ELSEVIER

Muscle uptake of vitamin E and its association with muscle fiber type

Mohsen Meydani, Roger A. Fielding,* Joseph G. Cannon,† Jeffrey B. Blumberg, and William J. Evans†

*Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA USA; *Department of Health Sciences, Sargent College of Allied Health Professions, Boston University, Boston, MA USA; and †Noll Physiological Research Center, Pennsylvania State University, University Park, PA USA*

The effect of 800 IU vitamin E (dl-α-tocopheryl acetate) supplementation for 30 days on α- and γ-tocopherol concentrations in gastrocnemius muscle and the relation between vitamin E concentrations and the distribution of Type I and II fibers was investigated in nine healthy adults (21 to 44 years). The plasma concentration of α-tocopherol increased by 300% (65.2 ± 7.7 versus 21.8 ± 2.3 μmol/L) and γ-tocopherol decreased by 74% (1.3 ± 0.1 vs. 5.2 ± 0.6 μmol/L) within 15 days of supplementation and was maintained at this plateau with continuous supplementation. Muscle biopsies taken before and after supplementation showed a significant 53% increase of α-tocopherol (57.3 ± 12.1 versus 37.6 ± 7.0 nmol/g) and a 37% decrease of γ-tocopherol (7.8 ± 1.1 versus 12.5 ± 1.1 nmol/g). There was a significant correlation between plasma and muscle concentrations of α- and γ-tocopherol (r = 0.71, P = 0.001 and r = 0.59, P = 0.009 respectively). Muscle α-tocopherol, but not γ-tocopherol, was inversely correlated with body mass index (r = −0.69, P = 0.008). The percentage of Type I fibers was inversely correlated with plasma concentrations of α-tocopherol (r = −0.69, P < 0.05), but this correlation with muscle concentrations of α-tocopherol was weak. It appears that individuals with a higher percentage of Type I fibers may utilize more α-tocopherol, perhaps to prevent oxidative damage from contractile activity. (J. Nutr. Biochem. 8:74–78, 1997.) © *Elsevier Science Inc. 1997*

Keywords: vitamin E; plasma; gastrocnemius muscle; type I fiber; humans

Introduction

Human skeletal muscle consists of two main fibers, Type I and Type II fibers, which are further broken down into subtypes. Type I fibers (slow-twitch) are rich in myoglobin and mitochondria enzymes, whereas Type II fibers (fast twitch) have a well-developed glycolytic capacity.[1–3] The percentage of distribution of fiber types varies among muscle groups and between individuals, especially in men.[4] Distribution of muscle fiber types does not appear to be greatly affected by physical training; however, it has been suggested that specific training or perhaps inactivity may alter the biochemical and/or physiologic properties of the fiber enough to transform one type into another.[4,5]

Differential glycolytic enzyme activity in muscle fibers provides a basis for distinguishing between the two types of fibers. However, there is an overlapping spectrum of enzyme activity for terminal substrate oxidation for Type I and II fibers. The adenosine triphosphatase (ATPase) activity of muscle fibers provides a biochemical link between the specific histochemical and functional properties to the myosin content of fiber types[6]; based on the differential pH sensitivity of myofibrillar ATPase activity, fiber types can be identified histochemically.[7,8] Type I fibers replenish their phosphocreatine more efficiently through oxidative phosphorylation than Type II fibers.[9] Furthermore, relative to Type II fibers, Type I fibers contain three times more triglyceride[10] and higher catalase activity.[11] The higher catalase activity in Type I fibers is likely necessary due to oxidative metabolism in the large number of mitochondria

Address reprint requests to Dr. Mohsen Meydani at Vascular Biology Laboratory, 711 Washington Street, Boston, MA 02111 USA.
This project has been funded at least in part with Federal funds from the United States Department of Agriculture, Agricultural Research Service under contract number 53-K06-01. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.

655 Avenue of the Americas, New York, NY 10010

0955-2863/97/$17.00
PII S0955-2863(96)00176-3

and the associated production of peroxides.[12,13] Therefore, the metabolism of fat-soluble antioxidant nutrients such as vitamin E may substantially affect and be affected by the different metabolic patterns present in Type I and II fibers. The present study was undertaken to investigate the effect of vitamin E supplementation on the increase of vitamin E in muscle and its association with muscle fiber distribution in human gastrocnemius muscle.

