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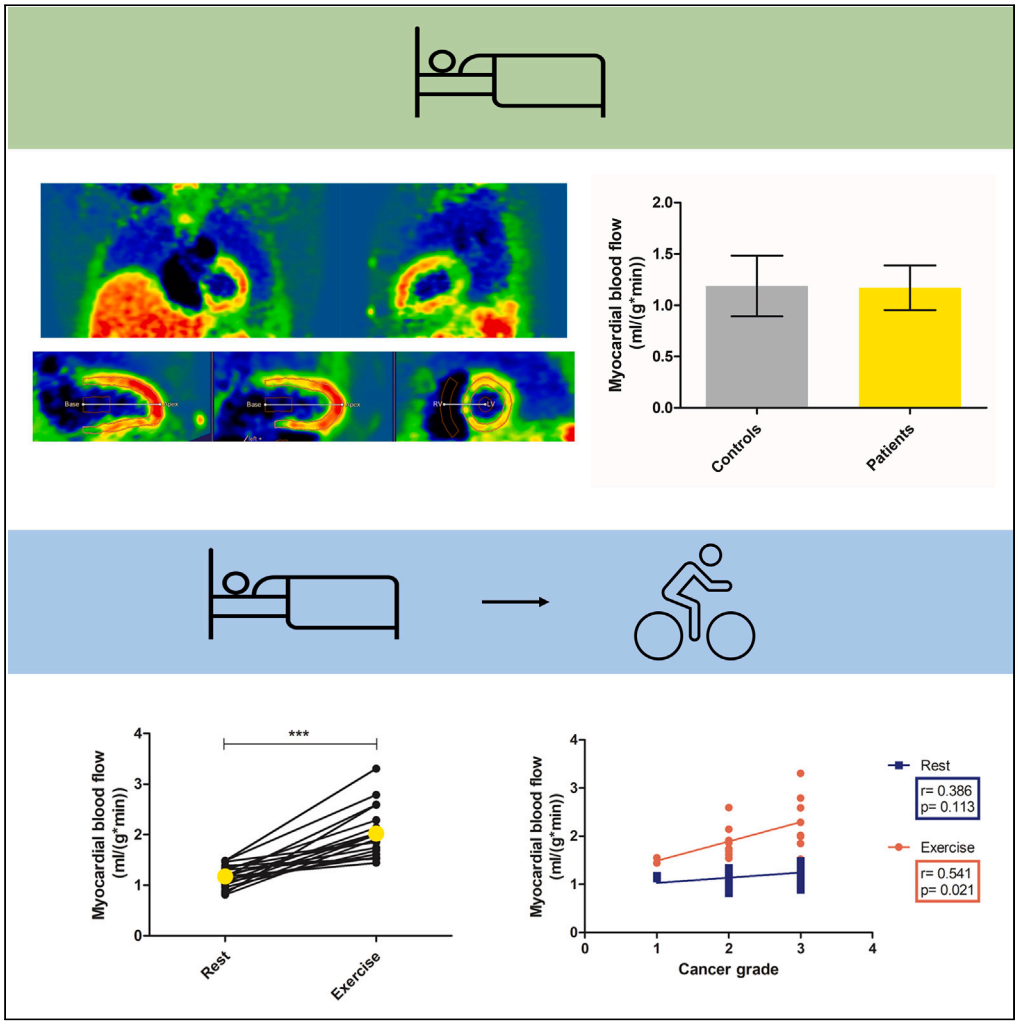

Tiia Koivula, Tytti-Maria Uurasmaa, Chunlei Han, ..., Heikki Minn, Juhani Knuuti, Ilkka Heinonen

tmkoiv@utu.fi

Highlights

Myocardial blood flow is similar in breast cancer patients and in healthy women

Exercising blood flow correlates positively with the histologic grade of cancer

Cancer does not affect cardiac capillary density in breast cancer mouse model

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Myocardial blood flow in newly diagnosed breast cancer patients at rest and during exercise

