Effects of Tai Chi Chuan on Insulin and Cytokine Levels in a Randomized Controlled Pilot Study on Breast Cancer Survivors

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Abstract:

Background-Tai Chi Chuan (TCC) is an integrative medicine mind-body practice with a physical activity component that has positive effects on aerobic capacity, muscular strength, and quality of life among cancer survivors, similar to the effects elicited by other modes of moderate intensity exercise. Inflammatory cytokines, and insulin and insulin-related signaling molecules may contribute to weight gain and affect cancer recurrence rates and survival; exercise can curb cancer- and treatment-related weight gain, increase survival, and reduce levels of insulin and inflammatory cytokines. Despite knowing the beneficial effects of conventional exercise interventions on these mediators, little is known about the physiologic effects of TCC, a mind-body practice with a physical activity component, on these pathways in breast cancer survivors.
Methods-We assessed the effects of a 12-week, moderately intense, TCC intervention (n=9) compared to a non-physical activity control (n=10) consisting of psychosocial support therapy (PST) on levels of insulin, IGF-1, IGFBP-1, IGFBP-3 and cytokines IL-6, IL-2, and IFN-γ in breast cancer survivors.
Results-Levels of insulin are significantly different in TCC and PST groups; levels remained stable in the TCC group, but increased in the PST control group ($p=0.099$). Bivariate analysis revealed novel and significant correlations (all $r > 0.45$, all $p \leq 0.05$) of both decreased fat mass and increased fat-free mass with increased IL-6 and decreased IL-2 levels.
Conclusions-This pilot study shows that TCC may be associated with maintenance of insulin levels and changes in cytokine levels that may be important for maintenance of lean body mass in breast cancer survivors.

Article:

INTRODUCTION

Following the diagnosis of breast cancer, many women gain weight. Several studies have shown that the mean weight gain during the first year after diagnosis is 2.5 to 6.2 kg (range from 1.0 to greater than 11 kg) (1–4). The lowest gains are observed in those receiving local radiation therapy or adjuvant hormonal therapies (e.g. tamoxifen) alone. Highest gains appear in those receiving adjuvant chemotherapy alone or combined with hormonal therapies. In all cases,

weight gain is persistent and still increasing 5 years after treatment (1–4). These levels of weight gain are greater than the weight gain experienced during normal aging and primarily represent an increase in fat mass and a loss of lean mass (fat-free mass), often referred to as sarcopenic obesity (5). While advances in oncology have dramatically increased survival for women with early-stage breast cancer, weight gain following diagnosis appears to negatively affect quality of life and recurrence rate (6). Additionally, weight gain post-diagnosis is associated with increases in all-cause mortality, breast cancer-specific mortality, and cardiovascular-related mortality; each 5-kg increase in weight post-diagnosis increases mortality by 10–12% (7).

Insulin and cytokine pathways may be key mediators of weight gain, recurrence, and survival for breast cancer survivors. Elevated fasting levels of insulin are associated with a 2-fold increased risk of breast cancer recurrence and a 3-fold decrease in survival (8). Indeed, insulin-related pathways involving insulin and insulin-like growth factors (IGFs) are associated with increased cell growth and proliferation (9), providing an explanation for the link between elevated insulin levels and tumor growth. Multiple pro-inflammatory signaling pathways involving cytokines have been implicated in cancer development, progression, and recurrence. These cytokines can alter proliferation, lead to malignant transformation, and promote metastasis (10).

Exercise interventions for breast cancer survivors have positive effects on survival (11–13), quality of life (14), and weight (15), and preliminary evidence suggests that exercise may also reduce the risk of cancer recurrence. Accumulating evidence suggests that exercise may elicit these beneficial effects by minimizing levels of circulating insulin and inflammatory molecules, thus providing positive effects on weight, recurrence, and survival. Regular exercise (both aerobic and strength training) can lower circulating insulin levels, reduce insulin resistance, and reduce weight gain (16, 17). Several recent randomized controlled trials using moderately intense exercise interventions have investigated the effects of exercise on possible insulin-related predictors of recurrence and prognosis in breast cancer survivors (15, 18–22). For example, Irwin et al. found statistically significant decreases in fasting insulin, IGF-1, and IGFBP-3 in postmenopausal women who followed a moderately intense walking-based intervention of 5 days/week for 6 months compared to non-exercisers (21). The 12-month intervention study by McTiernan et al. in postmenopausal breast cancer survivors showed that both exercising and stretching produced small reductions in IGF-1 and IGFBP-3; however, there were no significant differences in mean IGF-1 and IGFBP3 levels between the group that exercised and the group that stretched (20). Reductions in insulin are also associated with reductions in hip and waist circumference as shown in a mixed aerobic and strength training study by Ligibel et al., suggesting that decreased insulin levels may be associated with reductions in abdominal adiposity, which is strongly predictive of cardiovascular risk and risk of diabetes, in overweight survivors (15).

