Carnitine and Cardiac Dysfunction in Childhood Cancer Survivors Treated with Anthracyclines


Abstract

Childhood cancer survivors are at high risk of developing congestive heart failure (CHF) compared with the general population, and there is a dose-dependent increase in CHF risk by anthracycline dose. The mechanism by which this occurs has not been fully elucidated. Metabolomics, the comprehensive profile of small-molecule metabolites, has the potential to provide insight into the pathogenesis of disease states and discover diagnostic markers for therapeutic targets. We performed echocardiographic testing and blood plasma metabolomic analyses (8 pathways; 354 metabolites) in 150 asymptomatic childhood cancer survivors previously treated with anthracyclines. Median time from cancer diagnosis to study participation was 12.4 years (2.6–37.9 years); 64% were treated for a hematologic malignancy; median anthracycline dose was 350 mg/m² (25–642 mg/m²). Thirty-five (23%) participants had cardiac dysfunction—defined as left ventricular end-systolic wall stress >2SD by echocardiogram. Plasma levels of 15 compounds in three metabolic pathways (carbohydrate, amino acid, and lipid metabolism) were significantly different between individuals with cardiac dysfunction and those with normal systolic function. After adjusting for multiple comparisons, individuals with cardiac dysfunction had significantly lower plasma carnitine levels (relative ratio (RR), 0.89; \( P < 0.01 \)) in relation to those with normal systolic function. These findings may facilitate the development of primary prevention (treatment of carnitine deficiency before/during anthracycline administration) and secondary prevention strategies (screening and treatment in long-term survivors) in patients at highest risk for CHF. Cancer Epidemiol Biomarkers Prev; 23(6); 1109–14. ©2014 AACR.

Introduction

Anthracyclines are widely used in the treatment of childhood cancer (1). However, the use of anthracyclines is limited by their strong dose-dependent association with late-onset congestive heart failure (CHF; ref. 1). Outcome of patients with anthracycline-related CHF is poor with 5-year mortality rates exceeding 50% (1, 2). Anthracycline-related cardiotoxicity is mediated, in part, by direct myocardial injury due to formation of free radicals or toxic alcohol metabolites, disruption of myocyte fatty acid oxidation, or by topoisomerase II–directed disruption of the myocyte mitochondrial genome (1, 3). However, the exact mechanism of anthracycline-induced cardiotoxicity at the cellular level has not been completely elucidated.

Metabolomics refers to the comprehensive profiling of small-molecule metabolites found in biologic specimens. The metabolome could represent the cumulative end product of gene expression, environmental factors, or complex multifaceted interactions between them. Importantly, metabolomic analysis has the potential to elucidate molecular pathways involved in the pathogenesis of disease states such as anthracycline-related CHF and help identify druggable targets. In a recent study (4) of patients with primary dilated cardiomyopathy (DCM), decreased levels of plasma steroid metabolites, glutamine, histidine, threonine, and increased levels of lipid \( \beta \)-oxidation products were associated with disease severity. Importantly, clinical response to anti-congestive treatment such as furosemide and angiotensin-1–converting enzyme inhibitors corresponded with improvement in plasma metabolites such as glutamine, threonine, and steroid metabolites. These findings informed subsequent studies evaluating response-based pharmacologic therapy to prevent progression of cardiac dysfunction in DCM (5). Similar studies are lacking in childhood cancer survivors at high risk for anthracycline-related CHF. The current report...
describes how metabolomic profiling of anthracycline-exposed survivors may provide new information for the development of targeted primary or secondary prevention strategies.

