

LUTEIN AND ZEAXANTHIN STATUS AND ITS RELATION TO BODY FAT
PERCENTAGE AND BONE MINERAL DENSITY
by

EMILY R. BOVIER

(Under the Direction of Billy R. Hammond, Jr.)

ABSTRACT

Lutein (L) and zeaxanthin (Z) may prevent oxidative damage associated with macular degeneration and osteoporosis. It may be that LZ status is influenced by personal characteristics that influence the natural history of degenerative conditions. The purpose of this study was to correlate LZ status with total and regional body fat and areal bone mineral density in 63 young adults. Macular pigment was measured using heterochromatic flicker photometry. Serum LZ was quantified with high performance liquid chromatography. Body fat and aBMD were assessed using dual-energy X-ray absorptiometry. Body fat percentage was not related to LZ status (possibly due to a restriction in the range of body fat). Macular pigment was positively related to aBMD ($p < 0.05$). A relation between LZ status and conditions characterized by oxidative stress is consistent with the recommendation to increase intake of antioxidant-rich foods in order to help prevent the development of degenerative conditions.

INDEX WORDS: Macular pigment, lutein, zeaxanthin, bone mineral density, adipose tissue

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER	
1 INTRODUCTION	1
Overview	1
Lutein and Zeaxanthin	2
Lutein and Zeaxanthin Status and Body Fat Percentage	4
Lutein and Zeaxanthin Status and Bone Health.....	5
Summary and Hypotheses.....	7
2 METHOD	9
Subjects.....	9
Assessment of LZ Status: Macular Pigment & Serum LZ	9
Assessment of Body Fat Percentage and Bone Mineral Density	11
Assessment of Health Habits: Diet & Physical Activity	11
3 RESULTS	13
4 DISCUSSION	16
REFERENCES	22

LIST OF TABLES

	Page
Table 1: Risk factors common to both age-related macular degeneration and osteoporosis.....	29
Table 2: Empirical evidence for the association between tissue lutein and zeaxanthin and body fat percentage (data reported as mean \pm standard deviation).....	30
Table 3: Descriptive data (mean \pm standard deviation) for all subjects and stratified by sex	31
Table 4: Pearson-product moment correlation coefficients and significance values (r p) for associations between LZ status and dietary intake of fruits (F) and vegetables (V).	32
Table 5: Pearson-product moment correlation coefficients and significance values (r p) for associations between bone mineral density, LZ status, body fat percentage, and health habits	33
Table 6: Pearson-product moment correlation coefficients and significance values (r p) for associations between body fat percentage and LZ status.....	34

LIST OF FIGURES

	Page
Figure 1: Wavelengths of targets relative to the absorbance spectrum of macular pigment	35
Figure 2: Schematic of test stimulus seen by subject in HFP	36
Figure 3: Example full-body DXA scan illustrating regional distribution used for body fat percentage	37
Figure 4: The relationship between serum lutein/zeaxanthin (LZ) and macular pigment optical density (MPOD) at 7.5 minutes (7.5') retinal eccentricity	38
Figure 5: The relationship between body fat percentage and macular pigment optical density (MPOD) at 30 minutes (30') retinal eccentricity (b) and serum lutein/zeaxanthin for male and female subjects	39
Figure 6: Mean (\pm standard deviation) body fat percentage for the total body and regional distribution for male and female subjects	40
Figure 7: The relationship between skeletal mass of the lumbar spine and macular pigment optical density (MPOD) at 30' retinal eccentricity and serum lutein/zeaxanthin	41
Figure 8: The relationship between skeletal mass of the proximal femur and macular pigment optical density (MPOD) at 30' retinal eccentricity and serum lutein/zeaxanthin	42

CHAPTER 1

INTRODUCTION

Overview

Most of the major causes of morbidity and mortality are strongly influenced by lifestyle factors, many of which are linked to oxidative stress. Habits such as smoking and poor diet accelerate senescence by promoting free radical production and subsequent structural damage throughout the body. Environmental stress is associated with degenerative conditions such as age related macular degeneration (AMD) and osteoporosis, in the form of oxidative damage to the retina and skeleton, respectively. These seemingly disparate conditions are similar in that they share many etiological factors linked to oxidative stress (see Table 1). Certain risk factors, such as a diet deficient in antioxidants, can be modified to protect against oxidative damage. The carotenoids lutein (L) and zeaxanthin (Z), for instance, may provide protective benefits to both ocular tissues and bone. In fact, pharmaceutical companies are currently marketing custom made nutritional supplements with LZ to specifically target both AMD and osteoporosis (e.g., Mitamins[©]; Jarrow Formulas[©]).

It has, in fact, often been hypothesized that LZ protect against degenerative damage by quenching oxygen radicals (e.g., Cantrell, McGarvey, Truscott, Rancan, & Bohm, 2003) and reducing oxidative stress. This is particularly relevant to age-related macular degeneration, given the role of oxidative damage in its pathogenesis (see Bonnel, Mohand-Said, & Sahel, 2003 for a review) and the preponderance of LZ in retinal tissue (e.g., on the order of 140 ng; Handelman, Dratz, Reay, & Van Kuijk, 1988). With respect to osteoporosis, LZ may serve to

lower overall oxidative stress in the body, helping to maintain a proper antioxidant/oxidant balance necessary for bone health (e.g., Altindag, Ere Soran, Celik, & Selek, 2008). The ability to quantify LZ in tissue using noninvasive methods facilitates research designed to:

- a) assess the relationship between LZ status and biomarkers of aging and disease, and
- b) directly assess the effects of interventions with LZ supplementation.

The latter effect is important because not all individuals respond equally to supplementation (see the responder vs nonresponder distinction in Hammond, Johnson, et al., 1997). Hence, by measuring (noninvasively) tissue levels of LZ, we can study how LZ influence health at the level of the tissue it protects while bypassing issues such as compliance and differences in gut physiology. This study utilized this powerful tool in order to evaluate relations between LZ and skeletal mass (a marker of bone health).

Lutein and Zeaxanthin

LZ are two lipophilic pigments obtained exclusively from the diet. Common sources of LZ include dark green leafy vegetables such as spinach and kale. These nutrients are also found in egg yolk and a number of other fruits and vegetables (e.g., Sommerburg, Keunen, Bird, & Van Kuijk, 1998). Following consumption, LZ are absorbed in the gut and transported throughout the blood by lipoproteins (e.g., Connor, Duell, Kean, & Wang, 2007). They are then deposited in numerous tissues including retinal, kidney, lung, and liver tissues (e.g., Krinsky, Landrum, & Bone, 2003). LZ have also been found in the frontal and occipital cortex (e.g., Craft, Haitema, Garnett, Fitch, & Dorey, 2004).

LZ are likely to have a constellation of health effects throughout the body. Indeed, with respect to general wellness, higher tissue concentrations of LZ have been associated with a

reduced risk of cardiovascular disease, various cancers, and other acquired diseases (e.g., Mares-Perlman, Millen, Ficek, & Hankinson, 2002). Tissue LZ status can be quantified by measuring serum LZ and retinal LZ. When deposited in the central retina, LZ are referred to as the macular pigment (MP). The macula accrues LZ from the diet to the exclusion of other carotenoids. The mechanisms by which LZ are deposited into retinal tissue are not well understood, however positive associations between high-density lipoproteins and serum LZ (e.g., Loane, Nolan, & Beatty, 2010) and retinal LZ (e.g., Connor et al., 2007) have been reported.

Although dietary intake is the primary driver of tissue LZ levels, a number of other factors moderate this relationship (e.g., iris color and smoking behavior). Hammond et al. (1996) found significant differences in MP density between male and female subjects (females being lower), despite equivalent plasma concentrations and dietary intake of LZ. This finding was consistent with the general observation that conditions directly associated with LZ status, like AMD and cataracts, are also more prevalent in women. One possible reason for the observed sex differences in MP density (also reported by Broekmans et al., 2002 and Johnson et al., 2000) might be the fact that women generally have higher body fat percentages than men. Adipose tissue is, in fact, a storage site for LZ (e.g., Kaplan, Lau, & Stein, 1990). Increased body fat might absorb a higher fraction of circulating carotenoids making these pigments less available to tissues like the retina. Consistent with this view, Johnson et al., 2000 found that changes in adipose tissue L concentration were linked to changes in MP density (suggesting an interaction between adipose and retinal tissue in L metabolism). This interaction, however, was specific to sex: significant negative correlations were found between adipose tissue L concentrations and MP density for women, but a significant positive relation was found for men.