Tiia Koivula,[1,8,*] Tytti-Maria Uurasmaa,[1,2] Chunlei Han,[1] Teemu Maaniitty,[1] Shiva Latifi,[1,2,3] Salla Lempiäinen,[1,4] Kari Kalliokoski,[1] Carl Johan Sundberg,[5,6] Helene Rundqvist,[7] Katja Anttila,[2] Heikki Minn,[4] Juhani Knuuti,[1] and Ilkka Heinonen[1]

SUMMARY

The heart depends critically on continuous blood supply, but it is unknown whether cancer itself affects myocardial blood flow (MBF). This study investigated MBF in cancer patients and cardiac morphology in a cancer mice model. MBF was quantified with [^{15}O]H_2O positron emission tomography at rest in recently diagnosed breast cancer patients and age-matched female controls, and additionally during 10-min exercise in the cancer patients. Cardiac morphological changes were analyzed with a breast cancer mouse model and control mice without tumors. Resting MBF was similar in cancer patients and controls. MBF increased significantly during exercise in cancer patients, and exercising MBF correlated positively with cancer grade. In the mouse model, cancer did not affect heart weight, cardiomyocyte size, myocardial capillary density, or capillary-to-myocyte size ratio. Thus, resting MBF in humans or myocardial capillarity in mice appears not to be affected by breast cancer. The exercise-induced MBF increase in cancer patients with higher histologic grade requires further investigations.

INTRODUCTION

Breast cancer is the most commonly diagnosed cancer worldwide and is also among the primary causes of all cancer-related deaths.[1] Breast cancer also associates with increased risk for cardiovascular disease.[2,3] The heart muscle depends critically on continuous blood supply, which closely matches the myocardial energy demand and enables proper function of the myocardium.[4,5] In previous studies, myocardial perfusion defects have been detected in breast cancer and esophageal cancer patients after radiation therapy and in renal cancer and sarcoma patients after vascular endothelial growth factor inhibitor therapy.[6–8] However, direct effects of cancer itself, aside from treatment effects on the heart, remain incompletely studied and understood, and to the best of our knowledge myocardial blood flow (MBF) even at rest but particularly during exercise remains to be investigated in newly diagnosed cancer patients.[9–14] A retrospective patient study by Tadic et al. (2018) showed that treatment-naive patients with several cancer types (including patients with breast cancer) have lower longitudinal and circumferential cardiac strain, although otherwise largely maintained function, suggesting that cancer could affect cardiac mechanics in some cases.[15] Further, breast cancer-induced cardiac remodeling findings, such as increased left ventricle (LV) mass, were also reported in recent MRI studies.[16,17] However, in these studies majority (24/28) of the breast cancer patients were investigated after surgical removal of the tumor, which was also likely the main reason why breast cancer patients showed over 10 beats per minute higher resting heart rate (HR) at rest compared to their controls. Consequently, the primary aim of this study was to examine whether cancer itself affects MBF, independently of the LV mass, at rest or during acute exercise stress in breast cancer patients who have not started any cancer treatments. Patients were carefully chosen and studied in a short time window between diagnosis and initiation of any treatments. Additionally, the association between the severity of cancer and MBF is examined. To this end, we also aimed to investigate in a preclinical breast cancer mouse model whether breast cancer itself affects the myocardium at the tissue level, such as altering capillary density or capillary-to-myocyte size ratio, which contributes to tissue perfusion.

[1]Turku PET Centre, University of Turku and Turku University Hospital, 20520 Turku, Finland
[2]Department of Biology, University of Turku, 20520 Turku, Finland
[3]Department of Biosciences, Åbo Akademi, 20500 Turku, Finland
[4]Department of Oncology and Radiotherapy, Turku University Hospital, 20520 Turku, Finland
[5]Department of Physiology and Pharmacology, Karolinska Institutet, 171 77 Stockholm, Sweden
[6]Department of Learning, Informatics, Management and Ethics, Karolinska Institutet, 171 77 Stockholm, Sweden
[7]Department of Laboratory Medicine, Karolinska Institutet, 171 77 Stockholm, Sweden
[8]Lead contact
*Correspondence: tmkoiv@utu.fi
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Table 1. Baseline and resting characteristics of the study participants

	Breast cancer patients (n = 18)	Controls (n = 32)
Age, years	58 (11)	61 (7)
BMI, kg/m^2	28.3 (4.9)	26.0 (3.6)
Heart rate, bpm	66 (9)	63 (10)
Systolic blood pressure, mmHg	141 (32)	138 (20)
Diastolic blood pressure, mmHg	75 (12)	76 (12)
RPP, bpm·mmHg	9401 (2785)	8756 (2220)

Data presented as mean (SD).