Methods and materials

Subjects

Nine healthy volunteers between 21 and 44 years of age (x: 29.7 years) participated in this study. Subjects' weight ranged from 53.3 to 95.2 kg (x: 74.2 kg) with body mass index (BMI) (kg/m^2) between 19.8 and 27.8 (x: 23.4). Participants were free-living and were not taking vitamin supplements. The subjects supplemented their usual diet with 800 IU *dl*-α-tocopheryl acetate (vitamin E) taken as two capsules (400 IU in soybean oil) daily (gift from Hoffmann-La Roche, Nutley, NJ USA) for 30 days. Blood samples were obtained before and after 2, 15, and 30 days of vitamin E supplementation. Heparinized blood samples were collected from the antecubital vein of fasted (14 hr) subjects. Percutaneous needle biopsies were obtained from the lateral head of the gastrocnemius muscle[14] before and after 30 days of vitamin E supplementation. This study was reviewed and approved by the Tufts University/ New England Medical Center Human Investigation Review Committee, and informed consent was obtained from each subject.

Tissue preparation

Plasma was separated from red blood cells by centrifugation at 400 g for 20 min at 4°C, and aliquots were stored under nitrogen at −70°C. To obtain muscle needle biopsies, the skin was anesthetized with 1% lidocaine (Xylocaine), and a 2-cm incision was made in the skin and overlaying fascia. The muscle samples were separated from adipose tissue, divided in half, and weighed. One sample was immediately frozen in liquid nitrogen and stored at −120°C (Cryomed, New Baltimore, MI USA) until analysis. The other sample was placed in mounting medium, frozen in isopentane, and cooled to the temperature of liquid nitrogen for histochemistry. Samples collected at different time points from each subject were analyzed together for tocopherol content and muscle fiber types to eliminate interassay variation.

Analytical measurements

Vitamin E concentration in plasma was measured by a modified high-performance liquid chromatography (HPLC) as described by Meydani et al.[15] Tocol (gift from Hoffmann La-Roche) was used as an internal standard, and eluted peaks were detected with Perkin-Elmer 650-15 fluorescence detector (Perkin-Elmer, Norwalk, CT USA) set at 292 nM excitation and 330 nM emission. Concentrations of α- and γ-tocopherol were measured in muscle biopsy samples after saponification. Briefly, muscle tissue (10 to 30 mg) was homogenized at 4°C using a glass mortar and pestel homogenizer. After saponification with 60% KOH in the presence of pyrogallol, tocol was added as an internal standard and extracted with hexane for tocopherol analysis by HPLC as described.

Fiber type determination

Transverse sections (8 μm) were obtained from the muscle sample prepared for histochemistry and stained for myofibrillar ATPase activity at a preincubation pH of 4.3 to determine Type I and Type II fiber populations.[16] An average of 450 fibers per biopsy were counted under a microscope for determination of muscle fiber type distribution.

Statistical analysis

Univariate analysis of variance was performed for the significance of changes in plasma tocopherols, followed by paired Student's *t*-tests with Bonferoni correction for multiple comparisons. Paired Student's *t*-test was also used to determine significant differences in muscle concentrations of tocopherols and fiber types measured before and after vitamin E supplementation. Pearson correlation was used to determine associations between concentrations of tocopherols in plasma and muscle with BMI and muscle fiber types.

