Associations of Plasma Bioactive Adrenomedullin Levels with Cardiovascular Risk Factors in *BRCA1/2* Mutation Carriers

Zusammenhang zwischen bioaktivem Adrenomedullin-Spiegel und kardiovaskulären Risikofaktoren bei *BRCA1/2*-Mutationsträgerinnen

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Authors

Jacqueline Lammert[1], Maryam Basrai[2], Joachim Struck[3], Oliver Hartmann[3], Christoph Engel[4], Stephan C. Bischoff[2], Anika Berling-Ernst[5], Martin Halle[5], Marion Kiechle[1], Sabine Grill[1]

Affiliations

1 Department of Gynecology and Center for Hereditary Breast and Ovarian Cancer, University Hospital rechts der Isar, Technical University of Munich (TUM), Munich, Germany
2 Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany
3 Sphingotec GmbH, Hennigsdorf, Germany
4 Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany
5 Department of Prevention and Sports Medicine, University Hospital Klinikum rechts der Isar, Technical University of Munich, Munich, Germany

Key words

BRCA1, *BRCA2*, cardiovascular risk, breast cancer survivors

Schlüsselwörter

BRCA1, *BRCA2*, kardiovaskuläres Risiko, Brustkrebsüberlebende

received 19. 1. 2022
accepted after revision 28. 3. 2022

Bibliography

Geburtsh Frauenheilk 2022; 82: 601–609
DOI 10.1055/a-1811-2164
ISSN 0016-5751

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence

Jacqueline Lammert
Department of Gynecology and Center for Hereditary Breast and Ovarian Cancer, University Hospital rechts der Isar, Technical University of Munich
Ismaninger Str. 22, 81675 Munich, Germany
jacqueline.lammert@tum.de

ABSTRACT

Background Cardiovascular disease (CVD) is an important cause of morbidity and mortality in breast cancer survivors. Effective screening modalities to identify CVD risk are lacking in this population. Adrenomedullin (ADM) has been suggested as a biomarker for subclinical cardiac dysfunction in the general population. Levels of ADM have been proven to be responsive to lifestyle changes that lead to improved cardiovascular health. As *BRCA1/2* mutation carriers are deemed to be at an increased risk for CVD, the aim of this study was to examine plasma ADM levels in a cohort of *BRCA* mutation carriers and to assess their association with cardiovascular risk factors.

Methods Plasma ADM concentrations were measured in 292 female *BRCA1/2* mutation carriers with and without a history of breast cancer. Subjects were classified into high versus low ADM levels based on the median ADM level in the entire cohort (13.8 pg/mL). Logistic regression models were used to estimate the odds ratios (OR) of having elevated ADM levels by several cardiovascular risk factors.

Results Of all women (median age: 43 years), 57.5% had a previous diagnosis of breast cancer. The median time between diagnosis and study entry was three years (range: 0–32 years). Women presenting with metabolic syndrome had 22-fold increased odds of having elevated ADM levels ($p < 0.001$). Elevated ADM levels were associated with lower cardiorespiratory fitness (OR = 0.88, $p < 0.001$) and several parameters of obesity ($p < 0.001$). ADM levels were higher in women who have ever smoked (OR = 1.72, $p = 0.02$). ADM levels were not associated with a previous diagnosis of breast cancer ($p = 0.28$).

Conclusions This is the first study in *BRCA* mutation carriers that has linked circulating ADM levels to traditional cardiovascular risk factors. The long-term clinical implications of these findings are yet to be determined.

ZUSAMMENFASSUNG

Hintergrund Herz-Kreislauf-Erkrankungen (HKE) sind eine wichtige Ursache für Morbidität und Mortalität bei Brustkrebsüberlebenden. Es fehlt aber an effektiven Früherkennungsuntersuchungen, welche die HKE-Risiken in dieser Population identifizieren könnten. Adrenomedullin (ADM) wurde bereits als möglicher Biomarker für subklinische Herzerkrankungen in der Allgemeinbevölkerung vorgeschlagen. Es hat sich gezeigt, dass Lebensstiländerungen, die zu einer Verbesserung der kardiovaskulären Gesundheit führen, sich in ADM-Plasmakonzentrationen widerspiegeln. Da Trägerinnen von *BRCA1/2*-Mutationen ein erhöhtes HKE-Risiko haben, zielt diese Studie darauf ab, die ADM-Plasmakonzentrationen in einer Gruppe von *BRCA*-Mutationsträgerinnen zu messen und den Zusammenhang mit HKE-Risikofaktoren zu untersuchen.