RESULTS

Baseline and exercise characteristics

Baseline characteristics of the cancer patients and controls are presented in Table 1. Age, BMI, resting HR, blood pressure, and rate pressure product (RPP) were similar in both groups. In cancer patients, HR, blood pressure, and RPP increased significantly from rest to exercise. In cancer patients, HR was 66 $\pm$ 9 bpm at rest and 114 $\pm$ 17 bpm during exercise ($p < 0.001$). Systolic blood pressure was 141 $\pm$ 32 mmHg at rest and 158 $\pm$ 32 mmHg during exercise ($p < 0.001$), and diastolic blood pressure was 75 $\pm$ 12 mmHg at rest and 90 $\pm$ 30 mmHg during exercise ($p < 0.010$). RPP was 9,401 $\pm$ 2,785 bpm·mmHg at rest and 18,226 $\pm$ 4,819 bpm·mmHg during exercise ($p < 0.001$). Moreover, HR percentage from age-predicted maximal HR was 71% $\pm$ 10% during the exercise in cancer patients.

MBF

Resting global MBF did not differ between the groups being 1.2 $\pm$ 0.2 mL/(g·min) in cancer patients and 1.2 $\pm$ 0.3 mL/(g·min) in controls ($p = 0.84$) (Figure 1A). MBF increased to 2.0 $\pm$ 0.5 mL/(g·min) during exercise in cancer patients ($p < 0.000$) (Figure 1B). Moreover, exercising MBF, but not resting MBF, correlated positively with cancer histologic grade (Figure 1C). Exercising HR, RPP, rate of perceived exertion, or HR percentage from age-predicted maximal HR did not correlate with cancer grade ($p > 0.23$ in all correlations).

Resting MBF correlated positively with resting HR and RPP in both groups (Figure 2). In addition, when resting MBF and exercising MBF were clustered, MBF correlated positively with resting HR and RPP in breast cancer patients, but exercising MBF alone was not significantly associated with exercising HR or RPP (Figures 2A and 3B).

Cardiac morphology

There was no significant difference between the control mice and the breast cancer mice in relative body mass ($p = 0.81$, Figure 3A) and relative whole heart weight ($p = 0.73$, Figure 3B). There was also no significant correlation between the heart weight and the capillary density ($p = 0.12$, r = 0.10, Figure 3C). Similarly the groups did not differ in LV capillary density ($p = 0.74$, Figure 3D), cross-sectional cardiomyocyte size ($p = 0.30$, Figure 3E), or number of capillaries per cardiomyocyte ($p = 0.17$, Figure 3F). Further, there was no significant correlation between mouse tumor volume and capillary density or number of capillaries per cardiomyocyte.

DISCUSSION

Cancer patients often suffer from treatment-related cardiovascular side effects including arrhythmias, arterial hypertension, heart failure, and myocardial infarction.[18] It is, however, poorly understood whether a cancer disease itself has any negative effects on heart health and function. Here, newly diagnosed breast cancer patients and female control subjects without cancer were scanned with positron emission tomography (PET) and their MBF was measured to investigate cardiac function. We found that resting MBF was similar in breast cancer patients and in control subjects suggesting that untreated cancer does not have an impact on myocardial perfusion at resting state.