Lutein and Zeaxanthin Status and Body Fat Percentage

Table 2 lists data from studies with cross-sectional associations between tissue LZ and body fat percentage. A number of studies have reported that higher body fat levels, especially when approaching obesity, are linked to lower levels of retinal LZ (e.g., Hammond, Ciulla, & Snodderly, 2002). As shown in the table, the link between body fat and LZ status (in adipose, serum, and retinal tissue) appears to be different for men and women. For example, Broekmans et al. (2002) found that female subjects had 13% lower MP than males, despite significantly higher serum and adipose tissue L. Positive associations of adipose L with serum L and MP density were reported for male subjects. For females, adipose L was correlated with serum L, but not MP density. Conversely, Nolan et al. (2004) reported data they stated to be consistent with a competition between the retina and body fat for LZ, but only for males. In their sample, MP was inversely related to body fat percentage for men, but no effect was found for women.

Hammond et al. (2002) noted that men and women with high body fat also had low MP density, and therefore suggested the inverse relationship between fat and retinal LZ was not influenced by sex. The authors reported that subjects with body fat greater than 27% had 16% less MP compared to subjects with lower body fat; however, the inverse relationship between MP density and body fat was driven by obese subjects since the relationship was only significant for subjects with over 27% body fat, suggesting a nonlinear effect of adiposity on retinal LZ.

Taken together, one plausible inference is that the mechanism by which adiposity effects retinal accumulation of LZ for individuals within a healthy weight range is different for men and women. One source for this difference might be the well known differences in the distribution of body fat between the sexes (as opposed to just total body fat percentage). Regional adiposity was originally proposed as a possible mechanism for explaining the sex differences in

associations between adipose tissue L and MP by Johnson et al. (2000). The authors reported that their preliminary data indicated sex differences in adipose L concentrations at different bodily sites. Chung et al. (2009) reported, more specifically, that LZ was higher in adipose tissue in the abdomen compared to the buttocks and thighs. The relationship between serum LZ and adipose tissue was stronger for the abdomen compared to the buttocks. Differential accumulation of body fat within a sample may explain the inconsistencies in past data regarding the relationship between MP and body fat percentage, especially if abdominal body fat does accumulate L more readily. One goal then of the present study was to assess the relation between body fat distribution in males and females and LZ status.

Evaluating regional body fat percentage and LZ in both the serum and the retina will contribute to an understanding of differences between men and women and may explain variance in LZ status between individuals who are within a healthy range of body weight. As mentioned, public interest in LZ and the popularity of supplements containing LZ has increased given the nutrients' potential health effects. Considering factors that influence nutrient absorbance is particularly relevant to understanding the effectiveness of using LZ supplements to protect against degenerative conditions.

Lutein and Zeaxanthin and Bone Health

Degenerative damage from oxidative stress contributes to the pathogenesis of AMD and osteoporosis. For example, reactive oxygen species (ROS) are likely responsible for the deterioration of the fovea, resulting in the permanent loss of central vision (e.g., Zarbin, 1998; Bonnel et al. 2003). This is characteristic of advanced stages of AMD in which blood vessels form under the retina and hemorrhage. Excessive oxidative activity, however, also attenuates

bone mass over time (e.g., Wauquier, Leotoing, Coxam, Guicheux, & Wittrant, 2009). The production of ROS is a normal part of the bone remodeling process, which involves the coupling of osteoblast and osteoclast functioning. Osteoclasts form and remove bone, resulting in the production of ROS, followed by an increase in bone formation by osteoblasts. A proper balance between osteoclast and osteoblast activity can be maintained with a proper balance between antioxidants and oxidants (e.g., Sheweita & Khoshhal, 2007). However, if ROS production outweighs antioxidant mechanisms, subsequent increases in oxidative stress may result in accelerated bone loss. Deteriorated bone strength and increased susceptibility to fractures are primary symptoms of osteoporosis. Some empirical data supports the use of LZ to improve eye health based on associations of higher retinal LZ with reduced disease risk (e.g., Bone et al., 2001; Beatty et al., 2001; Gale, Hall, Phillips, & Martyn, 2003). Data linking LZ to bone health, however, is limited.

To date, the association between LZ status and areal bone mineral density (aBMD) has been addressed in two empirical studies. Wattanapenpaiboon, Lukiton, Wahlqvist, & Strauss (2003) reported cross-sectional associations, whereas Sahni, Hannan, Blumberg, Cupples, Kiel, & Tucker (2009) reported associations at baseline and after a four-year follow up. Wattanapenpaiboon et al. (2003) studied 205 subjects ranging from 26 to 86 years of age. For premenopausal women, higher aBMD of the lumbar spine was related to a greater dietary intake of LZ ($N = 47$, $r = 0.35$, $p < 0.05$). However, no effect was found for men ($N = 68$; $r = -0.18$) or postmenopausal women ($N = 90$, $r = 0.18$). Sahni and colleagues (2009) did not find significant cross-sectional associations between dietary LZ and aBMD (at the femoral neck, trochanter, spine, and radial shaft). Their cross-sectional study, however, included 976 subjects with an average age of approximately 75 years. Despite the absence of an effect at baseline, a higher

intake of LZ for male subjects (N = 193) was associated with less reduction in trochanter BMD after four years ($p = 0.008$). Both Wattanapenpaiboon et al. and Sahni et al. concluded the results of their studies offered support for protective associations of LZ with bone health (despite their mixed results and the acknowledgments that serum LZ may not adequately characterize long-term dietary intake).

If LZ do offer protection against bone loss, most likely via antioxidant mechanisms, then it would be useful to understand the association between LZ status and bone health prior to the onset of degeneration that is commonly seen in aging samples. Past research has included subjects in their 60s and 70s, whose bone health is likely to already reflect consequences of oxidative stress. If pharmaceutical companies are targeting at-risk populations by marketing customized supplements with LZ to prevent co-morbid degenerative conditions such as AMD and osteoporosis, it is important to know if a relationship exists between LZ status and skeletal mass in young, healthy individuals. This argument is supported by the concept of temporality – outlined by Mares-Perlman et al. (2002) – with regard to the role of LZ in protecting against disease. Generally speaking, to strengthen a protection hypothesis, markers of LZ should be measured prior to the onset or progression of a condition.

Summary and Hypotheses

LZ accumulate throughout bodily tissue and are generally thought to improve health and wellness. Individuals vary widely with respect to LZ status and tissue responses to dietary intake of foods rich in LZ and supplements. Variance in LZ status could be attributed to a number of variables, including dietary patterns, lipid profiles, and body composition. Adiposity, for example, has been shown to be related to serum and retinal LZ, possibly due to a competition

among tissues for uptake of LZ; however the effect appears to be different for men and women and data are inconsistent. By using a tissue marker of LZ - MP density, in addition to serum, the classic index – and assessing regional body fat percentage, it may be possible to gain a better understanding of the relationship between adiposity and LZ status in healthy individuals.

Another goal of the present study was to address whether individual variance in LZ status is related to skeletal mass, given that LZ are being marketed as a protective measure against both AMD and osteoporosis. Understanding this relationship in young, healthy individuals may support the use of LZ in protecting against both retinal and skeletal damage.

CHAPTER 2

METHOD

Subjects

A total of 63 subjects (Females, N = 39; Males, N = 24; average age of 22.5 years) were recruited from The University of Georgia and the surrounding Athens area. All subjects completed informed consent and an initial measurement of MP density at the Vision Sciences Laboratory. Within two to four weeks, subjects completed assessments of body fat percentage and aBMD at the UGA Bone Clinic and returned to the Vision Lab for a second assessment of MP density (an average over the two visits was used in all subsequent analyses). Additional assessments included a routine physical exam (for height and weight measures to assess body mass index; BMI, kg/m²) and a blood draw to determine serum LZ, at which time subjects completed questionnaires related to health habits.

Assessment of LZ Status: Macular Pigment & Serum LZ

Retinal LZ status was assessed noninvasively by measuring the optical density of the macular pigments using a well-validated psychophysical method (Wooten & Hammond, 1999). This technique, known as heterochromatic flicker photometry, involved measuring sensitivity to stimuli presented in free-view. A stimulus alternated in square-wave between a “blue” light maximally absorbed by MP (460nm) and a “green” light not absorbed by MP (540nm; see Figure 1). Given the differential absorbance of “blue” light compared to “green” light, there was discordance in the amount of energy that reached the photoreceptors. This difference in energy was perceived as a flicker. Subjects’ thresholds were obtained by having the subjects minimize

or eliminate the perception of flicker, a condition known as sensation luminance (Kaiser, 1988), by adjusting the radiance of the 460nm light (while the radiance of the 540nm light was held constant) until the energy of the two lights was perceptually the same.