Methoden ADM-Plasmakonzentrationen wurden in 292 *BRCA1/2*-Mutationsträgerinnen mit oder ohne frühere Brustkrebsdiagnose gemessen. Basierend auf der medianen ADM-Konzentration der Gesamtgruppe (13,8 pg/ml) wurden die untersuchten Frauen gemäß ihrer ADM-Konzentrationen in 2 Gruppen (hohe bzw. niedrige ADM-Konzentration) eingeteilt. Logistische Regressionsmodelle wurden verwendet, um das Chancenverhältnis (OR) verschiedener kardiovaskulärer Risikofaktoren in Abhängigkeit der Höhe der ADM-Konzentration zu schätzen.

Ergebnisse Bei 57,5 % der Frauen (Durchschnittsalter: 43 Jahre) wurde zuvor Brustkrebs diagnostiziert. Die mediane Zeit zwischen der Krebsdiagnose und die Aufnahme in dieser Studie betrug 3 Jahre (Spanne: 0–32 Jahre). Frauen mit metabolischem Syndrom hatten eine 22-fach höhere Wahrscheinlichkeit eines erhöhten ADM-Spiegels ($p < 0{,}001$). Erhöhte ADM-Spiegel waren mit niedriger kardiorespiratorischer Fitness (OR = 0,88, $p < 0{,}001$) sowie verschiedenen Übergewichtsparametern ($p < 0{,}001$) assoziiert. Der ADM-Spiegel war höher bei Frauen, die rauchten bzw. früher geraucht hatten (OR = 1,72, $p = 0{,}02$). Es gab kein Zusammenhang zwischen ADM-Konzentrationen und einer früheren Brustkrebsdiagnose ($p = 0{,}28$).

Schlussfolgerungen Dies ist die erste Studie von *BRCA*-Mutationsträgerinnen, welche die Verbindung zwischen ADM-Plasmakonzentrationen und traditionellen kardiovaskulären Risikofaktoren untersucht. Die langfristigen klinischen Implikationen der Befunde müssen noch ermittelt werden.

Introduction

With continual improvements in cancer outcomes, cardiovascular disease (CVD) is an important cause of morbidity and mortality in (early) breast cancer patients [1]. In fact, risk of death from cardiovascular causes surpasses the risk of death from breast cancer eight years after diagnosis [2, 3]. CVD can be caused or accelerated by a variety of breast cancer treatments, including anthracycline chemotherapy, Her2-targeted therapy, chest radiation therapy and long-term oestrogen suppression [4, 5]. Reciprocally, studies in mice and humans have shown that a serious cardiac event, such as a myocardial infarction, accelerates breast cancer outgrowth and cancer-specific mortality [6]. Additionally, there is a significant overlap of risk factors common to both diseases, including aging, physical inactivity and metabolic syndrome [7]. Thus, breast cancer survivors have been shown to have a higher prevalence of cardiovascular risk factors than age-matched, cancer-unaffected women [8, 9].

Secondary prevention of CVD is dependent upon the ability to identify high-risk individuals long before the development of cardiac injury.

Given the long latency periods between the initial diagnosis of breast cancer and manifest CVD of approximately five to seven years [10, 11], there is a window of opportunity to identify and treat CVD risk factors before any clinical signs or symptoms become evident.

One of the barriers to improving cardiovascular disease outcomes in breast cancer survivors is the lack of reliable, effective screening modalities. Traditional risk assessment tools, such as the Framingham Risk Score, significantly underestimate a breast cancer survivor's risk of developing CVD [9, 12], highlighting the importance of specific CVD assessment in these women [13].

An increasing number of biomarkers has been identified to predict cardiovascular events among the general population [14]. The value of blood-based biomarkers to identify preclinical CVD in breast cancer survivors has not yet been evaluated.

Adrenomedullin (ADM) represents one of the candidate markers that predict vascular changes, and it becomes elevated years before the onset of non-communicable diseases [15]. In particular, increased levels of ADM among healthy individuals are strongly associated with later development of CVD and cancer, as well as premature mortality [16]. Moreover, studies suggest that ADM is responsive to lifestyle and metabolic changes that lead to improved cardiovascular health [17 – 20].