These findings were also supported by the findings in our animal experiments, which demonstrated that heart weight, cardiomyocyte size, myocardial capillary density, and capillary-to-myocyte size ratio were similar in tumor-bearing mice and control mice. In accordance with our findings, a previous study by Hoffman et al. (2021) also demonstrated that untreated breast cancer-bearing nude mice had similar cardiac morphology and function 2 and 4 months after inoculation.[19]

Our findings suggest that largely other mechanisms, such as cancer therapies, may cause cardiac and cardiovascular dysfunction rather than the cancer itself. Hoffman et al. also showed that the chemotherapeutic treatment caused cardiac atrophy and damage to cardiac vasculature and function in nude mice with breast cancer rather than the cancer itself.[19] They found that breast cancer decreased the expression of endothelial cell tight junction proteins, but this decrease was more apparent in the treated animals. Additionally, with a mouse melanoma model, with known cardiac effects, it has been demonstrated that the cardiac effects of cancer alone were quickly reversible after cancer recovery whereas the chemotherapy-induced effects were irreversible or long lasting.[20] Further, recent breast cancer MRI studies also suggest that cancer itself could induce myocardial remodeling,[16,17] but interpretations of these findings are challenging. In these studies breast cancer patients were studied after surgical removal of the tumor in the majority of the patients (24/28), which was also most likely the reason why breast cancer patients showed over 10 beats per minute higher resting HR due to continued surgery-induced stress reaction. In the present

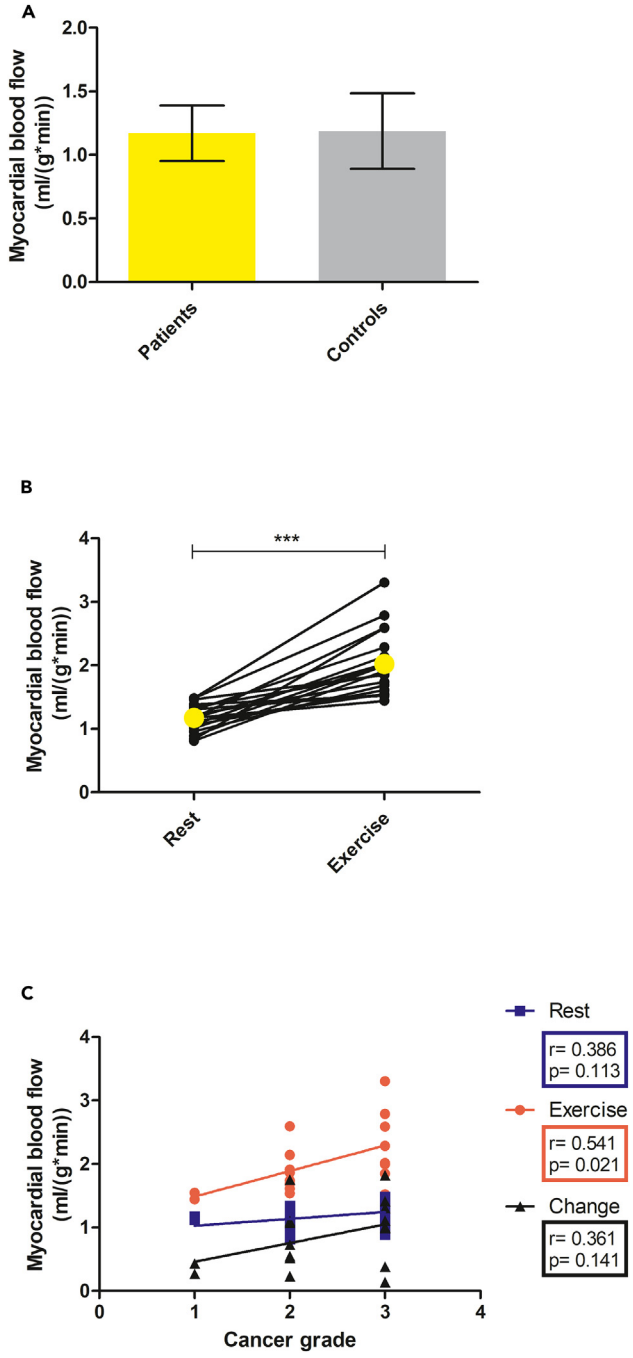

Figure 1. Myocardial blood flow in cancer patients and healthy controls

(A) Resting myocardial blood flow was similar in breast cancer patients and control group.

(B) Myocardial blood flow increased from rest to exercise in cancer patients.

