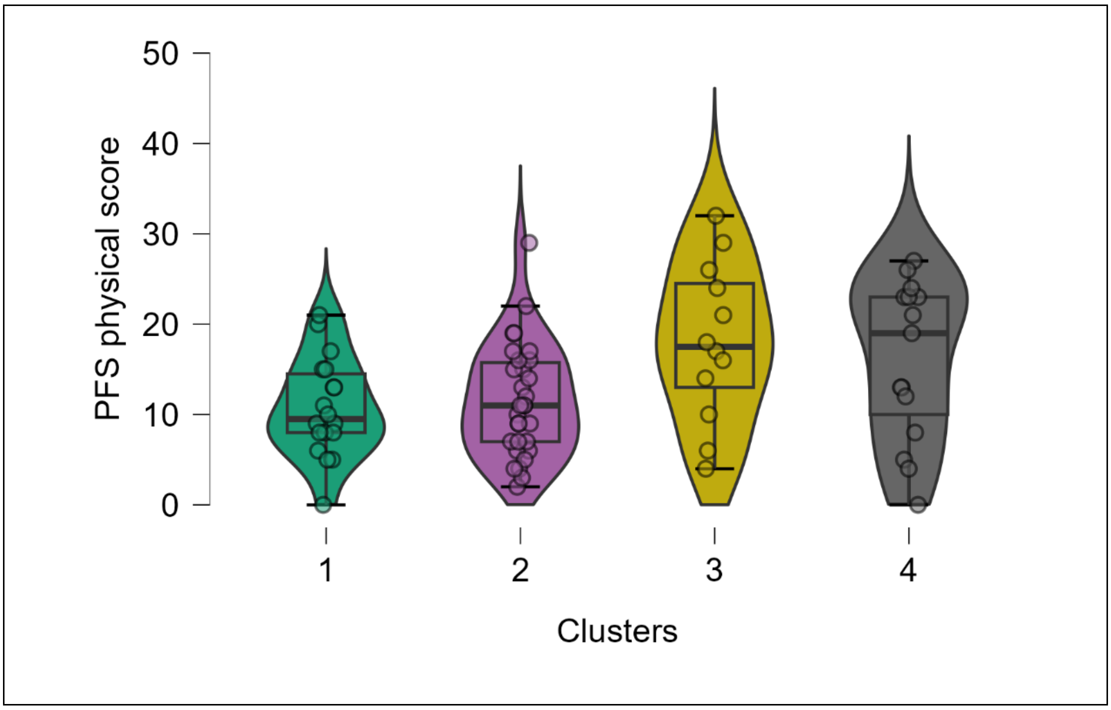

Figure 1C. Clusters for PFS physical score (median). **Abbreviations:** PFS, Pittsburgh Fatigability Scale.

Table 6. Contingency Table.

Sex	Clusters		PFS < 15 (0); PFS ≥ 15 (1)		Total
			0	**1**	
F	1	**Count**	3	0	3
		%within row	100%	0.00%	100.00%
	2	**Count**	10	1	11
		%within row	90.91%	9.09%	100.00%
	3	**Count**	2	7	9
		%within row	22.22%	77.78%	100.00%
	4	**Count**	4	6	10
		%within row	40.00%	60.00%	100.00%
Total count for Female			19 (57.58%)	14(42.42%)	33(100.00%)
M	1	**Count**	11	4	15
		%within row	73.33%	26.67%	100.00%
	2	**Count**	10	9	19
		%within row	52.63%	47.37%	100.00%
	3	**Count**	2	1	3
		%within row	66.67%	33.33%	100.00%
	4	**Count**	3	2	5
		%within row	60.00%	40.00%	100.00%
Total count for Male			26 (61.90%)	16(38.10%)	42(100.00%)

Abbreviations: PFS, Pittsburgh Fatigability Scale.

Discussion

Actually, it is unknown whether $\dot{V}O_2R$ and perceived physical fatigability may interact in their contribution to free-living AEE in older adults. For this reason, this study aims to examine how $\dot{V}O_2R$ and perceived physical fatigability influenced free-living AEE in older adults. Different statistical approaches were proposed, and the results showed the complexity of the interaction between self-reported and physiological measures. Given current scientific knowledge, combining self-reported and physiological measures is necessary to obtain a comprehensive understanding of age-related changes, not only for outcomes such as energy expenditure and physical activity level but also for emotional components (Pavic et al., 2024). The results showed that $\dot{V}O_2R$ may be a good indicator of physical functioning in older adults because it considers physiological decline in both $\dot{V}O_{2max}$ and RMR, and it was significantly linked with fatigability; this aspect was confirmed by data from older adults with a high level of $\dot{V}O_2R$ who reported a low level of perceived fatigability. Despite $\dot{V}O_2R$ and AEE was significantly correlated, no differences were found in the Wilcoxon rank-sum test between participants with low or high levels of perceived fatigability in terms of AEE.