It is well established that *BRCA1/2* mutation carriers have a high lifetime risk of developing breast cancer. Having a risk of 69–72 % of developing breast cancer and a risk of 17–44 % for developing ovarian cancer by age 80 years [21], *BRCA1/2* mutation carriers are exposed to cancer treatments and prophylactic bilateral salpingo-oophorectomy (PBSO) with detrimental short- and long-term effects on cardiovascular health [22]. Firstly, women with *BRCA*-associated breast cancers are typically diagnosed before age 50 years [21], which is substantially younger than the median age at breast cancer diagnosis of 64 years in the general population [23]. They also have a high risk of developing contralateral [24] or ipsilateral cancer [25]. Secondly, *BRCA*-associated cancers exhibit pathological features suggestive of an aggressive phenotype (e.g., G3 cancers, basal-like disease in *BRCA1* mutation carriers and luminal B tumours in *BRCA2* mutation carriers) [26, 27], and therefore, most patients undergo potentially cardiotoxic che-

motherapy. Thirdly, when diagnosed with ER-positive breast cancer, patients might benefit from an extended adjuvant endocrine therapy [28]. Additionally, *BRCA1/2* mutation carriers are advised to undergo PBSO after child-bearing age. Long-term oestrogen deprivation in women undergoing PBSO has been shown to increase CVD risk by two- to threefold as compared to women of the same age without surgical menopause [29, 30]. Preliminary evidence indicates that *BRCA1/2* mutation carriers are more prone to cardiovascular disease both at baseline and in response to cancer treatments [31 – 35]. Recent research suggests that the *BRCA* genes regulate cardiomyocyte survival and function, and that loss of function leads to increased susceptibility to cardiac damage [33 – 35]. Experimental findings in mice have demonstrated that *BRCA1* limits endothelial cell apoptosis, restores endothelial function, and attenuates atherosclerotic lesion development [36]. Moreover, loss of *BRCA2* has been shown to increase susceptibility to doxorubicin-induced heart failure [37]. Therefore, a biomarker to determine cardiovascular risk might be of particular relevance to *BRCA1/2* mutation carriers.

In this study, we investigated plasma ADM levels in *BRCA1/2* mutation carriers with and without breast cancer and their association with traditional cardiovascular risk factors.

Methods

Study population

The participants under investigation were enrolled in the randomized controlled LIBRE-2 trial (Lifestyle intervention study in women with hereditary breast and ovarian cancer) and the associated feasibility study LIBRE-1. The trials are registered at ClinicalTrials.gov (NCT numbers: NCT02087592 – registered on 14/03/2014, NCT02516540 – registered on 06/08/2015).

The LIBRE-2 trial is an ongoing, two-armed randomized (1 : 1) controlled multicentre trial conducted in Germany aimed at determining the impact of a structured one-year lifestyle intervention program on adherence to the Mediterranean Diet, cardiorespiratory fitness and BMI among *BRCA1/2* mutation carriers. The study cohort includes both women with a previous diagnosis of early-stage cancer in remission (diseased) or without a prior cancer diagnosis (non-diseased). Details on the study design have been published elsewhere [38, 39].

Of the 325 participants who had a blood sample available, we excluded those who had a previous history of ovarian cancer or other cancers than breast cancer. After these exclusions, a total of 292 participants were available for the current analysis. None of the participants had an overt CVD.

The study was approved by the institutional ethics review boards of both the host institutions (Technical University of Munich: Reference No. 5686/13, University Hospital Cologne: Reference No. 13-053 and University Hospital Schleswig-Holstein in Kiel: Reference No. B-235/13) and the participating study centers, and all study subjects provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Data collection

For this study, all measurements, including biologically active ADM, were captured at baseline.

At baseline, participants completed a standardized questionnaire to collect detailed information on medical history, demographic data as well as various reproductive, hormonal and lifestyle factors. Adherence to the Mediterranean Diet was captured by the Mediterranean Diet Adherence Screener (MEDAS), a validated questionnaire consisting of 14 items [40]. We calculated the MEDAS score ranging from 0 to 14 as a percentage of positively answered questions. At enrolment, all participants underwent physical examination to collect systolic and diastolic blood pressure, resting heart rate and anthropometric measurements (i.e., weight [kg], height [m], waist [cm], and hip circumferences [cm]). The four anthropometric measurements were used to calculate body mass index (kg/m^2) and waist-to-hip-ratio (waist circumference [cm]/hip circumference [cm]).

Specimen collection and analysis

All routine analyses were performed by affiliated laboratories of local institutions. Blood samples were withdrawn after an overnight fast for at least 12 hours for assessment of the serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, glucose, insulin and high-sensitivity C-reactive protein (hs-CRP) using standard procedures.

Insulin Resistance (IR) was calculated using the homeostasis model assessment (HOMA-IR) equation formula as follows: HOMA-IR = fasting insulin (µU/mL) multiplied by fasting glucose (mmol/L) divided by 22.5. IR was defined as HOMA-IR ≥ 2.5.