A schematic of the final target viewed by the subject is illustrated in Figure 2. Sensation luminance was measured in the fovea by having the subject fixate the center of the target. HFP samples MP density at the edge of a test stimulus (the edge effect; Hammond, Wooten, & Snodderly, 1997), therefore a target 60 minutes (i.e., 60') of arc would yield threshold at 30' retinal eccentricity. Parafoveal measures of sensation luminance were obtained by having the subject fixate a light to the left of the target, thus placing the target at 420' eccentricity in the parafovea. The difference between the log energy necessary to achieve sensation luminance in the parafovea from the mean log energy necessary to achieve sensation luminance in the fovea was used to derive macular pigment optical density (MPOD). The spatial distribution of the targets used to measure MPOD across the retina in the right eye corresponded to measures of MPOD at 7.5', 30', 60', and 105' retinal eccentricity.

Assessment of serum LZ required collection of blood into 10 mL lithium heparin coated vacutainers (BD) by a licensed phlebotomist. Plasma was separated by centrifugation at 1500g for 20 minutes at 4°C and then distributed into light protected Eppendorf vials tubes for storage at -80°C. The analysis of the blood was done by the analytical laboratories of DSM Nutritional Products Ltd., Kaiseraugst, Switzerland. Serum LZ were quantified with a normal-phase HPLC system after extraction with a n-hexane/chlorophorm 20% (v/v) mixture.

Assessment of Bone Mineral Density and Body Fat Percentage

Body composition was assessed with dual-energy X-ray absorptiometry (DXA; Delphi A, Hologic Inc., Waltham, MA, USA). The system used an X-ray generator that produced beams at two different energy levels which passed through the subject and were measured by a detector on a scanning arm located over the subject. As each beam passed through the subject, the amount of X-ray attenuation at high or low energy levels was predicated on the chemical composition through which it passed (e.g., bone or soft tissue). The unattenuated and attenuated energy levels of the high and low X-ray beams were used to solve for the amount of bone and soft tissue. Precision of DXA measurements have been reported as 99%, with an accuracy report of less than 1% error (Lukaski, 1993).

Two scans were completed to yield assessments of site-specific areal bone mineral density (aBMD) for the proximal femur and lumbar spine. An additional whole-body scan was completed to assess for body fat percentage. Analysis of the whole-body scan provided total body fat percentage and body fat percentage by different regions (i.e., arms, trunks, and legs; see Figure 3). Additional information included fat mass and fat free soft tissue (FFST).

Assessment of Health Habits: Diet & Physical Activity

A brief dietary screener was constructed for the purpose of determining general consumption of fruits and vegetables and consumption of foods rich in calcium and vitamin D. The values for the general and specific section of the dietary screener reflected an average number of servings per week of fruits/vegetables (general section) and foods rich in calcium and vitamin D (specific section). The items for the specific section of the dietary screener were

selected based on the foods with the highest concentrations of calcium and vitamin D according to the US Food and Drug Administration.

Physical activity was quantified using a seven-day physical activity questionnaire that estimated total caloric expenditure. The screener (e.g., Blaire et al., 1985) assessed the amount of time spent engaging in sleep and mild, moderate, and heavy physical activities. Examples of the activities that were considered mild, moderate, or heavy were listed on the questionnaire and explained to each subject. The screener included a formula to calculate the amount of calories expended over a seven-day period based on the responses from the subject.

CHAPTER 3

RESULTS

Table 3 lists the descriptive data (means and standard deviations) for LZ status (serum and MP density), body composition (body fat percentage and aBMD), and health habit screeners (physical activity levels and diet). Pearson-product moment correlations were conducted to determine associations between variables relevant to LZ status, body fat percentage, and aBMD of the proximal femur and the lumbar spine. Statistical significance was set at $p < 0.05$.

Comparisons between male and female subjects were made with independent samples t-tests.

As shown in Table 3, average MPOD across the retina for the sample was 0.57 at 7.5' eccentricity, 0.46 at 30', 0.30 at 60', and 0.12 at 105'. Mean serum LZ levels were 0.25 $\mu\text{mol/L}$. Table 4 lists the Pearson product moment correlation coefficients and significance values for associations between measures of LZ status (e.g., serum LZ and MPOD) and fruit/vegetable intake. The relationship between serum LZ and MPOD at 7.5' retinal eccentricity is illustrated in Figure 4. Serum LZ significantly correlated with MPOD at 7.5' ($r = 0.27$, $p = 0.03$) and 105' ($r = 0.24$, $p = 0.05$) retinal eccentricity, however associations at 30' and 60' did not reach statistical significance. As shown in Table 4, higher MPOD was associated with greater fruit and vegetable intake (quantified as number of servings per week) for more central eccentricities (i.e., 7.5', 30', 60').

Male subjects had significantly lower serum LZ compared to females ($t = 2.39$, $p = 0.02$). Sex differences between MPOD across the retina and fruit/vegetable intake were not statistically significant (see Table 3).

The average total body fat percentage for the sample was approximately 25%. Total or regional body fat percentage was not related to serum LZ or MPOD across the retina ($p > 0.05$; see Table 5). Figure 5 illustrates the relationships between total body fat percentage and tissue LZ status, separated by sex. Male subjects had significantly lower (i.e., approximately 19% fat) total and regional (e.g., trunk, arms, legs) body fat percentage ($p < 0.01$) compared to female subjects (i.e., approximately 29% fat). The distribution of fat within the body for both males and females is illustrated in Figure 6. Other measures of body composition, such as fat free soft tissue and body mass index, were not significantly related to serum LZ or MPOD.

As shown in Table 3, the average aBMD for the proximal femur and lumbar spine was 1.00 g/cm^2 and 1.03 g/cm^2 , respectively. The relationship between LZ status and aBMD of the proximal femur (PF) and lumbar spine (LS) is illustrated in Figures 7 and 8. As listed in Table 6, PF and LS aBMD were positively related to MPOD at the following retinal eccentricities: 7.5' (PF: $r = 0.32$, $p = 0.02$; LS: $r = 0.29$, $p = 0.02$), 30' (PF: $r = 0.30$, $p = 0.03$; LS: $r = 0.26$, $p = 0.04$), and 105' (PF: $r = 0.43$, $p < 0.01$; spine: $r = 0.28$, $p = 0.03$). Associations between aBMD and MPOD at 60' eccentricity did not reach statistical significance (PF: $r = 0.24$, $p = 0.08$; LS: $r = 0.22$, $p = 0.09$). No significant association was found between serum LZ and aBMD of the PF ($p = 0.65$) or LS ($p = 0.42$).

On average, subjects expended approximately 2574 calories per week. Greater physical activity was associated with higher aBMD of the PF ($r = 0.53$, $p < 0.01$) but not the LS ($r = 0.16$, $p = 0.28$). Dietary intake (total number of servings per week) of foods rich in calcium (mean intake of approximately 19 servings) was associated with aBMD ($p = 0.02$), however fruit and vegetable intake (mean intake of approximately 7 servings per week) was not related to skeletal mass ($p = 0.41$). Body fat percentage did not account for variance in PF or LS aBMD ($p = 0.68$

and $p = 0.30$, respectively). Male and female subjects did not have significantly different PF aBMD ($p = 0.06$) or LS aBMD ($p = 0.80$).

CHAPTER 4

DISCUSSION

Dietary supplements containing LZ are being marketed as a way to increase general wellness and protect against conditions linked to oxidative stress. Indeed, a diet deficient in antioxidants is likely to be associated with an elevated risk of degenerative damage. However, when promoting LZ as protective nutrients, it is important to consider that tissue LZ response – and therefore effectiveness of LZ supplementation – varies among individuals. The purpose of this study was two-fold: to understand individual differences in LZ status by investigating the influence of adiposity on tissue LZ status, and to determine if LZ status was related to skeletal mass (a relation already assumed as evidenced by the recent marketing of LZ as a protection against both age-related macular degeneration and osteoporosis). In general, tissue markers of LZ in the retina and serum were not related to overall body fat percentage or regional distribution of body fat. A significant relationship was found between MP and aBMD, although the relationship between aBMD and serum LZ did not reach statistical significance.

In our sample, subjects with more serum LZ had significantly higher MP density at more central eccentricities (e.g., 7.5' eccentricity; see Figure 4). Significant associations were also found at 105' eccentricity, however serum LZ did not correlate with MPOD at 30' and 60' eccentricity. This could be attributed to individual variation in the distribution of MP across the retina (e.g., Hammond et al., 1997). Tissue LZ status in general is primarily driven by dietary habit.