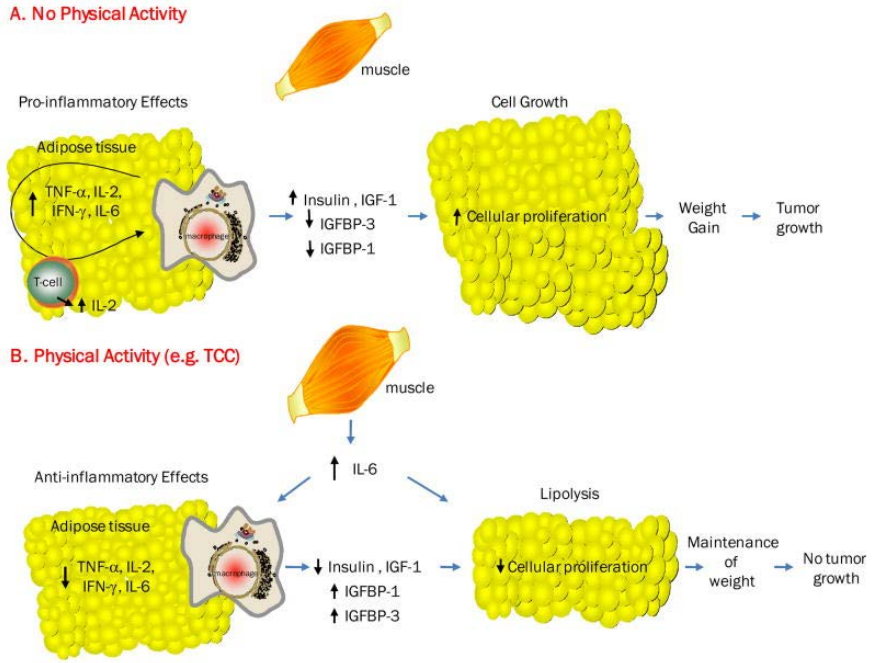
Numerous studies in healthy individuals have revealed that exercise can reduce chronic inflammation by inducing anti-inflammatory effects (23). On a molecular level, during exercise, IL-6 is rapidly produced by contracting skeletal muscle fibers (24) and acts as a myokine with anti-inflammatory effects to inhibit pro-inflammatory cytokine expression (25). Exercise can also reduce adiposity by IL-6-mediated lipolysis and by diminishing levels of circulating and adipose-derived cytokines (e.g. TNF-α, IL-1β) in adults (26–28).

These studies indicate that conventional exercise programs may represent a beneficial intervention for reducing fat mass, improving lean mass and altering insulin, insulin-related molecules, and inflammation to reduce recurrence risk in breast cancer survivors. However, whether integrative medicine approaches with a physical activity component, such as Tai Chi Chuan (TCC), may have the same beneficial effects on cancer patients and survivors remains unknown.

TCC is a traditional Chinese martial art that combines slow, fluid, weight-bearing physical movements with deep, controlled breathing exercises and relaxation techniques. Many cancer patients and survivors are trying integrative medicine approaches, such as TCC, as a way of reducing side effects of treatment such as weight gain (29, 30). TCC, which may result in an energy expenditure equivalent to that of brisk walking, improves aerobic capacity, flexibility, strength, mood and quality of life in breast cancer survivors (31–33). Whether TCC, which has both a meditative and a physical activity component, can produce similar physiologic effects in breast cancer patients and survivors as traditional exercise programs is unknown. In a previous randomized controlled trial conducted by our research group, breast cancer survivors used a 15-move yang-style TCC as a form of integrative medicine to control side effects from cancer treatments. We found that TCC significantly improved functional capacity, self-esteem, bone health and quality of life (32–35) and led to reduced fat mass compared to the non-exercising group, although this difference in fat mass was not statistically significant (34).

Little is known about the mechanisms linking exercise and weight gain in breast cancer survivors. Moreover, we do not know whether TCC, an integrative medicine intervention with a meditative and a physical activity component, elicits similar physiologic responses as traditional exercise programs already investigated on breast cancer survivors. Because hormone (e.g. insulin) and exercise-mediated anti-inflammatory mechanisms (e.g. IL-6) are thought to be the most plausible explanation for the protective effects of exercise on weight gain and recurrence, we investigated the physiologic effects of TCC on insulin and cytokine levels. Additionally, interventions with meditative components are thought to have anti-inflammatory effects (36). Specifically, the current post-hoc pilot study was designed to identify molecules that may be altered by TCC and to identify novel correlative relationships between insulin and insulin-related molecules, cytokines, and fat and fat-free mass. Based upon the effects of traditional exercise in breast cancer survivors and the anti-inflammatory properties of exercise, we developed a model to explain plausible effects of physical activity interventions, such as TCC, on insulin, insulin-related proteins, and cytokines) in breast cancer survivors (Figure 1). We hypothesized that TCC, like more traditional exercise interventions, would reduce insulin levels. We also hypothesized TCC would alter cytokine levels that may be linked with the reduction in fat mass and increase in fat-free mass induced by the TCC.

Figure 1: Proposed model of the effects of a physical activity intervention on insulin, insulin-signaling molecules and cytokines



We hypothesize that IL-6 may contribute to inflammation-mediated cellular proliferation and ultimately tumor growth and increased recurrence risk in an environment of no physical activity (A). However, in the presence of an intervention with a physical activity and meditative component (e.g. TCC), increased levels of skeletal-muscle-derived IL-6 act in an anti-inflammatory manner and induce lipolysis leading to maintenance of a healthy weight and reduced risk of recurrence (B).

PARTICIPANTS AND METHODS

Participants

The detailed methods of the original study assessing the effects of TCC on health-related quality of life, self-esteem, and functional capacity in breast cancer survivors have been previously described by Mustian and colleagues (32–34). Approval from the Institutional Review Board (IRB) was obtained prior to acquiring written consent and enrolling participants. Potential participants were required to meet the following criteria for inclusion in this study: (1) female; (2) primary diagnosis of breast cancer stages 0-IIIb; (3) treatment completed more than 1 month prior but less than 30 months prior; (4) no drainage tubes or catheters; (5) not taking part in moderate or vigorous physical activity more than once a week; (6) physician's permission for aerobic fitness testing and exercise; (7) physically able to participate in an physical activity regimen; and (8) no clinical diagnosis of any mental disorder, as defined by the use of psychotropic drugs and self-report (32).

