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Relationships Between Serum Cortisol, Vitamin D, Bone Mineral Density, and Body Composition in National Team Figure Skaters

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This thesis, Relationships Between Serum Cortisol, Vitamin D, Bone Mineral Density, and Body Composition in National Team Figure Skaters, by Monica B. Grages, was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the Byrdine F. Lewis School of Nursing and Health Professions, Georgia State University. The Master's Thesis Advisory Committee members, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.

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ABSTRACT

Title: Relationships Between Serum Cortisol, Vitamin D, Bone Mineral Density, and Body Composition in National Team Figure Skaters

Background: Studies have not examined the relationships between serum vitamin D (SVitD), serum cortisol (SCort), bone mineral density (BMD), and body fat percent (BF%) in elite figure skaters. However, studies of non-athletes have found that BMD is inversely related to SCort and directly related to SVitD, and BF% is inversely related to SVitD and directly related to SCort. It was, therefore, the purpose of this study to assess the relationships between SCort, SVitD, BMD, and BF% in elite figure skaters.

Methods: U.S. national team figure skaters were assessed at a national training camp during the summer, 2012. BMD and body composition were measured by dual energy x-ray absorptiometry (DEXA). Blood chemistry values for SVitD and SCort were obtained via venous puncture after an overnight fast, the same morning as the DEXA measurement. Georgia State University Institutional Review Board approval was obtained for the assessment of data collected at this training camp.

Results: 24 out of 39 training camp attendees (61.5%) volunteered to be assessed as part of this study. Subjects ranged from 17 to 34 years and included males (n=11) and females (n=12). In all skaters statistically significant negative correlations (2-tailed Spearman) were found between SCort and BMD of the spine ($r=-0.458$, $p=0.032$), pelvis ($r=-0.532$, $p=0.011$), ribs ($r=-0.517$, $p=0.014$), and trunk ($r=-0.538$, $p=0.010$). In females, SCort was negatively correlated with BMD of the pelvis ($r=-0.664$, $p=0.026$) and trunk ($r=-0.609$, $p=0.047$), and was positively correlated with total BF% ($r=0.657$, $p=0.020$) and trunk fat % ($r=0.708$, $p=0.010$). In males, SCort was significantly correlated with BMD of the ribs ($r=-0.627$, $p=0.039$). The 3 skaters (all female) with SCort > 28 mcg/dL had significantly lower mean BMD of the total body, left femoral neck, legs, trunk, and pelvis, and significantly greater BF% of the total body and trunk when compared to the 20 skaters with SCort 7-28 mcg/dL. No significant correlations between SVitD and BMD or BF% were found. A Mann-Whitney U test found no significant differences in BMD and BF% between the 8 skaters with SVitD ≥ 30 ng/mL compared to the 15 skaters with SVitD < 30 ng/mL ($p>0.05$). Females with SVitD ≥ 30 ng/mL had significantly higher BMD ($p=0.041$) of the right femoral neck when compared to those with lower SVitD.

Conclusions: Correlations consistently found negative associations between SCort cortisol and BMD in multiple assessment areas, particularly those composed of trabecular bone. Higher SCort was also associated with higher BF% in female skaters. Despite spending a great deal of time in indoor facilities, limiting vitamin D creation through sunlight exposure, no significant correlation between SVitD and BMD was found. Female athletes in ‘appearance’ sports, may be predisposed to restrained eating behaviors, which may be associated with elevated SCort. These findings suggest a need for further study of the interaction between SCort, BMD, and BF% in these athletes. The lack of a statistically significant relationship between SVitD and BMD suggests the need to investigate additional factors associated with bone injury risk in athletes.

Relationships Between Serum Cortisol, Vitamin D, Bone Mineral Density, and Body Composition in National Team Figure Skaters

by

Monica B. Grages

A Thesis

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ABBREVIATIONS

$1,25(OH)_2D$	1,25-dihydroxyvitFeramin D
25(OH)D	25-hydroxyvitamin D
BF%	Body fat percent
BMI	Body mass index
BMD	Bone mineral density
DEXA	Dual-energy X-ray absorptiometry
DRI	Dietary Reference Intake
g/cm2	Grams per square centimeter
kcal	Kilocalories
kg	Kilogram
L	Left
L1-L4	Lumbar vertebrae 1 through 4
ng/mL	Nanograms per milliliter
p	Probability value
r	Correlation coefficient
R	Right
SD	Standard deviation
TFEQ	Three-Factor Eating Questionnaire
U.S.	United States
USG	Urine specific gravity
WHR	Waist-to-hip ratio
µg/dL	Micrograms per deciliter

CHAPTER I

Introduction

Figure skaters have relatively high rates of stress fractures and have also been shown to be at increased risk for certain nutrition-related issues, including food and energy restriction and suboptimal intakes of micronutrients (Porter et al., 2007). Figure skating requires competitors to have low body weight and to maintain a fit appearance, but is also physically demanding, requiring difficult spins and jumps that produce high impact to bones through jump landings (Dubravcic-Simunjak et al., 2003). The aesthetic demands of the sport predispose figure skaters to the same dietary concerns that may have negative effects on performance and bone health. Vitamin D and cortisol are known to influence bone mineralization, and recent research suggests that both also play a role in body composition.

Vitamin D plays a clear role in increasing bone mineral density (BMD), particularly through the regulation of calcium homeostasis (Mawer & Davies, 2001). Dietary vitamin D as well as vitamin D synthesized by skin tissues from sunlight are activated by conversion to vitamin D3, which enters blood circulation and is either stored in adipose cells or travels to the liver to be hydroxylated to 25-hydroxyvitamin D (25(OH)D), the form indicative of vitamin D status (Holick, 2009). Then 25(OH)D travels to the kidneys to be hydroxylated to calcitriol, or 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), which works in the small intestine to regulate absorption of dietary calcium and at bone to influence bone-forming osteoblast and bone-degrading osteoclast activity and regulate uptake of calcium and phosphorus.