Fruit and vegetable intake – quantified as the number of servings per day – was positively related to MP density, however no association was found between diet and serum LZ. This may be due to the fact that dietary assessments tend to reflect long-term habits and MP is likely to reflect life-long LZ intake, whereas serum measures of LZ reflect more short-term intake of LZ.

The observed relationships between LZ status and body composition in this study could be a reflection of the sample itself. The use of primarily young (e.g., mean age approximately 23 years) college students in good health (e.g., non-smokers, mean body mass index of approximately 23 kg/m²) reduced the likelihood that measures of tissue LZ and body composition reflected long-term consequences of oxidative damage that is often characteristic of elderly samples. However, the variability in LZ status was limited and may not be representative of the young adult population. For example, MPOD at 30' eccentricity ranged from 0.16-0.80, with an average of 0.46 ± 0.16 . This average is comparable to Stringham & Hammond's (2007) data, which consisted of a sample of young college students from the same Southeastern region, however subjects from that study had a range of MPOD from 0.08 to 1.04 log units at 30' retinal eccentricity. Hence, it is possible that the observed relationships in this study may have been reduced due to a restriction in range. A larger sample size that includes members from a community sample, spanning across a wider age range, may be necessary to have a wider range of LZ status representative of the general population.

In the second phase of the study we examined the relation between adiposity (including distribution) on tissue LZ. The average body fat percentage of the sample was approximately 25%, and it ranged from approximately 12% to 38%. Total and regional (e.g., trunk, arms, legs) body fat percentage was not significantly related to MPOD or serum LZ. The absence of an effect is consistent with Hammond et al.'s (2002) study, in which MP density was not related to

body fat percentage for those subjects with an average body fat of below 27%. The sample size in this study is substantially smaller ($N = 62$ compared to $N = 400$ in the Hammond et al. study), which may explain the absence of an effect despite the fact that 30 of our 62 subjects had over 27% body fat.

The effect of body fat percentage on MP in obese subjects was independent of sex in the Hammond et al. (2002) study. In our sample, of the 30 subjects with over 27% body fat, only four were men. Females had approximately 10% more total body fat than males and higher serum LZ ($p = 0.01$). Despite these differences, male and female subjects had equivalent MPOD (e.g., 0.47 and 0.46 log units at 30' eccentricity, respectively; $p = 0.68$). Although statistical significance for the entire sample was not found, the relationship between LZ status and body fat percentage may be different for men and women (see Figure 5). Sex differences have been reported in the past (see Table 1), but data are inconsistent. For example, Johnson et al. (2000) provided evidence for the support of a competition between the retina and adipose tissue for L in women. However, Nolan et al (2002) suggested that the influence of adiposity on retinal LZ was only apparent for men, since there was no significant relationship between body fat percentage and MP in their sample of women. The influence of adipose tissue on accumulation of LZ throughout body, and how it differs between men and women, is still unclear.

Chung et al. (2009) suggested that the distribution of body fat – particularly abdominal fat - may be the key to individual differences in tissue LZ status. In our sample, subjects accumulated the least amount of fat in the trunk region relative to arms and legs, and body fat percentage in the abdominal region was not related to LZ status for these young, healthy individuals. It may be that the distribution of body fat changes with age such that more fat accumulates in the abdomen for older subjects. This may also be the case for obese subjects.

Therefore, further study is warranted using individuals with a wider distribution of age and body fat percentage. Furthermore, differences in adiposity, either with respect to the total amount of adipose tissue, or the regional distribution of body fat, may result in differential tissue response to dietary supplementation with LZ. Results from a dietary intervention may demonstrate if regional body fat influences tissue response to LZ supplementation.

Individuals with higher areal bone mineral density (aBMD) of the proximal femur and lumbar spine had significantly higher MPOD. The relationship between serum LZ and skeletal mass, however, was not statistically significant. This may be explained by the fact that macular pigment and bone density tend to reflect life-long habits, whereas serum LZ reflects more short-term dietary intake. The relationship between LZ status and skeletal mass is based on the theory that LZ maintain the balance between antioxidants and oxidants in the body. However, the association between LZ status and other nutrients that may have a more direct impact on bone health must also be considered. LZ status may coincide with greater intake of nutrients that promote bone health, such as lycopene (e.g., Rao et al., 2007), beta-cryptoxanthin (e.g., Yamaguchi, 2008), and beta-carotene (e.g., Sahni et al., 2009). Indeed, Broekmans et al. (2002) reported significant positive associations between MP density and serum beta-cryptoxanthin and beta-carotene. As with LZ, however, data regarding associations of these nutrients and skeletal mass are inconsistent (e.g., Maggio et al., 2006; Wolf et al., 2005).

In general, moderate levels of LZ may serve to preserve bone health by preventing excessive oxidative stress that promotes bone loss. This may be a more indirect mechanism, unlike beta-cryptoxanthin, which has a direct influence on bone resorption and bone formation (e.g., Yamaguchi, 2008). If LZ simply prevent bone loss by lowering oxidative stress, then increasing LZ status through dietary intervention may not improve bone density in young,

healthy individuals. These subjects most likely have reached peak bone mass and have not had significant bone loss. However, it may be that LZ are particularly influential in younger individuals who are not yet experiencing the effects of hormonal changes and accumulated oxidative stress that are often associated with degenerative damage. It would be worthwhile to note if higher doses of LZ could have an effect beyond preventing bone loss and serve to increase aBMD, particularly if LZ supplements are to be recommended to patients already suffering from early stages of degenerative damage.

Sahni and colleagues (2009) were able to see an effect of LZ in the diet after following a sample of elderly subjects for four years, even though baseline associations between dietary LZ and bone density were not significant. Subjects with greater LZ in their diet not only had reduced bone loss compared to other subjects, their bone density was actually higher than their baseline measurement. Results from the elderly sample indicate that LZ may not only prevent bone loss, but may promote bone formation as well. In order to strengthen the argument of LZ as a protective measure, it is necessary to know if increasing LZ in the diet could influence bone density in a younger population, according to the concept of temporality as outlined by Mares-Perlman et al (2002). The goal for the follow-up intervention is to compare skeletal mass between a group of individuals who show tissue responses to supplementation with LZ and a group of subjects on a placebo.

One significant relation we found in this study was that higher MP density was related to higher aBMD. The relationship between skeletal mass and serum LZ, however, was not statistically significant. The current findings do not support a relationship between serum and retinal LZ and overall body fat percentage in young, healthy individuals. This may be due to the

limited variability with respect to both tissue LZ measures and adiposity (our inclusion criteria excluded subjects with very low and very high body fat percentage).

Our data (like Johnson et al., 2000) do not preclude the possibility that total body fat percentage and distribution of fat could explain individual differences in response to a LZ intervention. As originally suggested by Johnson et al. (2000), for example, it is possible that body fat percentage influences uptake of LZ differently in females, possibly due to hormonal influences. We are currently conducting a dietary intervention with LZ and measuring serum, retina, and fat distribution to assess this possibility. Subjects are supplementing their diets for 12 months with 10mg of lutein and 2mg of zeaxanthin, nearly fifteen times the amount of lutein and seventeen times the amount of zeaxanthin normally consumed in the diet (e.g., Johnson, Maras, Rasmussen, & Tucker, 2010).

One general interpretation of our data and results is simply that we confined our sample to young healthy subjects with very limited variability in diet, adiposity, and bone density: to wit, the group least likely to show effects. Often nutritional relations are driven by the extremes, those deficient in intake, those showing loss, etc. Our strategy, however, was purposeful. Market studies (e.g., Dietary Supplement Barometer Survey, 2005) indicate, for instance, that 85% of Americans take supplements and that these are most often individuals who are already healthy and tend to be affluent and young. We wanted to assess whether normal variation in body fat and bone density was related to LZ intake within the average range of most Americans. To extend this study, our plan is to now go outside of this normal range and assess individuals with very high and low body fat percentage and greater variation in bone density, dietary intake, etc.

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Table 1: Risk factors common to both age-related macular degeneration and osteoporosis

Factor	Description of Risk	Age-Related Macular Degeneration	Osteoporosis
Age	Risk increases with age	Klein et al., 1992; The Eye Diseases Prevalence Research Group, 2004.	van der Voort et al., 2001
Males vs. Females	Females at higher risk compared to males	Vingerling et al., 1995	van der Voort et al., 2001
Smoking (<i>tobacco cigarettes</i>)	Risk increases for smokers	Delcourt et al., 1998; Age-Related Eye Disease Study Research Group, 2005; Klein et al., 2002.	Wong, 2007
Adiposity (<i>measured as body mass index or body fat percentage</i>)	Risk increases with greater adiposity	Schaumberg et al., 2001; Age-Related Eye Disease Study Research Group, 2005; Seddon et al., 2003	van der Voort et al., 2001
Dietary Intake of Carotenoids	Risk increases if diet is deficient of carotenoids	Seddon et al., 1994; Snodderly et al., 1995; Age-Related Eye Disease Study Research Group, 2007.	Sahni et al., 2009; Wattanapenpaiboon et al., 2003.