Design and Procedures

Participants were randomly assigned to either the TCC group or a psychosocial therapy (PST) control group for a period of 12 weeks. Both groups met for 60 minutes three times a week in separate classrooms in the same building at the same time of day for the duration of the trial. Randomization was achieved by the flipping of a coin, and group assignment was concealed from participants until the completion of all baseline assessments. All baseline assessments were

completed two days prior to the start of the intervention. Adherence and compliance in the trial were monitored through attendance records and personal records kept by each participant. The PST group sessions, facilitated by a trained counselor and exercise psychology graduate student, placed special emphasis on behavioral coping strategies, peer support, and group cohesion. Participants in the PST and TCC arms were instructed not to change their pattern of physical activity in any manner for the duration of the study.

The TCC group was led by an American College of Sports Medicine (ACSM) certified Health and Fitness Instructor. The instructor was extensively trained in Yang-style TCC and had more than six years of teaching experience. Each session consisted of a 10-minute warm-up, followed by a 40-minute session in which the participants learned and performed a 15-move short form of Yang-style TCC. The 15 moves used in this intervention comprise the first 15 moves of the traditional 104-move, long-form Yang-style TCC. In the final 10 minutes, participants were instructed to perform structured breathing, imagery, and meditation.

All participants completed bioelectrical impedance tests to assess body composition at baseline and at twelve weeks. Fasting blood draws for each patient were also taken at these time-points. No formal assignments were given to participants to perform at home, but they were encouraged to practice the TCC and behavioral coping strategies they learned during the intervention.

MEASURES

Demographics and Medical Information

Demographic and clinical information (i.e. participant's age, weight, stage of disease, surgery type, and treatment regimens) were obtained from the patient or her medical record at the time of entry into the trial. In addition, body mass index (BMI, kg/m^2) was calculated for each participant.

Measurements of Insulin, Insulin-Related Molecules and Cytokines

Fasting-state blood samples were collected at both pre- and post-intervention time-points (N=19). The blood samples were allowed to clot for ≥ 30 minutes, then centrifuged, and serum collected. Serum samples were then aliquotted and stored at −80°C until used for ELISA. Serum samples were shipped to a central reference laboratory and tested simultaneously to eliminate inter-assay variation. Serum concentrations of insulin were measured by radioimmunoassay (RIA) assay with commercial kits from Diagnostic Systems Laboratories, Inc. (Webster, TX, USA), and IGF-I, IGFBP-1, and IGFBP-3 were measured by immunoradiometric assay with commercial kits from Diagnostic Systems Laboratories, Inc. (Webster, TX, USA). Serum cytokines (IL-2, IL-6, and IFN-γ) were measured by OPT-EIA ELISA kits from BD Biosciences.

Body Composition

Body composition was assessed using bioelectrical impedance analysis (BIA, Quantum-II Desktop with a real-time resolution of 0.1 ohm) at both pre- and post-intervention as described in detail previously [10]. Patients were instructed to prepare for BIA by fasting 4 hours, not participating in physical activity for 12 hours, abstaining from alcohol and diuretics (unless prescribed) for 48 hours but remaining well hydrated. BIA measures opposition to the flow of an electric current through body tissues which is used to calculate total body water and fat-free mass. The resistance to flow is inversely related to both fat-free mass and total body water. Total

body electrical conductivity and body composition determined by densiometry, such as hydrostatic weighing, are highly correlated (37).

Statistical Analysis

Data analyses were conducted using SPSS version 16.0 software. Descriptive statistics for the participants' demographics and baseline values were calculated; percentages were calculated for categorical variables and means and standard deviations for continuous variables. Independent sample t tests were performed on all baseline characteristics. Means and standard deviations were calculated for all study outcome variables at pre-intervention and post-intervention and for change from pre- to post-intervention. Analyses of covariance (ANCOVA) with baseline values as a covariate were used to compare differences between groups at post-intervention in insulin, IGF-1, IGFBP-1, IGFBP-3, IL-2, IL-6 and IFN-γ. Pearson correlations were calculated to assess the association between changes in insulin, IGF-1, IGFBPs, serum cytokines, fitness outcomes, and body composition. All biomarker values are within assay range and are included in the analyses. Since this is a pilot study with a small sample size, two-sided p values of ≤ 0.10 were considered statistically significant in all cases. Preliminary effect sizes were determined using pre- and post-intervention means and standard deviations to calculate within-group Cohen's *d* values.

RESULTS

Thirty-one breast cancer survivors agreed to participate; 21 participants successfully completed the trial. Reasons for withdrawal were previously reported in our original study and included: not liking their treatment group, work, family, and too many side effects following treatment. Additionally, those in the TCC group had a 72% exercise attendance rate and those in the PST group had a 67% attendance rate. Compliance at each attended session was 100% in both groups (32, 34). Nineteen participants agreed to give blood samples and are included in these analyses. Adequate amounts of serum were available for measurement of insulin, IGF-I, and IGFBPs in all subjects and cytokines in most subjects. Table 1 describes the baseline characteristics of these study participants. All participants were female and Caucasian with a mean age of 53 years (range from 43 to 78 years). No significant differences were noted between the groups in any of the baseline variables assessed (Table 1). Additionally, no significant group differences ($p>0.10$) were observed for any serum biological marker assessed at baseline except IFN-γ ($p=0.086$).