Materials and Methods
Patient eligibility and recruitment
Childhood cancer survivors were recruited between October 2010 and September 2012 from the City of Hope or the Children’s Hospital Los Angeles Childhood Cancer Survivorship Clinics. Eligibility criteria included: (i) cancer diagnosis before 22 years of age, irrespective of current age; (ii) 2 or more years after completion of cancer treatment; and (iii) receipt of anthracycline chemotherapy. To ensure heterogeneity of anthracycline exposure, survivors at high risk (cumulative anthracycline dose ≥ 300 mg/m²) and low risk (1–299 mg/m²) of developing CHF per the Children’s Oncology Group Long Term Follow-up Guidelines (6) were targeted for recruitment. Furthermore, to enrich the cohort for those with cardiac dysfunction, patient recruitment disproportionately (2:1) targeted high-risk survivors over those who were at low risk. We excluded survivors who were actively being treated for cardiomyopathy as pharmacologic therapy could possibly alter metabolomic profile. The study was approved by the respective institutional review boards. All study participants or their parents/legal guardians provided informed consent.

Cardiac evaluation
Study participants underwent a detailed physical examination by their healthcare provider within the respective Survivorship Clinics, with special attention to signs and symptoms of CHF. Echocardiograms were performed on the day of clinical evaluation by a designated study technician and consisted of complete 2-dimensional (2D), M-mode, and Doppler evaluations, per the American Heart Association/American College of Cardiology (AHA/ACC) task force practice guidelines for the clinical application of echocardiography (7). Identical ultrasound machines (General Electric Vivid-7 echocardiography machine; General Electric) were used for all study-related echocardiographic evaluations at the 2 institutions. Left ventricular ejection fraction (LVEF) was calculated from the apical 4- and 2-chamber views using a modified Simpson biplane method (7). Left ventricular end-systolic wall stress (ESWS) was calculated from the formula \(1.35 \times \text{MAP} \times \text{LVESD} / [(4 \times \text{LVPWS}) (1 + \text{LVPWS} / \text{LVESD})]\) as described (8, 9) where MAP is the mean arterial pressure as obtained by Dinamap blood pressure machine, LVESD is the left ventricular chamber diameter in systole, and LVPWS is left ventricular wall thickness in systole. A digitized and anonymized copy of each echocardiogram was sent to the study core cardiology laboratory (University of Michigan, Ann Arbor, MI) for a reading by the study cardiologist (S.K. Gelehrter) who was blinded to the risk status of the study participant.

Cardiac dysfunction was defined as having ESWS ≥2 SDs of normal (≥70 g/cm²) on the echocardiogram (9, 10). The rationale for choosing ESWS over more conventional indices of left ventricular systolic function such as ejection fraction was that change in ejection fraction typically occurs in the later stages of chronic pathology, signaling an extensive loss of function beyond the compensatory capacity of the myocardium. ESWS is the best-studied echocardiographic index in childhood cancer survivors aside from ejection fraction and is a well-recognized precursor to anthracycline-related CHF (10–12). Importantly, increase in ESWS typically precedes clinically meaningful changes in ejection fraction (<50%; refs. 10–12) providing a reliable and prognostic echocardiographic index to study asymptomatic individuals at risk for CHF.

Metabolomic profile
Blood samples were collected on the day of the echocardiographic assessment, and plasma was extracted within 1 hour of sample collection. Plasma samples were stored at −80°C and shipped to Metabolon, Inc. for batched analytic studies. Detailed procedure for sample extraction and metabolomic analysis is described in a recent technical manuscript (13) and detailed in the Supplementary Appendix (Metabolon Platform). Briefly, approximately 200 μL of plasma was split across 3 analytic platforms: gas chromatography/mass spectrometry (GC/MS), ultra high-performance liquid chromatography (UHPLC)/MS-positive, and UHPLC/MS-negative. Following peak identification and quality control (QC) filtering, integrated peak ion counts for each compound in each sample were used for statistical analysis (Supplementary Appendix; Metabolon Platform). The dataset used for the current study included all detectable compounds of known identity (named biochemicals) that met established acceptance criteria for instrument and process variability; the final dataset included a total of 354 named biochemicals (Supplementary Appendix; Table 1).

Clinical data collection
Self-reported questionnaires were used to obtain baseline demographics data, and medical record review allowed abstraction of the following information: date of diagnosis, type of cancer, stage of disease (if applicable), cumulative dose of anthracycline exposure, and receipt of chest-directed radiation therapy. Lifetime cumulative anthracycline dose was calculated by multiplying the total dose of each anthracycline (doxorubicin, daunorubicin, epirubicin, idarubicin, and mitoxantrone) by a factor that reflects the cardiotoxic potential of each drug and then summing the products of the converted anthracyclines (14).