Results

After vitamin E supplementation, plasma concentration of α-tocopherol increased (*Figure 1A*) and γ-tocopherol decreased (*Figure 1B*). Two days after vitamin E supplementation, plasma levels of α-tocopherol increased by more than 100% (45.5 ± 3.3 versus 21.8 ± 2.3 μmol/L, $P < 0.001$). After 2 weeks, the concentration of plasma α-tocopherol increased by more than 200% (65.2 ± 3.3 μmol/L, $P < 0.01$) compared to baseline (*Figure 1A*), but the increase was not significantly different from levels recorded on day 2. Two more weeks of vitamin E supplementation did not further increase plasma levels of α-tocopherol (*Figure 1A*); however, the level remained higher ($P < 0.001$) than baseline. Plasma γ-tocopherol concentrations decreased by 41% (3.1 ± 0.5 versus 5.2 ± 0.6 μmol/L) after 2 days of vitamin E supplementation ($P < 0.01$), and after 2 weeks was 74% lower than baseline level (1.3 ± 0.3 μmol/L, $P < 0.01$) (*Figure 1B*). Plasma γ-tocopherol did not decrease further with 2 more weeks of vitamin E supplementation. Relative to day 2, concentrations of γ-tocopherol were lower at day 15 ($P < 0.001$) and at day 30 ($P < 0.01$).

After 30 days of vitamin E supplementation, the concentration of α-tocopherol in gastrocnemius muscle increased by 53% (57.3 ± 12.1 versus 37.6 ± 7.0 nmol/g, $P < 0.0001$),

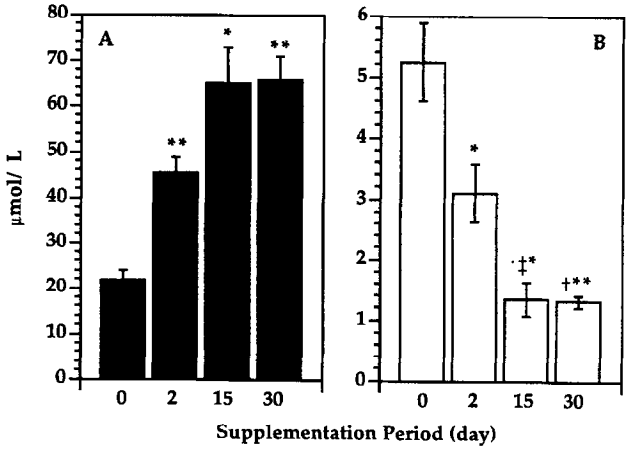

Figure 1 Effect of vitamin E supplementation (800 IU/d) on plasma α- and γ-tocopherol concentrations. A: α-tocopherol; B: γ-tocopherol. Data are mean ± SEM. $n = 9$. Significantly different from before supplementation at $^*P < 0.01$, $^{**}P < 0.001$. significantly different from day 2 at $\dagger P < 0.05$, $\ddagger P < 0.001$.

and γ-tocopherol decreased by 37% (12.0 ± 1.0 vs. 7.5 ± 1.1 nmol/g, $P < 0.0001$) (*Figure 2*), compared with baseline values. Muscle α- and γ-tocopherol concentrations were correlated with the plasma levels of these tocopherols (α-tocopherol: $r = 0.71$, $P = 0.001$; γ-tocopherol: $r = 0.59$, $P = 0.009$; *Figure 3*).

Muscle α-tocopherol concentrations were also inversely correlated with BMI, both at baseline ($r = -0.69$, $P = 0.04$) and after vitamin E supplementation ($r = -0.72$, $P = 0.03$). In contrast to α-tocopherol, muscle γ-tocopherol showed no significant relation to BMI.

Despite the high inverse correlation between muscle α-tocopherol and BMI, the correlation between plasma α-tocopherol and BMI ($r = -0.54$ at baseline, $r = -0.42$ after vitamin E supplementation) was not statistically significant. However, plasma γ-tocopherol was positively correlated with BMI at baseline ($r = 0.67$, $P = 0.05$), but this association diminished after vitamin E supplementation.

The distribution of Type I and II fibers in gastrocnemius muscle was unchanged by vitamin E supplementation (*Table 1*). The average percent distribution of Type I and II fibers among all biopsy samples obtained before and after vitamin E supplementation was 66.3 ± 8.0% and 33.7 ± 8.0%, respectively. There was an inverse relationship between the percent of Type I fibers and concentrations of α-tocopherol in plasma ($r = -0.69$, $P < 0.05$) before vitamin E supplementation (*Figure 4A*); for example, subjects with an initially higher level of plasma α-tocopherol had a lower percentage of Type I fibers in the gastrocnemius muscle. The relationship between muscle α-tocopherol and Type I fiber from pre-supplementation biopsy samples was weak ($r = -0.38$, $P < 0.3$; *Figure 4B*). No significant association was found between γ-tocopherol levels in plasma and muscle and muscle fiber type distribution (data not shown).