Although there are few total studies in this area, research has consistently shown an inverse association between serum vitamin D and body fat (Arunabh et al., 2003; Kremer et al., 2009; Lenders et al., 2009; Parikh et al., 2004; Snijder et al., 2005). Although the mechanism by which vitamin D may influence body fat accumulation is not completely understood, in vitro studies have suggested that adipocyte production is inhibited by 1,25$(OH)_2$D. Four in vitro studies, two using animal preadipocytes and two using human preadipocytes, observed that 1,25$(OH)_2$D had a significant negative effect on adipose cell differentiation (Kelly & Gimble, 1998; Kong & Li, 2006; Nimitphong et al., 2012; Shi et al., 2001). It is believed that a common parent cell gives rise to both osteoblasts and preadipocytes (Kelly & Gimble, 1998; Vu et al., 1996). In the presence of 1,25$(OH)_2$D, osteoblast production is increased and adipocyte production is inhibited. These findings suggest that the roles vitamin D plays in body fat and bone density regulation are interrelated.

Cortisol, which is released by the hypothalamic-pituitary-adrenal axis, is increased during physical and psychological stress (Schwarz et al., 2011). Additionally, a positive association has been observed between cortisol secretion and delayed or restricted eating patterns; the majority of research was performed with female adult subjects (Anderson et al., 2002; Bedford & Barr, 2010; McLean et al., 2001; Putterman & Linden, 2006; Rideout et al., 2006; Tomiyama et al., 2010). This phenomenon, although not fully understood, has been attributed to the role of ghrelin in cortisol production (Schwarz et al., 2011). Ghrelin, a hormone secreted during fasting, stimulates the release of adrenocorticotropic hormone from the pituitary gland, which signals the hypothalamic-pituitary-adrenal-axis to produce cortisol (Borer, 2003).

There are multiple mechanisms by which cortisol may act to lower BMD, including impairment of dietary calcium absorption in the small intestine, inhibition of calcium reabsorption at the renal tubules, stimulation of resorption of bone calcium, and, in females, inhibition of sex hormones (Canalis et al., 2007; Schwarz et al., 2011; Van Schoor et al., 2007). In vitro studies suggest that cortisol acts to inhibit periosteal cell proliferation and cell differentiation of osteoblasts (Canalis et al., 2007; Pereira et al., 2001).

The mechanism by which cortisol may influence body fat is not well understood. In vitro experiments have revealed that cortisol increases formation and activity of lipoprotein lipase (LPL), a hormone that aids in the catabolism of dietary triglycerides to one monoacylglycerol molecule and two free fatty acids (Mead et al., 2002). LPL works to promote the uptake of free fatty acids into cells, including adipocytes. Studies have shown that chronically elevated cortisol is associated with increased body weight and body fat, particularly of the abdomen (Dimitriou et al., 2003; Duclos et al., 2001; Purnell et al., 2004).

Past studies have examined the relationship between vitamin D, BMD, and body composition in some athlete populations. However, there are no studies that have examined the interaction between vitamin D, cortisol, BMD, and body composition in athletes, including no prior studies in elite figure skaters. It is, therefore, the objective of this thesis to assess the relationships between fasting serum cortisol, serum vitamin D, BMD, and BF% in elite figure skaters.

Hypotheses

1) Serum vitamin D is positively associated with BMD.

    $^{1-0}$Serum vitamin D is not positively associated with BMD.

2) Serum Vitamin D is inversely associated with BF%.

    $^{2-0}$Serum Vitamin D is not inversely associated with BF%.

3) Serum cortisol is inversely associated with BMD.

    $^{3-0}$Serum cortisol is not inversely associated with BMD.

4) Serum cortisol is positively associated with BF%.

    $^{4-0}$Serum cortisol is not positively associated with BF%.

CHAPTER II

Literature Review

Common Nutritional Risks Faced by Figure Skaters

Due to the aesthetic nature of the sport, figure skaters often use food/energy restriction as a weight-control method. Various studies have shown through analysis of self-reported food records that the caloric intake of figure skaters is below requirements. For example, the mean daily energy intake of 161 elite figure skaters aged 12-28 years was 36 kcal/kg for men and 33 kcal/kg for women, which is lower than the recommended >50 kcal/kg for males and 45-50 kcal/kg for females in this athlete population (Ziegler et al., 2001). An assessment of 49 male and female competitive figure skaters (mean age 19 years for males, 15.5 years for females) found average energy intakes of 31 kcal/kg among males and 32 kcal/kg among females during the competitive season (Jonnalagadda et al., 2004). Additionally, 4% of male subjects and 36% of female subjects in this study reported current use of dietary restriction to control weight, and 4% of males and 30% of females perceived themselves as overweight. The lowest reported average energy intake was seen in an analysis of 123 female synchronized skaters aged 14-30 years, who consumed only 26 kcal/kg (Ziegler et al., 2005). This analysis also found that the skaters' perceptions of their current body shape differed significantly from that which they considered ideal. Figure skaters (N=41) aged 11-18 years who competed in National Championships reported a mean intake of 37 kcal/kg for males and 33 kcal/kg for females (Ziegler et al., 1999). An assessment of 18 female competitive figure skaters aged 14-16 years reported a mean intake of 31 kcal/kg during the competitive season, a slight reduction from the 32 kcal/kg reported during the off-season (Ziegler et al., 2002). All studies saw energy intakes below estimated requirements for figure skaters, and females were generally more restrictive than males.

As is expected with dietary restraint, suboptimal intakes of multiple vitamins and minerals have also been observed in competitive figure skaters (Jonnalagadda et al., 2004; Ziegler et al., 1999; Ziegler et al., 2001; Ziegler et al., 2002; Ziegler et al., 2005). The studies examined above also assessed micronutrient intake in elite figure skaters. All but one reported calcium intakes less than 2/3 the dietary reference intake (DRI) values for females, but none found suboptimal calcium intake in males (Jonnalagadda et al., 2004; Ziegler et al., 1999; Ziegler et al., 2002; Ziegler et al., 2005). Calcium intake exceeding the DRI was observed by one study in both male and female elite skaters (N=161) (Ziegler et al., 2001). The four studies that assessed fat-soluble vitamin intake (two in male and female skaters, two in female skaters only) all found mean intakes less than 2/3 the DRI for the fat-soluble vitamins D and E (Jonnalagadda et al., 2004; Ziegler et al., 1999; Ziegler et al., 2002; Ziegler et al., 2005). Insufficient intake of most micronutrients was reported by at least one study, but calcium and vitamins D and E were consistently reported as problem nutrients for figure skaters. These dietary shortcomings can have adverse effects on the health of elite figure skaters, including loss of lean mass and increased fracture risk, and can negatively impact performance, which is of primary concern for competitive athletes.