(C) Cancer grade correlated positively with myocardial blood flow during exercise. ***$p < 0.001$ in paired t test. Error bars shown are ±SD.

study we show that particularly resting HR correlates tightly with MBF, in both cancer patients and controls, meaning that higher resting MBF could have been documented in the recent MRI studies if that had that been measured. In the present study breast cancer patients were carefully chosen and studied in a short time frame between the diagnosis and initiation of any treatments or surgery, and breast cancer patients showed similar resting HR compared to our matched controls.

In the current study breast cancer patients were also studied during acute bicycling exercise. As expected, MBF increased significantly from rest to exercise. Interestingly, the higher was the cancer histologic grade, the higher was also the MBF during exercise. We lack direct

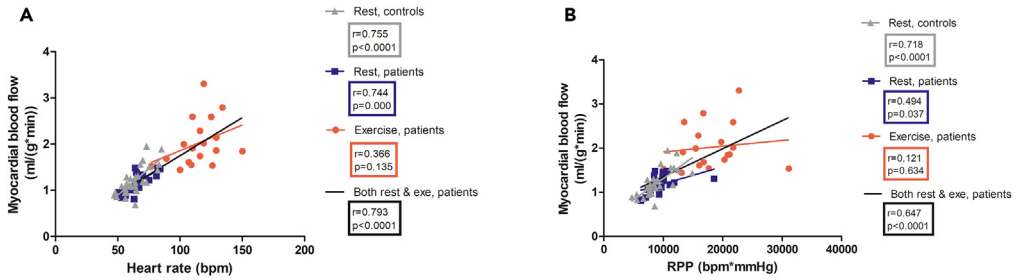

Figure 2. Correlations between heart rate and rate pressure product and myocardial blood flow

(A) Heart rate and (B) rate pressure product (RPP) correlated positively with resting myocardial blood flow in both cancer patients and healthy controls.

(patho)physiological explanations for this finding but can speculate a few options. One option could have been that strenuousness of exercise was more intense and myocardial oxygen demand was higher in breast cancer patients with higher histologic grade of cancer. However, this appeared not to be the case as HR, RPP, or HR percentage from age-predicted maximal HR did not correlate with cancer grade. While HR is the single most important determinant of myocardial oxygen demand, and RPP also takes account of the blood pressure effect (afterload), also wall stress and myocardial contractility (force of one heartbeat) are important determinants of myocardial oxygen consumption. Thus the second option might be that ventricular wall stress and/or contractility were higher in patients with higher cancer grade, which contributed to the higher MBF during exercise, but which cannot be addressed by any HR or RPP normalizations. Wall stress is taken into account and reflected in stroke work, which gives the best measure of myocardial oxygen demand, but it requires invasive measurements directly from the LV with pressure-volume catheters.[21–25] Further, myocardial oxygen demand (as well as efficiency of cardiac work) and MBF might still be affected as circulating energy substrates also contribute to the myocardial oxygen consumption, and thus MBF.[26] At present we do not know whether the higher stress MBF observed in patients with higher cancer histologic grade is detrimental or beneficial for the heart, but it warrants further investigation.

Limitations of the study

In the present study we were measuring MBF non-invasively with gold-standard PET methodology not only at rest but also during exercise for the first time in breast cancer patients. However, our study is not without limitations. Echocardiography has a well-established role in cardiac evaluation of breast cancer patients. Therefore, additional echocardiography could have provided us more insights but was not included due to logistic issues. Another limitation is that we do not know for how long breast cancer had manifested in patients before its diagnosis and our measurements. The duration of cancer before diagnosis may have contributed to the results. The patients in this study had grade 1, 2, or 3 breast cancers, and only a few of the cancers were metastatic. It is possible that in more widespread cancer, cardiovascular effects may be caused. Moreover, the exercise intensity was not controlled in a standardized manner that could allow the same relative exercise intensity for each patient. This may have led to variation in exercise responses. We were also unable to examine MBF in our control individuals during exercise, and thus we could not compare MBF responses in healthy subjects and cancer patients. Finally, there is also a possibility in regards to our preclinical model that the results could have been different in a spontaneous breast cancer model compared to the subcutaneous model applied here. However, the current study still offers important evidence that the presence of mammary tumor burden and its secreted factors alone do not induce changes in the murine cardiac structure at this time frame of tumor growth. Spontaneous tumor model resembles better human breast cancer, but subcutaneous model anyway mimics tumor that has spread from the tissue of origin to nearby tissues and it accounts for some tumor-secreted factors. Importantly subcutaneous model allows equal timing for the development of the tumors and more equal conditions for when tumors begin to develop while also avoiding potential genetic differences that a spontaneous model may have that could contribute to study outcomes.