Discussion

Vitamin E supplementation was found to increase the concentrations of plasma and muscle α-tocopherol in healthy adults. The magnitude of an immediate increase in plasma

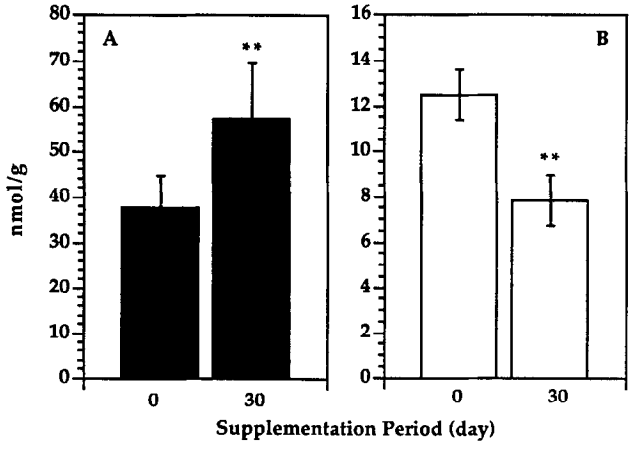

Figure 2 Effect of vitamin E supplementation (800 IU/d) in gastrocnemius muscle α- and γ- tocopherol. A: α-tocopherol; B: γ-tocopherol. Data are mean ± SEM. $n = 9$. Significantly different from before supplementation at $^{**}P < 0.0001$.

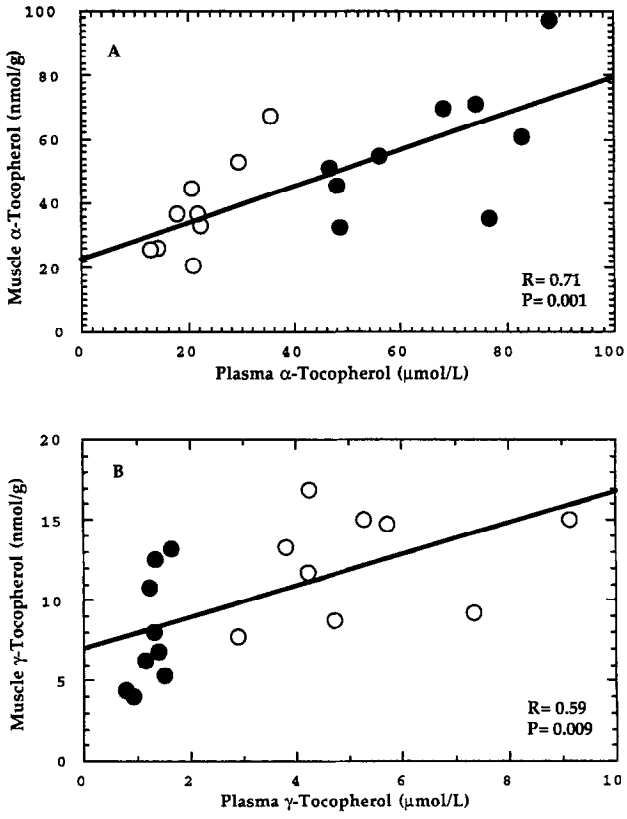

Figure 3 Correlation between plasma and muscle tocopherols. A: α-tocopherol; B: γ-tocopherol.