Table 2. Empirical evidence for the association between tissue lutein and zeaxanthin and body fat percentage (data reported as mean \pm standard deviation).

	Johnson et al. (2000)		Broeksman et al. (2002)		Hammond et al. (2002)		Nolan et al. (2004)	
	Males	Females	Males	Females	Males	Females	Males	Females
N	8	13	177	199	400		45	55
Age	62 \pm 5	63 \pm 4	42 \pm 15	41 \pm 13	29.6 \pm 13.1 ¹		42.78 \pm 11.67	
BMI	25.4 \pm 2.1	26.1 \pm 1.3	24.5 \pm 3.5	25.0 \pm 4.9	NR ²		26.54 \pm 2.9	24.71 \pm 3.70
Body Fat %	28 \pm 4	40 \pm 4	NR		15.6	23.6	23.11 \pm 5.9	36.18 \pm 6.09
Tissue LZ Status & Significant Associations								
MPOD	0.41 \pm 0.004 ⁵	0.48 \pm 0.004 ⁶	0.35 \pm 0.15 ⁵	0.31 \pm 0.14	Fat > 27% = 0.22 \pm 0.13 ⁴ Fat < 27 % = 0.26 \pm 0.13		0.33 \pm 0.19 ⁴	0.31 \pm 0.23
Serum L	[0.35 \pm 0.05 0.32 \pm 0.04] ⁵		0.16 \pm 0.07 ⁵	0.19 \pm 0.08 ⁵	NR ³		0.09 \pm 0.04	0.10 \pm 0.05 ⁴
Adipose L	0.09 \pm 0.03	0.36 \pm 0.10	0.32 \pm 0.17	0.47 \pm 0.31	NR		NR	

¹ Age reported as average from sample of 682 subjects, 400 of which completed assessments of body fat percentage

² Average BMI values not reported for subset of sample with assessments of body fat percentage

³ Serum data was not collected for those subjects who completed assessments of body fat percentage

⁴ Significant negative association with body fat percentage, $p < 0.05$

⁵ Significant positive association with adipose L, $p < 0.05$

⁶ Significant negative association with adipose L, $p < 0.05$

Table 3. Descriptive data (mean \pm standard deviation) for all subjects and stratified by sex.

	N = 63	Males (N = 24)	Females (N = 39)
Macular Pigment			
MPOD 7.5'	0.57 \pm 0.20	0.59 \pm 0.20	0.57 \pm 0.21
MPOD 30'	0.46 \pm 0.16	0.47 \pm 0.15	0.46 \pm 0.17
MPOD 60'	0.30 \pm 0.13	0.30 \pm 0.11	0.30 \pm 0.14
MPOD 105'	0.12 \pm 0.08	0.13 \pm 0.08	0.12 \pm 0.08
Serum ($\mu\text{mol/L}$)			
LZ	0.25 \pm 0.12	0.21 \pm 0.07	0.28 \pm 0.13
Lutein (L)	0.19 \pm 0.09	0.14 \pm 0.06	0.21 \pm 0.10
Zeaxanthin (Z)	0.07 \pm 0.03	0.06 \pm 0.02	0.07 \pm 0.03
Body Fat (percentage)			
Total	24.92 \pm 6.64	18.70 \pm 4.91	28.75 \pm 4.23
Arms	25.25 \pm 8.54	16.70 \pm 4.85	30.52 \pm 5.48
Trunk	21.60 \pm 6.23	17.24 \pm 5.88	24.28 \pm 4.80
Legs	29.87 \pm 8.62	20.85 \pm 5.08	35.42 \pm 4.76
Bone Mineral Density (g/cm^2)			
Proximal Femur	1.00 \pm 0.13	1.04 \pm 0.15	0.97 \pm 0.10
Lumbar Spine	1.03 \pm 0.12	1.03 \pm 0.13	1.04 \pm 0.11
Health Habit Screeners			
Calories Expended	2574 \pm 538	2818 \pm 486	2440 \pm 525
F/V Intake (serving/wk)	6.87 \pm 2.28	6.44 \pm 2.30	7.12 \pm 2.27
Calc/Vit D Intake (serving/wk)	18.95 \pm 6.39	19.88 \pm 8.30	18.42 \pm 5.09
Age (years)	22.52 \pm 3.71	21.71 \pm 4.09	23.03 \pm 3.41
Body Mass Index (kg/m^2)	22.87 \pm 2.59	23.47 \pm 2.33	22.51 \pm 2.70

Table 4. Pearson-product moment correlation coefficients and significance values (r | p) for associations between LZ status and dietary intake of fruits (F) and vegetables (V).

		Macular Pigment				Serum			
		MPOD 7.5'	MPOD 30'	MPOD 60'	MPOD 105'	LZ	Lutein	Zeaxanthin	
Macular Pigment	MPOD 7.5'	1.00 0.00	0.95 0.00	0.75 0.00	0.65 0.00	0.27 0.03	0.26 0.04	0.25 0.05	
	MPOD 30'	0.95 0.00	1.00 0.00	0.84 0.00	0.71 0.00	0.22 0.09	0.22 0.09	0.18 0.15	
	MPOD 60'	0.75 0.00	0.84 0.00	1.00 0.00	0.84 0.00	0.19 0.13	0.21 0.09	0.10 0.42	
	MPOD 105'	0.65 0.00	0.71 0.00	0.84 0.00	1.00 0.00	0.24 0.05	0.24 0.06	0.23 0.07	
Serum (mol/L)	LZ	0.27 0.03	0.22 0.09	0.19 0.13	0.24 0.05	1.00 0.00	0.99 0.00	0.90 0.00	
	Lutein	0.26 0.04	0.22 0.09	0.21 0.09	0.24 0.06	0.99 0.00	1.00 0.00	0.83 0.00	
	Zeaxanthin	0.25 0.05	0.18 0.15	0.10 0.42	0.23 0.07	0.90 0.00	0.83 0.00	1.00 0.00	
F/V Intake (servings/week)		0.30 0.04	0.30 0.04	0.29 0.05	0.22 0.15	0.12 0.44	0.10 0.50	0.15 0.33	

Table 5. Pearson-product moment correlation coefficients and significance values (r | p) for associations between bone mineral density, LZ status, body fat, and health habits.

		Bone Mineral Density (g/cm²)	
		Proximal Femur	Lumbar Spine
Macular Pigment	MPOD 7.5'	0.32 0.02	0.29 0.02
	MPOD 30'	0.30 0.03	0.26 0.04
	MPOD 60'	0.24 0.08	0.22 0.09
	MPOD 105'	0.43 0.00	0.28 0.03
Serum (mol/L)	LZ	0.06 0.65	0.10 0.42
	Lutein (L)	0.02 0.88	0.09 0.50
	Zeaxanthin (Z)	0.18 0.19	0.14 0.27
Total Body Fat Percentage		-0.06 0.68	0.13 0.30
Calories Expended		0.53 0.00	0.16 0.28
F/V Intake (serving/wk)		0.13 0.41	0.12 0.41
Calc/Vit D Intake (serving/wk)		0.38 0.02	0.34 0.02

Table 6. Pearson-product moment correlation coefficients and significance values (r | p) for associations between body fat percentage and LZ status.

		Body Fat Percentage			
		Total	Arms	Trunk	Legs
Macular Pigment	MPOD 7.5'	-0.12 0.33	-0.12 0.33	-0.11 0.40	-0.11 0.38
	MPOD 30'	-0.11 0.40	-0.08 0.55	-0.11 0.40	-0.09 0.47
	MPOD 60'	-0.13 0.31	-0.10 0.46	-0.14 0.26	-0.10 0.41
	MPOD 105'	-0.15 0.25	-0.15 0.23	-0.14 0.27	-0.13 0.31
Serum ($\mu\text{mol/L}$)	LZ	0.15 0.25	0.10 0.44	0.15 0.25	0.15 0.25
	Lutein (L)	0.17 0.19	0.13 0.30	0.15 0.24	0.17 0.17
	Zeaxanthin (Z)	0.06 0.62	-0.03 0.84	0.11 0.39	0.04 0.77

Figure 1. Wavelengths of HFP targets relative to the absorbance spectrum of macular pigment.