Statistical analysis
Clinical characteristics of individuals with and without cardiac dysfunction. Categorical variables were compared using \(\chi^2\) tests, and continuous variables were compared using independent 2-sample t tests. Data were analyzed using SPSS Version 18.0 (IBM). All statistical tests were 2-sided, and \(P < 0.05\) was considered statistically significant.
Metabolomic analyses. Data for each compound were normalized using the median values for each run (Supplementary Appendix; Metabolon Platform). This minimizes interday instrument gain drift but does not interfere with intraday sample variability. Welch 2-sample t test was performed on the log-transformed data. To account for multiple comparisons, false discovery rate (FDR) method was used (15); q value was calculated using the method of Storey and Tibshirani (16), a cutoff for statistical significance of $q < 0.05$ (i.e., $FDR < 5\%$) was used. The statistical analysis program "R" was used for the analysis of metabolites (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org).

Results

One hundred fifty-two individuals were approached for participation in the study and 150 participated (high risk: $n = 100$; low risk: $n = 50$). One individual refused participation and one did not complete the full battery of echocardiographic and blood testing (participation rate: 98.6\%).

The salient characteristics of cancer survivors are presented in Table 1. Median time from cancer diagnosis to study participation was 12.4 years (range, 2.6–37.9 years), and 63\% were treated for a hematologic malignancy at a median age of 10.6 years (range, 0.4–21.9 years). Median ejection fraction of study participants was 60\% (range, 50\%–85\%), and none had clinical symptoms or signs of CHF. Thirty-five (23\%) participants (29 high risk and 6 low risk) were found to have cardiac dysfunction at the time of evaluation; there was a dose-dependent increase in the prevalence of cardiac dysfunction by anthracycline dose (1–99 mg/m$^2$: 5.7\%, 100–299 mg/m$^2$: 11.4\%, 300–399 mg/m$^2$: 31.4\%, ≥400 mg/m$^2$: 48.6\%). The correlation between ESWS and ejection faction was $R = −0.48$, $P < 0.01$. Characteristics of patients with and without cardiac dysfunction are

<table>
<thead>
<tr>
<th>Table 1. Patient and treatment characteristics</th>
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<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>Sex, n (%)</td>
</tr>
<tr>
<td>Race/ethnicity, n (%)</td>
</tr>
<tr>
<td>Age at examination, y</td>
</tr>
<tr>
<td>BMI at examination</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
</tr>
<tr>
<td>Solid tumor</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
</tr>
<tr>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>Soft-tissue sarcoma</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
</tr>
<tr>
<td>Lifetime anthracycline, mg/m$^2$</td>
</tr>
<tr>
<td>Chest radiation, n (%)</td>
</tr>
<tr>
<td>Time since cancer diagnosis, y</td>
</tr>
</tbody>
</table>

*Represents matching criteria. 
$^b$Defined as $≥25$ kg/m$^2$ if age $≥18$ years or $≥85$th percentile for age $<18$ years.
presented in Table 2. For patients with cardiac dysfunction, median ESWS and ejection faction were 85 g/cm² (70–106 g/cm²) and 58% (50–67%), respectively. In patients with normal cardiac function, they were 55 g/cm² (30–69 g/cm²) and 62% (50–85%), respectively. Patients with cardiac dysfunction had significantly higher lifetime anthracycline exposure (400 vs. 300 mg/m², \( P < 0.01 \)) than those with normal function. The 2 groups were comparable with respect to sex, race/ethnicity, age at evaluation, body mass index (BMI), chest radiation exposure, mean arterial pressure and reported use of antihypertensive medications at the time of evaluation.