In conclusion, resting MBF was similar in breast cancer patients and control subjects without cancer. However, we found that MBF in response to exercise positively correlated with increasing histologic grade in breast cancer patients, which warrants further investigations to understand the clinical significance of this finding.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Tiia Koivula (tmkoiv@utu.fi).

Materials availability

This study did not generate new unique reagents.

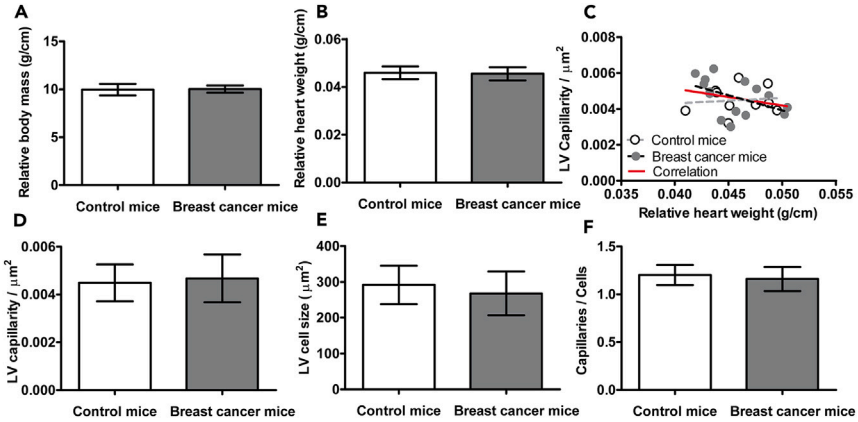

Figure 3. Cardiac morphology in breast cancer and control mice

The relative body mass (A) and relative heart mass (B) as well as the morphological variables of the left ventricles of female FVB/N mice without tumors (n = 10) or with subcutaneous breast cancer tumors (n = 15). The correlation of the heart mass to left ventricle capillary density (C) is shown with the correlation trend line for the control (light gray) and breast cancer group (dark gray) with the overall trend line for both groups shown in red. The left ventricle capillary density (D), cross-sectional cardiomyocyte size (E), and number of capillaries per cardiomyocytes (F) are shown above the exemplary images of the Schiff-stained left ventricles where the capillaries are indicated with black arrows. All error bars shown are ±SD.

Data and code availability

- Data and images that support the findings of this study are available on request from the lead contact.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

I.H. conceived and designed the research; T.K., S. Lempiäinen, T.-M.U., S. Latifi, and T.M. performed the experiments; T.K., T.-M.U., and C.H. analyzed data; T.K., T.-M.U., and I.H. interpreted the results of the experiments; T.K., T.-M.U., and C.H. prepared the figures; T.K., T.-M.U., and I.H. drafted the manuscript; all authors edited and revised the manuscript; all authors approved the final version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Cell lines		
Murine mammary 13TC cells derived from spontaneous tumors of PyMT-MMTV transgenic mice	Weiland et al.[27]	
Experimental models: Organisms/strains		
Mice: FVB/NHan®Hsd	Inotiv, Netherlands	
Software and algorithms		
Graphpad Prism v5	GraphPad Software, Inc.	https://www.graphpad.com/
Carimas	Turku PET Center	https://carimas.fi
ImageJ v1.53t		
NIS-Elements AR-23		

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Participants

The study included 18 women with recently diagnosed breast cancer who had not started their cancer treatments. Therefore, the patients were recruited during the first week after diagnosis and studied 1–3 weeks after diagnosis. There were 2 cases of histologic grade 1, 8 cases of grade 2, and 8 cases of grade 3. In addition, 32 age-matched female control participants were included. Inclusion criteria for cancer patients was newly diagnosed breast cancer and age over 18 years. Exclusion criteria were abnormal fatigue, anemia, or some physical dysfunction due to disease. Controls were participants from cardiac multimodality imaging studies[28,29] who were based on these imaging findings judged to be free of obstructive coronary artery disease. All patients provided a written informed consent after reviewing the study information sheet and hearing an explanation about the study from the investigators. The human study was approved by the Ethics Committee of the Hospital District of Southwestern Finland, and good clinical practice and the Declaration of Helsinki were followed.