General Factors Associated with Bone Mineral Density

Nutritional inadequacies as well as non-nutritional factors are known determinants of BMD in both athletes and non-athlete populations. Being older and being female have been associated with lower BMD, while having sufficient dietary intake of calcium, vitamin D, and energy and adequate sun exposure are associated with higher BMD in athletes as well as in the general healthy population (American Dietetic Association, 2005; Rodriguez et al., 2009). Stress to bone in the form of weight-bearing physical activity increases bone density by upregulating osteoblast activity. (Duncan et al., 2002; Etherington et al., 1996; Nichols et al., 2007). An assessment of 36 adolescent female figure skaters and 22 age-matched controls showed significantly higher calceneus BMD in skaters than in controls. The same assessment revealed no significant differences in calcaneus BMD between the 10 skaters who had sustained heel fractures and the 26 who had not (Oleson et al., 2002). No equivalent studies have been performed in male skaters. Out of 211 singles skaters assessed at two international competitions, 19.8% of females and 13.2% of males sustained at least one stress fracture by the age of 18 (Dubravcic-Simunjak et al., 2003). The same assessment found that 100% of male and female skaters had previously suffered some injury from overuse. Overuse syndrome, characterized by repeated exertion of the same body part, attenuates the positive effects of physical activity on bone and represents a significant contributor to stress fracture risk in athletes (Dubravcic-Simunjak et al., 2003; Porter et al., 2007). Abnormal menstrual status poses a threat to BMD as well. Low estrogen causes an increase in bone calcium resorption in both males and females (Syed & Khosla, 2005). Although no studies have examined the effects of abnormal menstrual status on BMD and fracture risk in figure skaters, this relationship is well documented in athletes of varied disciplines (Nichols et al., 2007; Redman & Loucks, 2005; Rencken et al., 1996).

Vitamin D and Bone Mineral Density

Vitamin D status is a known determinant of BMD in the general population (Mawer & Davies, 2001; Pekkinen et al., 2012) particularly through adolescence. In athletes, vitamin D may influence performance not only due to its role in bone metabolism; vitamin D also influences muscle function, prevention of acute and chronic illness, and body fat metabolism (Hamilton, 2011; Holick, 2009; Kremer et al., 2009; Lenders et al., 2009; Willis et al., 2008). Vitamin D status can be impacted by factors other than dietary intake of vitamin D. Increasing age and female gender are negatively associated with vitamin D status, as is having darker skin pigmentation (Angeline et al., 2013; Gennari, 2001). Lack of sunlight exposure caused by indoor training may predispose figure skaters to vitamin D insufficiency (Larson-Meyer & Willis, 2010). Most studies of athletes confirm the positive influence of vitamin D on bone (Angeline et al., 2013). However, not all studies of vitamin D and bone health show a clear relationship. An assessment of 18 male ballet dancers found no significant correlation between serum 25(OH)D and BMD, and no significant difference in fracture prevalence between dancers with sufficient and insufficient vitamin D status (Ducher et al., 2011). An assessment of 90 healthy females aged 16-22 years found no significant relationship between DEXA-measured BMD and serum 25(OH)D (Kremer et al., 2009). No studies have examined the relationship between vitamin D status and BMD in elite figure skaters.

Vitamin D and Body Composition

In non-athlete subjects, increased adiposity has been associated with vitamin D insufficiency, but the relationship in athletes is not clear. An assessment of 90 healthy average females aged 16-22 years revealed a strong inverse correlation between serum 25(OH)D and body fat mass measured by DEXA (Kremer et al., 2009). An assessment of 410 healthy women aged 20-80 years also found a significant inverse correlation between serum 25(OH)D and BF% measured by DEXA (Arunabh, Pollack, Yeh, & Aloia, 2003b). In 453 healthy males and females aged 65 years and older, serum 25(OH)D was found to be significantly negatively correlated with BMI, waist circumference, and skin-fold caliper measurements (Snijder et al., 2005). In 302 healthy men and women aged 18-71 years, serum 25(OH)D was significantly negatively correlated with BMI and body fat measured by DEXA, and significantly lower serum 25(OH)D levels were observed in the 152 obese subjects compared to those of normal weight (Parikh et al., 2004). An assessment of 58 obese adolescents aged 13-17 years revealed a significant negative correlation between serum 25(OH)D and fat mass measured by DEXA (Lenders et al., 2009). No studies have examined the relationship between vitamin D status and BF% in elite figure skaters.

Cortisol and Bone Mineral Density

The osteoporotic effects of glucocorticoid use are well established (Canalis et al., 2007; O'Brien et al., 2004). The majority of the research on the interaction between endogenous cortisol and bone focuses on aging, non-athlete populations. An assessment of 502 older men and women, as part of the Longitudinal Ageing Study Amsterdam, revealed a significant negative association between serum fasting cortisol and DEXA-measured BMD of the hip, femoral neck, trochanteric region, intertrochanteric region, and lumbar spine in women, but no relationship was seen in men (Van Schoor et al., 2007). An assessment of 132 healthy, normal-weight women aged 19-35 years found a significant inverse correlation between urinary cortisol and DEXA-measured total body and lumbar spine BMD values (Bedford & Barr, 2010). An assessment of 34 healthy men aged 61-72 years revealed significant inverse correlations between serum cortisol and BMD of the lumbar spine and three of five femoral sites, as well as significant positive correlations between serum cortisol and rates of lumbar, femoral, and trochanteric bone loss over four years (Dennison et al., 1999). An analysis of 82 healthy women aged 42-61 years reported a significant inverse correlation between fasting serum cortisol and BMD of the lumbar spine, total femur, and femoral neck (Osella et al., 2012). A cohort study of 247 healthy men and women aged 61-73 years observed a statistically significant positive relationship between elevated serum cortisol and decrease in lumbar BMD over four years in men as well as significantly lower mean BMD of the femoral neck in women with elevated cortisol compared to women with normal cortisol levels (Reynolds et al., 2005). There are no studies that examine the relationship between fasting serum cortisol and BMD in elite figure skaters.