Table 1: Baseline characteristics for TCC and PST randomized groups

Characteristic	TCC (n=9)	PST (n=10)	P-value
Age (years)	54.33 (10.64)	52.70 (6.67)	.0690
Surgery			0.868
Lumpectomy	55.60%	60.00%	
Unilateral Mastectomy	33.30%	30.00%	
Bilateral Mastectomy	11.10%	10.00%	
Radiation			0.387
Yes	100%*	90.00%	
No	0%*	10.00%	
Chemotherapy			0.123
Yes	66.70%	30.00%	
No	33.30%	70.00%	
Weight (Kg)	66.67 (14.87)	66.66 (9.84)	0.999
Fat Mass (Kg)	27.36 (8.52)	27.88 (6.38)	0.665
Fat Free Mass (Kg)	40.19 (6.67)	38.74 (5.34)	0.588
BMI (Kg/m2)	24.89 (5.78)	24.97 (4.39)	0.971

Values are represented as Means (Std. Dev.) or Frequencies

*missing 1 value

We previously reported the positive effects, although not significant, of TCC on BMI, weight, and fat mass in the 21 study participants who completed the original trial (34). We observed similar effects in the 19 individuals included in this analysis as in our previously reported results. Table 2 shows pre-intervention means, post-intervention means, and changes in weight, BMI, fat mass, and fat-free mass in TCC and PST groups. BMI in the TCC group decreased (Cohen's d = −0.07), while BMI in the PST group increased (Cohen's d = 0.06). Using ANCOVA, adjusting for baseline, BMI was significantly different between groups at post-intervention ($p < 0.10$). No other significant differences were observed. Weight and fat mass decreased in the TCC group (Cohen's d = −0.02 and −0.02, respectively) but increased in the PST group (Cohen's d = 0.05 and 0.04 respectively). Fat-free mass slightly decreased in the TCC group (Cohen's d = −0.004) and increased in the PST group (Cohen's d = 0.06).

Table 2: Pre-intervention, post-intervention, and change score means for body composition measures in TCC and PST Groups

Variable	Group	Pre-Intervention	Post-Intervention	Change
Weight (Kg)	TCC (9)	66.67 (14.85)	66.37 (14.68)	-0.30 (1.86)
	PST (10)	66.66 (9.85)	67.26 (10.33)	0.60 (1.43)
BMI (Kg/m^2)*	TCC (9)	24.89 (5.78)	24.47 (5.49)	-0.42 (0.75)
	PST (10)	24.97 (4.39)	25.26 (4.77)	0.29 (0.61)
Fat Mass	TCC (9)	26.29 (9.26)	26.13 (8.15)	-0.16 (2.91)
	PST (10)	27.88 (6.38)	28.16 (6.25)	0.28 (1.57)
Fat Free Mass	TCC (9)	40.27 (6.69)	40.24 (7.56)	-0.03 (3.05)
	PST (10)	38.74 (5.33)	39.1 (5.51)	0.36 (1.36)

*$p < 0.10$, representing between-group effect at post-intervention controlling for pre-intervention baseline value

Pre-intervention means, post-intervention means and mean changes in biomarkers insulin, insulin-related proteins, and cytokines were recorded (Table 3 and Figure 2). The significance of the differences between groups (main effect) at post-intervention was assessed with ANCOVA, incorporating baseline values as a covariate. There was a significant main effect for insulin ($p=0.099$); insulin levels remained relatively stable in the TCC group (mean change = 1.41; Cohen's d = 0.20) but increased in the PST group (mean change = 15.02; Cohen's d = 0.66;). The 15.02 increase represented a 1.80-fold increase in the PST group compared to a 1.12-fold increase in the TCC group. IGF-1 decreased in both groups; however, the decrease was greater in the TCC group (mean change = −27.32; Cohen's d = −0.80) than in the PST group (mean change = −16.64; Cohen's d = −0.23; $p = 0.495$ for the main effect). IGFBP-1 increased in both TCC (mean change = 3.76; Cohen's d = 0.10) and PST groups (mean change = 9.12; Cohen's d = 0.20; $p = 0.749$ for the main effect). IGFBP-3 increased in the TCC group (mean change = 0.89; Cohen's d = 0.13) but decreased in the PST group (mean change = −0.70; Cohen's d = −0.05;, $p = 0.299$ for the main effect).

The cytokine/myokine IL-6 increased in the TCC group (mean change = 2.00; Cohen's d = 0.35) but decreased slightly in the PST group (mean change = −0.02; Cohen's d = 0.01; $p = 0.297$ for the main effect). Both pro-inflammatory cytokines IL-2 (mean change = 4.59; Cohen's d = 0.46) and IFN-γ increased in the PST group (mean change = 2.32; Cohen's d = 0.19) but decreased in the TCC group (mean change = −8.82; Cohen's d = −0.54 and −0.17; Cohen's d = −0.08 respectively), although the main effect was not significant for either IL-2 ($p = 0.369$) or IFN-γ ($p = 0.831$).

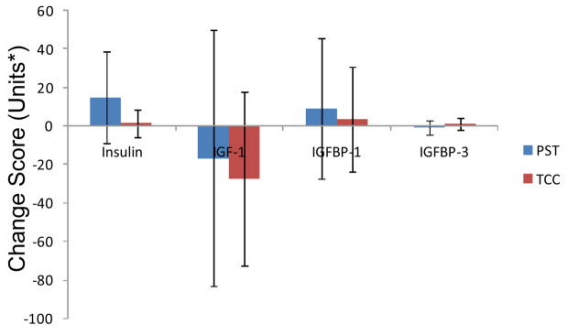
Table 3: Pre-intervention, post-intervention, and change score means for biomarker levels in TCC and PST Groups