Breast cancer mouse model

Ten-week old female mice of the strain FVB/NHanHsd (Inotiv, Netherlands) were divided into two groups, non-tumor bearing group (n = 10) and breast cancer tumor bearing group (n = 15) after minimum one week acclimation to the facilities. Tumor bearing animals were injected under isoflurane anesthesia (2%) subcutaneously with I3TC tumor cells 1.8 × 10^6 cells originating from the PyMT-MMTV transgenic tumor model[27,30] and the control animals were injected with same volume of PBS. Animals were housed two per cage and kept in standard conditions with 12 h light to dark cycle and *ad libitum* access to tap water and standard pellet feed. All the mice were handled similar amount to induce similar level of handling stress to the mice. The tumors were allowed to grow for a month unless the animals reached the endpoint criteria of tumor diameter exceeding 1.5 cm. During the study 7 out of 15 tumor bearing animals had to be euthanized before the end of the study for this reason. All the experiments were done with accordance to Finnish legislation under the ethical license 26508-2021.

Cell line

Prior inoculation the murine mammary tumor 13TC cells were cultivated at +37°C CO_2 5% in Duolbecco's Modified Eagle Medium (Thermo Fisher Scientific, 4.5 g/L D-Glucose, L-Glutamine ref. 41965-039) with 45 u/L penicillin/streptomycin (EuroClone ECB3001D) and 10% Fetal Bovine Serum (Sigma-Aldrich F7524-500mL). On passage 4 the cells were detached and suspended in PBS for inoculation. The mouse mammary tumor cells were previously authenticated and isolated from the transgenic PyMT-MMTV tumor model by Weiland et al.[27] and tested previously for mycoplasma contamination by Rundqvist et al.[30] before being sent to University of Turku for the current experiments.

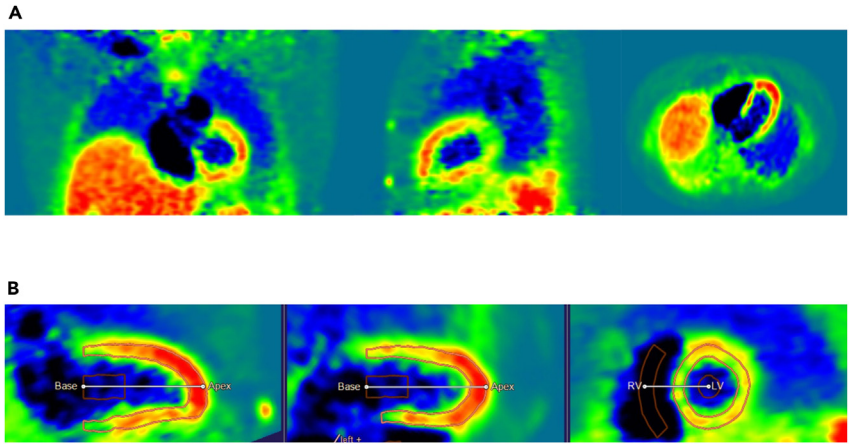
METHOD DETAILS

PET image acquisition and myocardial blood flow analysis

Both cancer patients and controls were imaged with a PET scanner at rest and cancer patients also during exercise. The PET scanners used to measure MBF with [^{15}O]H_2O radiowater tracer were GE Discovery VCT PET/CT (GE Healthcare, Milwaukee, WI, USA), Discovery 690 (D690) (GE Healthcare, Milwaukee, WI, USA), and Discovery MI PET/CT scanner (GE Healthcare, Milwaukee, WI, USA). Scanning was performed using

the GE Discovery STE VCT tomograph (General Electric Waukesha, WI, USA) using a standard protocols at the Turku PET Centre.[29,31] ^{15}O-labelled water (700-900MBq) was injected at rest and during exercise as an intravenous bolus over 20 s at an infusion rate of 10 mL/min and the venous line was then flushed for another 2 min. The data were reconstructed using the standard iterative reconstruction algorithm of the scanner manufacturer with a zoom of 2.0 to the 128 x 128 matrix (standard for PET myocardial perfusion imaging[32]; voxel size 2.73 × 2.73 × 3.27 mm).