Cortisol and Body Composition

The current understanding of the role of cortisol in athletes is mainly limited to its effects on bone. The interaction between cortisol and body fat has not been thoroughly investigated, especially in athletes. Most of the literature examines the relationship between cortisol and total body weight or the distribution of body fat. An assessment of 54 healthy men and women aged 18 years and older revealed a significant positive correlation between serum cortisol production and both BMI and BF% measured by underwater weighing (Purnell et al., 2004). An analysis of 300 healthy boys and girls aged 4-14 years found significant positive associations between glucocorticoid metabolite excretion and BMI, BF% and total fat mass (Dimitriou et al., 2003). In 50 premenopausal obese women, those with primarily abdominal fat had significantly greater cortisol excretion than those with primarily subcutaneous fat (Duclos et al., 2001). An assessment of 59 healthy premenopausal women aged 30-46 years reported that the 30 subjects with higher waist-to-hip ratio (WHR) had greater salivary cortisol secretion than the 29 subjects with lower WHR (Epel et al., 2000). A similar study by the same authors revealed no significant differences in cortisol secretion between men with high and low WHR (Epel et al., 1999). No studies have examined the relationship between fasting serum cortisol and BF% in elite figure skaters.

Cortisol and Energy Intake

There is a growing body of evidence, primarily in adult female subjects, that cortisol production is increased in energy-restriction. A randomized, controlled experiment of 121 healthy adult females found that subjects who restricted dietary intake to 1200 kcal/day for three weeks had significantly higher salivary cortisol than those who did not restrict intake and consumed, on average, 1700 kcal/day (Tomiyama et al., 2010). Interestingly, an assessment of 78 men and women aged 25-50 years revealed a significant direct correlation between self-reported dietary restraint, measured by Three-Factor Eating

Questionnaire (TFEQ) Restraint score, and fasting serum cortisol in men only (Therrien et al., 2008). In 123 healthy women aged 19-35 years, subjects with TFEQ Restraint scores in the upper 50th percentile had significantly higher urinary cortisol excretion than those with lower scores (Bedford & Barr, 2010). An assessment of 85 college-aged women observed a significant positive correlation between salivary cortisol levels and TFEQ Restraint scores (Anderson et al. 2002).

An inverse relationship between energy intake and cortisol has been observed in athletes as well. In 98 male and female marathon runners, consumption of a carbohydrate-containing beverage during a race prevented the increase in cortisol seen in runners who did not receive a caloric beverage (Nieman et al., 2001). These findings suggest that dietary restriction may contribute to cortisol-induced bone demineralization and possibly increases in body fat. Although no studies have assessed cortisol levels in relation to bone and body fat in figure skaters, energy restriction has been observed consistently in this population, indicating that skaters may be at increased risk for these negative effects of elevated cortisol.

CHAPTER III

Methods

Subjects

Twenty four out of a total of 39 skaters attending a national training camp for the top competitors on the U.S. national team volunteered to be assessed as part of this study. Subjects included male (n=12) and female (n=12) skaters competing in all disciplines, including ladies' and men's singles (n=11), pairs (n=9), and dance (n=4). Subjects ranged in age from 17-34 years and the racial background of subjects included Caucasians (n=20) and Asians (n=4).

Data Collection

U.S. Figure Skating organizes an annual national training camp for top U.S. national team figure skaters. During this training camp, 24 figure skaters volunteered to be assessed as part of the normal activities of the camp. This study represents a secondary analysis of the data collected, a procedure that was approved by the Georgia State University Institutional Review Board. DEXA was performed to measure total BF%, trunk fat %, and BMD of the total spine, L1-L4, L femur, R femur, L femoral neck, R femoral neck, arms, ribs, trunk, legs, and total body. Blood chemistry values for serum 25(OH)D and serum cortisol were obtained via venous puncture at the training camp in the morning after an overnight fast, the same morning as the DEXA measurement, and were analyzed by Quest Diagnostics Incorporated. Urine specific gravity (USG) was measured from urine collected at the same time as the blood sample. Serum cortisol was not obtained in one subject, serum vitamin D was not obtained in another subject, and USG was not obtained in 4 subjects; these subjects declined to be tested for the respective values. These cases were excluded on pairwise analyses. Information on reasons the other skaters chose not to participate in this study was not made available.

Statistical Analysis

All statistical analyses were performed using SPSS version 19. Descriptive statistics were reported as mean and standard deviation. Two-tailed Spearman correlations were used to assess relationships between serum cortisol, vitamin D, BMD, and body composition. Mann-Whitney U test was performed to assess differences in mean values between subjects with higher and lower serum vitamin D and cortisol. Significance was set at $p<0.05$ for all statistical tests.

CHAPTER IV

Results

Subjects

Subject demographic characteristics for all subjects, males, and females are shown in Table 1. Mean age of subjects was 22.7 years. Mean BF% of the total body was 17.5% (13.6% in males, 21.4% in females), and mean BF% of the trunk was 14.6% (12.1% in males, 17.0% in females). Mean BMD values varied between body areas and ranged from 0.688-1.494 g/cm^2. Mean serum 25(OH)D was 37.17 ng/mL (35.75 ng/L in males, 42.17 ng/mL in females). Mean serum cortisol was 18.278 µg/dL (16.245 µg/dL in males, 20.142 µg/dL in females). There were no significant correlations between USG and serum values of vitamin D or cortisol in either male or female skaters (See Table A in appendix). A Mann-Whitney U Test showed that subjects with USG z-score at or above the midpoint level (≥ 0.23408) had significantly higher BF% than subjects with USG z-scores in the lower 50% (p=0.028). A similar result was seen in male subjects (p=0.017), but in females, there was no significant difference in mean BF% between subjects with higher and lower USG. There was no significant difference in BMD measures of any area between subjects with normal and elevated USG. There was a strong positive correlation between BMD of the total body and the ratio of lean mass to height in all subjects (r=0.743, p=0.125).