Metabolic syndrome was defined according to the International Diabetes Federation (IDF) criteria by the presence of a waist circumference of ≥ 80 cm together with at least two of the following metabolic abnormalities:

a. fasting blood glucose ≥ 100 mg/dL;
b. systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg;
c. triglycerides ≥ 150 mg/dL;
d. HDL-cholesterol < 50 mg/dL.

The definition also considered treatment with the use of lipid-lowering, glucose-lowering, and antihypertensive drugs.

For blinded ADM analysis, EDTA samples were processed and stored at − 80 °C before transfer to the central laboratory of Sphingotec GmbH. Biologically active Adrenomedullin (bio-ADM) was measured using an immunoassay provided by Sphingotec GmbH, Hennigsdorf, Germany. Details on the assay have been published elsewhere [41, 42]. The analytical assay sensitivity was 2 pg/mL.

Physical activity assessment

Cardiorespiratory fitness was determined by peak oxygen uptake (VO_{2peak}) and assessed via cardiopulmonary exercise testing (CPET). The CPET was a ramp protocol (3 minutes sitting on the bicycle, 3 minutes steady state at 30 watts, continuous individual increase in wattage with the aim of achieving a maximal workload on the test person within 8 to 12 minutes, 5 minutes recovery

▶ **Table 1** Baseline characteristics by median Adrenomedullin levels.

Characteristic	bio-ADM < 13.8 pg/mL (n = 147)	bio-ADM ≥ 13.8 pg/mL (n = 145)
Age, years, mean ± SD	41.2 ± 9.7	43.8 ± 10.4
BRCA mutation status, n (%)		
▪ *BRCA1*	96 (33.2%)	87 (30.1%)
▪ *BRCA2*	46 (15.9%)	56 (19.4%)
▪ *BRCA1* and *BRCA2*	2 (0.7%)	2 (0.7%)
Parous, n (%)	90 (30.8%)	95 (32.5%)
Prophylactic bilateral salpingo-oophorectomy (PBSO), n (%)		
▪ Yes	44 (15.1%)	36 (12.3%)
▪ No	102 (34.9%)	110 (37.7%)
Age at PBSO, years, median (range)	45 (29–60)	45 (36–65)
Breast cancer		
Previous diagnosis of breast cancer, n (%)		
▪ Non-diseased	67 (22.9%)	57 (19.5%)
▪ Diseased	80 (27.4%)	88 (30.1%)
Age at breast cancer diagnosis, years, mean ± SD	39.3 ± 8	40.3 ± 8.3
Time between breast cancer diagnosis and study entry, years, mean ± SD	4.5 ± 6	4.9 ± 5.1
Tumour biology		
▪ Hormone receptor-positive	31 (18.4%)	38 (22.6%)
▪ HER2-positive	3 (1.8%)	3 (1.8%)
▪ Triple negative	46 (27.4%)	47 (28%)
Breast cancer treatments		
▪ Chemotherapy	63 (37.5%)	61 (36.3%)
▪ Chest radiation therapy	42 (25%)	46 (27.4%)
▪ Antihormonal treatment	31 (18.4%)	38 (22.6%)
▪ HER2-targeted treatment	3 (1.8%)	3 (1.8%)
Anthropometric measurements		
BMI, kg/m^2, mean ± SD	22.7 ± 3.3	27.7 ± 6.2
Waist circumference, cm, mean ± SD (cm)	76.5 ± 9.4	88.3 ± 15.2
Hip circumference, cm, mean ± SD (cm)	96.3 ± 9.6	107.6 ± 12.7
Waist-to-hip-ratio, mean ± SD	0.80 ± 0.078	0.82 ± 0.076
Metabolic variables		
Systolic blood pressure, mmHg, mean ± SD	113.4 ± 13.08	120.1 ± 14.9
Diastolic blood pressure, mmHg, mean ± SD	74.1 ± 8.5	78.8 ± 8.8
Fasting glucose, mg/dL, mean ± SD	85.2 ± 10.2	93.6 ± 28.3
Total cholesterol, mg/dL, mean ± SD	197.9 ± 38.9	199.5 ± 43.5
High-density lipoprotein cholesterol, mg/dL, mean ± SD	77.1 ± 17.9	66.5 ± 17.4
Low-density lipoprotein cholesterol, mg/dL, mean ± SD	114.5 ± 33.6	121.9 ± 40.5
Triglycerides, mg/dL, mean ± SD	73 ± 26.8	102.3 ± 47
Metabolic syndrome, n (%)		
▪ No	145 (49.7%)	111 (38%)
▪ Yes	2 (0.7%)	34 (11.6%)
hs-CRP, mg/L, mean ± SD	1.48 ± 2.85	2.96 ± 3.69
Insulin, µU/mL, mean ± SD	7.04 ± 4.51	11.22 ± 8.85
HOMA-IR score ≥ 2.5, n (%)	14 (4.8%)	43 (14.7%)
Other variables		
MEDAS score (percentage of positively answered questions), mean ± SD	0.5 ± 0.16	0.47 ± 0.15
VO_{2peak}, ml/min/kg, mean ± SD	28.2 ± 6.3	23.0 ± 6.2
Ever smoked, n (%)		
▪ No	85 (29.1%)	62 (21.2%)
▪ Yes	67 (22.9%)	78 (26.7%)
Number of pack-years smoked, mean ± SD	3.7 ± 6.3	5.9 ± 9.4