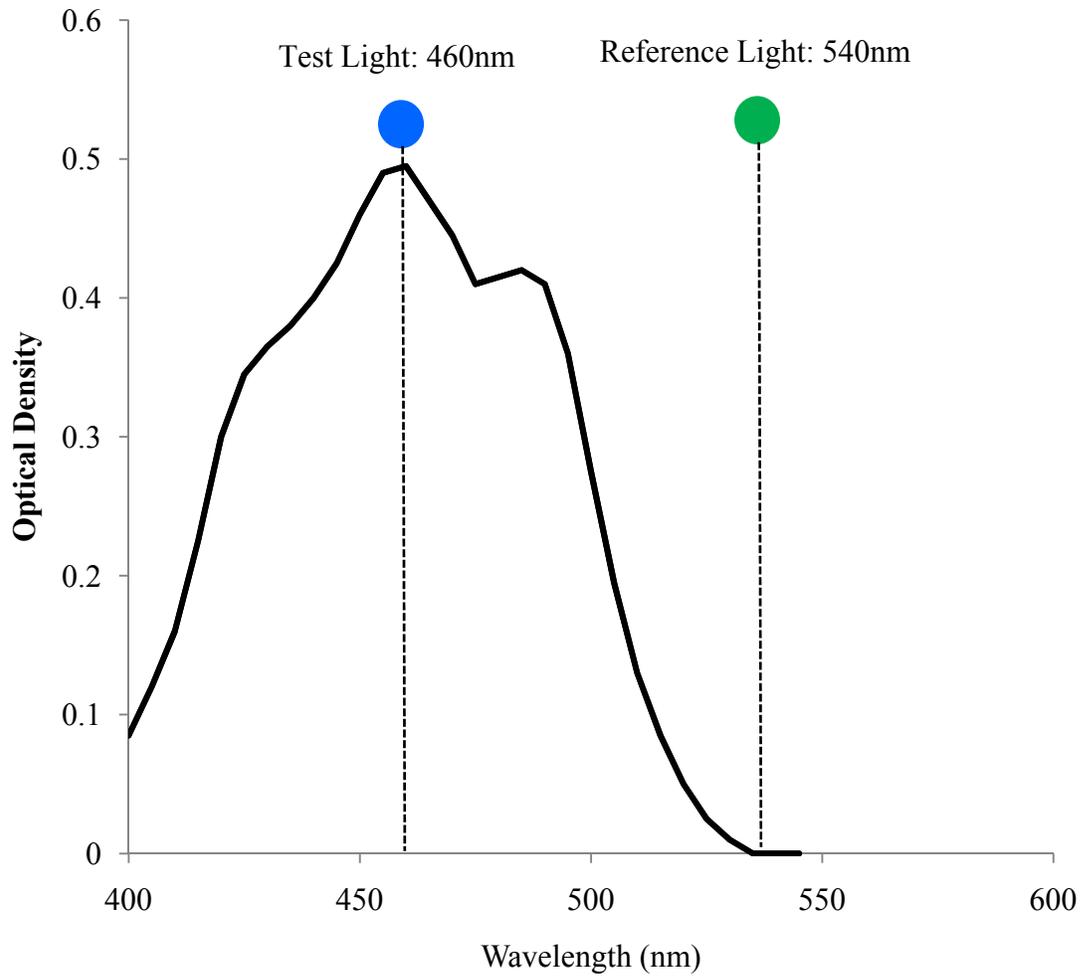


Figure 2. Schematic of test stimulus seen by subject in HFP.

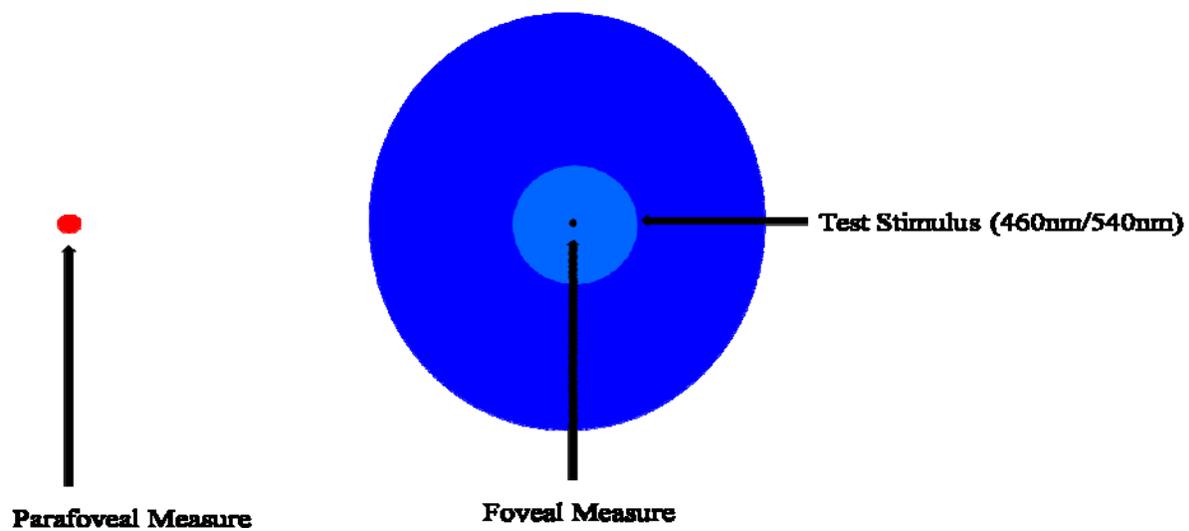


Figure 3. Example full-body DXA scan illustrating regional distribution used for body fat percentage.

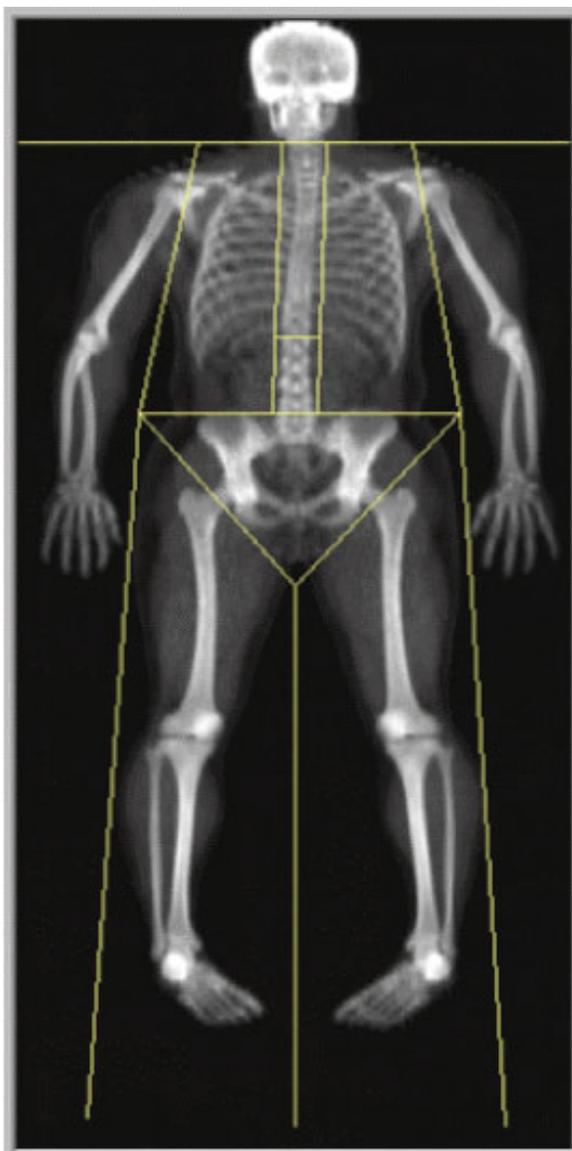


Figure 4. The relationship between serum lutein/zeaxanthin (LZ) and macular pigment optical density (MPOD) at 7.5 minutes (7.5') retinal eccentricity.