α-tocopherol concentration is more dependent on the dose than the duration of supplementation with vitamin E.[17] For example, after an early rapid increase, any further significant rise in plasma concentrations up to the saturation level may require a longer period with continued supplementation, depending on the dosage. Supplementation with 800 IU vitamin E increased the plasma levels of α-tocopherol close to the saturation level within 2 weeks, after which plasma levels remained relatively constant with continuous supplementation up to 30 days (*Figure 1*). However, long-term supplementation with the same dose can increase levels in adipose and other tissues where vitamin E accumulates with lipids.[18,19] Thus, tissue α-tocopherol concentration can vary significantly depending on the lipid content, metabolic turnover, and oxidative activity of tissue. Despite

Table 1 Distribution of Type I and II fibers in gastrocnemius muscle before and after vitamin E supplementation

	Type I		Type II	
	No. of fibers	%	No. of fibers	%
Before vitamin E supplementation	311 ± 71	65.6 ± 8.6	134 ± 30	34.4 ± 8.6
After vitamin E supplementation	333 ± 73	64.1 ± 6.9	126 ± 25	32.5 ± 7.7

Values expressed as mean ± SD. n = 9.

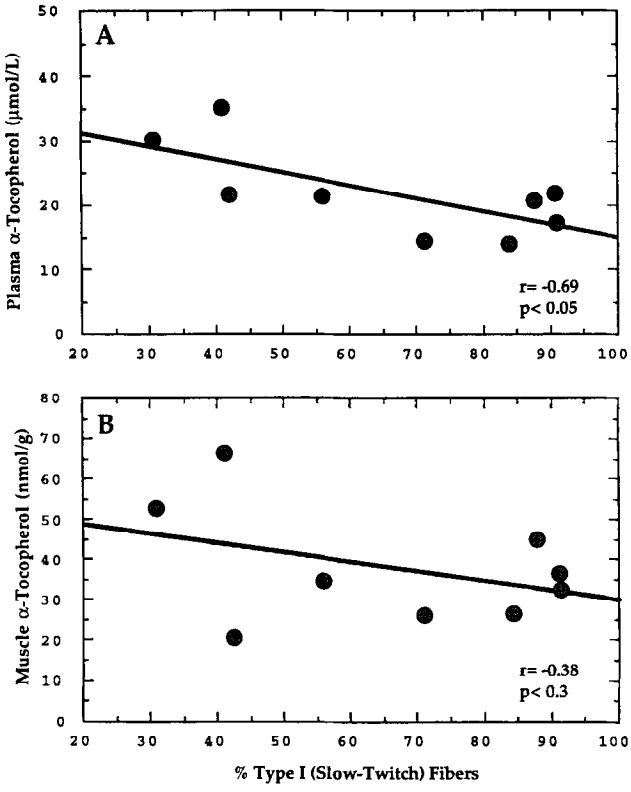

Figure 4 Relationship of presupplemental concentrations of α-tocopherol in plasma (panel A) and muscle (panel B) with Type I myofibrils of gastrocnemius muscle.

the low lipid content of muscle tissue relative to adipose tissue, concentration of α-tocopherol can be significantly increased with high intakes of vitamin E within 7 weeks.[20] The magnitude of the increase of vitamin E in the muscle may be attributed to the tissue's high oxidative metabolic activity, lipoprotein lipase, and low-density lipoprotein receptor activities, as well as to the non-specific exchange with plasma vitamin E.[10,21–23]

Concentration of α-tocopherol in the skeletal muscle has been reported to be about 32 to 37 nmol/g wet tissue.[19,20] Meydani et al.[20] reported that the concentration of α-tocopherol in the vastus lateralis muscle of young subjects supplemented with 800 IU/d vitamin E for 7 weeks was 44.6 ± 5.3 nmol/g muscle, comparable to the values obtained in this 4-week study. Even though muscle α-tocopherol concentration was not measured beyond 30 days of supplementation, 57.3 ± 12.1 nmol α-tocopherol/g of muscle likely approximates the maximum concentration that can be achieved by 800 IU vitamin E supplementation.

Despite the difference in the magnitude of the increase of vitamin E levels in plasma and muscle after supplementation, muscle α-tocopherol concentrations were strongly correlated with plasma concentrations. Thus, a close metabolic association between plasma and muscle vitamin E appears to exist.

There was a significant inverse correlation between muscle α-tocopherol concentrations and BMI in this study, which was not only evident at baseline, but also apparent after 30 days of supplementation with vitamin E. The findings of this study support the theory that in individuals with high BMI and thus, potentially high adiposity, low muscle lipoprotein lipase activity may limit lipid oxidation and favor deposition in adipose tissue[23] along with such fat-soluble vitamins as vitamin E. Our results indicate that in subjects with a higher BMI, α-tocopherol did not wholly accumulate in muscle tissue, but was most likely deposited in adipose tissue.