after exercise) with the target of being exhausted with a respiratory exchange ratio (RER) > 1.05.

Statistical analysis

Women were categorized into high vs. low plasma bio-ADM based on the median levels in the entire cohort (< 13.8 and ≥ 13.8 pg/mL). Baseline statistics are presented as mean ± standard deviation or as median and range (continuous variables) or as proportions (binary and categorical variables). Logistic regression analysis was performed to estimate the odds ratios (OR) and their associated 95% confidence intervals (95% CI) of having high circulating bio-ADM levels by different cardiovascular risk factors. A multivariate analysis was carried out to control for potential confounders. These analyses were adjusted for age (years) and history of breast cancer (diseased or non-diseased).

Statistical significance was defined at the level of $p \leq 0.05$, and all analyses were carried out using SPSS version 25.0 (IBM Corp., Armonk, NY).

Results

There were 292 women with a *BRCA1* and/or *BRCA2* mutation included in the current study. ▶ **Table 1** summarizes selected participant characteristics by median bio-ADM levels. The median bio-ADM level was 13.8 pg/mL. The median age of the entire study cohort was 43 years (range: 18–72 years). Of all women, 57.5% had a previous diagnosis of breast cancer. The median time between breast cancer diagnosis and study entry was three years (range: 0–32 years). 19.6% of all participants had undergone PBSO. The

▶ **Table 2** Associations between bio-ADM levels (low vs. high) and selected patient characteristics among *BRCA* mutation carriers (univariate logistic regression).

Predictor	OR [95% CI]	p
Age	**1.026 [1.002; 1.050]**	**0.03***
BRCA mutation status	1.29 [0.82; 2.02]	0.27
Parity	1.2 [0.75; 1.9]	0.45
PBSO	1.32 [0.79; 2.21]	0.29
Age at PBSO	1.01 [0.95; 1.09]	0.69
Previous diagnosis of breast cancer	1.29 [0.81; 2.06]	0.28
Age at breast cancer diagnosis	1.02 [0.98; 1.06]	0.42
Time between breast cancer diagnosis and study entry	1.01 [0.96; 1.07]	0.71
Time between breast cancer diagnosis and study entry ≥ 4 years	**1.91 [1.00; 3.66]**	**0.05***
BMI	**1.28 [1,19; 1,37]**	**< 0.001***
Waist circumference	**1.09 [1.06; 1.11]**	**< 0.001***
Hip circumference	**1.10 [1.07; 1.14]**	**< 0.001***
Waist-to-hip-ratio	**49.22 [1.98; 1225.97]**	**0.02***
Systolic blood pressure	**1.04 [1.02; 1.06]**	**< 0.001***
Diastolic blood pressure	**1.07 [1.04; 1.1]**	**< 0.001***
Fasting glucose	**1.04 [1.02; 1.07]**	**< 0.001***
Total cholesterol	1.001 [0.995; 1.007]	0.74
High-density lipoprotein cholesterol	**0.97 [0.95; 0.98]**	**< 0.001***
Low-density lipoprotein cholesterol	1.005 [0.999; 1.012]	0.09
Triglycerides	**1.02 [1.02; 1.03]**	**< 0.001***
Metabolic syndrome	**22.21 [5.22; 94.42]**	**< 0.001***
hs-CRP	**1.19 [1.01; 1.41]**	**0.04**
Insulin	**1.13 [1.07; 1.19]**	**< 0.001***
HOMA-IR score ≥ 2.5	**4.01 [2.08; 7.72]**	**< 0.001***
MEDAS score (continuous)	0.28 [0.06; 1.4]	0.12
MEDAS score > 0.5	0.64 [0.41; 1.02]	0.06
VO_{2peak}	**0.88 [0.84; 0.92]**	**< 0.001***
Ever smoked	**1.72 [1.08; 2.74]**	**0.02***
Number of pack-years smoked	**1.04 [1.01; 1.07]**	**0.02***

* Results are statistically significant at a p value of ≤ 0.05 (in bold).

median age at PBSO was 45 years (range: 29–65 years). Tumour biology and breast cancer treatments were similar between the two groups.