Variable[#]	Group (n)	Pre-Intervention	Post-Intervention	Change
Insulin	TCC (9)	15.34 (5.36)	16.75 (7.99)*	1.41 (7.05)
	PST (10)	15.83 (9.10)	30.85 (29.86)	15.02 (23.62)
IGF-1	TCC (9)	156.81 (19.58)	129.49 (43.83)	-27.32 (45.07)
	PST (10)	111.76 (82.64)	95.12 (58.65)	-16.64 (66.5)
IGFBP-1	TCC (9)	72.64 (25.64)	76.40 (42.76)	3.76 (27.32)
	PST (10)	92.22 (39.02)	101.34 (50.01)	9.12 (36.44)
IGFBP-3	TCC (9)	39.22 (6.26)	40.11 (7.29)	0.89 (3.12)
	PST (10)	40.81 (13.55)	40.11 (15.13)	-0.700 (3.77)
IL-6	TCC (9)	2.63 (3.96)	4.63 (6.97)	2.00 (5.53)
	PST (10)	2.44 (1.79)	2.42 (1.74)	-0.02 (1.51)
IL-2	TCC (9)	12.48 (22.15)	3.66 (5.83)	-8.82 (23.41)
	PST (9)	3.73 (4.96)	8.32 (13.22)	4.59 (12.64)
IFN-y	TCC (7)	1.34 (2.42)[+]	1.17 (1.78)	-0.17 (3.56)
	PST (9)	7.79 (9.70)	10.21 (15.55)	2.42 (6.45)

Values are represented as Means (Std. Dev.)
[#]Insulin Units = μIU/mL, IGF-1, IGFBP-1 and IGFBP-3 Units = ng/mL, IL-6, IL-2 and IFN-γ Units = pg/mL
[*]$p \leq 0.10$, representing between-group effect at post-intervention controlling for pre-intervention baseline value
[+]$p \leq 0.10$, representing the significant between-group difference at baselines

Figure2:

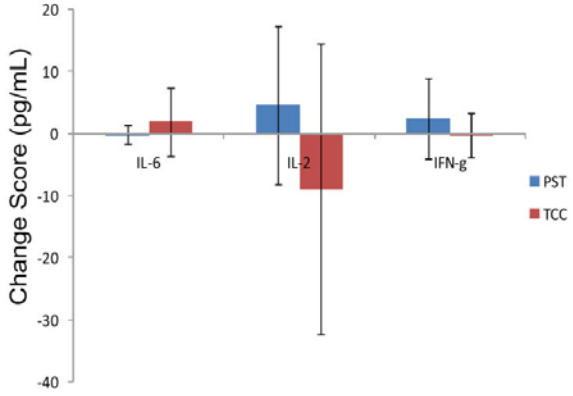
Figure 2A: Changes in mean insulin and insulin related protein concentrations in TCC and PST groups



*Insulin Units = μIU/mL
*IGF-1, IGFBP-1 and IGFBP-3 Units = ng/mL
*IL-6, IL-2 and IFN-γ Units = pg/mL

Error bars represent Standard Deviations of the Change Score values

Figure 2B: Changes in mean cytokines concentrations in TCC and PST groups



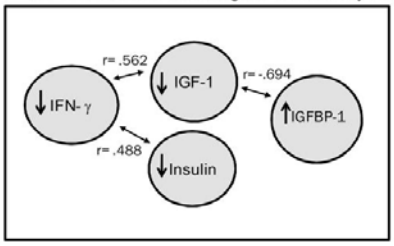
Error bars represent Standard Deviations of the Change Score values

In order to investigate possible linkages between fat mass, fat-free mass, insulin and insulin-related proteins, and cytokines, we performed correlations on all available subjects. These correlation analyses were performed for hypothesis-generating purposes to identify potential targets that may be investigated in larger studies of TCC in breast cancer survivors. We were interested in identifying novel patterns among changes (between pre-intervention and post-intervention means) for insulin and insulin-related proteins, cytokines and body composition.

We found that changes in IGF-1 were negatively correlated with changes in IGFBP-1 ($r=-0.694$, $p = 0.001$); as IGF-1 decreased, IGFBP-1 increased. Additionally, we found changes in IGF-1 were positively correlated with changes in IFN-γ ($r = 0.562$, $p = 0.024$); as IGF-1 increased, IFN-γ increased. Changes in IFN-γ were positively correlated with changes in insulin ($r = 0.488$, $p = 0.055$); as insulin decreased, IFN-γ also decreased. These data are summarized in Figure 3A. Additionally, we found that changes in fat-free mass were positively correlated with changes in IL-6 ($r = 0.474$, $p = 0.040$) and negatively correlated with changes in IL-2 ($r = -0.491$, $p = 0.038$). As fat-free mass increased, IL-6 also increased but IL-2 decreased. Further supporting this result, we found that changes in fat mass negatively correlated with changes in IL-6 ($r = -0.505$, $p = 0.028$) and positively correlated with changes in IL-2 ($r = 0.552$, $p = 0.018$). As fat mass decreased, IL-2 also decreased but IL-6 increased (Figure 4A).

Figure 3: Relationships between changes in IGF-1, IGFBP-1, IFN-gamma and insulin

A. Correlations including the entire study cohort.

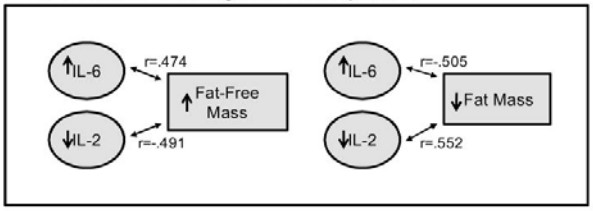


B. Correlations for participants in the TCC and PST groups.

Variables	PST	TCC
IGF-1 and IGFBP-1	r = -0.696, p = 0.025	r = -0.739, p = 0.023
IGF-1 and IFN-γ	r = 0.544, p = 0.130	r = 0.581 , p = 0.172
IFN-γ and insulin	r = 0.493, p = 0.177	r = -0.073, p = 0.876

Figure 4: Relationship between changes in cytokines IL-6 and IL-2 and fat mass and fat-free mass

A. Correlations including the entire study cohort.



B. Correlations for participants in the TCC and PST groups.

Variables	PST	TCC
IL-6 and fat-free mass	r = 0.084, p = 0.819	r = 0.584, p = 0.099
IL-2 and fat-free mass	r = -0.333, p = 0.381	r = -0.576, p = 0.104
IL-6 and fat mass	r = -0.359, p = 0.308	r = -0.538, p = 0.135
IL-2 and fat mass	r = 0.356, p = 0.346	r = 0.612, p = 0.800

We also assessed correlations by group to determine if differential patterns existed between intervention groups (Figures 3B, ,4B).4B). In both TCC and PST groups, changes in IGF-1 and changes in IGFBP-1 were negatively correlated ($r = -0.739$, $p = 0.023$ for TCC and $r = -0.696$, $p = 0.025$ for PST). The correlations for changes in IL-6, IL-2, fat mass and fat-free mass were higher for subjects in the TCC group than for those in the PST group (Figure 4B) suggesting that associations between the changes in these variables may result from the TCC intervention.