Plasma levels of 15 compounds in 3 metabolic pathways (carbohydrate, amino acid, and lipid metabolism) were significantly different between the 2 groups (Table 3). After adjusting for multiple comparisons, individuals with cardiac dysfunction had significantly lower plasma carnitine levels \( (\text{RR}, 0.89; P < 0.001) \) than those with normal function.

### Discussion

Anthracycline-related cardiac toxicity has emerged as one of the leading causes of morbidity and mortality in long-term survivors of childhood cancer (1), emphasizing the need for studies that examine the pathophysiology of cardiac injury. In the current study, metabolomic profiling of asymptomatic childhood cancer survivors revealed significantly lower plasma carnitine levels in individuals with cardiac dysfunction than those with normal function.

\( l \)-Carnitine (the biologically active stereoisomer of carnitine) is a quaternary amine essential for oxidation of long-chain fatty acids (LCFA), which are a major substrate for energy production in the myocardium (Fig. 1; ref. 17). Carnitine homeostasis is maintained through endogenous biosynthesis of \( l \)-carnitine, absorption of carnitine from dietary sources, and elimination and reabsorption by the kidneys (17). Cardiac myocytes contain relatively high concentrations of carnitine. Carnitine is actively transported into the cell, as myocytes are incapable of carnitine biosynthesis (17, 18). Clinically, both primary and secondary carnitine deficiency can result in cardiomyopathy and cardiac arrhythmias due, in part, to the accumulation of LCFA and acylcarnitines that are unable to be oxidized in the mitochondria and are unavailable for energy production (17, 19). In patients with a past history of myocardial infarction, administration of \( l \)-carnitine has been shown to lead to attenuation of left ventricular dilation, prevent left ventricular remodeling, and is associated with a lower incidence of chronic heart failure and cardiac death (20). These beneficial effects are thought to be due to the resumption of normal oxidative metabolism and restoration of myocardial energy reserves (16, 17).

Previous studies suggest that anthracyclines may exert, at least, part of their cardiotoxicity by inhibiting LCFA oxidation in the heart (21). In animal models, chronic anthracycline administration results in dose-dependent decrease in expression of heart fatty acid–binding protein (H-FABP) and organic cation/carnitine transporter.

### Table 3. Plasma metabolites altered in individuals with left ventricular dysfunction

<table>
<thead>
<tr>
<th>HMDBID</th>
<th>KEGG</th>
<th>Super pathway</th>
<th>Subpathway</th>
<th>Biochemical name</th>
<th>( RR^a ) (abnormal: normal)</th>
<th>( P )</th>
<th>( q )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMDB04136 C16884 Carbohydrate</td>
<td>Nucleotide sugars, pentose metabolism</td>
<td>Threitol</td>
<td>1.29</td>
<td>0.002</td>
<td>0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMDB00169 C00159 Carbohydrate</td>
<td>Mannose metabolism</td>
<td>Mannose</td>
<td>1.17</td>
<td>0.015</td>
<td>0.220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>N/A Amino acid</td>
<td>Glutamate metabolism</td>
<td>Pyroglutamine</td>
<td>1.13</td>
<td>0.003</td>
<td>0.117</td>
<td></td>
</tr>
<tr>
<td>HMDB00766 002847 Amino acid</td>
<td>Acetyl-L-Ornithine</td>
<td>Acetyl-L-Ornithine</td>
<td>1.09</td>
<td>0.002</td>
<td>0.081</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMDB00064 C00300 Amino acid</td>
<td>Creatine metabolism</td>
<td>Creatine</td>
<td>0.95</td>
<td>0.025</td>
<td>0.251</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMDB00736 N/A Amino acid</td>
<td>Valine, leucine, isoleucine metabolism</td>
<td>Isobuylcarnitine</td>
<td>1.07</td>
<td>0.046</td>
<td>0.328</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>C00318 Lipid</td>
<td>Carnitine metabolism</td>
<td>Carnitine</td>
<td>0.89</td>
<td>&lt;0.001</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>HMDB0231 N/A Lipid</td>
<td>LCFA</td>
<td>Eicosanoate</td>
<td>1.26</td>
<td>0.044</td>
<td>0.282</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMDB06547 C16300 Lipid</td>
<td>LCFA</td>
<td>Steardionate</td>
<td>1.62</td>
<td>0.004</td>
<td>0.176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMDB01043 C00219 Lipid</td>
<td>LCFA</td>
<td>Arachidonate</td>
<td>1.15</td>
<td>0.019</td>
<td>0.324</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>C16525 Lipid</td>
<td>LCFA</td>
<td>Dihomo-inooleate</td>
<td>1.34</td>
<td>0.030</td>
<td>0.229</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>N/A Lipid</td>
<td>Lysolipid</td>
<td>1-Stereoylalcoholphosphoinositol</td>
<td>1.44</td>
<td>0.012</td>
<td>0.365</td>
<td></td>
</tr>
<tr>
<td>HMDB01032 C04555 Lipid</td>
<td>Sterol/Steroid</td>
<td>Dehydroandrosterone sulfate</td>
<td>0.61</td>
<td>0.003</td>
<td>0.283</td>
<td></td>
<td></td>
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<tr>
<td>N/A</td>
<td>N/A Lipid</td>
<td>Sterol/Steroid</td>
<td>Pregnen-diol disulfate</td>
<td>0.76</td>
<td>0.011</td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>N/A Lipid</td>
<td>Sterol/Steroid</td>
<td>Pregen steroid monosulfate</td>
<td>0.66</td>
<td>0.035</td>
<td>0.599</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HMDBID, Human Metabolome Database ID; KEGG, Kyoto Encyclopedia of Genes and Genomes Compound Database; N/A, not available.