Anthropometric variables between the two groups differed substantially: Women with low bio-ADM levels had a lower BMI and smaller waist and hip circumferences compared to women with high bio-ADM levels. Women among the high bio-ADM levels group had higher systolic blood pressure, higher diastolic blood pressure, higher fasting glucose levels, higher triglyceride levels, and lower HDL levels as compared to the low bio-ADM levels group. Thus, metabolic syndrome was more prevalent among women with high bio-ADM levels (11.6% vs. 0.7%). Peak oxygen uptake was substantially higher in the low bio-ADM levels group (28.2 ml/min/kg vs. 23.0 ml/min/kg). Among the high bio-ADM levels group, there were more women who have ever smoked (26.7% vs. 22.9%).

Univariate analysis

▶ **Table 2** summarizes the odds ratios (OR) and associated 95% confidence intervals (95% CI) of traditional cardiovascular risk factors associated with low vs. high bio-ADM levels among *BRCA1* and *BRCA2* mutation carriers. Increasing age was associated with a tendency to higher bio-ADM levels (OR = 1.03, p = 0.03). Bio-ADM levels were not associated with *BRCA* mutation status (p = 0.27), a previous history of breast cancer (p = 0.28) or PBSO (p = 0.29).

However, women who received their breast cancer diagnosis at least four years prior to study enrolment had higher odds of having increased bio-ADM levels (OR = 1.91, p = 0.05). Women fulfilling the criteria of metabolic syndrome had over 22-times higher odds of having increased bio-ADM levels compared to those who did not meet the criteria (OR = 22.2, p < 0.001). Moreover, higher bio-ADM levels were significantly associated with a bigger body size, as determined by BMI (OR = 1.28; p < 0.001), waist circumference (OR = 1.09; p < 0.001), hip circumference (OR = 1.1; p < 0.001), and waist-to-hip-ratio (OR = 49.22, p = 0.02). Moreover, high bio-ADM levels were associated with insulin resistance (OR = 4.01, p < 0.001) and higher hs-CRP levels (OR = 1.19, p = 0.04). Although not statistically significant, there was a trend which suggested that adaptation of the Mediterranean diet at baseline was associated with lower bio-ADM levels (OR = 0.64, p = 0.06). Cardiorespiratory fitness as indicated by peak oxygen uptake was associated with lower bio-ADM levels (OR = 0.88, p < 0.001). Bio-ADM levels were higher in women who have ever smoked (OR = 1.7; p = 0.02), and increased with the number of pack-years smoked (OR = 1.04; p = 0.02).

Multivariate analysis

Results were similar in the multivariate analysis adjusting for potential confounders including age and previous history of breast cancer (as described in ▶ **Table 3**).

Discussion

There is a need for early detection of subclinical cardiac dysfunction in breast cancer survivors. This need is not yet reflected in an effective screening program [5]. Several CVD risk scores have been investigated in the general population but were not found to be suitable for breast cancer survivors [13]. In current practice, echocardiography is the most widely used technique in the diagnosis, prevention and risk stratification of CVD before, during and after cancer treatment. Yet, there is no clear consensus on follow-up cardiac monitoring in breast cancer survivors. While conventional echocardiography can detect significant structural and functional changes, global left ventricular systolic function often

▶ **Table 3** Associations between bio-ADM levels (low vs. high) and selected patient characteristics among *BRCA* mutation carriers (multivariate logistic regression).