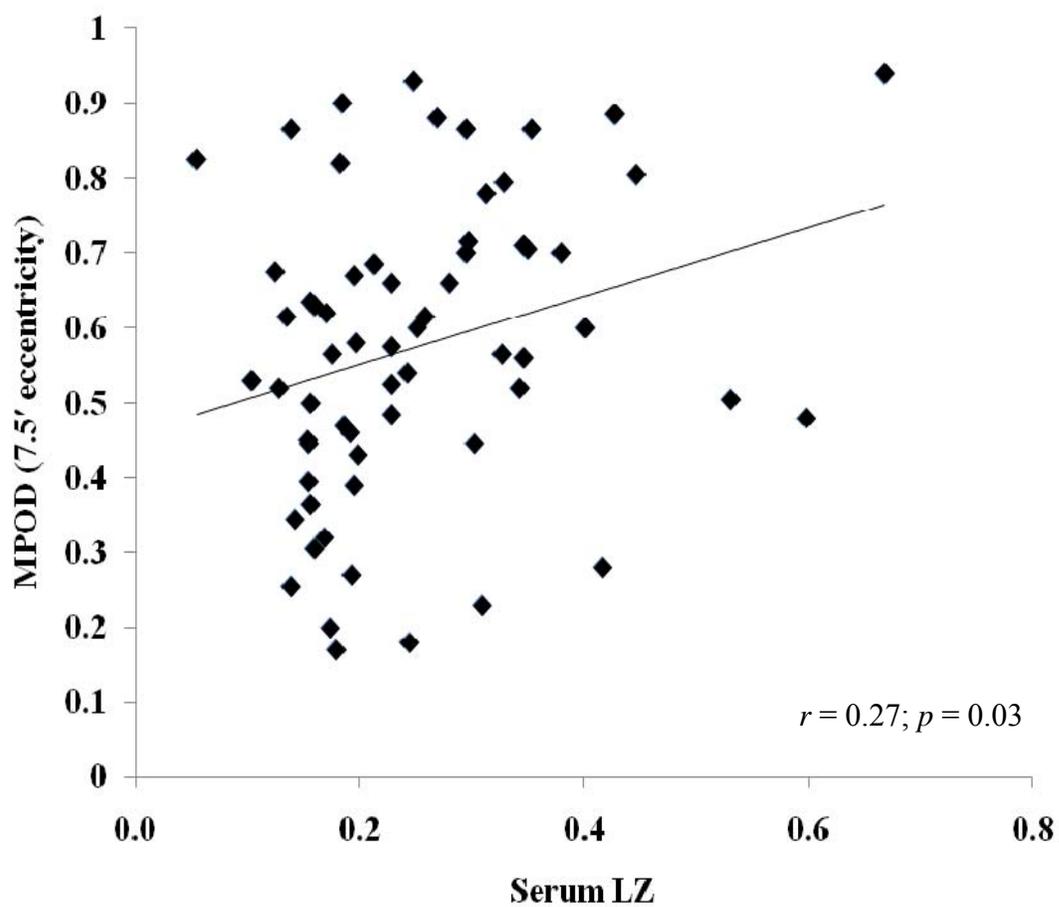
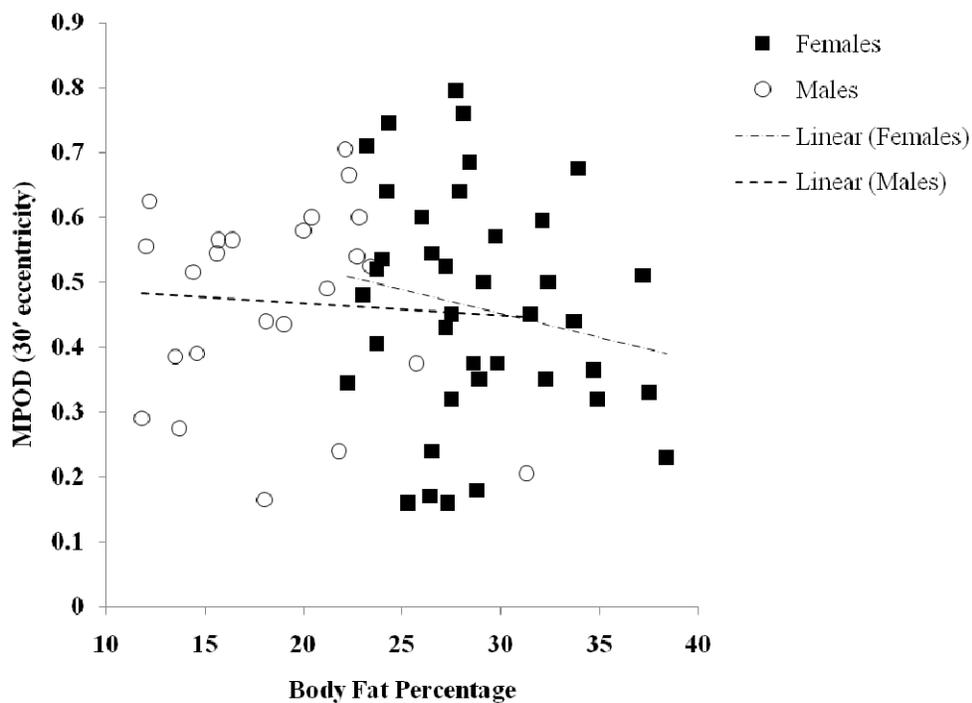


Figure 5. The relationship between body fat percentage and (a) macular pigment optical density (MPOD) at 30 minutes (30') retinal eccentricity (b) and serum lutein/zeaxanthin for male and female subjects.

(a)



(b)

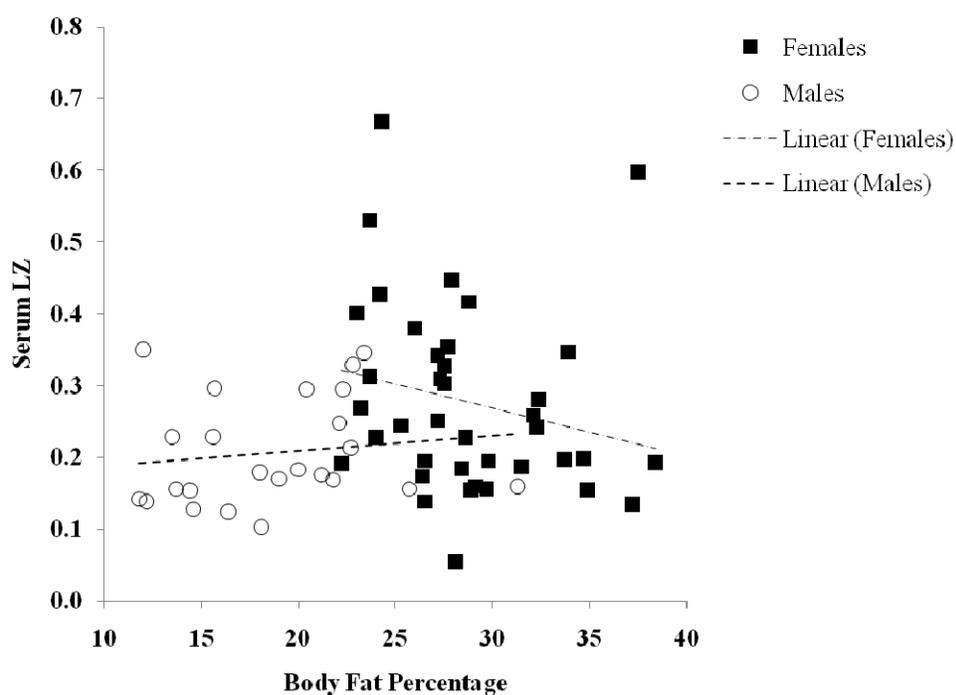


Figure 6. Mean (\pm standard deviation) body fat percentage for the total body and regional distribution for male and female subjects.