Dietary α-tocopherol supplementation decreased the concentrations of γ-tocopherol in plasma as well as in muscle. This inverse association of γ- with α-tocopherol in plasma and other tissues has been previously reported.[20,24] In contrast to the differential increase between plasma and muscle α-tocopherol, the decrease of γ-tocopherol concentrations in muscle was similar to that observed in plasma (37% and 41%, respectively). The presence of a specific high-affinity binding protein for α-tocopherol and its discrimination between α-tocopherol and γ-tocopherol may explain the selective excretion of γ-tocopherol from liver through bile.[24,25] α-Tocopherol binding proteins may be present in non-hepatic tissues, although this has not yet been demonstrated in skeletal muscle. The decrease of muscle γ-tocopherol reported here may be related to a direct metabolic exchange between plasma and muscle tissue. It is important to note that even though γ-tocopherol is fat soluble, we found no inverse relation between this tocopherol in muscle and BMI, either at baseline or after vitamin E supplementation. However, plasma concentrations of γ-tocopherol, in contrast to α-tocopherol, were positively correlated with BMI at baseline before supplementation. However, after 30 days of α-tocopherol supplementation, this relationship was diminished. This lack of association can be attributed to the inverse relationship of γ-tocopherol with α-tocopherol as discussed.

Tissue levels of α-tocopherol may have an influence on the oxidative metabolism in the exercising muscle.[20] Interestingly, we observed association of plasma and muscle α-tocopherol with the percentage of fiber types in the gastrocnemius muscle. Subjects with a higher percentage of Type I fibers had relatively lower plasma and, to some extent, lower muscle levels of α-tocopherol than those with a lower percentage of Type I fibers. It is important to note that the specific α-tocopherol concentrations in each fiber type were not determined in this study; rather, they were measured in muscle samples containing a combination of both fiber types. This may have contributed in part to a weaker association of percent Type I fiber with muscle α-tocopherol. As Type I fibers are dependent on the oxidative metabolism of their large number of mitochondria,[5] they may utilize more α-tocopherol (as an antioxidant) than Type II fibers, which produce energy primarily through anaerobic processes. Increases in oxidative stress induced by an intensive bout of eccentric exercise will utilize and reduce vitamin E concentration to prevent oxidative damage to exercising muscle as we have previously shown.[20]

Acknowledgments

The authors would like to thank Timothy S. McElreavy, M.A., for preparation of this manuscript.

References

1 Friden, J., Sjostrom, M., Ekblom, B. (1984). Muscle fiber type characteristics in endurance-trained and untrained individuals. *Eur. J. Appl. Physiol.* **52,** 266–271

2 Holloszy, J.O., Coyle, E.F. (1984). Addoptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* **56,** 831–838

3 Gollnick, P.D., Armstrong, R.B., Saubert, C.W., Piehl, K., Saltin, B. (1972). Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *J. Appl. Physiol.* **33,** 312–319

4 McArdle, W., Katch, F.I., Katch, V.L. (1986). *Exercise Physiology and Energy, Nutrition and Human Performance,* Lea & Febiger, Philadelphia, PA, USA

5 Rehunen, S., Naveri, H., Kuoppasalmi, K., Harkonen, M. (1982). High-enery phosphate compounds during exercise in human slow-twitch and fast-twitch muscle fibres. *Scand. J. Clin. Lab. Invest.* **42,** 499–506

6 Thomason, D.B., Baldwin, K.M., Herrick, R.E. (1986). Myosin isozyme distribution in rodent hindlimb skeletal muscle. *J. Appl. Physiol.* **60,** 1923–1931

7 Burke, R.E., Levine, D.N., Tsairis, P., Zajac, F.E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* (Lond.) **234,** 723–748

8 Drews, G., Engel, W.K. (1966). Reversal of the ATPase reaction in muscle fiber by EDTA. *Nature* (Lond.) **212,** 1551–1553