\( ^a \)Ratio of the mean value of each metabolite in individuals with abnormal cardiac function divided by that in the normal group.
(OCTN2) mRNA in cardiac tissues, corresponding to an increase in serum enzymes [lactate dehydrogenase (LDH), creatine phosphokinase isoenzyme (CK-MB)] of acute cardiac injury (22, 23). OCTN2 is a high-affinity carnitine transporter which mediates carnitine transport across the plasma membrane into the cells, whereas H-FABP plays an important role in eliminating toxic LCFA metabolites from the cytosol (23). Carnitine supplementation has been shown to restore H-FABP and OCTN2 gene expression to baseline and decrease serum LDH and CK-MB levels to control values (22, 23).

Small studies in patients with cancer receiving anthracyclines have found a dose-dependent decrease in plasma carnitine levels during treatment (24, 25), corresponding to acute impairment of left ventricular systolic and diastolic function (25). To date, there have been no studies that have evaluated the role of carnitine in long-term cardiac health of cancer survivors treated with anthracyclines. The findings from the current study, when confirmed in an independent cohort of anthracycline-exposed childhood cancer survivors, could facilitate the development of novel strategies for primary prevention (identification and treatment of carnitine deficiency around the time of anthracycline administration) and secondary prevention in cancer survivors at highest risk of CHF.

It is important to note that the current study used a cross-sectional design. As a result, we are not able to state whether the cardiac dysfunction was due to pre-existing anthracycline-mediated carnitine depletion. In addition, we cannot exclude unrelated causes of carnitine deficien-

![Figure 1. Role of carnitine in mitochondrial long-chain fatty acid oxidation.](http://www.aacrjournals.org)

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

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**Other:** D.R. Freyer supervised study site activities
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advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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References
Correction: Carnitine and Cardiac Dysfunction in Childhood Cancer Survivors Treated with Anthracyclines

In this article (Cancer Epidemiol Biomarkers Prev 2014;23:1109–14), which appeared in the June 2014 issue of Cancer Epidemiology, Biomarkers & Prevention (1), the authors regret that the labels for the supplementary data in the text are incorrect. Below are the correct names of these files, which are linked in the article.

Supplementary Appendix: Metabolon platform is now labeled Supplementary Materials and Methods, which also includes Supplementary Tables S1 and S2 and Supplementary Fig. S1.

Supplementary Appendix Table S1 is now labeled Supplementary Table S3.

Reference


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