This point may be explained by cluster 2 composed of "resilient" older adults with a low level of $\dot{V}O_2R$, a high level of physical fatigability, but a high level of AEE. It could be very interesting to evaluate why participants in this cluster maintain a certain level of AEE despite their levels of $\dot{V}O_2R$ and physical fatigability. In addition, $\dot{V}O_2R$, age, and % of fat mass were predictors of AEE, emphasizing the importance of individual physiological and body composition characteristics in daily energy expenditure (AEE). The mediation analysis confirmed a direct effect of $\dot{V}O_2R$ on AEE with no mediation of PFS physical score. Finally, 4 different clusters were detected, highlighting different older adult profiles which can contribute to the growth of knowledge of both physiology and behavior in the elderly, with these characteristics reported in this study. One of the key points concerned differences in perceived fatigability among women across clusters compared to men. According to the literature, these results suggested that perceived PFS physical scores were significantly different for women.

Strength and Limitations

This is the first study that combine $\dot{V}O_2R$, AEE, and physical fatigability in older adults (age 80.23±3.70 years [mean±SD]), including sex differences (43% female), and specific indicators such as pain perceived. In addition, DLW and Douglas bags were used as gold standard methods to measure TEE and $\dot{V}O_2$, and the validated Pittsburgh Fatigability Scale was used to measure fatigability. Statistical analyses investigated the relationship between physiological and self-reported outcomes from different points of view, given a new perspective to interpreting this relationship.

However, this work had some limitations: the first one was represented by the small sample size; it would be interesting to evaluate the same results in larger study; the second one was that the study included relatively well-functioning older adults (Guralnik et al., 1995) which may be no completely representative compared to older adults with severe limitation on physical functioning or very low level of $\dot{V}O_2R$ and AEE. This may limit generalizability.

Conclusions

This study contributes to the existing knowledge and understanding of complexity of the interaction between self-reported and physiological measures by demonstrating that AEE derived from both exercise (e.g., aerobic exercise and/or resistance training), non-exercise activities (e.g., cycling for transportation), and Activities of Daily Living (ADL) was related to specific characteristics and that different profile may exist in terms of perceived fatigability. Although fatigability does not seem to be a predictor or mediator, it was significantly associated to $\dot{V}O_2R$. Further studies are needed to explore the role of perceived fatigability in relation to daily energy expenditure among older adults.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Overall conclusions

This Thesis highlighted the complex interplay between self-reported outcomes and physiological components in two different cohorts, emphasizing that the integration of these measures may be essential for understanding overall Quality of Life (including fatigue, fatigability, and physical functioning) and cardiometabolic health to prevent breast cancer incidence and/or relapse and to promote healthy ageing.

Supervised and tailored aerobic exercise and a Mediterranean diet were confirmed as key strategies for improving overall Quality of Life, cardiometabolic health, glucose homeostasis, and tumor growth regulation in breast cancer survivors. For example, Study 2 showed that fasting glucose parameters differed significantly from non-fasting metrics of glucose control, highlighting an important aspect in preventing type-2 diabetes. These effects were related to food intake (i.e., different oligosaccharide intake after the lifestyle intervention) between active and non-active days.

Considering the side effects of cancer treatments and the survivors' need to receive follow-up care or support beyond routine check-ups aimed at detecting recurrences, these results confirm lifestyle interventions as effective strategies to improve both mental and physical health, which may be included as standard practice within the health care system.

In addition, engaging regularly in physical activity or in specific exercise training protocols, along with increased adherence to the Mediterranean diet, elicits the pleiotropic effect of a healthy lifestyle, influencing various aspects related to specific symptoms such as insomnia, cancer-related fatigue, emotional or cognitive functioning, and glucose homeostasis (thereby helping to prevent type-2 diabetes, which can result from sedentary behaviour combined with cancer treatment), as well as the balance between food intake and energy expenditure during aerobic exercise and tumor growth regulation. These aspects, which resulted extremely interrelated, may contribute to improving the quality of life of breast cancer survivors.

When overall quality of life is assessed, especially in older adults aged 65 and above, it is essential to consider the age-related physiological decline in specific outcomes (i.e., $\dot{V}O_{2max}$ and RMR), which can influence behavioural components such as physical activity level and adherence to a healthy diet, and consequently affect the daily energy expenditure.

For this reason, investigated outcomes such as *$\dot{V}O_2R$* and fatigability provide another perspective on how they may influence daily activities.

The significant correlation found between fatigability and activity energy expenditure, as well as *$\dot{V}O_2R$,* age, and fat mass percentage as predictors of daily energy expenditure, highlight the importance of the relationship between physiological parameters and self-reported outcome measures, not only in cancer survivors but also in older adults. Interestingly, four distinct functional older adult profiles were identified, revealing that perceived fatigability differs significantly between women and men. Additionally, the results showed that individuals with good physiological conditions (i.e., high *$\dot{V}O_2R$*) do not necessarily engage in greater activity energy expenditure than those with poorer physiological conditions. Although fatigability was not a predictor of activity energy expenditure, it was significantly associated with *$\dot{V}O_2R$*. These findings underline the importance of both physiological characteristics and predisposition to movement, despite ageing-related changes, with consideration of sex differences.