Table 1. Demographic Characteristics of U.S. National Team Figure Skaters

Characteristic	Mean (SD)		
	All Subjects	Males	Females
Age (years)	22.7 (4.2)	24.9 (4.4)	20.563 (2.817)
Body weight (kg)	63.59 (13.23)	75.06 (6.90)	52.13 (5.62)
Height (cm)	157.94 (5.79)	177.09 (6.20)	145.09 (4.49)
Total BF%	17.467 (6.032)	13.583 (3.445)	21.350 (5.595)
Trunk Fat %	14.550 (5.176)	12.117 (3.516)	16.983 (5.543)
25(OH)D (ng/mL)	37.17 (12.67)	35.75 (10.62)	42.17 (18.60)
Cortisol (µg/dL)	18.278 (7.177)	16.245 (4.046)	20.142 (8.956)
USG	1.019 (0.008)	1.024 (0.007)	1.015 (0.007)
BMD (g/cm^2)			
Total Spine	1.116 (0.141)	1.214 (0.107)	1.009 (0.086)
L1-L4	1.289 (0.130)	1.346 (0.110)	1.231 (0.126)
Pelvis	1.245 (0.131)	1.313 (0.081)	1.171 (0.138)
Left Femur	1.248 (0.137)	1.312 (0.141)	1.184 (0.103)
Right Femur	1.298 (0.166)	1.366 (0.159)	1.224 (0.146)
L Femoral Neck	1.250 (0.167)	1.362 (0.134)	1.139 (0.115)
R Femoral Neck	1.271 (0.172)	1.359 (0.141)	1.174 (0.154)
Arms	0.770 (0.103)	0.846 (0.083)	0.688 (0.037)
Ribs	0.905 (0.085)	0.942 (0.091)	0.865 (0.059)
Trunk	1.101 (0.105)	1.165 (0.074)	1.031 (0.088)
Legs	1.368 (0.168)	1.494 (0.110)	1.231 (0.096)
Total Body	1.236 (0.111)	1.308 (0.090)	1.158 (0.072)

Vitamin D and BMD

Table 2 shows 2-tailed Spearman correlations between serum 25(OH)D and BMD values in all subjects, males, and females. Correlations revealed no significant associations between serum vitamin D and BMD of any measured body area. As shown in Table 2, all correlations had p-values > 0.05 in all subjects as well as in males only and females only

Table 2. Spearman Correlations (2-Tailed) Between Serum 25(OH)D and BMD in All Subjects, Males, and Females						
	All Subjects		**Males**		**Females**	
Body Area	**r**	**p**	**r**	**p**	**r**	**p**
Total Spine	-0.106	0.631	-0.081	0.803	-0.082	0.810
L1-L4	0.026	0.904	0.207	0.519	-0.004	0.991
Pelvis	-0.061	0.783	0.326	0.301	0.027	0.936
Left Femur	-0.002	0.991	0.112	0.728	0.186	0.564
Right Femur	0.099	0.653	0.112	0.728	0.132	0.699
L Femoral Neck	0.092	0.670	0.234	0.465	0.270	0.397
R Femoral Neck	0.264	0.223	0.389	0.211	0.392	0.233
Arms	-0.159	0.468	-0.460	0.133	0.241	0.474
Ribs	-0.140	0.526	-0.200	0.533	0.087	0.800
Trunk	-0.130	0.556	0.000	1.000	-0.082	0.881
Legs	-0.154	0.483	-0.119	0.712	0.050	0.884
Total Body	-0.223	0.306	-0.077	0.811	-0.137	0.689

Mean BMD values of all subjects according to vitamin D status are displayed in Table 3. Eight subjects had serum 25(OH)D < 30 ng/mL and 15 subjects had serum 25(OH)D ≥ 30 ng/mL. A Mann-Whitney U test revealed no significant differences in mean BMD measures of any body area between subjects with vitamin D at or above the lower normal value of 30 ng/mL and those with vitamin D less than 30 ng/mL.

Table 3. Differences in Mean BMD Between All Subjects with Adequate and Inadequate Serum 25(OH)D as Determined by a Mann-Whitney U Test			
Body Area	**Mean BMD**		**p**
	25(OH)D < 30 ng/ml	**25(OH)D ≥ 30 ng/ml**	
	n=8	n=15	
Total Spine	1.123 (0.158)	1.112 (0.137)	0.949
L1-L4	1.265 (0.138)	1.301 (0.128)	0.501
Pelvis	1.221 (0.163)	1.257 (0.116)	0.796
L Femur	1.224 (0.210)	1.260 (0.874)	0.854
R Femur	1.262 (0.237)	1.317 (0.119)	0.439
L Femoral Neck	1.220 (0.219)	1.266 (0.140)	0.581
R Femoral Neck	1.195 (0.220)	1.311 (0.131)	0.138
Arms	0.790 (0.121)	0.760 (0.095)	0.699
Ribs	0.901 (0.079)	0.907 (0.091)	0.675
Trunk	1.092 (0.122)	1.106 (0.099)	0.949
Legs	1.363 (0.196)	1.371 (0.158)	0.897
Total Body	1.238 (0.106)	1.238 (0.106)	0.872

Differences in all mean BMD values between vitamin D status groups (< 30 ng/mL compared with ≥ 30 ng/mL) in male and female subjects combined had p-values > 0.05.

Mean BMD values of female subjects according to vitamin D status are displayed in Table 4. Three females had serum 25(OH)D < 30 ng/mL and eight females had serum 25(OH)D ≥ 30 ng/mL. According to results of a Mann-Whitney U test, females with serum 25(OH)D ≥ 30 ng/mL had statistically significantly greater BMD of the right femoral neck only compared to females with serum 25(OH)D < 30 ng/mL (1.233±0.124 g/cm2 vs. 1.018±0.116 g/cm^2, p=0.041). Differences in all other BMD values between females with serum 25(OH)D ≥ 30 ng/mL and < 30 ng/mL were not statistically significant (p>0.05).

Table 4. Differences in Mean BMD Between Female Subjects with Adequate and Inadequate Serum 25(OH)D as Determined by a Mann-Whitney U Test

Body Area	Mean BMD		p
	25(OH)D < 30 ng/ml	**25(OH)D ≥ 30 ng/ml**	
	n=3	n=8	
Total Spine	0.989 (0.090)	1.017 (0.089)	1.000
L1-L4	1.194 (0.130)	1.244 (0.130)	0.782
Pelvis	1.126 (0.234)	1.188 (0.102)	0.540
L Femur	1.116 (0.204)	1.207 (0.041)	0.405
R Femur	1.125 (0.233)	1.261 (0.095)	0.414
L Femoral Neck	1.064 (0.207)	1.164 (0.070)	0.405
R Femoral Neck	1.018 (0.116)	1.233 (0.124)	0.041
Arms	0.673 (0.024)	0.693 (0.041)	0.221
Ribs	0.851 (0.022)	0.870 (0.069)	0.221
Trunk	1.000 (0.129)	1.043 (0.076)	0.683
Legs	1.166 (0.147)	1.255 (0.066)	0.307
Total Body	1.122 (0.109)	1.171 (0.057)	0.414

Mean BMD values of male subjects according to vitamin D status are displayed in Table 5. Five males had serum 25(OH)D < 30 ng/mL and seven males had serum 25(OH)D ≥

30 ng/mL. According to results of a Mann-Whitney U test, no significant differences in mean BMD existed between males with serum 25(OH)D ≥ 30 ng/mL and males with serum 25(OH)D < 30 ng/mL. For all measured body areas, differences in BMD values between males with adequate and inadequate vitamin D status had p-values > 0.05.