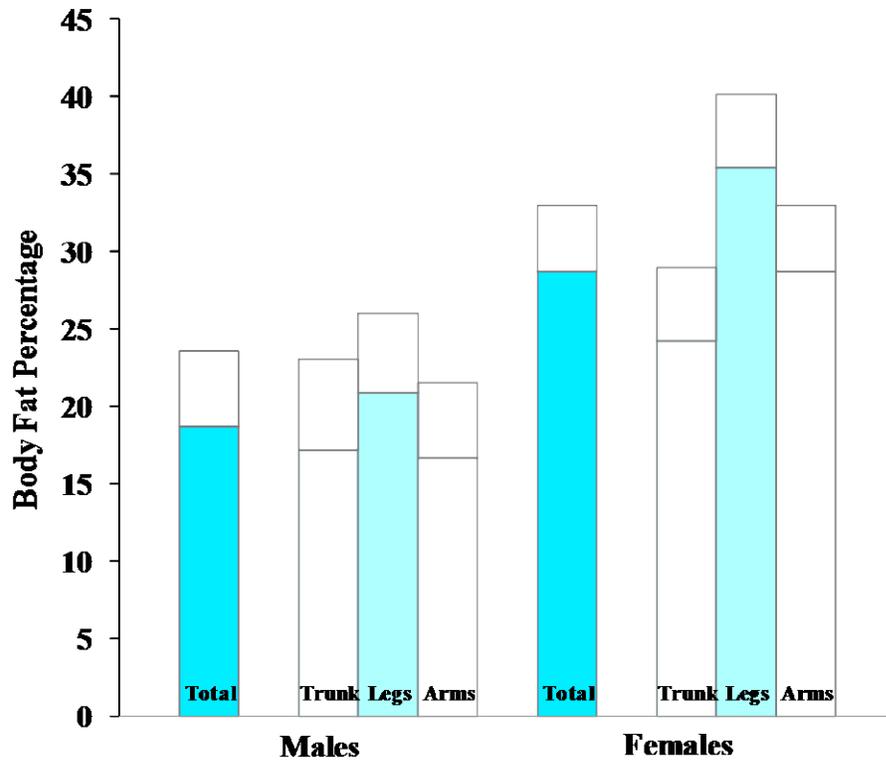
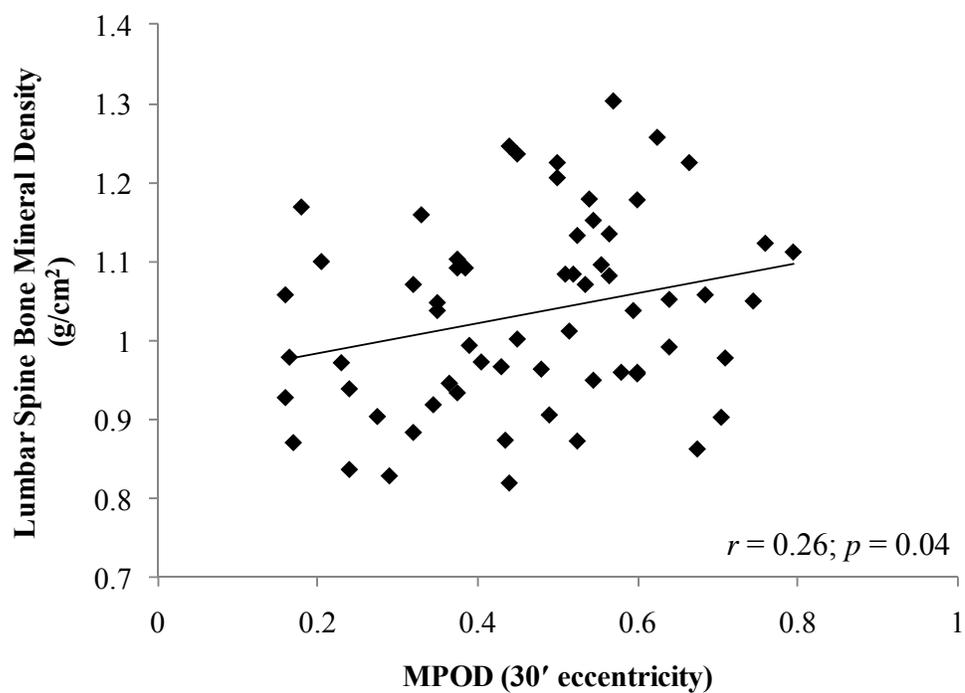


Figure 7. The relationship between skeletal mass of the lumbar spine and macular pigment optical density (MPOD) at 30' retinal eccentricity (a) and serum lutein/zeaxanthin (b).

(a)



b)

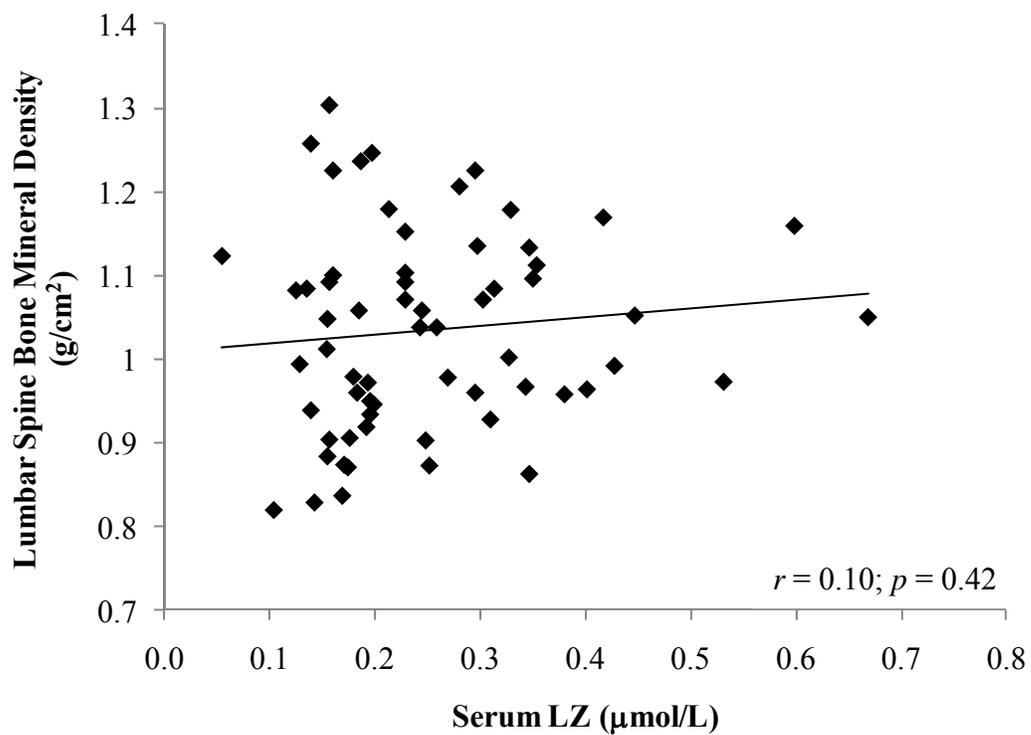
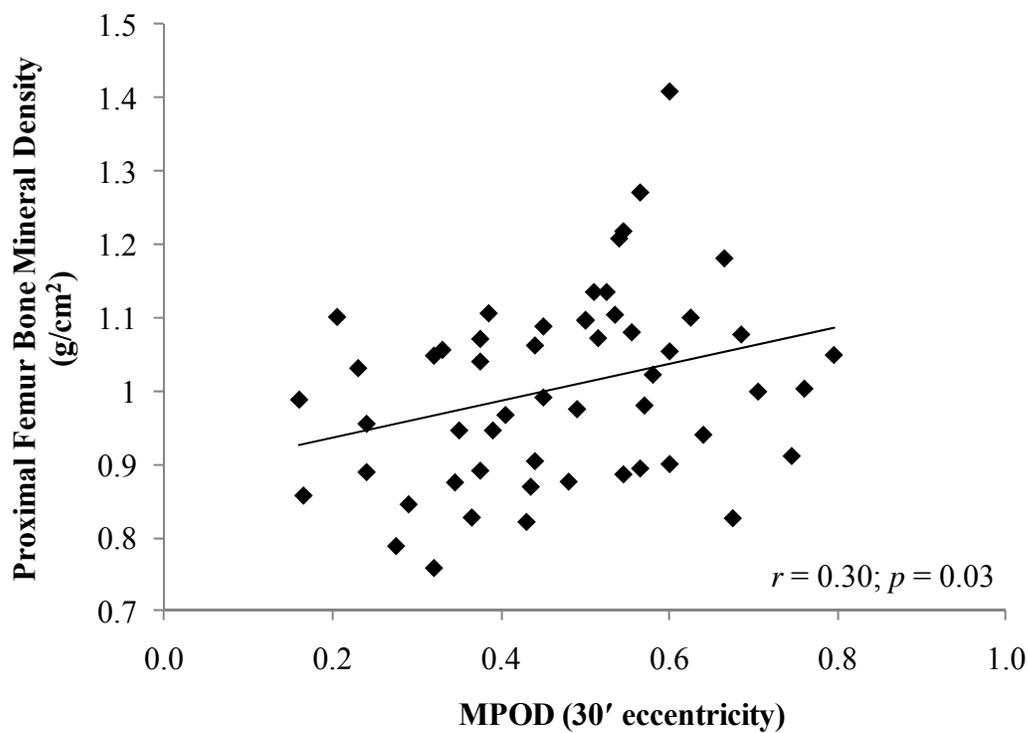


Figure 8. The relationship between skeletal mass of the proximal femur and macular pigment optical density (MPOD) at 30' retinal eccentricity (a) and serum lutein/zeaxanthin (b).

a)



b)