DISCUSSION

The goal of this study was to identify differential changes in biological marker profiles for insulin and cytokines in subjects who were randomized to a integrative medicine TCC intervention compared to a PST control, and to identify novel correlative relationships between insulin and insulin-related molecules, cytokines, and fat and fat-free mass. Of the insulin and insulin-related proteins and cytokines assessed for group differences at post-intervention (Table 2), only insulin showed a change that was significant. Levels were stable and within normal range in the TCC group but almost doubled in the PST group over the 12-week intervention period to a level that would be considered high. The increase in insulin in the non-exercising (PST) group and the stabilized levels of insulin observed in the TCC group in our study are consistent with reports from others who prospectively measured insulin levels in breast cancer survivors undergoing an exercise intervention (21). We hypothesize that the increases in insulin in the PST group may be related to increases in weight and BMI observed in that group but not in the TCC group; however, further study would be needed to assess this.

Because the TCC intervention led to stable levels of insulin over the 12-week period, this mind-body intervention with a physical activity component may represent an effective intervention to maintain stable insulin levels in breast cancer survivors, much like conventional exercise programs. Maintenance of stable insulin levels appears to be very important in view of recent research showing that increased insulin levels are associated with increased recurrence risk and reduced survival in breast cancer survivors (8).

This study revealed novel patterns in the relationships between changes in IL-6, IL-2, fat mass and fat-free mass, particularly those observed in the TCC group. We believe the positive correlation of changes in IL-6 and fat-free mass and inverse correlation of changes in IL-6 with fat mass, associations that are observed most strongly in the TCC group, might be indicative of physical activity-mediated changes in cytokines that are beneficial for maintenance of a healthy body weight in breast cancer survivors.

IL-6 is a pleiotropic molecule that has both pro- and anti-inflammatory effects that may be related to the cellular source of production. In clinical studies, higher circulating levels of IL-6 have been identified in those diagnosed with breast cancer than in healthy individuals (38). IL-6 secreted from adipose tissue has been implicated in promoting invasion of breast cancer cells (39), and in a chronic inflammatory setting, IL-6 may be secreted by T-cells leading to growth factor expression that may promote survival of tumor cells (40).

In the presence of physical activity, paradoxically, circulating IL-6 is markedly increased (41) and, at these levels, IL-6 can elicit anti-inflammatory effects due to its secretion from muscle (42). In our study, we believe that the elevated IL-6 levels are a marker of the positive effects of

TCC on fat-free mass. At this point, we are unsure whether TCC-induced IL-6 is a mediator that contributes or a responder that reflects fat reduction, and whether it may have direct anti-inflammatory effects on risk of recurrence. However, it is biologically plausible that TCC, which has both physical activity and meditative properties and can elicit similar positive effects on physical function as more traditional exercise programs, can dampen the pro-inflammatory state associated with cancer progression by increasing fat-free mass and reducing fat mass. Ultimately, we are interested in whether the association of IL-6 with lean body mass in breast cancer survivors produced by TCC could be a link between a healthy body weight and reduced risk of disease recurrence, a focus of future studies. We are also interested in whether the changes elicited by TCC are due to physical activity components, meditative components, or both.

Similar patterns of associations for IL-2, fat-free mass, and fat mass were observed herein as were observed for IL-6, which may indicate another mechanism of physical activity-mediated changes that are beneficial for maintenance of a healthy body weight. IL-2 is produced by T cells as a necessary proliferative factor; these cells accumulate in adipose tissue (43) and likely play a role in the inflammatory response within this tissue (44) and may ultimately promote tumor progression. Physical activity, therefore, by increasing fat-free mass and reducing fat mass may lead to reduced adiposity, leading to reduced accumulation of T cells within adipose tissue and lower levels of IL-2, which may ultimately lower the likelihood of tumor progression.

We found a positive correlation between changes in insulin and IFN-γ levels in the PST group; both insulin and IFN-γ increased in this group but remained relatively stable in the TCC group. Another study that assessed the effects of TCC on immune function found that TCC can enhance production of CD4+CD25+ regulatory T cells (45) which could mitigate IFN-γ production from inflammatory T cells in adipose tissue, thus providing another possible explanation for the difference in IFN-γ expression between groups. In a recent animal study of diet-induced obesity, IFN-γ promoted inflammation in adipose tissue and promoted insulin resistance (46), which provides a rationale for the positive correlation of IFN-γ and insulin observed in our non-exercising group. Further studies are needed to determine whether the correlation of IFN-γ and insulin is causal.