Table 5. Differences in Mean BMD Between Male Subjects with Adequate and Inadequate Serum 25(OH)D as Determined by a Mann-Whitney U Test

Body Area	Mean BMD		p
	25(OH)D < 30 ng/ml	25(OH)D ≥ 30 ng/ml	
	n=5	n=7	
Total Spine	1.204 (0.135)	1.222 (0.092)	0.570
L1-L4	1.308 (0.137)	1.374 (0.086)	0.223
Pelvis	1.278 (0.090)	1.337 (0.072)	0.223
L Femur	1.289 (0.206)	1.328 (0.085)	0.685
R Femur	1.301 (0.201)	1.382 (0.114)	0.685
L Femoral Neck	1.313 (0.184)	1.397 (0.083)	0.290
R Femoral Neck	1.301 (0.201)	1.401 (0.067)	0.372
Arms	0.860 (0.094)	0.836 (0.081)	0.685
Ribs	0.931 (0.088)	0.950 (0.098)	0.808
Trunk	1.147 (0.087)	1.178 (0.067)	0.464
Legs	1.481 (0.100)	1.503 (0.124)	0.808
Total Body	1.298 (0.090)	1.316 (0.096)	0.745

Vitamin D and Body Composition

2-Tailed Spearman correlations found no statistically significant associations between serum vitamin D and total BF% or trunk fat %, regardless of subdivision by gender (See Table B in Appendix). Additionally, Mann-Whitney U tests revealed no statistically significant differences in trunk fat % or total BF% between subjects, whether male or female, with serum vitamin D of at least 30 ng/mL compared to those with serum vitamin D < 30 ng/mL (See Table C in Appendix).

Cortisol and BMD

As shown in Table 6, an assessment of all skaters using 2-tailed Spearman correlations found statistically significant inverse correlations between fasting serum cortisol and BMD of the spine (r=-0.458, p=0.032), pelvis (r=-0.532, p=0.011), ribs (r=-0.517, p=0.014), and trunk (r=-0.538, p=0.010). In male and female subjects together, no significant correlations existed between fasting serum cortisol and BMD of L1-L4, either femur or femoral neck, the arms, legs, or total body. In female skaters, fasting serum cortisol was negatively correlated with BMD of the pelvis (r=-0.664, p=0.026), L femur (r=-0.587, p=0.045), and trunk (r=-0.609, p=0.047). In females, there were no significant correlations between fasting serum cortisol and BMD of the total spine, L1-L4, R femur, either femoral neck, arms, ribs, legs or total body. In male skaters, cortisol had a statistically significant negative correlation with BMD of the ribs only (r=-0.627, p=0.039); there were no significant correlations between fasting serum cortisol and BMD of the total spine, L1-L4, pelvis, R or L femur, R or L femoral neck, arms, trunk, legs, or total body in males.

Table 6. Spearman Correlations (2-tailed) Between Fasting Serum Cortisol and BMD in All Subjects, Males, and Females

	All Subjects		Males		Females	
Body Area	**r**	**p**	**r**	**p**	**r**	**p**
Total Spine	-0.458	0.032	-0.555	0.077	-0.384	0.244
L1-L4	-0.229	0.293	-0.427	0.190	-0.119	0.713
Pelvis	-0.532	0.011	-0.355	0.285	-0.664	0.026
L Femur	-0.371	0.082	-0.227	0.502	-0.587	0.045
R Femur	-0.319	0.148	-0.227	0.502	-0.400	0.223
L Femoral Neck	-0.351	0.100	-0.369	0.264	-0.510	0.090
R Femoral Neck	-0.235	0.291	-0.382	0.247	-0.173	0.612
Arms	-0.352	0.108	-0.409	0.212	-0.264	0.433
Ribs	-0.517	0.014	-0.627	0.039	-0.336	0.312
Trunk	-0.538	0.010	-0.491	0.125	-0.609	0.047
Legs	-0.386	0.076	-0.236	0.484	-0.573	0.066
Total Body	-0.369	0.091	-0.255	0.449	-0.491	0.125

No significant correlations existed between fasting serum cortisol and BMD of L1-L4, the R femur, either femoral neck, the arms, legs, or total body, regardless of subdivision of subjects by gender.

These correlational data were reinforced by results of a Mann-Whitney U test, which showed that the 3 skaters with serum cortisol above the upper normal morning fasting value of 28 μg/dL had lower mean BMD of the pelvis ($p=0.031$), left femur ($p=0.040$), left femoral neck ($p=0.028$), trunk ($p=0.031$), legs ($p=0.025$), and total body ($p=0.031$) when compared to the 20 skaters with serum cortisol within the normal range (7-28 μg/dL). As shown in Table 7, these differences only reached statistical significance when comparing the three skaters with cortisol ≥ 28 μg/dL, who were all female, to all skaters, both male and female, with cortisol < 28 μg/dL.