Predictor	OR [95% CI]	p
BRCA mutation status	1.26 [0.8; 2.0]	0.32
Parity	1.02 [0.61; 1.71]	0.95
PBSO	1.06 [0.78; 1,44]	0.69
Age at PBSO	0.996 [0.85; 1.17]	0.96
BMI	**4.33 [2.39; 7.85]**	**< 0.001***
Waist circumference	**1.08 [1.06; 1.11]**	**< 0.001***
Hip circumference	**1.1 [1.07; 1.14]**	**< 0.001***
Waist-to-hip-ratio	**31.1 [1.2; 805.8]**	**0.04***
Systolic blood pressure	**1.04 [1.02; 1.06]**	**0.001***
Diastolic blood pressure	**1.06 [1.03; 1.1]**	**< 0.001***
Fasting glucose	**1.04 [1.02; 1.07]**	**0.001***
Total cholesterol	0.999 [0.99; 1.01]	0.75
High-density lipoprotein cholesterol	**0.97 [0.95; 0.98]**	**< 0.001***
Low-density lipoprotein cholesterol	1.004 [0.997; 1.011]	0.26
Triglycerides	**1.02 [1.02; 1.03]**	**< 0.001***
Metabolic syndrome	**20.99 [4.91; 89.79]**	**< 0.001***
hs-CRP	**1.21 [1.02; 1.43]**	**0.03***
Insulin	**1.13 [1.07; 1.19]**	**< 0.001***
HOMA-IR score ≥ 2.5	**4.05 [2.09; 7.85]**	**< 0.001***
MEDAS score (continuous)	0.28 [0.06; 1.42]	0.124
MEDAS score > 0.5	0.63 [0.4; 1.01]	0.056
VO_{2peak}	**0.87 [0.83; 0.91]**	**< 0.001***
Ever smoked	**1.68 [1.05; 2.7]**	**0.03***
Number of pack-years smoked	**1.04 [1.00; 1.07]**	**0.04***

Adjusted for age (in years) and previous diagnosis of breast cancer (diseased vs. non-diseased).

* Results are statistically significant at a p value of ≤ 0.05 (in bold).

remains preserved until late in the course of CVD. Vasoactive peptides are directly related to the development and progression of CVD. Recent studies indicate that ADM might identify subclinical cardiac impairment prior to detectable changes in ejection fraction [43].

One way to make a screening program efficient is to apply it to a high-risk population. *BRCA1/2* mutation carriers are suggested to be at an increased risk for CVD, regardless of a previous cancer diagnosis [31, 33]. This is the first study to examine plasma bio-ADM levels among *BRCA* mutation carriers. In line with previous studies among the general population [44], high bio-ADM levels were associated with traditional cardiovascular risk factors, including age [45], BMI [45], insulin resistance [46, 47], metabolic syndrome [48], low cardiorespiratory fitness [49] and smoking [20, 50]. Central obesity (as measured by the waist-to-hip-ratio), rather than general obesity (as measured by BMI), was a strong predictor for high bio-ADM levels which corresponds to other investigations suggesting that adipose tissue is a major source of ADM [51 – 53]. Consistent with our findings, recent studies have shown that adipose tissue distribution outperforms BMI in identifying breast cancer survivors with a high risk for CVD [54]. As described previously in a cohort of cancer survivors [43, 50], we were able to confirm a significant association between the inflammatory marker hs-CRP and bio-ADM. Although not statistically significant in our baseline analysis conducted before intervention, there was a trend which suggested that adherence to the Mediterranean diet was associated with lower bio-ADM levels. After adjustment for age and history of breast cancer, the associations between bio-ADM levels and traditional cardiovascular risk factors remained stable.

Given its robust association with multiple CVD risk factors, our data suggest that bio-ADM might be useful in estimating the burden of CVD attributable to modifiable risk factors in *BRCA* mutation carriers.

ADM is an almost ubiquitously expressed peptide with vasodilatory and natriuretic properties. Previous studies have observed a link between high ADM levels and worse prognosis in patients with myocardial infarction and heart failure. With a prognostic value superior to that of brain natriuretic peptide [55], ADM plays a crucial role in the pathophysiology of major adverse cardiac events. More recently, studies among healthy individuals have shown that ADM levels become elevated years before the onset of CVD and cancer [16, 56]. Identification of the underlying mechanisms associated with this co-occurrence is of great public health importance.

Whilst ADM is a well-established biomarker for CVD, the role of ADM in breast cancer aetiology is less clear. ADM is expressed in sporadic breast cancer tissue [57, 58], and the degree of expression is associated with tumour growth [57, 59, 60], local tumour progression [58] and bone metastases [60, 61]. Preliminary evidence suggests that ADM influences the osteoclast differentiation mediated by Receptor Activator of NF-κB Ligand (RANKL) [61], an important signalling pathway in *BRCA1*-associated breast carcinogenesis [62, 63].