Based upon these preliminary data and the pertinent literature, we propose a model (Figure 1) whereby physically inactive breast cancer survivors will have a higher inflammatory status mediated by factors such as IL-2, IFN-γ, and TNF-α that are produced by T-cells, macrophages, and adipocytes. This inflammatory environment may directly enhance abnormal cellular proliferation which may increase weight gain and the risk of recurrence. Additionally, inflammation might promote insulin resistance and increased levels of insulin and IGFs in the blood that drive abnormal cellular proliferation and ultimately affect recurrence. We hypothesize, further, that the physical activity component of the TCC intervention could reduce the inflammatory state in breast cancer survivors through muscle release of IL-6, thus promoting anti-inflammatory processes and lowering insulin and IGF levels, which would maintain normal cellular proliferative processes, maintenance of a healthy body weight, and a reduced the rate of cancer recurrence.

The major limitation of this study is small sample size; all conclusions from these data are preliminary. Even though we had a small sample size in this pilot study, we were able to detect a

significant main effect of TCC on insulin levels. Higher powered confirmatory studies will allow for more precise determination of TCC-mediated effects on insulin and other biomarkers assessed in this study. Such studies should also assess the influence of menopausal status, pre-morbid weight (e.g. obesity), other co-morbid conditions and medication usage on these markers. Also, we did not follow the study subjects for long enough to obtain adequate recurrence information for correlation with biological markers assessed. We did not use a criterion gold standard for body composition such as hydrostatic weighing or dual-energy x-ray absorptiometry; given the positive findings of this study, future studies should incorporate a gold standard. Additional studies should also include a measure of overall muscle strength. We were concerned about exercise contamination in this study; however, we previously reported that only 20% of the PST group reported increasing their level of exercise, whereas 100% of the TCC group reported exercise due only to the intervention (34, 35). Future studies should include daily exercise diaries to more accurately reflect dose and intensity of any exercise. Lastly, our results pertain only to breast cancer survivors; studies of the effects of TCC in other cancer populations are needed.

A major strength of this study is our preliminary identification of a significant difference in post-intervention levels of insulin between TCC and PST groups; this difference suggests that the unique mind-body intervention, TCC, has a similar effect on insulin in breast cancer survivors as do traditional exercise regimens, making TCC a possible attractive alternative intervention. Furthermore, we identified novel relationships between cytokines, insulin, insulin-related molecules, and body composition that may explain the biological effects of our yang-style TCC intervention in breast cancer survivors. Future studies should investigate the relationships between these biological markers and recurrence rates in breast cancer survivors in a larger randomized controlled trial with a TCC intervention.

CONCLUSION

The results of this completed pilot study provide preliminary data suggesting that the integrative medicine intervention TCC can lower insulin levels in breast cancer survivors compared to a non-active control. We also found significant correlations between body composition and cytokines. While these significant findings are encouraging, given the small sample size, these analyses should be repeated in a larger study with higher power to be confirmatory.

These results are very positive, and suggest that non-traditional exercise interventions with a mindfulness component, such as Tai Chi Chuan, may elicit similar beneficial effects to breast cancer survivors as more traditional exercise programs. Larger randomized studies are needed to assess the effects of Tai Chi Chuan on biomarkers that are likely related to weight gain and recurrence. Such a study could have a tremendous impact for breast cancer survivorship research.

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REFERENCES

1. Demark-Wahnefried W, Rimer BK, Winer EP. Weight gain in women diagnosed with breast cancer. *J Am Diet Assoc*. 1997;97:519–26. 29. quiz 27–8.

2. Heideman WH, Russell NS, Gundy C, Rookus MA, Voskuil DW. The frequency, magnitude and timing of post-diagnosis body weight gain in Dutch breast cancer survivors. *Eur J Cancer*. 2009;45:119–26.

3. Boyd NF. Nutrition and breast cancer. *J Natl Cancer Inst*. 1993;85:6–7.

4. Tredan O, Bajard A, Meunier A, et al. Body weight change in women receiving adjuvant chemotherapy for breast cancer: A French prospective study. *Clin Nutr*. 2009

5. Demark-Wahnefried W, Peterson BL, Winer EP, et al. Changes in weight, body composition, and factors influencing energy balance among premenopausal breast cancer patients receiving adjuvant chemotherapy. *J Clin Oncol*. 2001;19:2381–9.

6. Caan BJ, Emond JA, Natarajan L, et al. Post-diagnosis weight gain and breast cancer recurrence in women with early stage breast cancer. *Breast Cancer Res Treat*. 2006;99:47–57.

7. Nichols HB, Trentham-Dietz A, Egan KM, et al. Body mass index before and after breast cancer diagnosis: associations with all-cause, breast cancer, and cardiovascular disease mortality. *Cancer Epidemiol Biomarkers Prev*. 2009;18:1403–9.

8. Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol*. 2002;20:42–51.

9. Kleinberg DL, Wood TL, Furth PA, Lee AV. Growth hormone and insulin-like growth factor-I in the transition from normal mammary development to preneoplastic mammary lesions. *Endocr Rev*. 2009;30:51–74.

10. Aggarwal BB, Gehlot P. Inflammation and cancer:how friendly is the relationship for cancer patients? *Curr Opin Pharmacol*. 2009;9:351–69.

11. Holmes MD, Chen WY, Feskanich D, Kroenke CH, Colditz GA. Physical activity and survival after breast cancer diagnosis. *JAMA*. 2005;293:2479–86.

12. Abrahamson PE, Gammon MD, Lund MJ, et al. Recreational physical activity and survival among young women with breast cancer. *Cancer*. 2006;107:1777–85.

13. Irwin ML, Smith AW, McTiernan A, et al. Influence of pre-and postdiagnosis physical activity on mortality in breastcancer survivors: the health, eating, activity, and lifestyle study. *J Clin Oncol*. 2008;26:3958–64.

14. Speed-Andrews AE, Courneya KS. Effects of exercise on quality of life and prognosis in cancer survivors. *Curr Sports Med Rep*. 2009;8:176–81.

15. Ligibel JA, Campbell N, Partridge A, et al. Impact of a mixed strength and endurance exercise intervention on insulin levels in breast cancer survivors. *J Clin Oncol*. 2008;26:907–12.

16. Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW. Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. *Diabetes Care*. 2003;26:557–62.

17. Schmitz KH, Ahmed RL, Yee D. Effects of a 9-month strength training intervention on insulin, insulin-like growth factor (IGF)-I, IGF-binding protein (IGFBP)-1, and IGFBP-3 in 30–50-year-old women. *Cancer Epidemiol Biomarkers Prev*. 2002;11:1597–604.

18. Fairey AS, Courneya KS, Field CJ, Bell GJ, Jones LW, Mackey JR. Effects of exercise training on fasting insulin, insulin resistance, insulin-like growth factors, and insulin-like growth factor binding proteins in postmenopausal breast cancer survivors: a randomized controlled trial. *Cancer Epidemiol Biomarkers Prev*. 2003;12:721–7.

19. Irwin ML, McTiernan A, Bernstein L, et al. Relationship of obesity and physical activity with C-peptide, leptin, and insulin-like growth factors in breast cancer survivors. *Cancer Epidemiol Biomarkers Prev*. 2005;14:2881–8.

20. McTiernan A, Sorensen B, Yasui Y, et al. No effect of exercise on insulin-like growth factor 1 and insulin-like growth factor binding protein 3 in postmenopausal women: a 12-month randomized clinical trial. *Cancer Epidemiol Biomarkers Prev*. 2005;14:1020–1.

21. Irwin ML, Varma K, Alvarez-Reeves M, et al. Randomized controlled trial of aerobic exercise on insulin and insulin-like growth factors in breast cancer survivors: the Yale Exercise and Survivorship study. *Cancer Epidemiol Biomarkers Prev*. 2009;18:306–13.

22. Speck RM, Courneya KS, Masse LC, Duval S, Schmitz KH. An update of controlled physical activity trials in cancer survivors: a systematic review and meta-analysis. *J Cancer Surviv*

23. Mathur N, Pedersen BK. Exercise as a mean to control low-grade systemic inflammation. *Mediators Inflamm*. 2008;2008:109502.

24. Hiscock N, Chan MH, Bisucci T, Darby IA, Febbraio MA. Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *FASEB J*. 2004;18:992–4.

25. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. *FASEB J*. 2003;17:884–6.

26. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev*. 2008;88:1379–406.

27. Stewart LK, Flynn MG, Campbell WW, et al. The influence of exercise training on inflammatory cytokines and C-reactive protein. *Med Sci Sports Exerc*. 2007;39:1714–9.

28. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. *J Biol Chem*. 2003;278:45777–84.

29. Yates JS, Mustian KM, Morrow GR, et al. Prevalence of complementary and alternative medicine use in cancer patients during treatment. *Support Care Cancer*. 2005;13:806–11.

30. Lengacher CA, Bennett MP, Kip KE, Gonzalez L, Jacobsen P, Cox CE. Relief of symptoms, side effects, and psychological distress through use of complementary and alternative medicine in women with breast cancer. *Oncol Nurs Forum*. 2006;33:97–104.

31. Mansky P, Sannes T, Wallerstedt D, et al. Tai chi chuan: mind-body practice or exercise intervention? Studying the benefit for cancer survivors. *Integr Cancer Ther*. 2006;5:192–201.

32. Mustian KM, Katula JA, Gill DL, Roscoe JA, Lang D, Murphy K. Tai Chi Chuan, health-related quality of life and self-esteem: a randomized trial with breast cancer survivors. *Support Care Cancer*. 2004;12:871–6.

33. Mustian KM, Palesh OG, Flecksteiner SA. Tai Chi Chuan for breast cancer survivors. *Med Sport Sci*. 2008;52:209–17.

34. Mustian KM, Katula JA, Zhao H. A pilot study to assess the influence of tai chi chuan on functional capacity among breast cancer survivors. *J Support Oncol*. 2006;4:139–45.

35. Peppone LJ, Mustian KM, Janelsins MC, et al. Effects of a structured weight-bearing exercise program on bone metabolism among breast cancer survivors: a feasibility trial. *Clin Breast Cancer*. 2010;10:224–9.

36. Oke SL, Tracey KJ. The inflammatory reflex and the role of complementary and alternative medical therapies. *Ann N Y Acad Sci*. 2009;1172:172–80.

37. Segal KR, Gutin B, Presta E, Wang J, Van Itallie TB. Estimation of human body composition by electrical impedance methods: a comparative study. *J Appl Physiol*. 1985;58:1565–71.

38. Jiang XP, Yang DC, Elliott RL, Head JF. Reduction in serum IL-6 after vacination of breast cancer patients with tumour-associated antigens is related to estrogen receptor status. *Cytokine*. 2000;12:458–65.

39. Walter M, Liang S, Ghosh S, Hornsby PJ, Li R. Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. *Oncogene*. 2009;28:2745–55.

40. DeNardo DG, Coussens LM. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. *Breast Cancer Res*. 2007;9:212.

41. Pedersen BK, Steensberg A, Fischer C, et al. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? *Proc Nutr Soc*. 2004;63:263–7.

42. Petersen AM, Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. *J Physiol Pharmacol*. 2006;57 (Suppl 10):43–51.

43. Kintscher U, Hartge M, Hess K, et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol*. 2008;28:1304–10.

44. Nishimura S, Manabe I, Nagasaki M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med.* 2009;15:914–20.

45. Yeh SH, Chuang H, Lin LW, Hsiao CY, Eng HL. Regular tai chi chuan exercise enhances functional mobility and CD4CD25 regulatory T cells. *BrJ Sports Med*. 2006;40:239–43.

46. Rocha VZ, Folco EJ, Sukhova G, et al. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. *Circ Res.* 2008;103:467–76.