Table 7. Differences in Mean BMD Between Subjects with Normal and Elevated Cortisol as Determined by Mann-Whitney U Test

Body Area	**Mean BMD (g/cm^2)**					
	All Subjects			**Females**		
	Cort ≥ 28 mg/dl	**Cort < 28 μg/dl**	p	**Cort ≥ 28 μg/dl**	**Cort < 28 μg/dl**	**p**
	n=3	n=20		n=3	n=8	
Total Spine	1.128 (0.139)	0.987 (0.087)	0.104	0.989 (0.090)	0.987 (0.087)	0.758
L1-L4	1.292 (0.123)	1.209 (0.157)	0.411	1.194 (0.130)	1.209 (0.157)	0.926
Pelvis	1.268 (0.112)	1.061 (0.121)	0.031	1.126 (0.234)	1.061 (0.121)	0.153
L Femur	1.263 (0.114)	1.074 (0.134)	0.040	1.116 (0.204)	1.074 (0.134)	0.116
R Femur	1.314 (0.138)	1.110 (0.208)	0.094	1.125 (0.233)	1.110 (0.208)	0.221
L Femoral Neck	1.269 (0.136)	1.024 (0.139)	0.028	1.064 (0.207)	1.024 (0.139)	0.116
R Femoral Neck	1.291 (0.147)	1.070 (0.207)	0.094	1.018 (0.116)	1.070 (0.207)	0.307
Arms	0.781 (0.106)	0.684 (0.403)	0.077	0.673 (0.024)	0.684 (0.040)	0.683
Ribs	0.916 (0.090)	0.856 (0.026)	0.114	0.851 (0.022)	0.856 (0.026)	0.414
Trunk	1.117 (0.097)	0.973 (0.082)	0.031	1.000 (0.129)	0.973 (0.082)	0.221
Legs	1.389 (0.145)	1.152 (0.124)	0.025	1.166 (0.147)	1.152 (0.124)	0.153
Total Body	1.251 (0.103)	1.106 (0.083)	0.031	1.122 (0.109)	1.106 (0.083)	0.221

Differences in BMD between male subjects with normal and elevated cortisol were not assessed because all male subjects had fasting serum cortisol within the normal range.

Cortisol and BF%

In females only, fasting serum cortisol was directly correlated with total BF% (r=0.657, p=0.020) and trunk fat % (r=0.708, p=0.010) (See Table 8). No significant correlations were found between fasting serum cortisol and BF% values in male subjects only or male and female subjects combined.

Table 8. Spearman's Correlations (2-tailed) Between Fasting Serum Cortisol and BF% in All Subjects, Males, and Females

	All Subjects		Males		Females	
Body Area	**r**	**p**	**r**	**p**	**r**	**p**
Trunk	0.377	0.077	-0.218	0.519	0.708	0.010
Total Body	0.304	0.158	-0.218	0.519	0.657	0.020

As shown in Table 9, the three skaters, who were all female, with serum cortisol above the upper normal fasting value of 28 μg/dL had significantly greater mean total BF% (p=0.008) and trunk fat % (p=0.006) when compared to the 20 skaters, both male and female, with serum cortisol within the normal range (7-28 μg/dL). These differences in mean total BF% and trunk fat % also reached statistical significance when the three female skaters with serum cortisol greater than 28 μg/dl were compared to the eight females with normal cortisol (p=0.021 and p=0.012, respectively). Differences in BF% between male subjects with normal and elevated cortisol were not assessed because all male subjects had fasting serum cortisol within the normal range.

Table 9. Differences in Mean BF% Between Subjects with Normal and Elevated Cortisol as Determined by Mann-Whitney U Test						
	Mean BF%					
	All Subjects			**Females**		
Body Area	**Cort ≥ 28 µg/dl**	**Cort < 28 µg/dl**	**p**	**Cort ≥ 28 µg/dl**	**Cort < 28 µg/dl**	**p**
	n=3	n=20		n=3	n=8	
Trunk	15.820 (4.364)	28.400 (5.575)	0.006	19.000 (3.228)	28.400 (5.575)	0.012
Total Body	13.105 (3.676)	23.733 (5.601)	0.008	14.733 (3.410)	23.733 (5.601)	0.021

There was no significant correlation between fasting serum cortisol and the ratio of lean mass to height in all subjects (r=-0.330, p=0.125), males (r=-0.218, p=0.519), or females (r=-0.420, p=0.175).

Summary

There were statistically significant inverse associations between serum cortisol and BMD of the spine, pelvis, ribs, and trunk in all subjects. In females, cortisol was inversely correlated with BMD of the pelvis, left femur, and trunk, and was directly correlated with total BF % and trunk fat %. In male skaters, cortisol was significantly inversely correlated with BMD of the ribs. These correlational data were reinforced with results of a Mann-Whitney U test, which indicated that the three skaters (all female) with serum cortisol above the upper normal morning fasting value of 28 mcg/dL had significantly lower mean BMD of the total body, left femoral neck, legs, trunk, and pelvis, and significantly higher BF % of the total body and trunk when compared to the 20 skaters with serum cortisol values that were in the normal range (7-28 mcg/dL). No significant correlations between serum 25(OH)D and BMD or body composition measurements were found. These results were supported by a Mann-Whitney U test, which revealed no significant differences in mean BMD and body composition measures between the eight skaters with serum vitamin D at or above the lower normal value of 30 ng/mL compared to the 16 skaters who had vitamin D less than 30 ng/mL. However, female skaters with serum vitamin D of at least 30 ng/mL had significantly higher BMD of the right femoral neck when compared to those with lower serum vitamin D.

CHAPTER V

Discussion and Conclusions

Urine Specific Gravity

Regardless of how the subject population was subdivided (all subjects, males, females), mean USG was between 1.000 and 1.030, indicating that the average hydration status of subjects was normal. The absence of significant correlations between USG and serum vitamin D or cortisol suggests that hydration status did not significantly influence these blood chemistry measures. The differences in mean BF% that were seen between subjects with normal USG and high USG indicate that skaters who were more dehydrated had greater body fatness. It is well documented that BF% measurement by DEXA is not significantly affected by hydration status, lending greater validity to this finding (Ackland et al., 2012). One possible explanation of these results is that skaters with greater BF% may be restricting both fluid and food intake in an attempt to control body weight and/or appearance.

Vitamin D and BMD

Despite spending a great deal of time training in indoor facilities, a factor likely to limit vitamin D creation through sunlight exposure, mean serum vitamin D was greater than 30 ng/ml in all subjects, in males, and in females. In eight of 24 subjects (33.3%), serum vitamin D was insufficient (25OHD < 30 ng/ml). Based on the lack of statistical relationship between vitamin D and BMD, we cannot reject the null hypothesis 1-0.

These results, in this athlete population documented to have a relatively high rate of stress fractures, should not diminish the important role of vitamin D in bone health (Dubravcic-Simunjak et al., 2003). Rather, these findings suggest the need to investigate additional predictive indicators of bone injury risk in figure skaters, such as insufficient dietary calcium intake, overproduction of cortisol, and overuse syndrome.