Contrary to expectations, history of breast cancer was not associated with elevated bio-ADM levels in our analysis. Nevertheless, we noted that women who were diagnosed with breast cancer at least four years before study entry had significantly higher bio-ADM levels, delineating them as a higher-risk cohort. Likewise, an older age was associated with a tendency to higher bio-ADM levels which might be attributable to longer oestrogen deprivation. However, due to the median age of the entire study cohort of 43 years, PBSO uptake was low in this population. Therefore, both PBSO and age at PBSO were not associated with higher bio-ADM levels. With respect to our study cohort, it is not entirely surprising that we found no association between circulating bio-ADM levels and history of breast cancer. In our cohort, the median time between breast cancer diagnosis and study entry was three years (range: 0–32 years), resulting in a selection bias for diseased women. Although this finding needs further confirmation, it is an interesting area of research with respect to the long latency periods between the initial diagnosis of breast cancer and the development of manifest CVD.

Strengths and limitations

Strengths associated with the current analysis include the comprehensive evaluation of cardiovascular risk factors using several objective measurements. After adjusting for age and prior history of breast cancer, the adjusted and unadjusted results did not differ significantly. Therefore, any additional confounding was likely small. Although our results provide an exciting direction for prevention research, this study had several limitations. The median age of our study cohort was 43 years. Thus, the prevalence of manifest CVD is expected to be low. The proportion of women who met the criteria of metabolic syndrome was 12.3% in our analysis. This compares to a prevalence of 18–21% among the general German population [64]. Considering the substantially lower prevalence of CVD risk factors among our study cohort, results obtained in this analysis likely underestimate the true associations between bio-ADM levels and outcomes attributable to modifiable risk factors. In order to estimate the association between ADM and traditional CVD risk factors, we used single measurements of bio-ADM at baseline only. Our study is limited by the fact that there is no reference cohort of *BRCA*-negative women. With regard to the lack of reference values for bio-ADM among the general population, we were not able to provide suitable bio-ADM thresholds for subclinical cardiac impairment. Pavo et al. have shown that patients with cancer and without prior cancer treatment had elevated levels of ADM even in the absence of overt CVD [43]. Although a continuous information would have been more informative and would have provided more decisive inference, we decided to dichotomize our outcome variable based on the median value of bio-ADM in order to increase robustness of our regression models. Given the prospective nature of the LIBRE trials, we will be able to elucidate the impact of a lifestyle intervention, namely physical activity and a healthy diet, on the change in bio-ADM levels over time. Finally, our cohort was not sufficiently powered to conduct analyses stratified by *BRCA* mutation type.

Conclusions

Identifying, monitoring and reducing CVD risk factors should be a priority for the long-term care of breast cancer survivors. Preliminary evidence suggest that *BRCA1/2* mutation carriers are more prone to CVD. In line with previous studies conducted in the general population, our results indicate that ADM is associated with several cardiovascular risk factors among *BRCA1/2* mutation carriers, irrespective of a previous breast cancer diagnosis. Further research is needed to define suitable bio-ADM thresholds for subclinical cardiac dysfunction. Moreover, the long-term clinical implications of reducing bio-ADM levels through lifestyle and/or medical interventions in women at high risk for breast cancer, complemented by mechanistic evidence, are yet to be determined.

Funding

JL gratefully acknowledges funding from the German Society for Nutritional Medicine (DGEM) and the German Society for Haematology and Medical Oncology (DGHO). The trial is funded in part by the German Cancer Aid (Deutsche Krebshilfe: http://www.krebshilfe.de) within the Priority Program “Primary Prevention of Cancer” (Grant no. 110013).

Trial registrations

NCT02087592; NCT02516540

Author Contributions Statement

JL conceptualized and designed the study, coordinated and conducted the acquisition and interpretation of data, carried out data analyses, and drafted the initial manuscript. MK conceived of the study, designed the study, coordinated the study and critically revised the manuscript. SG participated in the design of this study, was involved in the acquisition and interpretation of data, and gave final approval of the version to be published. JS and OH performed bio-ADM analyses and contributed to critical revision of the manuscript. MB, CE, SCB, ABE and MH were involved in the acquisition and interpretation of patient data and contributed to critical revision of the manuscript. All authors have read the manuscript and have given their final approval for publication of this study.

Ethics Statement

The study was approved by the institutional ethics review boards of both the host institutions (Technical University of Munich: Reference No. 5686/13, University Hospital Cologne: Reference No. 13-053 and University Hospital Schleswig-Holstein in Kiel: Reference No. B-235/13) and the participating study centers, and all study subjects provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations.

Data Availability Statement

Data is available upon reasonable request to the corresponding author.

Conflict of Interest

JS and OH are employed by Sphingotec GmbH, a company having patent rights in and commercializing the bio-ADM assay. MK and SG received grants from Sphingotec GmbH. JL, MB, CE, SCB, ABE and MH have nothing to disclose.

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