This Thesis revealed the complexity of the relationship between self-reported outcomes and physiological parameters across two distinct yet interrelated cohorts. The findings suggest that to fully understand the effect of lifestyle interventions, it is necessary to investigate both quality of life and cardiometabolic health, along with glucose homeostasis and tumor growth modulation, to gain a more comprehensive understanding of cancer survivors' overall health.

Additionally, improvements in physiological parameters are not necessarily associated with improvements in quality of life, and vice versa. Furthermore, older adults with good physiological conditions do not necessarily engage in greater activity energy expenditure than those with poor cardiorespiratory fitness levels.

The number of elderly women with a previous history of breast cancer diagnosis is expected to increase, and this study aimed to identify new insights that may be relevant to preventing breast cancer recurrence and promoting healthy ageing. It is important to note that both survivors and older adults experienced physiological declines in cardiorespiratory fitness (due to cancer treatments and age-related changes, respectively), and that individuals with a history of cancer or aged ≥ 65 perceived fatigue more intensely compared to younger or disease-free counterparts.

This work had some limitations, which were specifically described in each study presented in relation to specific outcomes analysed. Overall, the two main limitations were small sample sizes and the enrollment of relatively well-functioning participants, both of which may limit the generalizability of the results. It would be valuable to evaluate the same outcomes in larger, prospective studies with breast cancer patients and less well-functioning older adults.

Further studies should aim to understand how variables related to cardiometabolic health (e.g., $\dot{V}O_{2max}$ or $\dot{V}O_2R$, adherence to the MD, and glycemic homeostasis) and the regulation of key factors for tumor growth (e.g., IGF-1) may influence physical fatigability in older adults with a previous cancer diagnosis, and more specifically, in older women with a history of BC.

This future perspective could contribute to advancing current knowledge and enhance our understanding of the complex interaction between self-reported and physiological measures in older women with previous breast cancer, to maintain their overall quality of life and promote healthy ageing.

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List of abbreviations

ACSM	American College of Sports Medicine
ADL	Activities of Daily Living
AEE	Activity Energy Expenditure
AIOM	Associazione Italiana Oncologia Medica
BC	Breast Cancer
BCS	Breast Cancer Survivors
Bio	Metabolic and Cancer-related Biomarkers
BMI	Body Mass Index
CG	Control Group
CGMs	Continuous Glucose Monitoring Devices
CRF	Cardiorespiratory Fitness
CV	Coefficient of variation
DIT	Diet-Induced Thermogenesis
DLW	Doubly-Labeled Water
ELISA	Enzyme-Linked Immunosorbent Assay
EMM	Estimated Marginal
ENGAGE	ENerGetics in Old AGE
EORTCQLQ-C30	European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire Core 30
ER	Estrogen Receptor
FFM	Fat Free Mass
FIT	Cardiorespiratory Fitness
FM	Fat Mass
GH	Growth Hormone
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HR	Heart Rate
HRmax	Maximal HR
HRR	Heart Rate Reserve
HRR	Heart Rate Reserve
I2	High heterogeneity meta-analyses
IG	Intervention Group
IGF-1	Insulin-like Growth Factor-1
IGFBP1	Insulin-like Growth Factor-1 Binding Protein 1
IGFBP3	Insulin-like Growth Factor-1 Binding Protein 3
IPAQ-SF	International Physical Activity Questionnaire – Short Form
LI	Lifestyle Intervention
LMMs	Linear Mixed Models
MAGE	Mean Amplitude of Glycemic Excursions
MCID	Minimal Clinically Important Difference
MD	Mediterranean diet
MeDiet	Mediterranean Diet
MET	Metabolic Equivalent of Task
MLRs	Multiple Linear Regressions

MODD	Mean of Daily Differences
MoviS	Movement and health beyond care study
OS	On-Site Supervised
PA	Physical Activity
PFS	Pittsburgh Fatigability Scale
PROM	Patient-Reported Outcome measure
QLQ-C30 Summary score	Quality of Life Questionnaire Core 30 Summary score
QoL	Quality of Life
RCT	Randomized Controlled Trial
RMR	Resting Metabolic Rate
RS	Remotely Supervised
SD	Standard Deviation
SD	standard deviation
TAR	Time above glucose range
TBR	Time below glucose range
TEE	Total Energy Expenditure
TIR	Time in glucose range
$\dot{V}O_{2max}$	Maximal Oxygen Uptake
$\dot{V}O_2R$	Reserve of Oxygen Uptake
WCRF	World Cancer Research Fund
WHO	World Health Organization