9 Pette, D., Spamer, C. (1986). Metabolic properties of muscle fibers. *Federation Proc.* **45,** 2910–2914

10 Essen, B., Jansson, E., Henriksson, E., Taylor, A.W., Saltin, B. (1975). Metabolic characteristics of fibre types in human skeletal muscle. *Acta Physiol. Scand.* **95,** 153–165

11 Riley, D.A., Ellis, S., Bain, J.L. (1988). Catalase-positive microperoxisomes in rat soleus and extensor digitorum longus muscle fiber types. *J. Hisotchem. Cytochem.* **36,** 633–637

12 Jenkins, R.R., Newsham, D., Rushmore, P., Tengie, J. (1982). Effect of disuse on the skeletal muscle catalase of rats. *Biochem. Med.* **27,** 195–199

13 Lammi-Keefe, C.J., Hegarty, P.V.J., Swan, P.B. (1980). Effect of starvation and refeeding on catalase and superoxide dismutase activities in skeletal and cardiac muscles from 12-month-old rats. *Experientia* **37,** 25–27

14 Evans, W.J., Phinney, S.D., Young, V.R. (1982). Suction applied to a muscle biopsy maximizes sample size. *Med. Sci. Sports Exerc.* **14,** 101–102

15 Meydani, M., Cohn, J.S., Macauley, J.B., McNamara, J.R., Blumberg, J.B., Schaefer, E.J. (1989). Postprandial changes in the plasma concentration of α- and γ-tocopherol in human subjects fed fat-rich meals supplemented with fat-soluble vitamins. *J. Nutr.* **119,** 1252–1258

16 Padykula, H., Herman, E. (1955). The specificity of the histochemical method for adenosine triphosphatases. *J. Histochem. Cytochem.* **3,** 170–183

17 Meydani, S.N., Meydani, M., Blumberg, J.B., Leka, L.S., Siber, G., Loszewski, R., Thompson, C., Pedrosa, M.C., Diamond, R.D., Stollar, B.D. (1997). Vitamin E supplementation enhances in vivo immune response in healthy elderly: A dose-response study. *JAMA* In press

18 Bjorneboe, A., Bjorneboe, G.A., Drevon, C.A. (1990). Absorption, transport and distribution of vitamin E. *J. Nutr.* **120,** 233–242

19 Gall-Torres, H.E. (1980). Transport and metabolism. In Machlin L.J. *Vitamin E: A Comprehensive Treatise* (L.J. Machlin ed.), p. 193–267, Marcel Dekker, New York, NY, USA

20 Meydani, M., Evans, W.J., Handelman, G., Biddle, L., Fielding, R.A., Meydani, S.N., Burrill, J., Fiatarone, J., Fiatarone, M.A., Blumberg, J.B., Cannon, J.G. (1993). Protective effect of vitamin E on exercise-induced oxidative damage in young and older adults. *Am. J. Physiol.* **33,** R992–R998

21 Traber, M.G., Cohn, W., and Muller, D.P.R. (1993). Absorption transport and delivery of tissues, In *Vitamin E in Health and Disease* (L. Packer and J. Fuchs, eds.) p 35–51, Marcel Dekker, New York, NY

22 Gurusinghe, A., de Niese, M., Renaud, J.F., Austi, L., (1988). The binding of lipoproteins to human muscle cells: Binding and uptake of LDL, HDL, and alpha-tocopherol. *Muscle Nerve* **11,** 1231–1239

23 Ferraro, R.T., Eckel, R.H., Larson, D.E., Fontrielli, A-M., Rising, R., Jensen, D.R., Ravussin, E. (1993). Relationship between skeletal muscle lipoprotein lipase activity and 24-hour macronutrient oxidation. *J. Clin. Invest.* **95,** 1846–1853

24 Traber, M.G. and Kayden, H.J. (1989). Preferential incorporation of α-tocopherol vs. γ-tocopherol in human lipoproteins. *Am. J. Clin. Nutr.* **49,** 517–526

25 Verdon, C., Blumberg, J.B. (1988). An assay for the alpha-tocopherol-binding protein mediated transfer of vitamin E between membranes. *Ann. Biochem.* **169,** 109–120