Vitamin D and BF%

We were unable to disprove the null hypothesis 2-0, as there was no significant correlation between serum 25(OH)D and BF% or trunk fat % in this athlete population. Additionally, no differences in BF% or trunk fat % existed between subjects with serum vitamin D above and below 30 ng/ml. Because mean vitamin D status was normal and mean BF% was relatively low, these athletes may not be affected by the inverse relationship between serum vitamin D and body fat that has been observed in other populations (Arunabh et al., 2003; Kremer et al., 2009; Lenders et al., 2009; Parikh et al., 2004; Snijder et al., 2005).

Cortisol and BMD

Inverse correlations were consistently found between fasting serum cortisol and BMD in multiple areas, particularly those composed of trabecular bone. Additionally, skaters with elevated cortisol ($\geq$28 μg/dl) had significantly lower BMD of multiple areas (pelvis, L femur, L femoral neck, ribs, legs, and total body) compared to skaters with normal serum cortisol levels. Based on these results, we can reject the null hypothesis 3-0. These findings are consistent with those of prior studies, which show an inverse relationship between cortisol and BMD in non-athlete populations (Bedford & Barr, 2010; Dennison et al., 1999; Osella et al., 2012; Reynolds et al., 2005; Van Schoor et al., 2007). Because a direct relationship has been observed between restrained eating patterns and elevated cortisol, and figure skaters are known to have relatively high rates of restrained eating behaviors, further study is necessary to determine whether dietary restriction is a common cause of cortisol-induced bone demineralization in figure skaters (Anderson et al., 2002; Bedford & Barr, 2010; Jonnalagadda et al., 2004; Therrien et al., 2008; Tomiyama et al., 2010; Ziegler et al., 2001).

Cortisol and BF%

Higher serum cortisol was positively associated with total BF% and trunk fat % in female skaters, and the three female skaters with elevated cortisol had significantly higher total BF% and trunk fat % than the 20 skaters (male and female) with normal cortisol. Although the relationship between fasting serum cortisol and the ratio of lean mass to height was not statistically significant, correlation coefficients were negative in all subjects, males, and females, and the relationship was slightly stronger in females than in males. This tells us that the impact of cortisol on body composition is mainly related to its interaction with body fat, not lean tissue. Based on these results, we can reject the null hypothesis 4-0. These findings were expected, as elevated cortisol has been previously associated with increased adiposity, particularly in females (Dimitriou et al., 2003; Duclos et al., 2001; Epel et al., 2000; Purnell et al., 2004). This is the first comparison of fasting serum cortisol and BF% in elite figure skaters. These findings suggest a need for further study of the interaction between restrained eating patterns, cortisol, and body composition in athletes.

Limitations

The greatest limitation of this study was likely in the methods used to collect data. Because these results represent a secondary analysis of information collected as part of the normal activities of a figure skating training camp, the researchers had little control over the methods used for data collection. Blood chemistry, USG, and DEXA values were collected primarily for personal use by figure skaters, not for research purposes, and the accuracy of the measurement processes used is unknown. While the serum cortisol and serum vitamin D values were obtained in a "fasting" state in the morning, we are reliant on the skaters' assurances that they were, indeed, in a fasting state at the time of measure. Additionally, further information about the subjects, such as diet history and prior fracture history was not made available. This analysis was based on a small sample of primarily Caucasian figure skaters and can only be considered as preliminary evidence upon which to base further research.

Conclusions

In an unexpected finding, there was no significant relationship between serum vitamin D and BMD in elite figure skaters. Additionally, there was no significant relationship between vitamin D and BF%. As hypothesized, serum cortisol was inversely associated with BMD measures, particularly in trabecular areas. Higher serum cortisol was associated with greater BF% in female skaters only. Although dietary behaviors were not assessed in this subject population, figure skaters, both male and female, are known to be energy restrictors, and cortisol is known to be elevated in energy restriction, suggesting a possible interaction between dietary restraint, increased BF%, and decreased BMD. Because dietary information was not available, we cannot make conclusions based on eating patterns. However, when considering the previous findings that figure skaters often use energy restriction as a weight control method and that energy restriction is positively correlated with cortisol production, the results of this study suggest that the possible interactions between dietary behaviors, bone density, and body composition in figure skaters require further research. Future studies in this area may include additional assessments of the relationship between serum vitamin D and bone health as well as investigations into the role that dietary restriction may play in the interaction between cortisol, BMD, and body composition in figure skaters, other athletes, and the general population.

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APPENDIX 1

Table A. Spearman Correlations (2-Tailed) Between Urine Specific Gravity and Serum Values of 25(OH)D and Cortisol in All Subjects, Males, and Females

	All Subjects		Males		Females	
Measure	**r**	**p**	**r**	**p**	**r**	**p**
25(OH)D	0.046	0.847	0.312	0.380	-0.030	0.934
Cortisol	0.158	0.505	0.116	0.116	0.456	0.185

APPENDIX 2

Table B. Spearman Correlations (2-Tailed) Between Serum 25(OH)D and BF% in All Subjects, Males, and Females						
Body Area	**All Subjects**		**Males**		**Females**	
	r	**p**	**r**	**p**	**r**	**p**
Trunk	-0.128	0.550	-0.291	0.358	-0.344	0.274
Total Body	-0.003	0.988	-0.284	0.371	-0.333	0.291

APPENDIX 3

Table C. Differences in Mean BF % Between Subjects with Adequate and Inadequate Serum 25(OH)D as Determined by Mann-Whitney U Test

Body Area	Mean BF% (SD)								
	All Subjects			Males			Females		
	Vit D < 30 ng/ml	Vit D ≥ 30 ng/ml	p	Vit D < 30 ng/ml	Vit D ≥ 30 ng/ml	p	Vit D < 30 ng/ml	Vit D ≥ 30 ng/ml	p
Trunk	16.613 (5.616)	17.894 (6.363)	0.935	13.100 (1.639)	13.929 (4.432)	0.308	22.467 (4.772)	20.978 (6.060)	0.951
Total Body	14.100 (4.194)	14.775 (5.719)	0.935	11.640 (1.635)	12.457 (4.534)	0.405	18.200 (3.985)	16.578 (6.127)	0.540