The reconstructed rest and exercise PET dynamic images were quantitatively analyzed using Carimas software (developed in Turku PET Center, Turku University Hospital of Finland). After images were loaded, the orientation of the heart was identified manually, then myocardium was automatically detected. The volume-of-interest (VOI) of left ventricular myocardium and cavity could be manually adjusted if necessary. An implemented mathematical model for quantitative global left ventricular MBF (in unit of ml/g/min) based on one-tissue compartment model was performed[33] for both rest and exercise studies. The below figure illustrates the PET images and VOIs.



Analysis of the myocardial blood flow with PET imaging
(A) PET imaging of the heart and (B) volume of interests (VOIs) of left ventricular myocardium.

Exercise study

The breast cancer patients did a 10-min exercise session with supine bicycle ergometer (Tunturi E30R, Hungary) in a PET scanner during which exercising MBF was measured. Patients' upper body was supported and was immobile, and their feet were attached to the pedals of the ergometer placed as an extension of the PET scanner. MBF imaging was initiated after 4-min cycling, which represents steady-state exercise and MBF is thus average value for the measured 6-min period. Each patient tested pedaling of the ergometer one to few days before the study day. During that testing, pedaling power (watts) was determined for each patient individually, so that patients were able to pedal for 10 min without exhaustion, but also so that it was high enough to raise heart rate significantly, typically above 100bpm. Workload ranged from 15 to 75 W between patients.

Heart rate and blood pressure measurements and derived calculations

Heart rate (HR) and blood pressure were measured at rest in both groups and during exercise in cancer patients. Rate pressure product (RPP) was calculated with the following formula: $RPP = SBP * HR$, SBP being systolic blood pressure. Mean arterial pressure (MAP) was calculated with the following formula: $MAP = \frac{2*DBP+SPB}{3}$, DBP being diastolic blood pressure. Age predicted maximal heart rate was calculated with the following formula: $HRmax = 220 - age$.

Cardiac morphological analysis

The relative body mass and relative heart weight of the mice were calculated by dividing the body mass and the wet heart weight with tibia length. After recording whole heart weight a transversal tissue piece was cut from middle of the mouse hearts and fixed in 10% formalin for 48 h and stored in 70% ethanol at +4°C until further processing. The hearts were dehydrated in increasing ethanol series and UltraClear (J.T.Baker) and infiltrated and embedded in paraffin. Microtome was used to cut 5 μm sections from the hearts which were rehydrated and stained using Periodic Acid-Schiff Stain using 35-min Schiff's reagent staining (1.2 mM basic fuchsin; 0.1 M HCl; 2.1 mM sodium meta-bisulfite) according to Andersen[34] for the capillary vessel detection. All sections were imaged using Nikon Eclipse Ni-E with a Nikon pE-300ultra camera and NIS-Elements AR-23 software at 20x magnification. The capillary density and cell size were calculated from left ventricles by manually counting capillaries and cells within minimum area of ~4000 μm^2 using ImageJ software (version 1.53t).

QUANTIFICATION AND STATISTICAL ANALYSIS

Unpaired t-test was used to study differences between healthy subjects and cancer patients and differences between mouse groups. Change in variables between rest and exercise in cancer patients were analyzed using paired t-test. Correlations between MBF and HR and RPP, heart weigh and capillary density, and tumor volume and capillary density and number of capillaries were analyzed with Pearson, and correlations between cancer grade and MBF and HR and RPP were analyzed with Spearman. Significance was determined at $p < 0.05$. All statistical analyses were performed with Graphpad prism 5.0.

ADDITIONAL RESOURCES

The study is part of a clinical study registered in the international register of clinical trials (Clinicaltrial.gov NCT4416087).